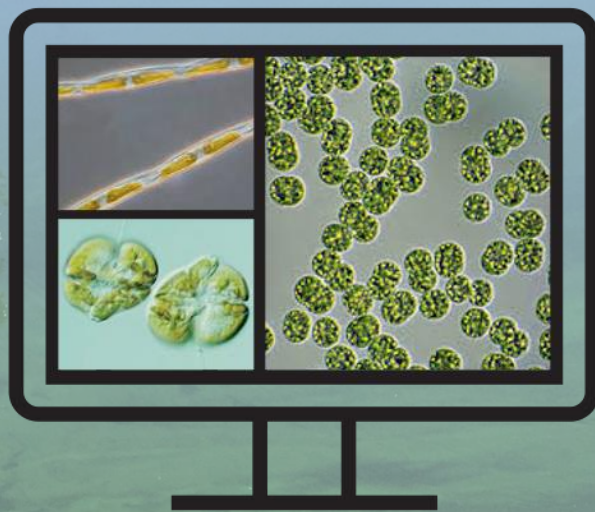# 10.5 U.S. Symposium on Harmful Algae

Emerging Voices and Blooming Careers
May 25-27, 2021 • Virtual

Conference Program

10.5 U.S. SYMPOSIUM ON HARMFUL ALGAE

SYMPOSIUM CHAIR

Jayme Smith — Southern California Coastal Water Research Project

SCIENTIFIC PROGRAM CHAIR

Keith Bouma-Gregson — California State Water Quality Control Board
Alan Wilson — Auburn University

STEERING COMMITTEE

Holly Bowers — Moss Landing Marine Labs
Timothy Davis — Bowling Green State University
Quay Dortch — NOAA National Ocean Service
H. Dail Laughinghouse IV — University of Florida IFAS
Rebecca Gorney — New York State Department of Environmental Conservation
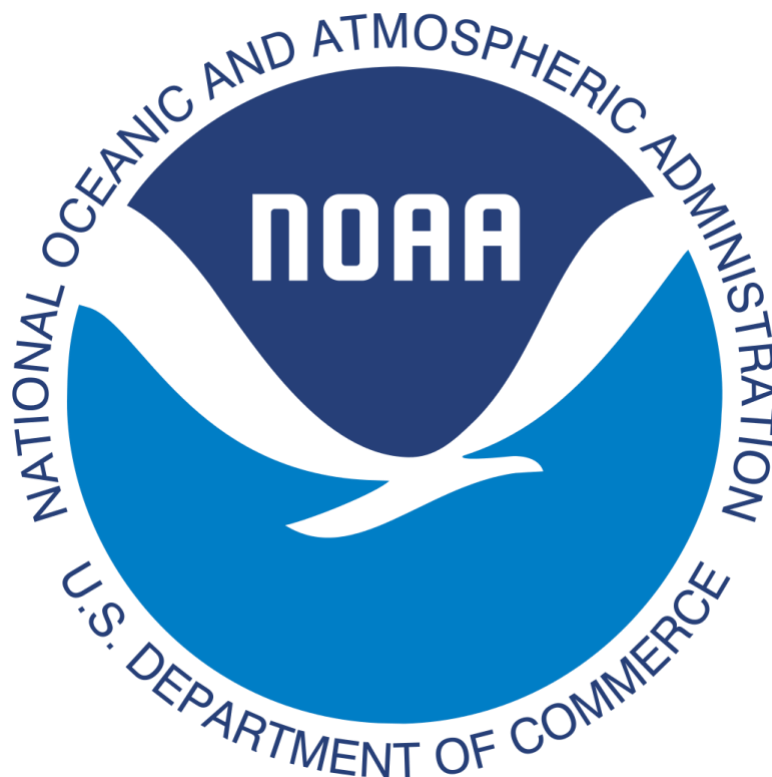Mindy Richlen — Woods Hole Oceanographic Institution
Mary Kate Rogener — NOAA National Ocean Service

SYMPOSIUM SPONSOR

SESSION CHAIRS AND MODERATORS

Monitoring and Management
Chairs: Dianne Greenfield and Silvia Angles

SPEED Talks: Bloom Dynamics and Drivers
Chair: Jayme Smith

Bloom Dynamics 1 & Predictive Models, Forecasting and Climate
Chairs: H. Dail Laughinghouse and Forrest Lefler

Ecophysiology, Food Web Dynamics & Impacts
Chair: Rebecca Gorney

Speed Talks: Microbial Interactions, Food Web Dynamics & Impacts, Mitigation & Management and Cell & Molecular Technology Advances
Chair: Mindy Richlen

Bloom Dynamics 2 & Microbial Interactions
Chair: Keith Loftin

Mitigation and Control
Chairs: Kaytee Boyd And David Berhold

Speed Talks: Human & Animal Public Health, Mitigation & Control and Monitoring & Management
Chairs: Quay Dortch and Mary Kate Rogener

Human and Animal Public Health
Chairs: Lorraine Backer and Amy Lavery

Closing Remarks
Donald Anderson

Public Careers Panel
Moderator: Lesley D'Anglada

Private Careers Panel
Moderator: Stephanie Moore

Hybrid Careers Panel
Moderator: Marc Suddleson

Plenary Moderators
Alison Robertson, Beth Stauffer, and Alan Wilson

"Emerging Voices and Blooming Careers"

On behalf of the National HAB Committee (NHC), we would like to welcome everyone to the US HAB Symposium on Harmful Algae 10.5! As most are aware, our 11th symposium was scheduled to be held in New York later this year, however, due to many conferences having to postpone, specifically ICHA, the NHC in consultation with NOAA and the ICHA organizers, made the decision to push back the 11th symposium to Fall 2022. This delay would create a 3-year gap between US HAB symposia. We realized that this extended gap would primarily impact students, post-docs, and early career scientists in the field, so we made the decision to hold an interim virtual meeting to showcase the work exclusively from these groups. *The response has been overwhelming – more than 430 registrants!* This online venue also provides a way to engage more of the management community, many of whom have been unable to travel to past US HAB meetings due to budget constraints and this has been reflected in the fact that 21% of registrants are joining in from State Management Agencies!

We would like to extend a special thanks to Dr. Jayme Smith for accepting the challenge of leading this unique effort and for putting together a dedicated steering committee to carry out the tasks needed for another successful US HAB meeting!

The planning team has made every effort to incorporate young investigators throughout the planning and execution of this meeting in support of the theme. It is our hope that participants take full advantage of the free registration and enjoy all of the online oral and poster sessions that span our traditional meeting topics. We also urge folks to arrange offline chats during and subsequent to the meeting in order to offer much needed networking opportunities for our young scientists. This meeting serves to support continued collaborations within the research, management and policy communities by creating a forum to exchange scientific information.

While we are looking forward to our next in-person meeting, we are happy to be able to offer this opportunity and are excited to hear about the cutting-edge research from the next generation of HAB scientists!

Holly Bowers
NHC Co-chair

Timothy Davis
NHC Co-chair

TABLE OF CONTENTS

MEETING PLATFORMS AND LOGISTICS

The 10.5 US Symposium on Harmful Algae will be held using a combination of the Zoom Webinar and Meeting platforms. For the best attendance experience, please use the most recent version of the zoom application to your computer. You can install the latest version of the Zoom application by visiting the Zoom website (www.zoom.com)

Oral presentations, career panels and plenaries will be held using the **Zoom Webinar** platform. You must register using the link provided via email to you in order to attend. Registration will provide you with a customized Zoom Webinar link for you to use throughout the meeting to access all Zoom Webinar events. The Zoom Webinar platform only allows for panelists (presenters and session chairs) and hosts (meeting moderator) to be seen and heard. This functionality protects against unintended interruptions during the live presentations. Questions for speakers can be made using the Q&A box. The session chair or moderator will try to address as many questions as possible with available time. We encourage reaching out to presenters with the provided contact information in the meeting program to address additional questions.

Poster sessions and social hours will be held using the **Zoom Meetings** platform using breakout rooms. These sessions require a different link that the oral presentations, career panels and plenaries. This link is provided to you by the conference organizers via email. Once you are admitted to the Zoom Meeting, you can navigate to the breakout room of your choice. The breakout room options can be accessed by navigating to the bottom of your screen and looking for the icon of four squares titled "Breakout". Clicking on the icon will open a pop-up window that lists each breakout room. For the poster sessions, the breakout rooms will be listed by the last name of the poster presenter. For the social hours the breakout rooms will be listed based on HAB genus names.

SOCIAL MEDIA POLICY

Best practice guidelines for using social media during the conference.

- NO recording of any kind (video, audio, photography, etc) scientific content from any session during the week (unless you receive permission from the authors/presenters) to respect presenters that wish to withhold audio-visual material from being recorded and/or posted on social media. This includes photos and screen shots of posters and slides.

- Conference attendees may (and are encouraged to) openly discuss general conference activities on social media. Please use the meeting hashtag #USHAB2021

CAN'T WAIT TO SEE YOU IN NEW YORK!

Pack your antibodies, because the 11th US HAB Symposium will return to a mostly in-person format!
Join us in Fall 2022, when the leaves are colorful in upstate New York.

New York is home to two Great Lakes, extensive Atlantic marine and estuary shoreline and thousands of inland lakes. HAB research and management efforts are extensive in New York.

Through a combination of speakers, interactive workshops, networking, and information and idea sharing, we look forward to your participation and attendance. As always, there will be ample social activities.

Stay tuned for more details soon.

Steering/Host committee:
NYSDEC, NEIWPCC, & USGS

PLENARY PRESENTATIONS

All Plenary Presentations will be hosted via Zoom Webinar

ARE ALGAL BLOOMS GETTING WORSE?

Grace Wilkinson
Assistant Professor, University of Wisconsin –Madison Department of Integrative Biology

Severe algal blooms threaten ecosystem and human health, resulting in billions of dollars in economic loss. The interaction between eutrophication and climate change is hypothesized to be driving widespread intensification of blooms in inland waters, although little work has been done to evaluate this hypothesis. In this talk, I will present recent research exploring trends in algal bloom magnitude and severity from hundreds of lakes across the United States and the conditions that are driving these trends. We found that bloom intensification in inland waterbodies, defined as increasing trends in chlorophyll-a of increasing bloom magnitude, severity, or duration, has not been widespread for hundreds of lakes in the United States. Only 10.8% of the 323 waterbodies analyzed had significant bloom intensification. Conversely, 16.4% of the waterbodies had significant decreasing trends during the same period. Interestingly, the interaction of between severe precipitation and trophic state of a lake was indicative of a waterbody's bloom trajectory. While it is encouraging that bloom intensification isn't currently widespread, continued efforts towards aquatic ecosystem protection and restoration are imperative for maintaining ecosystem services into the future.

Bio: Dr. Grace Wilkinson is a limnologist and ecosystem ecologist with a research focus on aquatic-terrestrial linkages. Her research interests are at the intersection of nutrient cycling, water quality, and ecosystem resilience. In 2021, she started as an assistant professor in the Center for Limnology at the University of Wisconsin – Madison. Prior to that time, she was an assistant professor at Iowa State University.

ADVANCING OBSERVATION NETWORKS IN SUPPORT OF HARMFUL ALGAL BLOOM (HAB) FORECASTING FROM POLAR TO SUB-TROPICAL SYSTEMS

Kate Hubbard
Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute (FWC-FWRI), katherine.hubbard@myfwc.com

Persistent and recurring harmful algal blooms (HABs) negatively impact human, ecosystem, and economic health. Accurate bloom tracking and forecasting can provide resource managers, health departments, and the public with timely information to inform decision-making. This requires several key components*: 1) a sustainable, multiparameter, regional HAB observation network that is highly leveraged, 2) geographically and temporally rich historical observations; 3) integration of new observing datastreams;* and *4) development and advancement of targeted forecasting capabilities.* State-of-the-art observing technology has proven to be critical in timely data collection during bloom events and, moreover, for revealing phenomena that might otherwise be overlooked, such as the importance of diel changes in *Karenia brevis* and *Pseudo-nitzschia* species transitions across environmental gradients. At the same time, repeated and sustained sampling is critical for building time series that can be used to inform and validate predictive models. Implementing a multi-partner (and thus highly leveraged) and innovative network helps ensure that in situ observations of cells and toxins occur routinely throughout the year – capturing key bloom stages and shifts in the physical and chemical environment. This would advance bloom forecasting, recognizing that abiotic and biotic factors such as nutrients, trace metals, and cell physiology are important parameters to include. Recognizing the ecological flexibility displayed by many HABs in lab and field studies, examples will be provided that demonstrate how expanding observational and modeling infrastructure is essential for forecasting and hindcasting the response of HABs to environmental changes occurring across event to multi-decadal time scales, with a focus on polar, temperate, and sub-tropical systems. Also critical to our success in building these comprehensive networks is the ability to communicate scientific results to broad audiences and to creatively span gaps among research, monitoring, and management communities.

Bio: Dr. Kate Hubbard leads the HAB monitoring and research program for the FWC-FWRI. As a research scientist, she works closely with a broad network of partners to sustain and advance comprehensive HAB monitoring. Her expertise in genomics and ecology has also helped evaluate drivers of HABs and other species. Recent enhancements to Florida's HAB monitoring network under her direction have focused on new, enhanced, and/or sustained biological, chemical, and physical observation capabilities. As a Co-Investigator of the NSF/NIEHS-funded Woods Hole Center for Oceans and Human Health at the Woods Hole Oceanographic Institution, Hubbard is involved in HAB detection and forecasting projects across the US to better understand and predict blooms and their impacts and is committed to working at the interface of HAB research and management. She also prioritizes engaging junior scientists in research and communication. She received her B.A. in Biology from New College of Florida and M.S. and Ph.D. degrees in Biological Oceanography from the University of Washington. She has served on the National HAB Committee since 2017.

HARMFUL ALGAL BLOOMS AND OCEAN ACIDIFICATION: DEFINING A RESEARCH AGENDA

Halle Berger
Department of Marine Sciences, University of Connecticut, Groton, CT 06340
National Oceanic and Atmospheric Administration (NOAA), Silver Spring, MD 20910

Harmful algal blooms (HABs) and ocean acidification (OA) are threats to marine ecosystems and human communities. HABs and OA have common drivers in coastal areas and often co-occur in space and time. Although many studies have provided insight into HAB dynamics, only a few of these have included OA. Similarly, OA studies have examined biogeochemistry and impacts to marine resources and have started to expand into multi-stressor studies, but less is known about the influences of HAB-OA interactions and cascading impacts to coastal ecosystems and economies.

The NOAA Ocean Acidification Program (OAP) and National Centers for Coastal Ocean Science (NCCOS) Competitive Research Program (CRP) held a virtual workshop to identify research needs at the intersection of HABs and OA. This presentation will focus on the regional and national grand challenges, research priorities, and research products highlighted at the workshop. Research areas of high priority at the national level include modeling for prediction, attribution, and sensitivity testing; leveraging monitoring assets to measure HAB and OA parameters simultaneously; data management to foster integration; data product development; and enhanced communication and outreach efforts. Importantly, the workshop identified a need for increased collaboration between HAB and OA scientists. Interdisciplinary approaches will be required to disentangle the complexities of HAB-OA interactions and address stakeholder needs. This effort will be facilitated by the NOAA OAP and NCCOS CRP through the development of a community of practice and a federal funding opportunity.

Bio: Halle Berger is a 2021 Sea Grant Knauss Marine Policy Fellow serving as a Coastal Stressors Program Coordinator with the NOAA Ocean Acidification Program and National Centers for Coastal Ocean Science Competitive Research Program. She is working on building a research community to address the overlapping challenges between harmful algal blooms and ocean acidification. Halle is also currently a PhD Student at the University of Connecticut. Her research assesses the vulnerability of important shellfisheries to changing ocean conditions.

Plenary Session will be followed by a Q&A panel with Halle and NOAA Program Managers Quay Dortch, Maggie Broadwater and Erica Ombres.

CAREER PANELS

All Career Panels will be hosted via Zoom Webinar

Students and early career scientists are usually quite familiar with the academic career path, particularly at doctorate granting institutions. These career panels are meant to provide attendees with insight into other career opportunities and highlight how different personal experiences and interests can aid in determining a career path. Panelists will each share a brief description of their positions, an overview of their background, and some key influential experiences. After these introductions, there will be time for questions from the audience!

TUESDAY MAY 25, 2021
3:15-4:00 PM Pacific, Zoom Webinar
Public Careers Working on Harmful Algae
Description: This workshop is focused on career options in public service working for governmental agencies at the federal, state or local level, or with tribal governments.

Panel Moderator: Lesley D'Anglada

Panelists:

Maggie Broadwater, Program Manager, NOAA	Bio: Dr. Maggie Broadwater is a program manager in the Competitive Research Program at NOAA's National Centers for Coastal Ocean Science (NCCOS). She previously worked as a research scientist in the NCCOS Marine Forensics and Marine Biotoxins Programs in Charleston, SC. Dr. Broadwater holds a B.S. in Biochemistry from the College of Charleston, M.S. in Biomedical Sciences, and a Ph.D. in Biochemistry from the Medical University of South Carolina.
Mandy Michalsen, HAB Program Coordinator, US Amry Corps of Engineers	Bio: Dr. Mandy Michalsen is the U.S. Army Engineer Research Development Center's (ERDC's) Harmful Algal Bloom Program Coordinator, currently stationed in Seattle, WA. Mandy's research interests have included novel applications of groundwater remediation technologies to accelerate cleanup of explosives- and chlorinated solvent-contaminated aquifers, as well as use of polymeric samplers for measuring freely dissolved contaminants in sediment porewater. Since joining USACE in 2008, Mandy has served as Principal Investigator and lead engineer on field-scale technology demonstrations and full-scale groundwater remedy optimization projects, resulting in multiple peer-reviewed research papers. She received her bachelor's degree in Civil Engineering from University of Iowa in Iowa City, IA and both her master's and doctorate degrees in Civil Engineering from Oregon State University in Corvallis, OR. Prior to joining ERDC in November 2014, Mandy was Chief of Soils at Seattle District USACE.
Meredith Howard, Environmental Program Manager,	Bio: Dr. Meredith Howard received her B.A. in Finance from Lehigh University in 1995, B.S. in Biology from Rutgers University in 2001 and Ph.D. in Ocean Science from the University of California, Santa

Central Valley Regional Water Quality Control Board	Cruz in 2007. Dr. Howard joined the Southern California Coastal Water Research Project in 2007 and her research focused on examination of the environmental factors that influence phytoplankton blooms, with a special emphasis on characterizing the effects of anthropogenic nutrient sources on blooms and HABs. Her work has challenged conventional ideas about the impacts of natural versus anthropogenic nutrient sources in coastal California. Her research focused on the transport of cyanotoxins across the freshwater-to-marine continuum and the improvement of monitoring tools that can address the challenges of monitoring inter-connected waterbodies and watersheds. In 2018 she moved into a regulatory and policy making position as an Environmental Program Manager at the Central Valley Water Board. Through this position, she focuses on shaping management discussions and decisions related to anthropogenic inputs and changes to the way that inland and coastal water quality is assessed, monitored, and ultimately regulated. She works with water quality regulatory agencies (federal, state, and local), California Tribes, and the scientific community to develop mitigation and management strategies for HABs.
Chris Whitehead, Environmental Program Manager, Sitka Tribe of Alaska	Bio: Chris Whitehead completed his degree in Environmental Marine Science at the University of Hawaii Manoa where he focused on marine biology, fisheries management, and environmental policy. He has over 18 years of expert experience working with non-profit, city, state, federal, and Tribal government agencies writing and managing grants, designing research projects, establishing water quality programs, developing shellfish aquaculture operations, managing fisheries, building environmental laboratories, and creating large regional networks of researchers, Tribal environmental staff, citizen scientist, and resource managers.

WEDNESDAY MAY 26, 2021
3:15-4:00 PM Pacific, Zoom Webinar
Private Careers Working on Harmful Algae

Description: This workshop is focused on career options in private industry including opportunities with private companies that do research & development, or environmental monitoring and consulting.

Panel Moderator: Stephanie Moore

Panelists:

Sarah Bickman, Senior Scientist, Product Manager, LightDeck Diagnostics	Bio: Dr. Sarah Bickman has worked at LightDeck for more than 8 years as a senior scientist and the product manager for the food and water products. Her recent work has focused on detection of toxins generated by harmful algal blooms including detection in fresh and salt water and shellfish. Additionally, she contributed to the design and understanding

	of the LightDeck system, optimizing system performance, and statistical analysis. Prior to coming to LightDeck, Dr Bickman worked at Vescent Photonics designing lasers and optics and at the National Institutes of Standards working on developing optical clocks. She has a PhD from Yale University in atomic, molecular, and optical physics and a BS from Amherst College in both physics and anthropology.
Tom Fougere, Senior Engineer, McLane Research Laboratories	Bio: Tom Fougere is currently the Engineering Team Manager in charge of Imaging Flow CytoBot (IFCB) & Environmental Sample Processor (ESP) production at McLane Research Labs. He was born and raised on Cape Cod and as such I have always felt a close connection to the ocean & marine environment. Fougere graduated from of the University of Rhode Island Ocean Engineering program (Class of 2005). He has enjoyed almost 10 years of employment at McLane. Prior to his current employment, he spent 7 years working for General Dynamics in the ship building industry.
Ellen Preece, Senior Limnologist, Robertson-Bryan Inc.	Bio: Dr. Ellen Preece received her M.S. and Ph.D. from Washington State University. Dr. Preece investigates cyanotoxins in freshwater, estuarine, and marine ecosystems and applies this research to determine cyanobacterial harmful algal bloom risks to human health. An important component of her research in to ensure that results are available to local, state and federal groups to sustain the ecological integrity of freshwater ecosystems while protecting human users, especially underserved communities.
Tim Otten, Director Bend Genetics	Bio: Dr. Tim Otten has been involved in harmful algal bloom research since 2007. He first became interested in the nexus between environmental quality and human health while working towards a Master of Public Health degree from George Washington University. He subsequently enrolled at UNC-Chapel Hill and earned a PhD for work focused on understanding the environmental factors that select for toxigenic strains of cyanobacteria. Afterward, he spent three years as a postdoc in the Microbiology Department at Oregon State University where he learned how to apply metagenomic tools to further investigate toxic algal blooms throughout the West. Recognizing that there was an ever-growing demand for HAB-related analyses, yet relatively few commercial laboratories dedicated to the field, Dr. Otten founded Bend Genetics in 2015 in order to offer customers with rapid and integrated analyses for all aspects of harmful algal bloom monitoring.

THURSDAY MAY 27, 2021
3:15-4:00 PM Pacific, Zoom Webinar
Hybrid Careers Working on Harmful Algae

Description: This workshop is focused on career options that do not neatly fit into the categories of public or private, such as NGOs, extension agents, regional observing associations, etc., careers that involve multiple sectors, and academic positions in non-doctorate-granting institutions.

Panel Moderator: Marc Suddleson

Panelists:

Mary Kate Rogener, Program Analyst, NOAA under CSS-Inc contract	Bio: Dr. Mary Kate Rogener is under contract with CSS-Inc, in support of NOAA's National Centers for Coastal Ocean Science, Competitive Research Program as a Coastal Ecology Program Analyst. She informs and guides relevant HAB and hypoxia science priorities, policies, and projects through identification and application of stakeholder needs. Prior to NOAA, Mary Kate was a 2018 John A. Knauss Policy Fellow where she was placed with the Office of the Oceanographer of the Navy. While with the Oceanographer, Mary Kate participated in many ocean policy groups and served as the science expert on interagency and internal Department of Defense documents relating to ocean observations, climate change, environmental security, science and technology, and research and development. She graduated from the University of Georgia with a Ph.D. in marine sciences and a B.A in marine sciences from Boston University. Her doctoral research largely focused on human impacts to the marine ecosystem (Gulf of Mexico hypoxic zone, Deepwater Horizon impacted locations, coastal Georgia, and the Arctic Ocean), with emphasis on greenhouse gas emissions, nutrient dynamics, and low oxygen conditions.
Clarissa Anderson, Executive Director, Southern California Coastal Ocean Observing System	Bio: Dr. Clarissa Anderson is a biological oceanographer with expertise in ecological forecasting and remote sensing. After receiving a B.A. in Biology and Art History at UC Berkeley and a Marine Science Ph.D. at UC Santa Barbara, she completed several postdoctoral appointments before transitioning into a professional research position at UC Santa Cruz. The majority of her research has focused on the prediction of harmful algal blooms and toxins in estuarine and coastal ecosystems as well as the fate and transport of harmful toxins to deeper waters and sediments. During her time as research faculty at UC Santa Cruz, she worked to establish the California Harmful Algae Risk Mapping (C-HARM) system with NASA Applied Science support. She is now at Scripps Institution of Oceanography directing the Southern California Coastal Ocean Observing

	System (SCCOOS) and continuing to conduct research on phytoplankton ecology in coastal California. She is an elected member of the UNESCO SCOR GlobalHAB Scientific Steering Committee, the Science Advisory Team for the CA Ocean Protection Council (OPC), the U.S. National HAB Committee (NHC), and the Steering Committee for the Harmful Algal Bloom Monitoring and Alert Program (Cal-HABMAP).
Heather Raymond, Director of Water Quality Initiative, OSU CFAES	Bio: Heather Raymond is the Water Quality Initiative Director for The Ohio State University's College of Food Agricultural and Environmental Sciences. In this role she helps coordinate applied interdisciplinary water quality research that addresses the needs of local, state, and federal partners and integrates research findings into extension outreach. Prior to accepting her position at OSU, Heather served as the State of Ohio Harmful Algal Bloom (HAB) Coordinator where she led development of the nation's first HAB monitoring and reporting rules, assisted public water systems and lake managers respond to HABs, conducted applied research on HAB treatment in coordination with U.S. EPA and University partners, and taught webinars and workshops on HAB response. She serves on the National HAB Committee and Great Lakes HABs Collaborative Steering Committee and is a contributing author to state, federal, and international HAB guidance. She has over twenty years of water quality related government experience and earned master's degrees in science and public administration from Ohio University.
Morgan Steffen, Associate Professor, James Madison University	Bio: Dr. Morgan Steffen is an Associate Professor of Biology at James Madison University in Harrisonburg, Virginia. Her research focus is on microbial interactions in freshwater cyanobacterial harmful algal blooms, with a focus on *Microcystis* blooms around the world. Currently the lab is trying to understand how heterotrophic bacteria support *Microcystis* bloom formation and success. JMU is a primarily undergraduate institution, so her lab is mainly comprised of undergraduate researchers who participate in every level of research from field sample collection to manuscript writing.

SYMPOSIUM SCHEDULE

TUESDAY MAY 25, 2021

All oral sessions will take place on the Zoom Webinar Platform

8:30   Welcome and Opening Remarks
Jayme Smith and TJ Lowdermilk

ORAL SESSIONS 1: Monitoring and Management
Chair: **Dianne Greenfield and Silvia Angles**

Time (Pacific Time)	Presenter	Title
8:45	Nora Straquadine	Ability of Asian Clams (*Corbicula Fluminea*) to Feed on and Differentiate Between Toxic Bloom-Forming Cyanobacteria and Other Algae
9:00	Edna Fernandez-Figueroa	Commercially Available Unoccupied Aerial Systems for Monitoring Harmful Algal Blooms: A Comparative Study
9:15	Courtney Hart	Using Cyst Mapping to Uncover Trends of PSP Toxicity in Southeast Alaska Geoduck Harvest Areas
9:30	Angelea Belfiore	Zooplankton Improve Water Quality in Farm Pond Aquaculture
9:45	Seth Buchholz	Rapid Cyanotoxin Detection Technology in Routine Monitoring and Citizen Science Groups

SPEED TALK SESSIONS 1: Bloom Dynamics and Drivers
Chair: **Jayme Smith**

10:00	Evangeline Fachon	Characterizing *Pseudo-nitzschia* Species Diversity Across Environmental and Spatial Gradients in The Alaskan Arctic
10:05	Colleen Yancey	Structural Variation of the mcy Operon During a *Microcystis*-Dominated Cyanobacterial Bloom Reveals Dynamics of Novel and Known Genotypes
10:10	Malcolm Barnard	Macronutrient and Vitamin Influences on Chowan River (North Carolina, USA) CyanoHABs

10:15	Aubrey Trapp	Eavesdropping on Predator-Prey Dynamics - The Case for Including Zooplankton in HAB Toxin Forecasting
10:20	Claudia Carrion	Ciguatera Toxicity Patterns in Red Hind, *Epinephelus Guttatus*, on Coral Reefs of St. Thomas, US Virgin Islands
10:25	Kyra Florea	Community domination by toxic *Microcystis* alters the microbial community structure in Clear Lake, CA
10:30-10:45	**Break**	

10:45-11:30   **Plenary, Grace Wilkinson, University of Wisconsin - Madison: ARE ALGAL BLOOMS GETTING WORSE?**
Moderator: Alison Robertson

11:30-12:00   **Break**

12:00-1:00   **Poster Session 1:**
Bloom Dynamics; Monitoring & Management

See poster schedule below for list of poster presentations

1:00-1:15   **Break**

ORAL SESSIONS 2: Bloom Dynamics 1 & Predictive Models, Forecasting and Climate
Chairs: **Dail Laughinghouse and Forrest Lefler**

1:15	Rachel Brewton	Extreme Primary Producer Shifts in Florida's Northern Indian River Lagoon and Banana River
1:30	Jacqueline Chrabot	Vertical Swimming Speeds of the Dinoflagellate Species *Margalefidinium polykrikoides* in the Lafayette River
1:45	Matthew Gladfelter	Available Dissolved Nitrogen Form Mediates Phycocyanin Content in Cyanobacteria
2:00	Kyla Kelly	*Pseudo-nitzschia multiseries* Toxicity Increases Under Projected Future Ocean Scenarios: A Multiple Stressor Approach
2:15	Gihong Park	Grazer-Induced Toxin Production Under Climate Change in the Neurotoxic Dinoflagellate *Alexandrium catenella*

2:30	Brendan Turley	O Hypoxia, Where Art Thou? Linking Hypoxia to Red Tide on the West Florida Shelf
2:45	Shiqi Fang	Assessing and Predicting Microcystin Variability in Western Lake Erie

3:00-3:15 **Break**

3:15-4:00 **CAREER PANEL 1:** **Public Careers Working on Harmful Algal Blooms**
Moderator: Lesley D'Anglada
Panelists: Maggie Broadwater (NOAA), Mandy Michalsen (US Army Corps of Engineers), Meredith Howard (Central Valley Regional Water Quality Control Board), Chris Whitehead (Environmental Program Manager, Sitka Tribe of Alaska)

4:15-5:15 Virtual Happy Hour (Zoom Meeting Platform) – Speed Dating

WEDNESDAY MAY 26, 2021

All oral sessions will take place on the Zoom Webinar Platform

ORAL SESSIONS 3: Ecophysiology, Food Web Dynamics & Impacts
Chair: **Rebecca Gorney**

Time (Pacific Time)	**Presenter**	**Title**
8:30	Clayton Bennett	Kinetics of an Experimental Depuration of Caribbean Ciguatoxin in Fish (*Lagodon rhomboides*)
8:45	Michelle Edwards	Multiple Harmful Algal Bloom Toxins Detected in A Top Predator in the Indian River Lagoon, Florida
9:00	Megan Ladds	Use of a Combined High Throughput Sequencing and Fluorometry Method to Assess Grazing on Cyanobacteria by *Daphnia* spp. and the Natural Protozooplankton Community During Cyanobacterial Blooms in Western Lake Erie, NY
9:15	Andrea Jaegge	Impacts of Non-Toxic *Microcystis aeruginosa* on Oyster Feeding

9:30	So Hyun (Sophia) Ahn	Dynamic Photophysiological Responses of Dinoflagellate *Karenia mikimotoi* Under Interactions Between Different Nitrogen Forms, Amounts, and Temperatures
9:45	Erik Broemsen	*Karlodinium veneficum* Ecotypes: Implications of Temperature Effects on Cell Division Time

SPEED TALKS SESSION 2: Microbial Interactions, Food Web Dynamics & Impacts, Mitigation & Management and Cell & Molecular Technology Advances
Chair: **Mindy Richlen**

10:00	Cong Fei	*Karenia brevis*-Associated Bacterial Community Composition and its Influence on Algal Growth and Biodegradation of Brevetoxin
10:05	Amanda Muni-Morgan	No Cells, No Toxins, Not So Fast. Why Mitigation of *Karenia brevis* Requires a Two-Pronged Approach
10:10	Ewaldo Leitao	Allelopathy Effects on *Alexandrium catenella* by Non-HAB Phytoplankton is Dampened Under Greenhouse Conditions
10:15	Ernest Williams	In-vitro and In-vivo Biochemical Methods to Separate Lipid and Toxin Biosynthesis in Dinoflagellates
10:20	Miranda Judd	A Strategy for Gene Knockdown in Dinoflagellates

10:25-10:45	**Break**
10:45-11:30	**Plenary, Kate Hubbard, Fish and Wildlife Research Institute: ADVANCING OBSERVATION NETWORKS IN SUPPORT OF HARMFUL ALGAL BLOOM (HAB) FORECASTING FROM POLAR TO SUB-TROPICAL SYSTEMS** **Moderator: Alan Wilson**
11:30-12:00	**Break**
12:00-1:00	**Poster Session 2:** **Cell & Molecular Technology Advances; Ecophysiology, Food Web Dynamics & Impacts; Microbial Interactions; Engaging Citizens & Stakeholders**

See poster schedule below for list of poster presentations

1:00-1:15   **Break**

ORAL SESSIONS 4: Bloom Dynamics 2 & Microbial Interactions
Chairs: **Keith Loftin**

1:15   Benjamin Kramer   Nitrogen and Phosphorus Sources and Limitation Significantly Alters the Growth, Nitrogen Fixation, Anatoxin-a Content, and Transcriptome of the Bloom-Forming Cyanobacteria, *Dolichospermum*

1:30   Kevin Tyre   Septic System – Groundwater – Surface Water Interactions in Aging Residential Finger Canals Contribute to Harmful Algal Blooms in the Caloosahatchee River Estuary

1:45   Daniel Hoffman   Internal Nitrogen Loading Supports Harmful Cyanobacteria Blooms and Toxicity in Western Lake Erie

2:00   James Fiorendino   Growth Response of a New North American Isolate of *Dinophysis* to Temperature, Salinity, and Irradiance

2:15   Kai Wang   Coordinated Diel Gene Expression of Cyanobacteria and Their Microbiome

2:30   Malia Gardner   Tracing Nitrogen Utilization in the *Microcystis* Phycosphere

2:45   Dominique Lockwood   Bacterial Community Structure of Non-Axenic Cyanobacterial Cultures

3:00-3:15   Break

3:15-4:00   CAREER PANEL 2:   Private Careers Working on Harmful Algal Blooms
Moderator: Stephanie Moore
Panelists: Sarah Bickman (LightDeck), Tom Fougere (McLane), Ellen Preece (Robertson-Bryan Inc.) and Tim Otten (Bend Genetics)

4:15-5:15   Virtual Happy Hour (Zoom Meeting Platform) – Choose your own breakout room!

THURSDAY MAY 27, 2021

All oral sessions will take place on the Zoom Webinar Platform

ORAL SESSIONS 5: Mitigation and Control
Chair: **Kaytee Boyd and David Berhold**

Time (Pacific Time)	Presenter	Title
8:30	Dominique Derminio	Preliminary treatments using UV-C, nanobubbles, and ultrasonification on lake water for remediation of cyanobacterial harmful algal blooms
8:45	Peter Sylvers	Mitigation of harmful algal blooms caused by *Alexandrium catenella*, and saxitoxin accumulation in bivalves, using cultivable seaweeds
9:00	Taylor Armstrong	The Use of Brewer's Spent Grain and various flavonoids in Inhibiting Harmful Algal Blooms
9:15	Yanfei Wang	Molecular and metabolomic insights of the effects of an algicidal bacterial derivative IRI-160AA on dinoflagellate *Karlodinium veneficum*
9:30	Jens Wira	The Use of Flavonoids and Cyclodextrins in Inhibiting Harmful Algal Blooms
9:45	Samuel Putnam	Water column release of toxins from Lyngbya wollei as a consequence of remediation

SPEED TALKS SESSION 3: Human & Animal Public Health, Mitigation & Control and Monitoring & Management
Chair: **Quay Dortch and Mary Kate Rogener**

10:00	Wannes Van Hassel	Molecular Origin of Microcystins in Dietary Supplements Originating from the Klamath Lake, Oregon.
10:05	Gretchen Johnson	Optimizing Potency of Algicidal Exudates Against *Karenia brevis*
10:10	Morgan Petrovich	Use of Genetic Methods to Monitor Toxic Cyanobacteria Blooms

10:15	Edith Martinez	Assessment of Cavitation as a Destructive Technology for Cyanobacteria and Their Toxins
10:20	Sarah Zetterholm	Novel Graphene-Based Composite Materials for Management of Harmful Algal Blooms
10:25	Victoria Roberts	Clay Treatment of Red Tide: Impacts on Blue Crab

10:30-10:45	**Break**
10:45-11:30	**Plenary, Halle Berger, John A. Knauss Fellow, 2021: HARMFUL ALGAL BLOOMS AND OCEAN ACIDIFICATION: DEFINING A RESEARCH AGENDA – Followed by Q&A with Quay Dortch, Maggie Broadwater and Erica Ombres** **Moderator: Beth Stauffer**
11:30-12:00	**Break**
12:00-1:00	**Poster Session 3:** **Predictive Models, Forecasting & Climate** **Human & Animal Public Health; Mitigation & Control; Innovation In HAB Detection** **See poster schedule below for list of poster presentations**
1:00-1:15	**Break**

ORAL SESSIONS 6: Human and Animal Public Health
Chairs: **Lorraine Backer and Amy Lavery**

1:15	Kathleen Aarvig	Examining Trends in Harmful Algal Bloom-Associated Emergency Department Visits in the United States from 2017-2020
1:30	Muhammad Thuneibat	The One Health Harmful Algal Bloom System: Public Health Surveillance for Harmful Algal Bloom Events and Associated Human and Animal Illnesses for Years 2016-2019
1:45	Tabitha Phillips	Hunting the Eagle Killer: A Cyanobacterial Neurotoxin Causes Vacuolar Myelinopathy
2:00	Haley Plaas	Harmful Cyanobacterial Bloom Aerosolization Dynamics and Microcystin Production in the Chowan River, NC

2:15	Isadora Nogueira	Challenges of Implementing a California HAB Health Impact Surveillance System, 2018-2020

2:30-3:00   **Closing Remarks, Don Anderson**

3:00-3:15   **Break**

3:15-4:00   **CAREER PANEL 3:**   **Hybrid Careers Working on Harmful Algal Blooms**
Moderator: Marc Suddleson
Panelists: Clarissa Anderson (SCCOOS), Heather Raymond (OSU CFAES), Mary Kate Rogener (NOAA Affiliate) and Morgan Steffen (JMU)

POSTER PRESENTATION SCHEDULE

POSTER SESSION 1: TUESDAY MAY 25, 2021 – 12:00-1:00PM PACIFIC

*All Poster Session will occur via the provided Zoom Meeting link. The poster number will correspond to the breakout room dedicated to each poster. *Indicates a poster with a corresponding Speed Talk*

BLOOM DYNAMICS

Name	Title	Breakout Room #
Zabdiel Roldan Ayala	Report on Harmful Algal Bloom Species Detected in Long Island Sound During the Summer Of 2020	1
Christina Chadwick	Multi-Year Sampling Provides Novel Insight into Linkages Between *Pseudo-nitzschia* Species Dynamics and Domoic Acid in The Nearshore Gulf of Maine	2
Evangeline Fachon	Characterizing *Pseudo-nitzschia* Species Diversity Across Environmental and Spatial Gradients in The Alaskan Arctic	*3
Claudia Carrion	Ciguatera Toxicity Patterns in Red Hind, *Epinephelus Guttatus*, on Coral Reefs of St. Thomas, US Virgin Islands	*4
Colleen Yancey	Structural Variation of the mcy Operon During a *Microcystis*-Dominated Cyanobacterial Bloom Reveals Dynamics of Novel and Known Genotypes	*5
Malcolm Barnard	Macronutrient and Vitamin Influences on Chowan River (North Carolina, USA) CyanoHABs	*6
Aubrey Trapp	Eavesdropping on Predator-Prey Dynamics - The Case for Including Zooplankton in HAB Toxin Forecasting	*7

| Kyra Florea | Community domination by toxic *Microcystis* alters the microbial community structure in Clear Lake, CA | ***8** |

MONITORING & MANAGEMENT

Name	Title	Breakout Room #
Dana Shultz	Cyanobacterial Blooms and Eutrophication in Recreational Lakes Near Los Angeles, California	**9**
Savannah Mapes	Using a FlowCam to Facilitate HAB Cell Counts: Comparing Cell Enumeration Methods	**10**
Alicia Hoeglund	Integrating HABscope into Florida's Harmful Algal Bloom Monitoring Network	**11**
Katelyn Brown	Investigation into the Environmental Drivers of Microcystin and Saxitoxin in Harmful Algal Blooms in Chautauqua Lake, NY	**12**

POSTER SESSION 2: WEDNESDAY MAY 26, 2021 – 12:00-1:00PM PACIFIC

All Poster Session will occur via the provided Zoom Meeting link. The poster number will correspond to the breakout room dedicated to each poster. *Indicates a poster with a corresponding Speed Talk

CELL AND MOLECULAR TECHNOLOGY ADVANCES

Name	Title	Breakout Room #
Ewaldo Leitao	Allelopathy Effects on *Alexandrium catenella* by Non-HAB Phytoplankton is Dampened Under Greenhouse Conditions	***1**
Ernest Williams	In-vitro and In-vivo Biochemical Methods to Separate Lipid and Toxin Biosynthesis in Dinoflagellates	***2**

| Ricardo Colon | Redox Proteomics and The Essential Components of The Xanthophyll Cycle in High and Low Toxic *Karenia brevis* | **3** |

ECOPHYSIOLOGY, FOOD WEB DYNAMICS & IMPACTS

Name	Title	Breakout Room #
Amanda Muni-Morgan	No Cells, No Toxins, Not So Fast. Why Mitigation of *Karenia brevis* Requires a Two-Pronged Approach	***4**
Ewaldo Leitao	Allelopathy Effects on *Alexandrium catenella* by Non-HAB Phytoplankton is Dampened Under Greenhouse Conditions	***5**
Emily Healey	Assimilation of Nitric Oxide by *Heterosigma akashiwo* in the Presence of Other Nitrogen Sources	**6**
Jordana Cutajar	Spatial-Temporal Variability in Phytoplankton biomass and Composition in Texas Residential Canals	**7**

MICROBIAL INTERACTIONS

Name	Title	Breakout Room #
Cong Fei	*Karenia brevis*-Associated Bacterial Community Composition and its Influence on Algal Growth and Biodegradation of Brevetoxin	***8**
Jennifer Harper	Spatial Distribution of Microbial Communities from the 2018 Lake Erie HABs Grab	**9**
Lucy Utz	Bacterial Antagonism in a Lake Tai (Taihu) *Microcystis* Bloom	**10**

ENGAGING CITIZENS AND STAKEHOLDERS

Name	Title	Breakout Room #

| Daniela Maizel | Study of Environmental Exposure and Long-Term Health Impacts of Cyanobacterial Harmful Algal Blooms in Waters from South Florida | **11** |

POSTER SESSION 3: THURSDAY MAY 27, 2021 – 12:00-1:00PM PACIFIC

*All Poster Session will occur via the provided Zoom Meeting link. The poster number will correspond to the breakout room dedicated to each poster. *Indicates a poster with a corresponding Speed Talk*

PREDICTIVE MODELS, FORECASTING AND CLIMATE

Name	**Title**	**Breakout Room #**
Allison Moreno	Estimating *Pseudo-nitzschia* Domoic Acid Production in a Changing World	**1**
Johnathan Evanilla	Forecasting Shellfish Toxicity in Coastal Maine Using Toxin Data, Environmental Covariates, And Deep Learning	**2**
Tryston Metz	Modeling Degradation of Paralytic Shellfish Toxins in Freshwater Systems	**3**

HUMAN AND ANIMAL PUBLIC HEALTH

Name	**Title**	**Breakout Room #**
Wannes Van Hassel	Molecular Origin of Microcystins in Dietary Supplements Originating from the Klamath Lake, Oregon	***4**
Muriel Dittrich	Using Harmful Algal Bloom Monitoring to Understand PSP Risks and Mitigate Testing Burdens at a Shellfish Farm in Southeast Alaska	**5**

MONITORING, MANAGEMENT, MITIGATION AND CONTROL

Name	Title	Breakout Room #
Gretchen Johnson	Optimizing Potency of Algicidal Exudates Against *Karenia brevis*	***6**
Morgan Petrovich	Use of Genetic Methods to Monitor Toxic Cyanobacteria Blooms	***7**
Edith Martinez	Assessment of Cavitation as a Destructive Technology for Cyanobacteria and Their Toxins	***8**
Sarah Zetterholm	Novel Graphene-Based Composite Materials for Management of Harmful Algal Blooms	***9**
Victoria Roberts	Clay Treatment of Red Tide: Impacts on Blue Crab	***10**
Byran Fuhrmann	A Novel Phosphorus Remediation Technology for Flowing Waters	**11**

INNOVATION IN HAB DETECTION

Name	Title	Breakout Room #
Elizabeth Mudge	Boronate Affinity Polymers for Selective Extraction, Concentration, and Cleanup of Ciguatoxins in Fish Extracts	**12**
Rosaly Castorena	Methods Towards Measuring the Impacts and Cycling of Domoic Acid in Benthic Environments	**13**

ORAL PRESENTATION ABSTRACTS
(Ordered alphabetical by author)

EXAMINING TRENDS IN HARMFUL ALGAL BLOOM-ASSOCIATED EMERGENCY DEPARTMENT VISITS IN THE UNITED STATES FROM 2017-2020

Kathleen E Aarvig, Amy M. Lavery, Lorraine Backer

Centers for Disease Control

Introduction: We previously demonstrated the value of using electronic health records (EHR) to enhance public health surveillance of harmful algal bloom (HAB)-related outcomes. As a continuation of this work, we analyzed EHR reporting HAB-related exposures from 2017-2020 and compared the records from the first three years with those reported during the COVID-19 pandemic to assess changes in geographic distribution and frequency of records over time.
Methods: The CDC's National Syndromic Surveillance Program (NSSP) captures data from emergency departments across the country. A query comprising HAB-associated key terms and HAB exposure diagnostic codes was developed to identify HAB-associated ED visits from January 1, 2017 to December 31, 2020. Descriptive analyses summarized data by demographic characteristics, location, and time.
 Results: A total of 440 HAB-associated ED visits were identified from 2017-2020, of which 48 (11%) occurred in 2017, 270 (61%) in 2018, 75 (11%) in 2019, and 47 (11%) in 2020. In the 2020 NSSP dataset, nearly half of HAB-associated ED visits (46.8%) occurred from June - August. Of all 47 visits in 2020, most patients were aged 18-44 years (68.1%) and received care from hospitals in the Southeast region (Region IV) of the United States (29.8%). These same patterns were observed in 2017-2019 NSSP datasets.
Conclusion: The total number of HAB-associated ED visits differed from year to year. Fewer ED visits in 2020 could reflect hesitancy to seek care during the COVID-19 pandemic. However, ED visits over the year still maintained a seasonal pattern consistent with the previous three years.

DYNAMIC PHOTOPHYSIOLOGICAL RESPONSES OF DINOFLAGELLATE KARENIA MIKIMOTOI UNDER INTERACTIONS BETWEEN DIFFERENT NITROGEN FORMS, AMOUNTS, AND TEMPERATURES

[1]So Hyun (Sophia) Ahn, [1]Patricia Glibert, [2]Stacie Flood, [2]Cynthia Heil

[1]*University of Maryland Center for Environmental Science, Horn Point Laboratory*
[2]*Mote Marine Laboratory*

Karenia mikimotoi is a toxic dinoflagellate found in worldwide coastal waters, including in Florida coastal waters, co-occurring with *K. brevis*. Although it is a mixotroph, *K. mikimotoi* is primarily photo-autotrophic and prefers chemically reduced nitrogen (N). Here we examined the photosynthesis-irradiance responses of exponentially growing *K. mikimotoi* culture to different temperatures (15, 20, 25, 30°C) and when pulsed with different nitrogen forms (nitrate, ammonium, urea) and amounts (1, 5, 10, 20, 50 µM). Using PAM fluorometry, several patterns emerged. First, the fluorescence parameters all suggested substantial stress when the cells were exposed to 30°C even for 1 hr, although the stress response was relatively less for the urea treatment samples. After overnight exposure to these temperatures, cells did not recover, indicating photodamage. These results suggest that sensitivity of this species to high temperature may limit its success in warm summer waters of the Gulf of Mexico. Second, even though cultures had residual N in the media, maximum Photosystem Ⅱ (PS Ⅱ) photochemical efficiency, Fv/Fm, the yield of PS Ⅱ photochemical efficiency under the light, Y(Ⅱ), and relative electron transfer rate (rETR) increased with the pulsed additions of N (all N forms) in the 15, 20, 25°C treatments, but this effect was largest with cells pulsed with ammonium and smallest with urea. These results indicate the dynamic response of photosynthesis to changes in N supply and suggest that the pull of metabolism may buffer the stress of high light, but the extent of photoprotection depends on N form

THE USE OF BREWER'S SPENT GRAIN AND VARIOUS FLAVONOIDS IN INHIBITING HARMFUL ALGAL BLOOMS

Taylor Armstrong, Allen Place

University of Maryland Center for Environmental Science-Institute of Marine and Environmental Technology

Alternatives to synthetic algicides are necessary to avoid indirect negative ecosystem consequences when treating Harmful Algal Blooms. In recent years, the use of natural secondary metabolites produced by plants and bacteria have received considerable attention as alternative control mechanisms. For instance, barley straw bales are regularly used for growth control of cyanobacteria as a widespread management technique in lakes. Barley likely inhibits cyanobacteria due to the release of flavonoids and other phenolic content during the breakdown of the barley. However, barley straw dispersal can be time consuming, labor intensive, and its effectiveness is dependent on deploying bales ~12 weeks prior to the bloom formation. Thus, it is better as a preventative measure than a remediation technique. Brewer's Spent Grain (BSG) is the insoluble part of barley grain husks that constitutes as much as 85% of breweries total byproducts. Since the husk contains much of the phenolic components of barley grain, the phenolic acids and flavonoids are an estimated fivefold concentration higher in BSG to that of barley straw. Here, we: 1. Investigate the impact of BSG and various purified flavonoids on *Microcystis aeruginosa*, *Karlodinium veneficum*, *Karenia brevis*, and *Scenedesmus obliquus*, 2. Separate and identify some of the BSG algicidal compounds using HP-20 resin and HPLC, and 3. Understand the mode of action of the purified flavonoids and BSG algicidal fraction on Microcystis aeruginosa photosynthesis, membrane integrity, esterase activity, and production of reactive oxygen species.

ZOOPLANKTON IMPROVE WATER QUALITY IN FARM POND AQUACULTURE

Angelea P Belfiore, Riley P. Buley, Edna G. Fernandez-Figueroa, Matthew F. Gladfelter, Alan E. Wilson

Auburn University

Warming temperatures and extended growing seasons conducive for algal blooms are further exacerbated in shallow aquaculture ponds due to high nutrient loading from fish feed and nitrogenous waste. In pond aquaculture, toxin production and off-flavor compounds by cyanobacteria can negatively affect fish health and production. Although chemical and physical methods for control can be effective in aquaculture, such treatments may be short-lived and can negatively impact non-target organisms, including aquaculture species. Food web manipulations have a long history in lake and fisheries management to improve water quality, but have been rarely considered in aquaculture. This study from August 2018 to June 2020 examined zooplankton and phytoplankton communities, cyanobacterial toxins, and nutrients, in nine catfish aquaculture farm-ponds in west Alabama, USA. During this project, farm managers reduced planktivorous fish abundance in select ponds to create a large-scale field experiment that addressed the role of zooplankton control of phytoplankton in hypereutrophic catfish aquaculture ponds. There was a strong negative effect of zooplankton on phytoplankton, including cyanobacteria, despite high nutrient concentrations. Although high zooplankton ponds sustained elevated zooplankton biomass during much of this study, including when pond temperatures exceeded 30 °C, the effect of zooplankton on phytoplankton was most pronounced during the non-growing season (November to April). In addition, total ammonia nitrogen was significantly higher in high zooplankton ponds, which could lead to ammonia toxicity in fish at elevated temperature and pH. Our findings suggest that biomanipulation may be an efficient method to control algal blooms in farm-pond aquaculture.

KINETICS OF AN EXPERIMENTAL DEPURATION OF CARIBBEAN CIGUATOXIN IN FISH (LAGODON RHOMBOIDES)

Clayton T Bennett, Alison Robertson

University of South Alabama/ Dauphin Island Sea Lab

Ciguatoxins bioaccumulate in tropical marine food webs from benthic dinoflagellates and cause the neurotoxic illness known as ciguatera. A major gap remains in interpreting the risk period for ciguatera following a dinoflagellate bloom. To understand kinetics for C-CTX-1, a major congener in the western Atlantic, we performed a two-phase, 17 week feeding trial *in Lagodon rhomboides* (Pinfish). Fish were fed a known amount of C-CTX-1 for 6, 10, or 20 days and depurated for 5, 10, 20, 40, 70, or 99 days. Whole muscle, brain, liver, gonad, and other pooled visceral contents (heart, spleen, gall bladder, intestine) were dissected separately for toxin analysis by mouse neuroblastoma assay and LC-MS/MS. Bioaccumulation: CTX was detectable in *L. rhomboides* muscle, liver, and viscera by day 6 with the highest levels on day 20. Visceral extracts (minimum and maximum in replicate fish in standard concentration: pg CTX-3C eq. mg-1 or ppb $\pm$ standard deviation; 3.26 ± 1.5 to 5.74 ± 1.78) were the most toxic for all fish while muscle was least toxic (0.02 ± 0.00 to 0.07 ± 0.00). Depuration: Visceral extract toxicity steadily declined during depuration to study end (1.86 ± 0.22 to 2.79 ± 1.82). Liver toxicity remained relatively stable to study end. However, muscle toxicity exhibited a wave-like effect with highest levels observed at study end (min: 0.07 ± 0.03 to 0.11 ± 0.04). Somatic growth rates were similar between bioaccumulation and depuration end (0.23 vs 0.24 g day-1 respectively), so growth dilution had no apparent effect on lowering toxicity.

EXTREME PRIMARY PRODUCER SHIFTS IN FLORIDA'S NORTHERN INDIAN RIVER LAGOON AND BANANA RIVER

Rachel A. Brewton, Diana Baladi, Kevin Tyre, Brian Lapointe

Harbor Branch Oceanographic Institute, Florida Atlantic University

Eutrophication of coastal lagoons can lead to shifts in the distribution and abundance of primary producers, including seagrass, phytoplankton, and macroalgae, that can potentially lead to bottom-up ecological effects. The highly urbanized Indian River Lagoon (IRL) on Florida's east-central coast is an example of a eutrophic lagoon that has experienced multiple harmful algal blooms (HABs), including macroalgal blooms since the 1980s and severe phytoplankton blooms over the last decade. These HABs has resulted in catastrophic seagrass losses throughout the northern IRL and Banana River (BR), which have been followed by large-scale blooms of the rhizophytic macroalgae *Caulerpa prolifera*. To better understand the ecological implications of the *C. prolifera* blooms, monitoring of water quality and community composition of macroinvertebrates began in the northern IRL and BR in March 2020. Initially, the sites surveyed had high water clarity (secchi depth > 1.0 m), extensive C. prolifera cover, and supported dense macroinvertebrate and resident fish populations. After the initial monitoring event, COVID concerns led to a temporary delay in field work. When these sites were able to be revisited in November 2020, water clarity was greatly reduced (secchi depth < 0.2 m) due to an ongoing, widespread nanocyanobacteria HAB, there was compete loss of C. prolifera, and few macroinvertebrates or fishes were collected. These preliminary results provide evidence that the benthic, ephemeral *C. prolifera* blooms can respond quickly to changes in temperature, light, and nutrient availability and oscillate with other HABs.

KARLODINIUM VENEFICUM ECOTYPES: IMPLICATIONS OF TEMPERATURE EFFECTS ON CELL DIVISION TIME

[1]Erik L. J. E. Broemsen, [1]Matthew W. Parrow, [2]Allen R. Place

[1]*The University of North Carolina at Charlotte, Department of Biological Sciences*
[2]*University of Maryland Center for Environmental Research, Institute of Marine and Environmental Technology*

Estimating growth rates of microalgae in natural systems is made difficult by factors such as physical convection and cell mortality, which lead to unmeasured population gains and losses. Methods for circumventing these effects typically do so by calculating in situ growth rates based on the proportion of dividing cells in a population. The most reliable of these methods, the mitotic index technique, also requires determination of the duration of cell division (td). In the case of *K. veneficum*, td must be determined empirically from dividing cell frequencies and growth rates in asynchronously growing cultures. In order to better understand the intraspecific variability and the effect of temperature on td, four *K. veneficum* strains of distinct geographic origin (CCMP 1975 (lower Chesapeake Bay), 2010IH (upper Chesapeake Bay), CCMP 416 (Norway), and CCMP 426 (Florida Everglades)) were grown asynchronously under continuous light at 15 °C, 20 °C, and 25 °C. The frequency of dividing cells was measured hourly over 27 hours of mid-log growth. Calculated td values ranged between 1.36 ± 0.36 h and 5.78 ± 1.76 h, and growth rates ranged from 0.13 ± 0.04 days-1 to 0.35 ± 0.08 days-1. Two-way ANOVA analysis of both td and growth rate revealed significant interaction effects between strain and temperature ($p = 0.02$ and $p < 0.001$, respectively). These interactions indicate differences in temperature optima among these strains, suggestive of the existence of ecotype species within *K. veneficum*. The observed td values will be useful for establishing limits in future field studies.

RAPID CYANOTOXIN DETECTION TECHNOLOGY IN ROUTINE MONITORING AND CITIZEN SCIENCE GROUPS

Seth D Buchholz

Bowling Green State University

Current sampling methods are insufficient to quantitatively measure cyanotoxins, such as microcystins and other emerging cyanotoxins of concern such as saxitoxins and cylindrospermopsins. The second generation LightDeck MC/CYN HAB Toxin Detection System is a user-friendly technology capable of accurate measurements of microcystins and cylindrospermopsins that will yield toxin data more efficiently and conveniently than the current EPA-validated Microcystin ELISA method. The LightDeck Toxin Detection System is being implemented by citizen science groups in western Lake Erie as well as university-led Lake Erie water quality monitoring programs. These coordinated efforts not only provide data to validate LightDeck against the validated laboratory method, but also assess the practicality of the instrument itself. A total of 302 comparison samples were collected and analyzed between collaborators. Laboratory experiments investigated potential sources of assay interferents, including silica from diatom frustules and instrument component contamination, that may be contributing to false positive microcystin results. However, there are no definitive conclusions regarding the source of discrepancies between analytical methods.

VERTICAL SWIMMING SPEEDS OF THE DINOFLAGELLATE SPECIES MARGALEFIDINIUM POLYKRIKOIDES IN THE LAFAYETTE RIVER

Jacqueline B. Chrabot, Margaret R. Mulholland, Michael Echevarria, Peter Bernhardt, Sophie Clayton

Old Dominion University/Department of Ocean and Earth Sciences

Margalefidinium polykrikoides is a dinoflagellate HAB species that has bloomed almost annually in the lower Chesapeake Bay tributaries since its introduction. *M. polykrikoides* is known to migrate vertically within the water column which is thought to provide them with a competitive advantage. As a mixotroph, it has been hypothesized that by migrating. vertically, *M. polykrikoides* can take advantage of both the high light levels near the surface, and the higher organic and inorganic nutrient concentrations near the bottom, allowing them to outcompete purely photoautotrophic organisms which cannot access the deeper nutrient pool. Recent lab-based work has shown that *M. polykrikoides* has a maximum swimming speed of ~1.4 mm s-1, making it one of the fastest dinoflagellate swimmers. However, only one study has produced in situ vertical migration rates for *M. polykrikoides* and these rates are for the Korean ribotype. In this study we analyzed in situ mooring data collected from a long-term HAB time series station in the Lafayette River, a sub-tributary of the lower Chesapeake Bay, to determine *M. polykrikoides* swimming speeds and examine how rates might be influenced by environmental conditions (e.g. wind and tides). We combined data from continuously deployed surface and bottom data sondes, diel vertical profile studies and microscopic cell counts to estimate *M. polykrikoides* swimming speeds. Observations show that upward migration in the water column starts at around 0630 and with biomass concentrated in the surface layer by 1120, a distance of approximately 4.5-6 meters, depending on the tidal stage. We estimate a range of vertical migration velocities between ~1.22 meters per hour to upwards of ~6.05 meters per hour.

PRELIMINARY TREATMENTS USING UV-C, NANOBUBBLES, AND ULTRASONIFICATION ON LAKE WATER FOR REMEDIATION OF CYANOBACTERIAL HARMFUL ALGAL BLOOMS

Dominique S. Derminio, Gregory Boyer, Jason Dean

EGET LIBER/State University of New York College of Environmental Science and Forestry/Keuka College

Cyanobacterial harmful algal blooms (cHABs) are an environmental and ecological concern. Most efforts focus on prevention of blooms, but methods to remediate and remove existing blooms are also being investigated. Here, we examined a novel treatment that uses the combination of UV-C light at 36 watt seconds, nanobubbles, and 28 kHz ultrasonification in an optical chamber to combat blooms. Unlike other chemical and biological methods that have been proposed, this approach will not have a lasting effect on the ecosystem. Total chlorophyll was measured by in vitro Turner Designs 700 fluorometer equipped with the Welschmeyer filter set, as well as a bbe Fluoroprobe for in vivo chlorophyll. Microcystins were calculated using a Waters ZQ4000 mass spectrometer. Laboratory experiments using a mixture of UV-C light, nanobubbles, and ultrasonification in various combinations showed this approach limited the growth of cyanobacterial by at least 20% using chlorophyll levels in lake water under controlled conditions of temperature and light. Application of UV-C and nanobubbles on their own reduced cyanobacterial chlorophyll to near 0 µg/L after 17 days. Microcystin levels were decreased in comparison to the control for all samples over 17 days. Future work will test these combinations of UV-C, sonication, and nanobubbles in a natural lake setting to better evaluate their ability to remove cHABs from natural water systems.

MULTIPLE HARMFUL ALGAL BLOOM TOXINS DETECTED IN A TOP PREDATOR IN THE INDIAN RIVER LAGOON, FLORIDA

Michelle L. Edwards, Michael McCallister, Adam M. Schaefer, Matthew J. Ajemian

Harbor Branch Oceanographic Institute, Florida Atlantic University

Florida's Indian River Lagoon (IRL) has experienced large-scale harmful algal blooms (HABs) of potentially toxic species almost annually since the early 2000s. Sentinel, or indicator, species can provide an integrative picture of contaminants in the environment and may be useful to understanding the presence of HAB toxins in the IRL. This study aims to evaluate presence of toxins in the IRL by using the bull shark (*Carcharhinus leucas*) as a sentinel species. Baseline concentrations of toxins were assessed from samples collected from 50 bull sharks captured in the Indian River Lagoon between Brevard and Martin counties from 2018 - 2020. Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) was used to measure HAB toxins in shark stomach contents, plasma and liver. Analysis of samples (n = 123) demonstrated the presence of multiple toxins (microcystins, nodularin, lyngbyatoxin, domoic acid, okadaic acid, and brevetoxin) in the tissues of bull sharks during non-bloom periods. However, all detected toxins were in low prevalence and in relatively low concentrations. This study will provide valuable information on background presence of multiple HAB toxins in this iconic estuary.

ASSESSING AND PREDICTING MICROCYSTIN VARIABILITY IN WESTERN LAKE ERIE

[1]Shiqi Fang, [2]Dario Del Giudice, [3]Justin D. Chaffin, [4]Thomas Bridgeman, [5]Arthur Zastepa, [6]Mary Anne Evans , [7]Thomas Johengen, [8]Daniel Obenour

[1]*Department of Civil, Construction & Environmental Engineering, NC State University*
[2]*F. T. Stone Laboratory and Ohio Sea Grant, The Ohio State University*
[3]*Department of Environmental Sciences and Lake Erie Center, University of Toledo*
[4]*Environment and Climate Change Canada, Canada Centre for Inland Waters*
[5]*Department of Biological Sciences, Bowling Green State University*
[6]*U.S. Geological Survey, Great Lakes Science Center*
[7]*Cooperative Institute for Great Lakes Research (CIGLR)*
[8]*Center for Geospatial Analytics, NC State University*

Cyanobacterial harmful algal blooms (cHABs) are prevalent worldwide and pose threats to ecosystems and human health, particularly due to the production of cyanotoxins. One of the most common group of cyanotoxins are microcystins (MCs), which have severely affected critical water resources such as the Laurentian Great Lakes. Despite decades of research into cHABs, our ability to assess and predict cyanotoxin concentration remains limited. With a space-time geostatistical approach, we characterize the inter- and intra-seasonal variability of MCs from 2008-2019 in Western Lake Erie (WLE). Then, using statistical variable selection, we developed multiple linear regressions for predicting MC concentration: model 'A' establishes relationships between MCs and in-lake water quality, while model 'B' predicts MC using mechanistic eutrophication model output, so that it is less reliant on in-lake data. Both models explain over 70% of the temporal variability in MC. The models indicate that MC is positively related to chlorophyll concentration, 30-day ahead moving average water temperature, 70-day ahead moving average Maumee River total nitrogen, and depletion of in-lake inorganic nutrients. Furthermore, model B demonstrates the potential to develop accurate seasonal MC forecasts.

COMMERCIALLY AVAILABLE UNOCCUPIED AERIAL SYSTEMS FOR MONITORING HARMFUL ALGAL BLOOMS: A COMPARATIVE STUDY

Edna G Fernandez-Figueroa, Alan Wilson and Stephanie Rogers

Auburn University

Reliable remote sensing platforms and methods for monitoring phytoplankton are needed for mitigating the detrimental impacts of cyanobacterial harmful algal blooms (CyanoHABs) on small inland waterbodies. Commercial unoccupied aerial systems (UAS) present an affordable high-resolution solution for rapid assessment of cyanobacterial abundance in small (<30 m) aquatic systems by recording the reflectance of photosynthetic pigments found in all phytoplankton (i.e., chlorophyll-a) and those unique to cyanobacteria (i.e., phycocyanin). This study evaluates the performance of four sensors, including visible light spectra (RGB) sensors on the Phantom 4 and Phantom 4 Professional platforms, the MAPIR Survey3W modified multispectral (near-infrared, green, blue) sensor, and the Parrott Sequoia multispectral (green, red, near-infrared, red-edge)sensor for estimating cyanobacterial abundance. Each sensor's performance was determined by comparing 26 vegetation indices to chlorophyll-aand phycocyanin measurements of 54 ponds that varied in productivity. Vegetation indices that included the red and near-infrared wavelengths generated from Parrot Sequoia aerial images provided the best chlorophyll-a(i.e., NDVI, $r2 = 0.78$, p<0.0001) and phycocyanin (i.e., EVI2, $r2 = 0.57$, p<0.0001) estimates. The RGB sensors were moderately effective for estimating chlorophyll-a, whereas the MAPIR Survey3W generated poor estimates of both pigments due to differences in recorded wavelengths.

GROWTH RESPONSE OF A NEW NORTH AMERICAN ISOLATE OF DINOPHYSIS TO TEMPERATURE, SALINITY, AND IRRADIANCE

[1]James M. Fiorendino, [2]Juliette L Smith, [1]Lisa Campbell

[1]Texas A&M University
[2]Virginia Institute of Marine Science, College of William & Mary

Dinophysis blooms in the United States are an increasingly common threat to shellfish fisheries. Novel blooms of *Dinophysis* and harvesting closures occurred in New York, Texas, and Washington State in recent years. Continuous monitoring of the coastal phytoplankton community with Imaging FlowCytobots deployed along the Texas coast for over a decade have revealed that blooms of *Dinophysis ovum* appear seasonally between January and April. Predicting and mitigating impacts of mixotrophic kleptoplastidic *Dinophysis* blooms is difficult because of their dependence on their ciliate prey *Mesodinium rubrum* and its cryptophyte prey *Teleaulax amphioxeia*. Seasonality of blooms suggests physical factors may also influence *Dinophysis* bloom dynamics. *Dinophysis ovum* from the Gulf of Mexico was successfully established in laboratory culture for the first time. The growth responses of this novel *D. ovum*, a temperate isolate of *Dinophysis acuminata* from Chesapeake Bay*, M. rubrum* and *T. amphioxeia* were evaluated at a range of temperature, salinity, and irradiance treatments to identify possible drivers of *Dinophysis* blooms. Results suggested temperature most impacted *Dinophysis* growth. Warming waters between winter and spring may therefore promote *Dinophysis* bloom formation in the Gulf of Mexico. In comparison with the temperate *D. acuminata* isolate*, D. ovum* growth was less affected by irradiance and suppressed at temperatures below 18 °C. Additionally, toxin profiles were evaluated for both *Dinophysis* isolates. The toxin profile of the subtropical *D. ovum* was markedly distinct and toxin per cell was two orders of magnitude greater than for the temperate *D. acuminata* isolate.

TRACING NITROGEN UTILIZATION IN THE MICROCYSTIS PHYCOSPHERE

Malia I. Gardner, Alexa Hoke, Dominique Lockwood, Isabella Becker, Louie Wurch, Morgan Steffen

James Madison University

Microcystis spp. are cyanobacteria that cause destructive cyanobacterial harmful algal blooms (cHABs) worldwide. During cHABs, heterotrophic bacteria are present within *Microcystis'* phycosphere that can have a growth promoting effect on *Microcystis*. While the mechanism behind growth promotion in the *Microcystis* phycosphere is largely unknown, genomic evidence suggests it involves nutrient exchange, specifically the exchange of nitrogen (N), which can be an important limiting nutrient during blooms. To identify the phycosphere bacteria that can actively use the N sources urea and nitrate, a stable-isotope probing (SIP) metagenomics protocol was adapted using non-axenic lab cultures of *Microcystis aeruginosa* NIES 843 and CCMP 3462. The two non-axenic strains of *Microcystis aeruginosa* were incubated with 15N stable isotopes, urea and potassium nitrate as the only source of N. Members of the non-axenic cultures that were able to utilize the 15N stable isotopes incorporated the isotope into their DNA, providing a direct measure of N metabolic function. The isotopically labeled DNA was separated from the unlabeled DNA using a CsCl density gradient and ultracentrifugation. Targeted 16S metagenomes of the V3-V4 region were compared between the labeled and unlabeled populations, providing new insight into the N uptake capabilities of bacterial cHAB partners in lab culture populations of *Microcystis*, which can serve as a proxy of bloom bacterial communities.

AVAILABLE DISSOLVED NITROGEN FORM MEDIATES PHYCOCYANIN CONTENT IN CYANOBACTERIA

Matthew F. Gladfelter, Riley P. Buley, Angelea P. Belfiore, Edna G. Fernandez-Figueroa, Bridget L. Gerovac, Nicole D. Baker, Alan E. Wilson

Auburn University

A two-week field experiment was carried out using 24, 1100L plastic limnocorrals to examine how different forms of dissolved nitrogen (i.e., nitrate, ammonium, and urea) impact the phycocyanin pigment content of cyanobacterial cells. Results showed that upon nitrogen introduction, cells quickly incorporated the nitrogen into excess phycocyanin as a possible means of nitrogen storage, peaking at 72h after fertilization. Ammonium and urea treatments had significantly more phycocyanin per cell than nitrate or control treatments at 72h. After 72h, phycocyanin content quickly decreased as cells degraded the pigment as a source of nitrogen. Despite the decrease in light-harvesting pigments, total number of cyanobacterial cells increased in the ammonium and urea treatments after two weeks. Nitrate treatment caused an initial growth of cyanobacteria followed by a rapid decline to starting concentrations. Cyanobacterial toxins (microcystins) peaked 72h after nitrogen fertilization. Urea and ammonium treatments resulted in the most toxic communities and had significantly more toxins than the control, however the nitrate toxin concentrations were not different from any other treatment. Results from this experiment add to the growing understanding of the advantages that cyanobacteria gain from growth on reduced forms of nitrogen (e.g., ammonium and urea). Results also suggest that simple pigment extractions may be under-estimating the amount of cyanobacteria present during a bloom depending on ambient dissolved nitrogen concentrations.

USING CYST MAPPING TO UNCOVER TRENDS OF PSP TOXICITY IN SOUTHEAST ALASKA GEODUCK HARVEST AREAS

[1]Courtney Hart, [2]Dr. Elizabeth Tobin, [3]Kari Lanphier, [4]Kate Sullivan, [1]Ginny Eckert

[1]*University of Alaska Fairbanks, Juneau Fisheries Center*
[2]*Jamestown S'Klallam Tribe, Natural Resources*
[3]*The Sitka Tribe of Alaska Environmental Research Lab*
[4]*Southeast Alaska Regional Dive Fisheries Association*

In Southeast Alaska, the commercial geoduck harvest is a small but lucrative wintertime fishery with annual ex-vessel values averaging US$4.7 million (2010-2018). In recent years, this fishery has been hampered by paralytic shellfish toxins (PSTs); failed PST tests causes substantial economic loss to the geoduck fishery through increased sampling costs and by delaying or closing harvests. In the 2018-2019 commercial season, 25% of harvestable biomass was left in the ground due to PST closures. Clam toxicity within a harvest area varies substantially from week-to-week, fluctuating well above and below the regulatory limit. Geoduck clams (*Panopea generosa*) are filter feeders and can acquire PSTs by ingesting the toxin-producing phytoplankton, *Alexandrium catenella*. Like many dinoflagellate species, *A. catenella* alternates between a dormant, benthic stage, and a motile vegetative cell. The dormant cyst-stage persists in sediment during commercial clam harvests and we suspect they may influence wintertime toxicity in clams. To better understand the variation of geoduck toxicity, we are investigating spatial and temporal patterns of A. catenella cyst abundance through cyst-mapping. In collaboration with our partners, we collected over 350 sediment samples with cyst quantities ranging from zero to over 5000 cysts/cc wet sediment and we have identified various hotspots where elevated cyst densities persist. Uncovering spatial and temporal patterns of cyst densities as they relate to trends in geoduck PSP toxicity may provide valuable data for managers to mitigate toxicity in commercial clams.

INTERNAL NITROGEN LOADING SUPPORTS HARMFUL CYANOBACTERIA BLOOMS AND TOXICITY IN WESTERN LAKE ERIE

[1]Daniel K. Hoffman, [1]Mark McCarthy, [2]Timothy Davis, [3]Ashlynn Boedecker, [1]Justin Myers, [1]Silvia Newell

[1]*Wright State University*
[2]*Bowling Green State University*
[3]*Baylor University*

Ammonium (NH_4^+) is biologically important for cyanobacteria, especially non-N-fixing taxa (e.g., *Microcystis*), but its relationship to cyanotoxin (e.g., microcystins) production and prevalence during blooms is confounded by low NH_4^+ concentrations when bloom biomass is high. Over three field seasons (April - October, 2015 - 2017), we sampled western Lake Erie with the NOAA Great Lakes Environmental Research Laboratory and conducted incubation experiments to quantify NH_4^+ regeneration and potential uptake rates in the water column. Potential uptake rates followed spatial and seasonal patterns, with the greatest rates measured nearest the Maumee River inflow and during peak bloom months (August and September). Regeneration followed a similar spatial pattern but was greatest in early summer (June and July). When extrapolated to the basin, internal regeneration of NH_4^+ may exceed the seasonal external N load and contribute 60 - 76% of the annual load of N to the western basin as an internal source. We also combined measured rates with frequently monitored physicochemical and biological water quality metrics to explore a preliminary regression model, which suggests that nitrogen availability, estimated as turnover/regeneration rates, is important for predicting microcystin concentration. Our results indicate that water column NH_4^+ concentrations do not accurately reflect availability and reinforce previous studies suggesting that measuring NH_4^+ turnover rates is necessary to understand the dynamics that promote microcystin production in freshwater HABs.

IMPACTS OF NON-TOXIC MICROCYSTIS AERUGINOSA ON OYSTER FEEDING

[1]Andrea C Jaegge, [2]Sandra Casas, [2]Jerome La Peyre, [3]Megan La Peyre, [1]Beth A. Stauffer

[1]*Department of Biology, University of Louisiana at Lafayette*
[2]*School of Animal Sciences, Louisiana State University Agricultural Center*
[3]*U.S. Geological Survey, Louisiana Cooperative Fish and Wildlife Research Unit, School of Renewable Natural   Resources, Louisiana State University Agricultural Center*

Changes in estuarine conditions due to warming temperatures, increased precipitation, eutrophication, and large-scale river engineering is contributing to the expansion of freshwater cyanobacteria in these environments. These altered physicochemical conditions also have direct physiological impacts on ecologically and economically important estuarine consumers such as the eastern oyster (*Crassostrea virginica*). These consumers may experience additional indirect impacts through shifting planktonic prey communities which increasingly include nutritionally poor, and often toxic, species of cyanobacteria. To examine these indirect food web impacts on oysters, we conducted four population-level feeding experiments using natural phytoplankton assemblages collected from Terrebonne Bay, Louisiana and assemblages amended with a non-toxic strain of *Microcystis aeruginosa*. Population-level clearance rate, change in chlorophyll a concentration in three size fractions ($< 5\mu$m, $5 - 20\mu$m, and $> 20\mu$m), and change in *M. aeruginosa* cell abundance were quantified through time. In all experiments, the addition of *M. aeruginosa* significantly increased the quantity of food available to the oysters, especially in the $< 5\mu$m size fraction, but these cells were not consistently filtered or avoided. The response varied between experiments depending on the salinity and composition of the natural phytoplankton assemblage present. However, clearance rates were generally reduced with the addition of *M. aeruginosa* across experiments and at lower salinities (5). These preliminary results suggest that low estuarine salinities will impact oysters indirectly through food web effects, independent of toxin production, and that those effects will depend on the physicochemical conditions and phytoplankton community already present.

PSEUDO-NITZSCHIA MULTISERIES TOXICITY INCREASES UNDER PROJECTED FUTURE OCEAN SCENARIOS: A MULTIPLE STRESSOR APPROACH

[1]Kyla J. Kelly, [1]Kristin H. Art, [2,3]Monica Thukral, [1]Michelle A. DeMers, [1]Fei-Xue Fu, [1]David A. Hutchins

[1]*University of Southern California*
[2]*Scripps Institution of Oceanography at the University of California San Diego*
[3]*J. Craig Venter Institute*

Harmful algal blooms are increasing with climate change, but studies of bloom dynamics in the lab have been limited by our ability to accurately represent complex in situ oceanic conditions. We used a scenario-based multiple driver approach to holistically examine how climate change may impact *Pseudo-nitzschia multiseries* blooms. Cells were simultaneously exposed to pCO2, temperature, irradiance, and silicate/phosphate (Si/P) co-limitation at levels projected for 2100 and 2200, relative to today. Replete growth rates increased incrementally in future ocean scenarios yet were reduced by concurrent Si/P limitation. Cellular domoic acid (DA) quotas were low in present day and 2100 replete scenarios (0 and 0.01 pg cell^{-1}) but increased to 1.24 pg cell^{-1} in 2200. Adding Si/P limitation nearly doubled these DA quotas. This aligns with previous findings that increased nutrient limitation, pCO2, irradiance, and especially warming promote DA biosynthesis. Despite increased cell-specific toxicity, DA production rates were suppressed under future, nutrient-limited conditions due to low growth rates. If nutrient limitation restricts cell division, toxic bloom biomass accumulation will be reduced. In contrast, under replete conditions both growth and toxicity were enhanced, suggesting that cells with moderate DA levels could divide faster to form a highly toxic bloom. The future severity of toxic *P. multiseries* blooms will therefore be dependent on the complex interplay between toxin biosynthesis, growth, and oceanic conditions, with consequences for ecosystem and human health.

NITROGEN AND PHOSPHORUS SOURCES AND LIMITATION SIGNIFICANTLY ALTERS THE GROWTH, NITROGEN FIXATION, ANATOXIN-A CONTENT, AND TRANSCRIPTOME OF THE BLOOM-FORMING CYANOBACTERIA, *DOLICHOSPERMUM*

Benjamin J. Kramer, Christopher J. Gobler

Stony Brook University Southampton, School of Marine and Atmospheric Sciences

Freshwater diazotrophic cyanobacteria have traditionally been thought to be solely limited by the availability of phosphorus, as they are capable of N_2-fixation. This study, however, characterized how various nitrogen sources as well as nitrogen and phosphorus colimitation alter the growth, toxin production, N_2-fixation, and gene expression of an anatoxin-a producing strain of *Dolichospermum*. Cultures amended with nitrogen exhibited considerable transcriptomic similarity with each other and the control. Growth rates were significantly higher when cultures were grown on exogenous N sources relative to the control with growth on NH_4^+ and urea being significantly greater than growth on NO_3^-. Conversely, NH_4^+ and urea significantly reduced N_2-fixation while cultures amended with nitrate exhibited nitrogenase activity comparable to that of cultures grown without exogenous nitrogen and/or phosphorus. Relative to the control, the abundance of *nif* gene transcripts were significantly lower in cultures grown with NH_4^+ and urea, while transcripts for genes such as the NO_3^- reductase encoding gene *narB* exhibited significantly greater abundance when cultures were grown on NO_3^- or deprived of N and P. Finally, cultures exhibited the lowest anatoxin-a concentrations when grown on NH_4^+, and growth and toxin content were negatively correlated. Transcripts of several anatoxin synthesis genes were also highest under N and P - deplete conditions. Our findings indicate that bloom-forming diazotrophic cyanobacteria may grow more rapidly using reduced forms of exogenous nitrogen for growth, but that such growth comes at the expense of lowered N_2-fixation and anatoxin-a production.

USE OF A COMBINED HIGH THROUGHPUT SEQUENCING AND FLUOROMETRY METHOD TO ASSESS GRAZING ON CYANOBACTERIA BY *DAPHNIA* SPP. AND THE NATURAL PROTOZOOPLANKTON COMMUNITY DURING CYANOBACTERIAL BLOOMS IN WESTERN LAKE ERIE, NY

Megan Ladds, Christopher J. Gobler

Stony Brook University Southampton, School of Marine and Atmospheric Sciences

Harmful algal blooms (HABs) are becoming an increasing problem worldwide and one that will continue to increase in the future. The most common freshwater HAB is cyanobacteria, which has been linked to increased nutrients and decreased grazing pressures. Many types of cyanobacteria that can make up these blooms pose health risks, however responses to grazing pressures and nutrients of specific genera within the overall cyanobacterial community are not well understood. The use of 16S and 18S sequence analyses combined with fluorometry can resolve changes in genera of phytoplankton when grazing pressure is either relaxed or increased as well as when excessive nutrients are added. Grazer addition experiments using two daphnids, *Daphnia magna* and *Daphnia pulex,* as well as a dilution series were performed within the epicenter and within the periphery of the 2015 cyanobacterial blooms in Maumee and Sandusky Bay, Lake Erie. *Planktothrix*, *Microcystis*, *Synechococcus*, and *Dolichospermum* were the dominant genera in both Bays. *Daphnia* spp. addition reduced growth of *Synechococcus* at a majority of sites, *Planktothrix* in Sandusky Bay, and *Dolichospermum* in Maumee Bay, but grazing rates on diatoms (when measurable) were significantly greater than on cyanobacteria. Dilution enhanced growth of *Planktothrix* at all sites and *Synechococcus* at a majority of sites, suggesting the natural community was actively grazing these cyanobacteria. In stark contrast *Microcystis* was not affected by either daphnid addition or dilution. The use of this novel combined method was able to establish differences in grazing pressure and growth on different members of the overall phytoplankton community. It also highlighted a method for the persistence of *Microcystis* in Lake Erie blooms via combined resistance to grazing and enhancement with nutrient additions.

BACTERIAL COMMUNITY STRUCTURE OF NON-AXENIC CYANOBACTERIAL CULTURES

[1]Dominique J Lockwood, [2]Guadalupe Reynoso, [3]Morgan Smith, [1]Morgan Steffen, [1]Louie Wurch

[1]*James Madison University*
[2]*Virginia Polytechnic Institute and State University*
[3]*Texas A&M University*

Toxic cyanobacteria blooms are a global problem, causing adverse effects to numerous lakes and rivers. Combatting these blooms is difficult because the combination of factors contributing to the success of bloom-forming cyanobacteria is not fully understood. Here we focus on biotic factors, and the potential role of heterotrophic bacteria, in supporting blooms of toxic *Microcystis*. Anecdotally, *Microcystis* grows more robustly in non-axenic cultures relative to axenic cultures. To explore this, we generated 16S libraries to analyze community structure in cultures of two non-axenic strains of *Microcystis aeruginosa* (NIES 843 and CCMP 346). We then compared this to the bacterial community associated with another toxic, bloom-forming cyanobacteria species, *Cylindrospermopsis raciborskii* CS-505, to identify species that were unique to *Microcystis*, and species that were shared among the different cyanobacteria. Finally, we compared community structure to that of a marine eukaryotic alga that also forms high-biomass blooms (*Aureococcus anophagefferens* CCMP1984) to use as an outgroup. We analyzed both alpha and beta diversity to explore differences within and between the samples, respectively. We found differences in bacterial composition even between the two species of *Microcystis* both taxonomically and in terms of beta diversity. These data provide a foundation for further studies in isolating and characterizing bacteria that may have beneficial effects on the growth and physiology of toxic, bloom-forming cyanobacteria.

CHALLENGES OF IMPLEMENTING A CALIFORNIA HAB HEALTH IMPACT SURVEILLANCE SYSTEM, 2018-2020

[1]Isadora Nogueira, [1]Susan Paulukonis, [2]Rebecca Stanton, [2]Shannon Murphy

[1]*Public Health Institute*
[2]*Office of Environmental Health Hazard Assessment*

In 2018, California formed an interagency workgroup tasked with creating the first centralized statewide system for investigating and reporting harmful algal bloom (HAB) related illnesses. As part of the Centers for Disease Control and Prevention's One Health Harmful Algal Bloom System (OHHABS) monitoring effort, we reported 68 freshwater HAB-related illnesses in humans, fish and wildlife, and domestic animals between 2018 and 2020. In 2020, we began reporting illnesses associated with marine HABs, including 8 red tide mortality events, and 277 domoic acid-related marine animal strandings that occurred between 2019 and 2020. This presentation discusses the challenges of California's nascent HAB health impact surveillance system with the aim of delineating gaps that limit the reach and effectiveness of our current efforts. Gaps in California's ability to capture illnesses associated with HABs are the result of legal, informational, and technical barriers. Legally, our surveillance efforts are restricted by the absence of a legislative mandate to monitor HABs, report HAB events and illnesses, or post waterbody signage when toxins are discovered. Our work encounters significant education and outreach challenges, including limited awareness amongst the general public about HABs, misconceptions or absence of knowledge within the healthcare and veterinary communities about HABs and related illnesses, and the lack of scientific consensus regarding different routes of exposure and the sequelae, severity, and spectrum of HAB-related symptoms. Finally, our work is subject to time delays associated with reporting party communications, workgroup follow up efforts, sample collection, and data analysis.

GRAZER-INDUCED TOXIN PRODUCTION UNDER CLIMATE CHANGE IN THE NEUROTOXIC DINOFLAGELLATE ALEXANDRIUM CATENELLA

Gihong Park, Ewaldo Leitao Jr., and Hans G. Dam

University of Connecticut, Department of Marine Sciences

Grazer-toxic phytoplankton interactions depend on the cell growth and the cell toxin content of the prey. An open question is how these interactions will unfold under climate change. It has been shown that toxin production dramatically increases in *Alexandrium catenella* in the presence of copepod grazers, but incurs a fitness cost. We tested for grazer-induced toxin production in *A. catenella* lineages adapted to ambient (16°C, 400 ppm CO2) and greenhouse conditions (20°C, 2000 ppm CO2) after ~80 generations of selection. *Alexandrium* growth rate increased under greenhouse conditions relative to ambient conditions. Grazer-induced toxin production was evident in both ambient and greenhouse conditions, but it was lower in the latter. The trade-off of net growth rate versus toxin production is less under greenhouse conditions. Reciprocal transplants (from greenhouse to ambient conditions and vice versa) indicate that the growth rate response to greenhouse conditions was plastic. By contrast, the grazer-induced toxin production response reflected adaptation to greenhouse conditions. The implication of our work is that *A. catenella* blooms may be more prevalent in the future.

HUNTING THE EAGLE KILLER: A CYANOBACTERIAL NEUROTOXIN CAUSES VACUOLAR MYELINOPATHY

[1]Tabitha J Phillips, [2]Steffen Breinlinger, [1]Susan Wilde, [2]Timo Niedermeyer

[1]*Warnell School of Forestry and Natural Resources, University of Georgia*
[2]*Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany*

Vacuolar Myelinopathy is a fatal neurologic disease initially discovered during a mysterious mass mortality of the iconic bald eagle in Arkansas, US. The cause for this wildlife disease eluded scientists for decades while its occurrence has continued to spread throughout freshwater reservoirs in the southeastern United States. Recent studies demonstrated that Vacuolar Myelinopathy is induced by consumption of the epiphytic cyanobacterial species *Aetokthonos hydrillicola* growing on aquatic vegetation, primarily invasive *Hydrilla verticillata*. Here, we describe the identification and biological activity of aetokthonotoxin, a pentabrominated biindole alkaloid that is produced by the cyanobacterium *A. hydrillicola*. We identified this novel cyanobacterial neurotoxin as the causal agent of Vacuolar Myelinopathy, and discuss environmental factors, especially bromide availability, promoting toxin production.

HARMFUL CYANOBACTERIAL BLOOM AEROSOLIZATION DYNAMICS AND MICROCYSTIN PRODUCTION IN THE CHOWAN RIVER, NC

[1]Haley E Plaas, [2]Karsten Baumann, [3]Ryan W. Paerl, [4]Kimberly J. Popendorf, [5]Colleen Karl, [6]Jill Paxson, [3]Naomi Y. Chang, [3]Joel Sanchez, [3]Nathaniel Curtis, [3]Hwa Hwang, [1]Malcolm A. Barnard, [4]Daniela Maizel, [1]Nathan S. Hall, [1]Karen Rossignol, [1]Amy Bartenfelder, [1]Jeremy Braddy, [1]Randolph Sloup, and [1]Hans W. Paerl

[1]*Institute of Marine Sciences, University of North Carolina at Chapel Hill*
[2]*University of North Carolina Gillings School of Global Public Health*
[3]*Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University*
[4]*Rosenstiel School of Marine and Atmospheric Science, University of Miami*
[5]*Chowan Edenton Environmental Group*
[6]*North Carolina Department of Environmental Quality*

The environmental health of the Chowan River-Albemarle Sound (CR-AS) estuary is threatened by the expansion of harmful cyanobacterial blooms (cHABs) linked to the production of microcystin (MC), a potent cyanotoxin associated with human liver disease. With the NC Department of Environmental Quality (DEQ) and community scientists of the Chowan Edenton Environmental Group (CEEG), our pilot field campaign (summer 2020), was the first to analyze cHAB DNA and MC in aerosol of the CR-AS airshed to evaluate inhalation as a potential exposure pathway to MC in coastal NC. Aerosol (PM2.5, particulate matter with diameter <2.5 µm) samples were collected using medium-volume impactors. Offline analytical methods were applied to quantify DNA and MC in environmental (water and aerosol) samples. Water quality parameters, ambient PM2.5 mass concentrations, and meteorological conditions were measured in parallel and used as predictor variables in generalized additive models to evaluate the environmental factors most strongly associated with MC production and aerosolization. From June – October 2020, cyanobacteria dominated the phytoplankton community (~45% of the algal biomass) in the CR-AS, but maximum MC concentrations were low (<1 µg/L), yielding aerosol concentrations below the limits of quantification. However, two congeners demonstrated to be most easily enriched in aerosol, MC-LA (leucine-alanine) and MC-LR (leucine-arginine), were quantified in water samples. Airborne DNA findings are still being evaluated. Summer 2021 we will follow up with improved aerosol collection approaches to better characterize the physicochemical properties of airborne cHAB cells and MC, with the goal to protect human respiratory health in this region.

WATER COLUMN RELEASE OF TOXINS FROM LYNGBYA WOLLEI AS A CONSEQUENCE OF REMEDIATION

Samuel Putnam, Tryston Metz, Judson Riser, Carl Bussells, Casey Moorer, John Ferry

University of South Carolina

Dean Swamp is an impoundment directly connected to Lake Marion, a large reservoir that is part of the Santee River watershed. This area has a persistent bloom of the harmful benthic algae *Lyngbya wollei*, which is known to produce several toxins in the saxitoxin family. Due to residential property surrounding the area, the water was treated several times with a copper herbicide to control the *Lyngbya wollei*. Water and algae samples were collected before, during, and after these treatments along with continuous water quality data. Extractions of the algal samples coupled with liquid chromatography and mass spectrometry allowed for detection and quantification of the *Lyngbya wollei* toxins (LWTs) 1, 4, 5, and 6. The toxin in the algal biomass was tracked for the course of the study. A biological oxygen demand experiment was performed in the presence of *L. wollei* material to determine the ability of the system to biodegrade the material. Changes in pH and dissolved oxygen, while statistically significant, were not proportionate to the biomass of *L. wollei* present. This suggests that *L. wollei* may be more biologically resilient than other cyanobacteria in the presence of herbicide. This field study was followed by a laboratory-controlled experiment where live algae collected from the field was dosed with various commonly used pesticides at recommended treatment doses, and toxin was measured over time. Toxin release into the water column shows how different pesticides can influence the toxicity of the surrounding environment and the side effects of management practices. Our work presents a novel opportunity to examine the effects on management practices on a large scale when applied to *L. wollei* and explores the impacts of various chemical remediation techniques.

ABILITY OF ASIAN CLAMS (*CORBICULA FLUMINEA*) TO FEED ON AND DIFFERENTIATE BETWEEN TOXIC BLOOM-FORMING CYANOBACTERIA AND OTHER ALGAE

Nora R.W. Straquadine, Christopher J. Gobler

Stony Brook University Southampton, School of Marine and Atmospheric Sciences

Cyanobacterial harmful algae blooms (cHABs) are increasingly disrupting freshwater ecosystems and introducing toxins into aquatic food webs. The invasive and edible Asian clam (*Corbicula fluminea*) can be found in waterways afflicted with cHABs across the US. This study focused on how quickly *C. fluminea* could become a possible vector of cHAB toxins by investigating feeding on cHAB species. We conducted laboratory experiments to quantify *C. fluminea* clearance rates and particle selection using algal cultures (microcystin-producing *Microcystis aeruginosa,* saxitoxin-producing *Dolichospermum circinale*, and using the non-toxic green microalga *Raphidocelis subcapitata* as a control). The first aim was to investigate clearance rates of each algae species at three concentrations mimicking typical dense bloom conditions and smaller blooms: 1×10^6 cells mL^{-1}, 5×10^5 cells mL^{-1}, and 2.5×10^5 cells mL^{-1}. The second aim was to explore particle selectivity with five ratios of *Microcystis* or *Dolichospermum* and *Raphidocelis* in the above cell concentrations/biovolumes. Clearance rates were statistically higher ($p<0.05$) on algal type in both the *Microcystis* and *Dolichospermum* monoculture experiments compared to the control algae. Clams did not produce pseudofeces in the mixed *Microcystis* experiments, indicating ingestion of cells, but did produce pseudofeces in the *Dolichospermum* experiments, indicating possible cell rejection. Understanding how rapidly *C. fluminea* consumes cyanobacterial cells and toxins has implications for human health and could be used for management decisions on harvesting this species.

MITIGATION OF HARMFUL ALGAL BLOOMS CAUSED BY *ALEXANDRIUM CATENELLA*, AND SAXITOXIN ACCUMULATION IN BIVALVES, USING CULTIVABLE SEAWEEDS

Peter H. Sylvers, Christopher J. Gobler

Stony Brook University Southampton, School of Marine and Atmospheric Sciences

Alexandrium catenella is a HAB dinoflagellate that causes significant damage to the shellfish industry due to its synthesis of paralytic shellfish toxins. To evaluate the potential of seaweed aquaculture to mitigate *A. catenella* blooms, we determined the effects of three cultivable seaweeds - *Saccharina latissima, Chondrus crispus*, and *Ulva* spp. - on *A. catenella* in culture- and field-based experiments. Co-culture growth assays of *A. catenella* with each seaweed showed that all species save low levels of *C. crispus* caused cell lysis and significant reductions in *A. catenella* densities relative to control treatments of 17-74% in 2-3 days and 42-96% in ~one week ($p<0.05$). In a toxin accumulation experiment, *S. latissima* significantly lessened ($p<0.05$) saxitoxin accumulation in blue mussels (*Mytilus edulis*), keeping levels below US closure limits (80 µg STX 100g^{-1}) compared to the untreated control. Bottle incubations of field populations of *A. catenella* experienced significant reductions in cell densities (up to 95%) when exposed to all seaweeds ($p<0.005$). The stocking of aquacultured *S. latissima* within mesocosms containing a bloom population of *A. catenella* reduced the population of *A. catenella* by 73% over 48 h ($p<0.005$) while *Ulva* addition caused a 54% reduction in *A. catenella* over 96 h ($p<0.01$). Among the three seaweeds, their relative ability to inhibit *A. catenella* was *S. latissima* > *Ulva* spp. > *C. crispus*. These results suggest that the integration of seaweed with shellfish-centric aquaculture should be considered as a measure to mitigate *A. catenella*-caused damage to the shellfish industry.

THE ONE HEALTH HARMFUL ALGAL BLOOM SYSTEM: PUBLIC HEALTH SURVEILLANCE FOR HARMFUL ALGAL BLOOM EVENTS AND ASSOCIATED HUMAN AND ANIMAL ILLNESSES FOR YEARS 2016-2019

Muhammad M Thuneibat, Marissa Vigar, MPH, Jonathan S. Yoder, MPH, Virginia A. Roberts, MSPH

ORAU (CDC), National Center for Emerging and Zoonotic Infectious Diseases

The Centers for Disease Control and Prevention has conducted national public health surveillance for harmful algal blooms (HABs) and associated human or animal illnesses through the One Health Harmful Algal Bloom System (OHHABS) since 2016. A One Health approach recognizes the connection between the health of humans, animals, and the environment. OHHABS reports for 2016–2019 that had been entered by January 8th, 2021 were analyzed using SAS (version 9.4; SAS institute) to describe HAB exposures, laboratory testing, associated illnesses, and recent system adoption. Data from 2019 are preliminary. A total of 22 states voluntarily reported 669 HAB events, 452 human illnesses, and 480 animal illnesses. HAB events primarily occurred during May-October (93%) and in freshwater settings (84%). Laboratory testing results were provided for most HAB events (92%); microcystins were detected in 70% of these HAB events. Human illnesses occurred most often (97%) during June-September; at least 40% of ill persons were aged <18 years. Reported signs or symptoms included gastrointestinal (69%), generalized (e.g., headache) (48%), and dermatologic (27%). Domestic pets (n = 78), livestock (n = 53), and wildlife (n = 349) were affected. Reported signs (n = 128 animals) included generalized (e.g., weakness) (57%), gastrointestinal (52%), and neurologic (25%). The number of reports increased annually (range: 111-242 reports); five states started reporting in 2019 and one state each in 2020 and early 2021. This analysis demonstrates how a One Health approach can be used to characterize HAB events and associated health outcomes to further inform illness prevention efforts.

O HYPOXIA, WHERE ART THOU? LINKING HYPOXIA TO RED TIDE ON THE WEST FLORIDA SHELF

[1]Brendan D Turley, [1]Chris Kelble, [2]Mandy Karnauskas

[1]*Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School for Marine and Atmospheric Science, University of Miami*
[2]*US National Oceanic and Atmospheric Administration*

Red tides on the west Florida shelf are a nearly annual occurrence causing widespread ecological and economic harm. Effects range from minor respiratory irritation and localized fish kills to events causing massive mortalities to marine organisms. The region has not historically been reported to have significant areas of hypoxia because the broad shelf has regular wind-induced mixing limiting the conditions necessary for hypoxia formation. Several research cruises over the past 15 years identified areas of hypoxia coincident with red tide and mass mortality events suggesting that hypoxia is linked to red tide. We examined 20 years of oceanographic data to determine the frequency of hypoxia and to assess their association with red tide. Hypoxia was present in 5 of the 17 years data were available and was always shoreward of the 50-meter bathymetry line. There were 2 clusters of recurrent hypoxia: offshore of the Big Bend coast and near the mouth of the Caloosahatchee River. We identified 3 hypoxic events that were characterized by multiple CTD casts and occurred concurrently with an extreme red tide. The vertical extent of the hypoxia ranged from 1-10 meters off the seafloor and indicate possible severe stress to demersal organisms. We had difficulty in assessing the spatiotemporal extent of these events due to limited data availability and uncertainty about true seafloor sampling. While we cannot unequivocally explain the association between extreme red tide events and hypoxia on the west Florida shelf, there is sufficient evidence to suggest a causal linkage between them.

SEPTIC SYSTEM - GROUNDWATER - SURFACE WATER INTERACTIONS IN AGING RESIDENTIAL FINGER CANALS CONTRIBUTE TO HARMFUL ALGAL BLOOMS IN THE CALOOSAHATCHEE RIVER ESTUARY

[1]Kevin N Tyre, [1]Rachel Brewton, [2]Lisa Kreiger, [1]Kevin Tyre, [1]Diana Baladi, [1]Lynn Wilking, [1]Laura Herren, [1]Brian Lapointe

[1]*Florida Atlantic University - Harbor Branch Oceanographic Institute*
[2]*Lee County Division of Natural Resources*

Water quality in Florida's Caloosahatchee River and Estuary has degraded over the last 30 years, while the occurrence of harmful algal blooms (HABs) has increased. *Karenia brevis* blooms originate offshore before being advected toward Florida's southern Gulf Coast, while blooms of *Microcystis* spp. often originate from Lake Okeechobee before flowing into the Caloosahatchee River. These HABs are enhanced by terrestrial nutrient inputs from urbanized watersheds. North Fort Myers (NFM) is a heavily developed low-lying area on the Caloosahatchee River serviced by septic systems that experiences ongoing water quality issues, such as recurring bacterial contamination. To identify sources of pollution within the NFM watershed, this multi-year study (2017-2020) examined septic system- groundwater- surface water interactions in NFM through analysis of water table depth, nutrients (N and P), stable nitrogen isotopes ($\delta 15N$) of groundwater and particulate organic matter (POM), POM elemental composition (C:N:P), and chemical tracers (sucralose, pharmaceuticals). In 2017-2018, POM was also collected during *K. brevis* and *Microcystis* HABs. Depth to water table was low (< 1m). High concentrations of NH_4^+ and NO_3^-, up to 1,094 µM and 482 µM respectively, were found in groundwater and surface waters. $\delta 15N$ values were frequently enriched (>3 ‰) at study sites and in downstream HABs. N:P ratios in POM were low (<16), indicating N-limitation. Additionally, sucralose, carbamazepine, and acetaminophen were detected near septic systems. These data suggest that groundwater and surface water in the NFM watershed are contaminated by septic system leachate, which is contributing to the maintenance and intensification of downstream HABs.

MOLECULAR AND METABOLOMIC INSIGHTS OF THE EFFECTS OF AN ALGICIDAL BACTERIAL DERIVATIVE IRI-160AA ON DINOFLAGELLATE KARLODINIUM VENEFICUM

Yanfei Wang, Kathryn Coyne

University of Delaware

IRI-160AA is an algicidal compound(s) secreted by the algicidal bacterium *Shewanella* sp. IRI-160 that can specifically control the growth of dinoflagellates. Studies revealed IRI-160AA inhibited photosynthesis, induced cell cycle arrest, DNA degradation, and reactive oxygen species production, implying a programmed pathway leading to cell death (PCD). No research has been focused on the transcriptomic or metabolomic mechanisms behind these effects. Recent research indicated ammonium is among the active compounds in IRI-160AA, while impacts by ammonium differed from the algicide with respect to dinoflagellate photobiology. Here, transcriptomic analyses were conducted on dinoflagellate *Karlodinium veneficum* exposed to IRI-160AA vs. ammonium to illustrate and differentiate the impacts of IRI-160AA and ammonium on dinoflagellates at the molecular level. *K. veneficum* exposed to IRI-160AA was also subjected to a metabolomic analysis to elucidate the accompanied metabolic changes. Results demonstrated over 7,000 genes were highly differentially expressed (DEGs; p<0.001, fold-change > 4) across the treatments and controls. Gene ontology enrichment analysis revealed impacts by the IRI-160AA regulated DEGs on biological processes involved in RNA silencing, cell adhesion, pseudouridine synthesis, and chloroplast-nucleus signaling pathway. DEGs involved in photoinactivation/phororepair, oxidative stress response, DNA damage response, cell cycle checkpoint activation, and PCD were also identified in the algicide treatments. Metabolites that are indicative for oxidative stress, DNA damage, and PCD also increased in the cell pellets of *K. veneficum* treated with the algicide. Results of this research further differentiated the impacts of ammonium from the algicidal effects of IRI-160AA and illustrated the cellular mechanisms behind the algicidal effect.

COORDINATED DIEL GENE EXPRESSION OF CYANOBACTERIA AND THEIR MICROBIOME

Kai Wang, Xiaozhen, Mou

Kent State University

Circadian rhythms in cyanobacterial metabolisms have been well recognized. However, whether this programmed activity of cyanobacteria could elicit a coordinated diel gene expression in their co-occurring microorganisms (microbiome) and how responses of the microbiome in turn impact cyanobacterial metabolism are unknown. To address these questions, a microcosm experiment and metatranscriptome sequencing of cyanobacteria and their microbiome were performed over two day-night cycles. During the experiment, 1205 *Microcystis* genes showed diel patterns in the whole water (WWs) microcosm. Over 99% (1195 genes) of these diel *Microcystis* genes retained the diel feature but had significantly higher expressions in the *Microcystis* (MCY) microcosm, where the microbiome communities were removed from the whole water. A total of 4779 microbiome genes showed diel expression pattern in the WW microcosm, they were affiliated with diverse phyla, including prokaryotic bacteria, eukaryotic algae, ciliates, and Fungi. A similar taxonomic composition of the microbiome was found in the microbiome (MIB) microcosm, where the *Microcystis* were removed from the whole water; however, no diel microbiome gene was identified there. Correlation analyses showed that expressions of diel genes of *Microcystis* and the microbiome were significantly coordinated in the WWs, including those for C-processing, nutrient transformation (i.e., N and P), and micronutrient metabolism (i.e., iron and vitamin B12). Overall, our results suggest that the diel fluxes of OC and vitamin B12 in *Microcystis* largely impact the diel expression of microbiome genes. Meanwhile, the microbiome communities may support the growth of *Microcystis* by supplying them with recycled nutrients but compete with *Microcystis* for iron.

THE USE OF FLAVONOIDS AND CYCLODEXTRINS IN INHIBITING HARMFUL ALGAL BLOOMS

Jens Wira, Taylor Armstrong, Allen Place

University of Maryland Center for Environmental Science-Institute of Marine and Environmental Technology

Lake Williston is a dammed lake in the headwaters of the Choptank River, a major tributary to the Chesapeake Bay. The lake is used recreationally by a Girl Scout troop in Camp Todd. The lake drains agricultural land fertilized by chicken litter and synthetic fertilizers, and the soil and water are usually enriched in nutrients. In 2009 the lake experienced blooms of *Microcystis aeruginosa* that closed the lake to recreational use, persisting for three years. Starting in 2012, the lake was drained and bales of barley straw (*Hordeum vulgare*) were deployed to mitigate the blooms. With these efforts, blooms no longer developed to levels that would trigger state recreational use advisories. Water samples taken in winter to summer 2018 and assayed for their polyphenol, flavonoid and tannin contents over time. The assays showed polyphenol and flavonoid levels were raised with the application of the barley bales which decreased after flushing. Laboratory tests of pure flavonoids at matching concentrations revealed inhibition of *M. aeruginosa* and *Karenia brevis* growth in culture. Analyses were also developed for the detection and isolation of flavonoids via HPLC and LC-MS/MS. In order to improve delivery of flavonoids which have little water solubility, the use of cyclodextrins were investigated. Cyclodextrins are cyclic oligosaccharides that have been recognized as pharmaceutical adjuvants. We found that the flavonoids could be bound to the cyclodextrins and inhibit *M. aeruginosa* and *Karenia brevis* growth but more surprisingly, the cyclodextrins themself inhibited growth, with gamma cyclodextrin being most effective.

SPEEDTALK PRESENTATION ABSTRACTS
(Ordered alphabetical by author)

MACRONUTRIENT AND VITAMIN INFLUENCES ON CHOWAN RIVER (NORTH CAROLINA, USA) CYANOHABS

[1]Malcolm A. Barnard, [1]Haley E. Plaas, [2]Ryan W. Paerl, [3]Colleen M. Karl, [4]W. Christopher Holland, [4]D. Ransom Hardison, [1]Nathan S. Hall, [1]Amy N. Bartenfelder, [1]Karen L. Rossignol, [1]Jeremy S. Braddy, [1]Randolph S. Sloup, [2]Joel J. Sanchez, [2]Nathaniel Curtis, [2]Hwa Hwang, [1]Hans W. Paerl

[1]*Institute of Marine Sciences, University of North Carolina at Chapel Hill*
[2]*Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University*
[3]*Chowan Edenton Environmental Group*
[4]*National Oceanic and Atmospheric Association, National Centers for Coastal Ocean Science*

Cyanobacterial harmful algal bloom (CyanoHAB) proliferation impacts ecosystem and human health on a global scale. The Chowan River-Albemarle Sound Estuary, NC has been plagued by a resurgence of toxigenic CyanoHABs since 2015 after prolific CyanoHABs in the 1970s and 1980s. While the Chowan River (CR) blooms has been impacted by both non-N2-fixing and N2-fixing cyanobacteria, the summer 2020 CyanoHABs were dominated by N2-fixing *Dolichospermum* spp. To understand the nutritional drivers of these CyanoHABs, we investigated N2-fixation rates using acetylene reduction assays in addition to nitrogen (N), phosphorus (P), and B vitamin limitation using F/200 vitamin addition bioassays in the summer of 2020. Cyanobacteria are generally thought to not require external B-vitamins, though the opportunistic use of external vitamins may increase metabolism, or, alternatively, increased supply of B-vitamins may stimulate the cyanobacterial 'microbiome' and in-turn stimulate cyanobacterial metabolism. The CR CyanoHABs were found to be N-limited with a secondary vitamin limitation in NH4 addition treatments. The secondary vitamin limitation is likely due to vitamin addition boosting non-diazotrophic populations during the summer blooms. Furthermore, given the high N2-fixation rates in the CR, further research is required to 1) understand if N reduction thresholds will be effective in reducing the severity of the blooms and 2) to evaluate the budgetary importance of N2-fixation relative to external N inputs and N regeneration in the CR. The results demonstrate the importance of N in supporting CR CyanoHABs and the need to better understand multiple nutritional drivers of the recurring blooms.

CIGUATERA TOXICITY PATTERNS IN RED HIND, *EPINEPHELUS GUTTATUS*, ON CORAL REEFS OF ST. THOMAS, US VIRGIN ISLANDS

[1]Claudia I Carrión, [2]Alison Robertson, [3]Mindy Richlen, [1]Sarah Heidmann, [1]Tyler B. Smith

[1]*University of the Virgin Islands*
[2]*Dept. Marine Sciences, University of South Alabama*
[3]*Woods Hole Oceanographic Institution*

Dinoflagellates of the genus *Gambierdiscus* are found in coral reef ecosystems globally and several species produce neurotoxins associated with ciguatera poisoning (CP) in humans. CP is the most common non-bacterial seafood-borne illness in the world, causing a range of severe gastrointestinal, neurological, and/or cardiovascular symptoms that can last from weeks to years. Preventing CP is challenging, as toxicity of reef fishes is impossible to establish *in-situ*. In this study, we investigated patterns of ciguatera toxicity in red hind (*Epinephelus guttatus*) from different habitats in St. Thomas, USVI. Using baited hook and line on a rod and reel, 96 individuals were collected for ciguatoxin analysis across five coral reef sites, along with benthic sampling for environmental and *Gambierdiscus* community data. Of the 96 fish tested, 83% had detectable levels of ciguatoxin and mean toxicity of fish were significantly different among sites. Toxicity patterns among sites indicated greatest toxicity in offshore sites with intermediate depth (20-22 m), relative to the shallow (10-15 m) inshore site and mesophotic (30+ m) offshore sites. Fish length and weight were not correlated to toxicity both within and among sites (p > 0.05), suggesting that ciguatera toxicity of *E. guttatus* was not associated with size. Stable isotope analyses of *E. guttatus* tissues are underway to compare composite dietary influences in fish among sites. These data will be used to identify environmental toxicity indicators and can help identify locations where fish are most toxic to reduce future cases of CP on St. Thomas.

CHARACTERIZING *PSEUDO-NITZSCHIA* SPECIES DIVERSITY ACROSS ENVIRONMENTAL AND SPATIAL GRADIENTS IN THE ALASKAN ARCTIC

Evangeline Fachon[1], Katherine A Hubbard[1,2], Maria Célia Villac[2], Christina Chadwick[2], Alexandra A Desmidt[2], Josée N Bouchard[2], Mindy Richlen[1], Robert Pickart[1], Peigen Lin[1], Michael Brosnahan[1], Mrunmayee Pathare[1], Calvin Mordy[3,4], Eric Wisegarver[4], Dean Stockwell[5], Donald M Anderson[1]

[1]*Woods Hole Oceanographic Institution*
[2]*Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute*
[3]*Joint Institute for the Study of the Atmosphere and Ocean, University of Washington*
[4]*Pacific Marine Environmental Laboratory*
[5]*College of Fisheries and Ocean Sciences, University of Alaska Fairbanks*

Recent evidence confirming the presence of domoic acid (DA) in the Alaskan Arctic food web has raised concerns about the impacts of the harmful alga, *Pseudo-nitzschia,* for regional environmental and public health. To learn more about Arctic/Subarctic *Pseudo-nitzschia* species and DA production, samples were collected during polar cruises in summer (August) and fall (October-November) of 2018. Genetic methods (Automated Ribosomal Intergenic Spacer Analysis and sequencing) utilized genus-specific primers to track 17 distinct taxa, including *Pseudo-nitzschia* species previously reported in the Arctic and Northeast Pacific, one *Fragilariopsis* species, as well as suspected novel diversity. Sequencing as part of the present study identified *P. delicatissima, P. obtusa, P. arctica, P. granii, P. pungens,* and two types of *P. seriata.* Multivariate statistical analysis revealed considerable structure over spatial, temporal, and environmental gradients, with taxa demonstrating varying temperature/salinity correlations and associations with major water masses. *P. delicatissima* was prominent in both seasons but dominated the majority of summer samples. One transect, near Barrow Canyon, that had elevated DA was instead dominated by *P. obtusa*. During the fall cruise, *P. arctica, P. granii,* and *P. seriata* were observed in the eastern Chukchi Sea and along the Beaufort shelf, where low levels of DA were also observed. Notably, during the same survey, a second genetically distinct population of *P. seriata* was observed in the Bering Sea and southern Chukchi Sea. These findings provide critical baseline information given shifting climate regimes and have implications for understanding potential drivers of DA in a changing Arctic.

KARENIA BREVIS-ASSOCIATED BACTERIAL COMMUNITY COMPOSITION AND ITS INFLUENCE ON ALGAL GROWTH AND BIODEGRADATION OF BREVETOXIN

Cong Fei, Shady Amin

New York University Abu Dhabi, United Arab Emirates

Harmful algal blooms (HABs) are a phenomenon that has detrimental effects on coastal areas, including compromising drinking water sources, and harming water-reliant industries such as fishing and tourism. HABs dynamics are influenced by multiple factors such as light intensity, water temperature, and nutrient levels. In addition to these physical and chemical factors, recent findings suggest that bacteria play an important role in regulating growth and physiology of phytoplankton species, including many HAB species. However, the microbial composition of HAB species and its influence on algal growth, toxicity and growth during blooms is largely unknown. Here, we characterized the attached and free-living bacterial communities associated with the blooming dinoflagellate *Karenia brevis* (New Pass strain) using 16S rRNA amplicon sequencing. Bacteroidia and Alphaproteobacteria were the dominant classes of both attached and free-living bacterial communities. Several families dominated the free-living community, such as Thalassospiraceae and Rhodobacteraceae, while Marinobacteraceae was more abundant in the attached bacterial community. Subsequently, we isolated bacteria from this culture and were able to cultivate the potentially algicidal bacteria *Paracoccus homiensis* and *Thalassospira* sp. Prior research has shown both strains have the ability to disrupt membranes of *K. mikimotoi* cells. Current work is examining the algicidal function of these bacteria on *K. brevis* and their ability to degrade brevetoxin, the major neurotoxin produced by *K. brevis*.

METAGENOMIC ANALYSIS OF CYANOHABS IN CLEAR LAKE, CA: THE SEARCH FOR THE TOXIN PRODUCER

Kyra M. Florea, Eric Webb

University of Southern California

Clear Lake, CA is a hypereutrophic lake that experiences annual toxic cyanoHABs. Clear Lake is an important drinking water resource for the region and as such there is a need to identify which cyanobacterial genera are producing cyanotoxins and the quantity of cyanotoxins being produced. To this end, it is important that we have a thorough profile of the microbial community in Clear Lake including identification of the dominant taxa and which cyanobacteria in the lake possess toxin production genes. 20 locations were sampled in August of 2019, five of which were used for metagenomic sequencing. One of these five locations (Site 12) was positive for microcystin at the time of sampling (0.562 µg/L). Metagenomic assembly resulted in 124 high quality metagenome assembled genomes (MAGs). Six high quality cyanobacteria MAGs were assembled, three of which are known microcystin producers: *Microcystis aeruginosa, Dolichospermum ciricnale, and Gloeotrichia* sp. We utilized the programs anti-SMASH, Anvi'o, and BLAST to identify *Microcystis aeruginosa* as the toxin producer. Comparison of the microbial and protistan communities at each of the five sites revealed distinct differences in community composition between the toxic and non-toxic sites raising further questions about how microbial community interactions may impact toxin production.

OPTIMIZING POTENCY OF ALGICIDAL EXUDATES AGAINST KARENIA BREVIS

[1]Gretchen I Johnson, [1]Yanfei Wang, [1]Kathryn Coyne, [2]Vince Lovko, [2]Devin Burris, [2]Dana Wetzel, [2]Christelle Abadia

[1]*University of Delaware*
[2]*Mote Marine Laboratory*

The bacterium *Shewanella* sp. IRI-160 produces algicidal exudates that affect photochemistry, ultrastructure, and viability of dinoflagellates, but not other algal groups. Development of this taxon-specific algicide as a tool for mitigation of harmful dinoflagellate blooms in the field involves 1) optimizing its effectiveness and 2) characterizing algicidal components in the exudate. To serve this effort, we conducted an exometabolomics experiment to identify potential algicidal compounds in the bacterial filtrate. Bacterial growth conditions were then optimized to enhance production of these compounds for use against the toxic dinoflagellate, Karenia brevis, by providing bacterial cultures with different carbon sources and incubation at different temperatures prior to harvesting the algicidal exudate. To reduce toxic effects of ammonium, which was present at varying concentrations in the algicidal exudate, each batch was diluted to the same concentration of ammonium before analysis. Algicidal activity of each diluted batch was then evaluated using a bioassay approach and compared to activity for samples prepared under control conditions. This work is ongoing, but preliminary experiments indicated that addition of arginine and/or histidine to the bacterial growth medium significantly increased algicidal activity of the exudate. Samples of each batch of algicide were then analyzed by collaborating chemists at Mote Marine Lab to identify putative algicidal components. Results of this project will support the development of bacterial algicides for prevention, control and mitigation of harmful algal blooms.

A STRATEGY FOR GENE KNOCKDOWN IN DINOFLAGELLATES

Miranda M. Judd and Allen Place

University of Maryland Center for Environmental Science-Institute of Marine and Environmental Technology

Dinoflagellates are unicellular protists that display unusual nuclear features such as large genomes, condensed chromosomes and multiple gene copies organized as tandem gene arrays. Genetic regulation is believed to be controlled at the translational rather than transcriptional level. An important player in this process is the initiation factor eIF4E which binds the 7-methylguanosine cap structure (m7G) at the 5´-end of mRNA. Transcriptome analysis of eleven dinoflagellate species has established that each species encodes between eight to fifteen eIF4E family members, a number surpassing that found in any other eukaryotes, including other alveolates. Determining whether all eIF4E family members are important requires a method of knocking down their expression. In all other eukaryotes this is usually accomplished using translational blocking oligos or morpholinos. Morpholinos act by binding to complementary strands of RNA, therefore inhibiting the mRNA processing and translation. Unmodified morpholinos lack the ability to pass through cell membranes, however peptide-based reagents have been used to deliver substances into the cytosol of cells by an endocytosis-mediated process without damaging the cell membrane. We have successfully delivered GeneTools, LLC fluorescently-tagged standard morpholinos to the cytosol of *Ampnhidinum carterae* (CCMP 1314) by using a specific cell penetrating peptide. Our goal is to use this delivery system to introduce customized morpholinos into *A. carterae* cells targeting eIF4e sequences to inhibit translation. Specific eIF4e knockdown success will be characterized via quantitative RT-PCR, western blot analysis and inhibition of general protein translation.

ALLELOPATHY EFFECTS ON ALEXANDRIUM CATENELLA BY NON-HAB PHYTOPLANKTON IS DAMPENED UNDER GREENHOUSE CONDITIONS

Ewaldo Leitao, Hans Dam, Gihong Park

University of Connecticut

Some HAB species are likely to become more prevalent under climate change. However, we lack understanding on how ecological interactions, such as allelopathy between HAB and non-HAB species, will be affected under elevated temperature and CO2 levels (greenhouse conditions). We tested for allelopathic interaction of the HAB species *Alexandrium catenella* (strain BF-5, ~40 fmol.cell^{-1}) and non-HAB phytoplankton species (the green algae *Tetraselmis* sp., the cryptomonad *Rhodomonas salina*, and the diatom *Thalassiosira weissflogii* at various ratios between species, at ambient (400 ppm CO2 and 16°C) and greenhouse (2000 ppm CO2 and 20°C) conditions. All three non-HAB species increased their growth rate in greenhouse relative to ambient conditions, but their growth rates were unaffected by the presence of A. catenella. Contrary to our expectations, non-HAB species decreased *Alexandrium* growth, and the effect was density-dependent. Under greenhouse conditions, however, only the diatom had a negative effect on *Alexandrium* growth rate, and the effect was less strong when compared to ambient conditions. This dampening of allelopathic effects on *Alexandrium* under greenhouse conditions may further exacerbate HABs under climate change. Next steps will be to test the generality of the results with other *Alexandrium* strains (CCPM2304 ~70 fmol.cell^{-1}, and CTGN16 ~5fmol.cell^{-1}), to investigate whether constitutive toxicity may play a role in allelopathic interactions.

ASSESSMENT OF CAVITATION AS A DESTRUCTIVE TECHNOLOGY FOR CYANOBACTERIA AND THEIR TOXINS

Edith Martinez, Victor F Medina, John Brasher, Jose Mattei-Sosa, Catherine Thomas

Army Engineer Research and Development Center

Cyanobacterial blooms can be highly problematic during the summer and autumn time periods throughout the United States. These blooms cause typical problems associated with eutrophication resulting in masses of decaying algae, including oxygen depletion and associated anaerobic gas generation, pH depression and associated metals mobilization, formation of taste and odor compounds, and the formation of unpleasant slimes. In addition, cyanobacteria can form toxins that can poison livestock, pets, and wild animals, and make water dangerous to drink for humans. The U.S. Army Engineer Research and Development Center (ERDC) is studying cavitation as a means for destroying cyanobacteria. The implosion of cavitation microbubbles can disrupt the physiology of cyanobacteria, causing the cells to sink or rupture. In addition, cavitation can form reactive oxygen species that can degrade the toxins. The ERDC has conducted previous studies, which will be presented as background. New laboratory studies are focused on precisely relating measurable properties to cavitation that can be used to better design field systems. This laboratory system and its results will be presented. The ERDC will also represent a design and conceptual application of a field system.

NO CELLS, NO TOXINS, NOT SO FAST. WHY MITIGATION OF KARENIA BREVIS REQUIRES A TWO-PRONGED APPROACH

[1]Amanda Muni-Morgan, [2]Cynthia A. Heil, [2]Richard Pierce, [2]Patricia Blum, [2]Samantha Harlow, [3]Mark Leone, [4]Jordyn White-Rowell

[1]*University of Florida*
[2]*Mote Marine Laboratory*
[3]*Florida Gulf Coast University*
[4]*Tennessee State University*

Karenia brevis, the unarmored, toxic dinoflagellate that reoccurs annually in the eastern Gulf of Mexico, is easy to kill. However, effective control of *K. brevis* blooms requires elimination of both cells and associated brevetoxins. We examined the efficacy of 13 products identified from the literature as a potential for red tide mitigation. Of the products, the macroalgae species *Gracillaria lemaneiformis* produced allelopathic chemicals that eliminated 100 % of cells and 61 % total brevetoxin after 48 hours of exposure. Many products tested decreased cell abundance after 24 and 48 hours but resulted in an increase of dissolved brevetoxins in the media, specifically PbTx-3 and PbTx-2-carboxylic acid. Of the products tested, the most effective in eliminating both cells and brevetoxins were the macroalgal species. These results indicate that future mitigation strategies require concomitant monitoring of both cells and brevetoxin composition and concentration when evaluating the effectiveness of mitigation compounds against *K. brevis*.

USE OF GENETIC METHODS TO MONITOR TOXIC CYANOBACTERIA BLOOMS

Morgan Petrovich, Kendra Brown

Exponent

Genetic methods are gaining traction for their use in monitoring harmful cyanobacteria blooms. These methods include targeted analyses that look for a sequence of DNA associated with a particular organism, such as *Microcystis* species, or with a particular toxin-producing gene, and allow for measurement of their presence or assessing their population density in a water sample. Genetic approaches also include community analyses, such as 16S rRNA sequencing, which allow for characterization of all bacterial genera in a sample. Some of these methods have already been incorporated into state monitoring programs. For example, the state of Ohio routinely screens water samples for elevated toxin levels using quantitative polymerase chain reaction (qPCR) to measure total cyanobacteria, as well as genes associated with the production of the toxins microcystin, saxitoxin, and cylindrospermopsin. We will present tiered approaches that incorporate genetic methods to efficiently monitor drinking or recreational water and that align with U.S. EPA's public health-based guidance. We will highlight the opportunities for and challenges of using increasingly sophisticated genetic methods as part of monitoring programs.

CLAY TREATMENT OF RED TIDE: IMPACTS ON BLUE CRAB

Victoria Roberts, Emily Hall, Kristy Lewis

University of Central Florida

Harmful algal blooms of toxic *Karenia brevis*, also known as red tide, are an active risk to marine ecosystems in the Gulf of Mexico. The use of clay as a control for harmful algal blooms is prevalent in China and South Korea and is gaining traction in the United States. To contribute to ongoing environmental safety assessments, we observed the impacts of clay treatment of *K. brevis* on mortality and reflexes of blue crab (*Callinectes sapidus*). Crabs were exposed over eight days to either *K. brevis* alone (1×10^6 cells L^{-1}), clay alone (0.5 g L^{-1}), or clay combined with *K. brevis*, which were compared to crabs in a seawater control (n = 12). Results indicated clay treatment reduced concentrations of cells ($p < 0.0001$) and brevetoxin PbTx-3 ($p < 0.01$) from the water column. Crabs in clay treatment also performed better in eyestalk reflex tests than crabs in *K. brevis* ($p < 0.01$), indicating reduced stress. Less than 17% mortality occurred in all groups, and no mortality occurred in the combined clay and *K. brevis* treatment. Given that crabs displayed high tolerance for brevetoxins and that clay alone does not appear to have negative impacts on their survival, this method may be a viable option to treat blooms in areas where this species is present. The potential environmental impacts of clay treatment will be evaluated further by scaling up this experimental design to multi-species communities in mesocosms.

EAVESDROPPING ON PREDATOR-PREY DYNAMICS - THE CASE FOR INCLUDING ZOOPLANKTON IN HAB TOXIN FORECASTING

Aubrey Trapp[1,3], Jan Heuschele[2], Erik Selander[3]

[1]*Department of Ocean Sciences, University of California Santa Cruz*
[2]*Section for Aquatic Biology and Toxicology, Department of Biosciences, University of Oslo, Norway*
[3]*Department of Marine Sciences, University of Gothenburg, Sweden*

Recent innovations and technological advancements have rapidly improved harmful algae bloom forecasting. However, the toxin level that accumulates in shellfish during a harmful algae bloom is often variable and difficult to predict. While grazers play an important role in HAB dynamics, data from grazing is rarely included in forecasting models. The present study draws on two proposed mechanisms for top-down grazing effects, induction of algal toxins by copepod cues and selective grazing by copepods, to explore the relationship between grazer density and toxin content in farmed shellfish. Results from generalized additive models using eight years of monitoring data from the west coast of Sweden show that models including copepod density were better at predicting okadaic acid in blue mussels (*M. edulis*) than models with *Dinophysis* sp. cell counts alone. We then focus more narrowly on copepodamides, a group of copepod exudates known to induce toxins in key species of harmful algae. During a short-term monitoring study, copepodamides were measured from Swedish blue mussels over 8 months and compared to routine biotoxin measurements from the same samples. Adding copepodamides to the forecasting model improved on the models using copepod density and extended prediction lead time to about two months. Results suggest that including zooplankton information through copepodamides or grazer density may offer a cost-efficient way to improve accuracy and lead time for predicting the toxin accumulation in shellfish.

MOLECULAR ORIGIN OF MICROCYSTINS IN DIETARY SUPPLEMENTS ORIGINATING FROM THE KLAMATH LAKE, OREGON

Wannes H.R. Van Hassel, Anne-Catherine Ahn, Bart Huybrechts, Julien Masquelier, Mirjana Andjelkovic, Annick Wilmotte

Sciensano/uLiège, Belgium

Cyanobacteria can proliferate to high cell densities (blooms) in lakes, (artificial) ponds or closed-system cultures and are used as dietary supplements. Certain cyanobacterial species are also capable of producing toxins, for instance, hepatotoxins (e.g., microcystins, nodularin and cylindrospermopsin) or neurotoxins (e.g., anatoxin-a and saxitoxin). During a screening for eight microcystin congeners (MCs) and nodularin in cyanobacteria- and *Chlorella*-based dietary supplements sold on the Belgium market, nine products were found to be contaminated. These products should only contain harvested *Aphanizomenon flos-aquae*, a species that blooms in the Upper Klamath Lake (South Oregon, US)[1]. As it is not clear if this species can produce MCs, the possible occurrence of other species in the blooms could be responsible for the MCs presence.

After quantifying the MCs with a validated LC-MS/MS method, the presence of genes involved in the production of MCs was tested. The mcyE gene was amplified using PCR and sequenced by the Sanger method. MCs concentrations ranged between 39.7 and 4837.4 µg/kg MC-LR equivalent. Two dietary supplements contained concentrations higher than 1 µg/kg MC-LR equivalent which is proposed as regulatory limit by Gilroy et al.[2]. The phylogenetic affiliation of the mcyE sequences could be traced back to *Microcystis* sp. for seven products. The contamination of the supplements might be explained by the presence of *Microcystis* during the harvest of *A. flos-aquae* dominated blooms, as already observed in the past[2].

1. Carmichael, et al., J. Appl. Phycol. 12, 585–595 (2000).
2. Gilroy, et al., Environ. Health Perspect. 108, 435–439 (2000).

IN-VITRO AND IN-VIVO BIOCHEMICAL METHODS TO SEPARATE LIPID AND TOXIN BIOSYNTHESIS IN DINOFLAGELLATES

Ernest P Williams, Ernest P Williams, Tsvetan R Bachvaroff, Allen R Place

Institute for Marine and Environmental Technologies, University of Maryland Center for Environmental Science

Dinoflagellates are common perpetrators of harmful algal blooms because their natural products can intoxicate animals and humans indirectly. These toxins also have potential therapeutic uses but their biosynthesis is poorly understood for reasons including large genome size, gene duplication, and non-canonical gene arrangement. Although biosynthetic gene clusters are well characterized in bacteria and fungi, those in dinoflagellates are distantly related making this information less applicable. To begin identifying the genes directly involved toxin biosynthesis we tabulated and binned candidate genes from dinoflagellate transcriptomes using *Amphidinium carterae* (CCMP 1314) as a model. We then focused on genes where analogous lipid synthesis domains could be differentiated from natural product synthesis. We chose the carrier proteins that scaffold the synthesis of toxins and the enzymes that activate them because they are low in copy number and diversity. After producing recombinant proteins in *E. coli* and in-vitro, we used a variety of direct and indirect biochemical methods to characterize these proteins. We found that the enzymes that activate carrier proteins for natural product biosynthesis were ubiquitous amongst toxic and non-toxic dinoflagellates but that they were expressed during the day, while lipid synthesis activators were expressed into the night. Also, there were differences in the pH optima of these two groups that may give clues as to their cellular locations and functions. More importantly these results demonstrate how a variety of classic techniques can be adapted to describe natural product biosynthesis in dinoflagellates beyond what sequence-based predictions can provide.

STRUCTURAL VARIATION OF THE MCY OPERON DURING A MICROCYSTIS-DOMINATED CYANOBACTERIAL BLOOM REVEALS DYNAMICS OF NOVEL AND KNOWN GENOTYPES

Colleen E Yancey, Colleen E. Yancey, Derek J. Smith, Paul Den Uyl, Osama G. Mohamed, Fengan Yu, Timothy W. Davis, Steven A. Ruberg, Justin Chaffin, Kelly Godwin, Ashootosh Tripathi, David H. Sherman, Gregory J. Dick

University of Michigan

Cyanobacterial harmful algal blooms (CHABs) occur annually in fresh waters around the world. In temperate environments, warm weather CHABs are often dominated by *Microcystis aeruginosa*, which is responsible for the synthesis of microcystins (MC). Here, we characterized the relative abundance and sequence variation of the mcy gene cluster encoding for MC biosynthesis in western Lake Erie in 2014 and 2018. Sampling was conducted across several spatial and temporal scales including different bloom phases within the same year, extensive spatial coverage on the same day, and frequent temporal sampling over a two-week period via an autonomous sampler. Mapping of metagenomic and metatranscriptomic sequences to a reference genome revealed three main *Microcystis* genotypes in terms of mcy gene content: complete (all genes present, mcyA-J), partial (truncated mcyA, plus mcyB, mcyC), and absent (no mcy genes). We also detected two different forms of mcyB, which result from recombination and that may influence the MC congeners produced. We found that the abundance of the complete mcy genotypes correlates with pH and nitrate concentration whereas the abundance of the mcy-absent genotype was negatively correlated with pH. The partial operons were expressed in situ, suggesting potential for synthesis of a truncated MC. Overall, our results reveal that natural *Microcystis* populations contain several mcy genotypes that demonstrate spatiotemporal shifts in abundance and may influence the diversity of MC congeners produced in western Lake Erie.

NOVEL GRAPHENE-BASED COMPOSITE MATERIALS FOR MANAGEMENT OF HARMFUL ALGAL BLOOMS

Sarah G Zetterholm, Luke A. Gurtowski, P.E., Chris S. Griggs, PhD, Jose A. Mattei-Sosa, Sheila McLeod, Angela Evans

U.S. Army Corps of Engineers Engineer Research and Development Center

Graphene- and graphene oxide-based composite materials are currently being evaluated as a management strategy for harmful algal blooms (HABs) caused by various cyanobacteria. Adsorption of the cyanobacteria onto the surface of the composite materials has been observed, and antimicrobial characteristics of the composites indicate that the adsorbed cyanobacteria may be exterminated after contact. Adsorption capacities and rates of different graphene- and graphene-oxide based composite materials prepared with chitosan against cyanobacterial cultures and relevant toxins were investigated in this study. Analysis of the base materials for these composites as separate treatment technologies was also investigated for comparison to determine the mechanism(s) of removal. Results of this study will inform scaling efforts and further evaluation of this novel, freshwater HAB management technology.

POSTER PRESENTATION ABSTRACTS
(Ordered alphabetical by author)

INVESTIGATION INTO THE ENVIRONMENTAL DRIVERS OF MICROCYSTIN AND SAXITOXIN IN HARMFUL ALGAL BLOOMS IN CHAUTAUQUA LAKE, NY

Katelyn Brown, Timothy Davis, Jay DeMarco, George Bullerjahn

Bowling Green State University

Chautauqua Lake is a eutrophic lake located in western New York that is frequently impaired by harmful algal blooms (HABs). HABs generally occur in systems with high nutrient concentrations, but other factors including lake morphology and temperature also contribute to growth and development of cyanobacteria. HABs often result in production of hepatotoxins and neurotoxins like microcystin and saxitoxin, that are harmful to living organisms. This study focuses on the environmental drivers of toxin production in Chautauqua Lake through nutrient addition experiments to find what conditions favor toxin production. This will help in lake management efforts, specifically in reducing toxin concentrations. This project consists of long-term nutrient diffusing substrata trays deployed at the benthos and short-term nutrient amendment bottle experiments. Both experiments will be analyzed for chlorophyll-a, toxin concentration, community composition, and toxin related genes. It was found that when *Gloeotrichia* was the main component of algal community, the bottle nutrient additions did not have an impact on algal concentrations. However, once the bloom shifted to *Microcystis* there was clear nitrogen limitation. In addition, the nutrient diffusing trays deployed July through August showed phosphorus limitation at the benthos. This study will identify the taxa that are producing toxins, if nutrient additions favor toxin production, and overall assist in developing nutrient management plans that will reduce toxins in Chautauqua Lake.

METHODS TOWARDS MEASURING THE IMPACTS AND CYCLING OF DOMOIC ACID IN BENTHIC ENVIRONMENTS

[1,2]Rosaly Castorena, [1]Dana Shultz, [3]Vanh Phonsiri, [3]Violet Renick, and [1]Jayme Smith

[1]*Southern California Coastal Water Research Project Authority*
[2]*Vanguard University*
[3]*Orange County Sanitation District*

The algal biotoxin domoic acid (DA) has caused major socioeconomic impacts, including closures of key fisheries, health advisories, and marine wildlife mortalities. DA is produced by species of the marine diatom genus Pseudo-nitzschia and enters the planktonic food web via bioaccumulation. Currently, there is a limited amount of research looking the impacts of DA on benthic communities and the cycling of DA in the benthic environment. This projected aimed to explore approaches for measuring domoic acid in marine sediments and to develop a better understanding of cycling of domoic acid in benthic environments. This project worked to optimize a sediment analysis protocol using ELISA. The efficiency of this approach was compared to published LC-MS approached. The other goal of this project was to better understand the persistence of DA in the marine environment. An experiment was conducted with treatments to mimic ocean conditions in the surface ocean and benthic environments to observe which conditions may be more susceptible to degradation. DA concentrations were monitored via ELISA over a period of weeks to determine which conditions may be more susceptible to degradation. Interestingly, although we hypothesized that DA would persist longer in the treatments mimicking the benthic environment than in surface ocean conditions, we found minimal differences among our experimental treatments. Together, the findings of this work will help to underpin future efforts to study the extent, magnitude, and impacts of DA in benthic environments.

MULTI-YEAR SAMPLING PROVIDES NOVEL INSIGHT INTO LINKAGES BETWEEN *PSEUDO-NITZSCHIA* SPECIES DYNAMICS AND DOMOIC ACID IN THE NEARSHORE GULF OF MAINE

Christina Chadwick[1], Farrell, A[4], Disney, J[4], Kanwit, K[3], Lewis, B[3], Nash, C[5], Dejadon, B[5], Chin, T[5], Clark, S[2], DeSmidt, A[1], Shankar, S[1], Villac, C[1], Bouchard, JN[1], Denny, E[1], Robert, M[1], Flewelling, L[1], Granholm, A[1], Joseph, M[1], Ralston, D[2], Brosnahan, M[2], Anderson, D[2], McGillicuddy, D[2], Hubbard, K[1,2]

[1]*Florida Fish and Wildlife Conservation Commission/Fish and Wildlife Research Institute*
[2]*Woods Hole Oceanographic Institution*
[3]*Maine Department of Natural Resources*
[4]*Mount Desert Island Biological Laboratory*
[5]*New Hampshire Department of Environmental Services*

The toxic diatom genus *Pseudo-nitzschia* occurs globally and consists of >52 described species. Some species produce the neurotoxin domoic acid (DA) which can cause Amnesic Shellfish Poisoning (ASP) in humans. In 2016, the Gulf of Maine experienced its first ASP closure in eastern Maine, coincident with the first observance of *Pseudo-nitzschia australis* in this region. To better understand toxic *Pseudo-nitzschia* bloom dynamics across years with and without ASP closures, ~weekly time series samples were collected in Bar Harbor, Maine from 2013-2020 and Hampton Harbor, New Hampshire from 2019-2020. Samples were collected for analysis of particulate DA, species composition, cell abundance, and dissolved nutrients. *Pseudo-nitschia* species diversity was analyzed using Automated Ribosomal Intergenic Spacer Analysis (ARISA), a DNA fingerprinting method that showed dynamic interannual, seasonal and event-scale transitions in *Pseudo-nitzschia* species assemblages. For example, *P. australis* was only observed from 2016-2020, typically initiated during September of those years, and was associated with Maine shellfish harvest closures each year except 2020. Another species, *P. plurisecta*, typically dominated in late July and August, produced total particulate toxin levels similar to that of *P. australis* at Bar Harbor, but has not caused any US harvest closures to date. In contrast, *P. delicatissima* sometimes formed abundant blooms, most often in spring, that were not associated with DA. This ongoing research is critical for developing qualitative and quantitative prediction capabilities for toxic blooms that account for changes in species diversity, leading to early warning systems and improved understanding of the potential triggers of toxin production.

REDOX PROTEOMICS AND THE ESSENTIAL COMPONENTS OF THE XANTHOPHYLL CYCLE IN HIGH AND LOW TOXIC KARENIA BREVIS

Ricardo Colon, Kathleen Rein

Florida International University

The cellular toxin content of *K. brevis* is highly variable between or even within strains. Herein, we investigate physiological differences between high (KbHT) and low (KbLT) toxin producing cultures both derived from the Wilson strain, related to energy-dependent quenching (qE) by photosystem II, and reduced thiol content of the proteome. We demonstrate that gene and protein expression of the xanthophyll cycle enzyme diadinoxanthin de-epoxidase (Dde) and monogalactosyldiacylglycerol (MGDG) synthase are not significantly different in the two cultures. Using redox proteomics, we report a significantly higher reduced cysteine content in the low toxin proteome, including plastid localized thioredoxin reductase (Trx) which can result in inactivation of Dde and activation of MGDG synthase. We also report significant differences in the lipidomes of KbHT and KbLT with respect to MGDG, which facilitates the xanthophyll cycle. We also analyze trends in the differences of protein expression and redox status between KbHT and KbLT relating to polyketide synthases and protein kinases.

SPATIAL-TEMPORAL VARIABILITY IN PHYTOPLANKTON BIOMASS AND COMPOSITION IN TEXAS RESIDENTIAL CANALS

Jordana Cutajar, Michael Wetz

Texas A&M University

Harmful algal blooms (HABs) are a natural phenomenon that appear to be increasing worldwide alongside the spread of urbanization and cultural eutrophication (Anderson et al., 2002). As a result, HABs are being documented in previously pristine locations, presenting new environmental and human health challenges (Verity, 2010). Many regions around the globe, including in Texas, are seeing a major increase in the number of residential canal systems along their coastline (Waltham & Connolly, 2011). These canals pose unique attributes that may enhance conditions for HABs, namely shallow depths, high susceptibility to urban runoff, reduced mixing, and long residence time. Despite this, there has been little research on the water quality and phytoplankton composition of these systems. In this study, I am quantifying water quality and phytoplankton biomass/composition along a mouth-interior gradient of a canal system on Padre Island (Corpus Christi, Texas). I hypothesized that sites toward the interior of the canal system will experience increased nutrient availability, stratification, reduced flow, and increased phytoplankton biomass/HAB occurrence.
Ultimately, this research will provide a novel assessment of the health of a residential coastal canal system and will determine if more intensive monitoring is needed due to presence of water quality/HAB issues.

USING HARMFUL ALGAL BLOOM MONITORING TO UNDERSTAND PSP RISKS AND MITIGATE TESTING BURDENS AT A SHELLFISH FARM IN SOUTHEAST ALASKA

Muriel Dittrich, Courtney Hart, David Tallmon

University of Alaska Southeast

In the state of Alaska, fishing and mariculture industries are integral to sustaining local and regional communities and play an important role in boosting state economic activity. One of the challenges to developing a profitable and safe mariculture industry, is mitigating the threat of harmful algal blooms (HABs) in coastal waters. Blooms of the HAB-species *Alexandrium catenella* result in the bioaccumulation of paralytic shellfish toxins (PSTs) in many species of shellfish; in Southeast AK, 80% of shellfish tests submitted are above the regulation limit for PSTs (Trainer et al. 2014). Our goal is to develop a metric for understanding how the presence and abundance of *A. catenella* cells in the water column relates to the toxicity in oyster tissues. We are collaborating with Juneau's first and only oyster farm, Salty Lady Seafood, to establish a monitoring site at the farm and track bloom dynamics, presence of PSTs in the water column, and toxin load in marketable oyster tissue. Over the next year we will conduct on-site HAB monitoring and collect environmental data including salinity and temperature, whole water samples at 1m and 5m and a three-minute horizontal surface tow (1-5 meters). Motile cells will be quantified using visual counts, PS's in the water column will be quantified using ELISA and the PSTs in the oyster tissue will be quantified with receptor binding assays. We hope to track the number of *A. catenella* cells in the water column to determine if there is a correlation with PSTs in oyster tissues.

FORECASTING SHELLFISH TOXICITY IN COASTAL MAINE USING TOXIN DATA, ENVIRONMENTAL COVARIATES, AND DEEP LEARNING

Johnathan Evanilla, Nicholas R. Record, Stephen D. Archer, Benjamin Tupper, Kohl Kanwit

Bigelow Laboratory for Ocean Sciences

Harmful algal blooms, also known as "red tides," are a challenge for shellfish aquaculture in coastal Maine, as their resulting biotoxin accumulation in shellfish causes harvesting closures. Forecasting shellfish toxicity, at a site-specific scale, is an opportunity for both farmed and wild coastal seafood industries to mitigate economic loss from unforeseen area closures. A novel shellfish toxicity forecasting system has been developed to predict paralytic shellfish poisoning (PSP) related toxin levels for aquaculture and harvesting sites in coastal Maine. The system uses an artificial neural network developed with Keras, a deep-learning API that runs on top of TensorFlow. Weekly shellfish toxin testing data, starting with the 2014 season, has been used for training and testing model configurations. The forecast had ~95% accuracy through 2017, predicting both the onset and ending of closures, but variable conditions in recent years have decreased accuracy. Adding environmental covariates to the toxin covariates has had mixed results in improving forecast accuracy. Transitioning from testing to an operational forecast involves regular input and feedback from shellfish growers, buyers, and managers. A live, experimental run of the forecast is being tested during the 2021 "red tide" season, informed by user feedback.

A NOVEL PHOSPHORUS REMEDIATION TECHNOLOGY FOR FLOWING WATERS

Byran C Fuhrmann, West Bishop, Katrina Spinelli, Chris Asselin, Greg Armel

SePRO Corporation

Cultural eutrophication is the process in which human activity leads to excessive and potentially toxic algal growth in aquatic resources like lakes and rivers. This algal growth is primarily driven by high nutrient input to the waterbody from external sources such as stormwater, agricultural runoff, wastewater treatment plant effluent, and septic system leaks. Proactive strategies to limit algal growth and protect water quality generally rely on limiting the availability of nutrients. Many species of cyanobacteria are capable of fixing gaseous atmospheric nitrogen, therefore, the majority of nutrient mitigation strategies to prevent HABs have focused on reducing phosphorus. Phosphorus mitigation technologies such as alum, Phoslock®, and oxygenation have been shown to be effective methods to prevent the release of phosphorus from the sediment, however, they are not easily implemented to reduce phosphorus levels in flowing waters. This presentation provides case studies and an overview of EutroSORB®, a novel new phosphorus filtering technology which is designed for use in flowing waters. EutroSORB® is an all-natural formulation which rapidly and selectively binds phosphate from many water types with environmentally relevant water chemistries and effective at short contact times spanning various flow rates. EutroSORB® can effectively remove more than 10 mg phosphorus per gram of material (> 1%) which allows small filter bags to treat large volumes of water or waters with high phosphorus concentrations. EutroSORB® media can also be used as a soil ammendment to enhance the quality of soils after the phosphorus binding capacity has been exhausted.

SPATIAL DISTRIBUTION OF MICROBIAL COMMUNITIES FROM THE 2018 LAKE ERIE HABS GRAB

Jennifer Harper[1], Kaitlin Plate[1], Justin Chaffin[2], Thomas Bridgeman[3], Ed Verhamme[4], Jorge Santodomingo[5], Timothy W. Davis[1], Christopher S. Ward[1]

[1]*Bowling Green State University*
[2]*Ohio State University*
[3]*University of Toledo*
[4]*LimnoTech*
[5]*Environmental Protection Agency*

Annual harmful algal blooms (HABs) threaten the ecological and economical well-being of western Lake Erie and the surrounding region. To reveal the patterns of an entire bloom at high spatial resolution, researchers from over 11 academic institutions, agencies and private sector groups coordinated a single-day survey of the 2018 HAB in the Western Lake Erie ('HABs Grab', August 9th). Here, we present the preliminary results from 16S rRNA gene amplicon sequencing of 100 samples, towards determining how the microbial community varied spatially and in relation to environmental conditions within the bloom. In libraries with high cyanobacterial abundances, GpI (*Anabaena/Aphamizomenon*), GpIIa (*Synechococcus*), GpVI (*Pseudanabaena*), and GpXI (*Microcystis*) were dominant groups whose abundances varied greatly across the sampling area and whose maximum relative abundances approached 50%. Bacillariophyta (mainly *Aulacoseira*) and Cryptophyta (*Teleaulax*) were prevalent throughout the dataset even in samples where cyanobacterial groups were abundant. Major heterotrophic groups were Flavobacteriia (Cryomorphaceae, Flavobacteriaceae), Planctomycetia (Planctomycetaceae), and Sphingobacteriia (Chitinophagaceae, Saprospiraceae). Ongoing analysis is focused on the environmental and biotic drivers of microbial group abundance and distribution. Insights from this study will improve our understanding of how the microbiome contributes to bloom formation, persistence, and senescence.

ASSIMILATION OF NITRIC OXIDE BY HETEROSIGMA AKASHIWO IN THE PRESENCE OF OTHER NITROGEN SOURCES

[1]Emily M. Healey, [2]Stacie Flood, [3]Robinson W. Fulweiler, [1]Joanna York, [1]Kathryn J. Coyne

[1]*University of Delaware*
[2]*Mote Marine Laboratory*
[3]*Boston University*

Heterosigma akashiwo is a globally distributed harmful algal bloom species. Annual blooms in Delaware's inland bays, DE, indicate that this species can outcompete other phytoplankton including other HAB species. *Heterosigma akashiwo* possesses a hybrid nitrate reductase enzyme, NR2-2/2HbN, which combines nitric oxide dioxygenase activity with nitrate reductase to assimilate NO. We investigated the capacity of *H. akashiwo* to utilize NO as a source of nitrogen, perhaps giving this species a competitive advantage when other N-sources are limiting. In previous research, nitrate reductase activity in *H. akashiwo* was inhibited by the presence of excess ammonium. To understand the molecular and biochemical response of *H. akashiwo* to NO in the presence of other nitrogen sources, *H. akashiwo* (CCMP 2393) was grown in continuous culture with 100:0 µM nitrate: ammonium, 50:50 µM nitrate: ammonium, or 0:100 µM nitrate: ammonium. Subsamples of each treatment were then spiked with 15N-labeled NO. Controls received 15N-labeled nitrate, 15N-labeled ammonium, or unamended media. *Heterosigma akashiwo* response was evaluated for the relative expression of genes involved in nitrogen assimilation (nitrate reductase, NR, glutamine synthase, GS, and glutamine:2-oxoglutarate amidotransferase, GOGAT), uptake rate of 15N, and nitrate reductase activity. Analysis is ongoing, but preliminary results revealed significant differences in the expression of key genes in the nitrogen assimilation pathway for cultures spiked with 15N-NO and 15N-NO_3^- compared to cultures spiked with 15N-NH_4^+ or unamended media. Results will inform future environmental microcosm studies and provide insight on regulation of NO assimilation by *Heterosigma akashiwo* and other marine algae.

INTEGRATING HABSCOPE INTO FLORIDA'S HARMFUL ALGAL BLOOM MONITORING NETWORK

[1]Alicia Hoeglund, [2]Barbara Kirkpatrick, [2]Robert D. Currier, [2]Grant Craig, [3]William C. Holland, [3]D. Ransom Hardison, [4]R. Wayne Litaker, [4]Michelle Tomlinson, [4]Richard Stumpf, [1]Katherine A. Hubbard

[1]*Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute*
[2]*Gulf of Mexico Coastal Ocean Observing System, Department of Oceanography, Texas A & M University*
[3]*NOAA/National Ocean Service, National Centers for Coastal Ocean Science*
[4]*National Oceanic and Atmospheric Administration/National Ocean Service, National Centers for Coastal Ocean Science*

Blooms of the harmful alga, *Karenia brevis*, occur nearly annually in southwest Florida but are spatially and temporally patchy. Each year, thousands of water samples are enumerated by the Fish and Wildlife Research Institute (FWRI) via traditional light microscopy (LM) to determine *K. brevis* cellular abundance. In March 2018, weekly HABscope sampling was initiated at five of FWRI's routine monitoring sites in the greater Tampa Bay area in response to recent, recurring blooms there (i.e., 2015-2018). HABscope is a new quantification tool developed by the Gulf of Mexico Coastal Ocean Observing System (GCOOS) and National Oceanic and Atmospheric Administration (NOAA) that couples LM with recorded video and artificial intelligence to rapidly enumerate *K. brevis* cells in a water sample. In 2018—during the 16-month bloom that impacted southwest Florida from 2017 to 2019—paired FWRI LM/HABscope monitoring captured the onset and departure of *K. brevis* from Tampa Bay and repeatedly sampled a bloom offshore of Boca Grande as part of a NOAA-supported event response effort. We continue to conduct targeted validation and veracity testing, drawing upon these efforts and ongoing Tampa Bay time series sampling. So far, these samples have contained *K. brevis* abundances spanning eight orders of magnitude, up to 10^5 cells L^{-1} of other *Karenia* species, additional harmful algal blooms and high abundance algal blooms, as well as numerous samples that did not contain *K. brevis*. Analysis informs the further expansion and integration of the HABscope into citizen science monitoring of *K. brevis* blooms and forecasting related impacts.

STUDY OF ENVIRONMENTAL EXPOSURE AND LONG-TERM HEALTH IMPACTS OF CYANOBACTERIAL HARMFUL ALGAL BLOOMS IN WATERS FROM SOUTH FLORIDA

[1]Daniela Maizel, [2]Alberto Caban-Martinez, [1]Cassandra Gaston, [1]Haley Royer, [1]Raymond Leibensperger III, [1]Kaycie Lanpher, [3]Helena Solo-Gabriele, [1]Larry Brand, [2]Grace Zhai, [4]Natasha Solle, [5]Nichole Klatt, [1]Katerina Santiago, [1]Mohamed Diop, [1]Addison Testoff, [1]Kimberly Popendorf

[1]University of Miami - RSMAS
[2]University of Miami Miller School of Medicine- Public Health Sciences
[3]Civil, Architectural and Environmental Engineering
[4]University of Miami Miller School of Medicine-Medical Oncology
[5]Microbiology Research Facility

Harmful Algal Blooms (HABs) caused by blue green algae can produce a wide range of toxins that have both short- and long-term health effects on humans, constituting a major public health concern.

In our study, a cohort of Floridians was established with the primary goal to examine environmental exposures to cyanobacterial toxins and its association to long-term human health. We quantified environmental exposure doses of toxins in the water for communities living, working and recreating in and near Florida lakes, rivers and canals; and we collected and characterized human biomonitoring data for the purpose of better understanding the health risks for people with variable exposure to the toxins.

In addition, laboratory experiments were conducted to determine the effectiveness of different types of air conditioner and face mask filters for HAB aerosol transmission prevention. We used a bubbler apparatus to generate aerosols from HAB cultures under controlled conditions. We measured the transmission prevention afforded by each filter both by quantifying the aerosolized microcystin toxins and quantifying the size distribution and abundance of particles. Our tests of a commonly used disposable surgical mask found that it removed more than 90% of particles across a wide particle size spectrum and reduced the HAB toxin concentration in the air to levels below detection.

USING A FLOWCAM TO FACILITATE HAB CELL COUNTS: COMPARING CELL ENUMERATION METHODS

[1]Savannah A Mapes, [2]Savannah Judge, [1]Kimberly Reece

[1]*Virginia Institute of Marine Science*
[2]*Fluid Imaging Technologies, Inc.*

Consecutive blooms of *Margalefidinium polykrikoides* and *Alexandrium monilatum* have occurred in the York River near-annually since 2007. Both species are considered harmful and therefore are monitored by the Virginia Department of Health assisted by the Reece lab at VIMS. These blooms, particularly those of the toxin-producing species *A. monilatum*, have occasionally coincided with kills of finfish, shellfish, or other aquatic organisms, and therefore blooms are reported to local aquaculturists. The Reece lab has previously quantified these species by visual cell counts and quantitative PCR (qPCR). Visual cell counts are a standard for enumerating cells but is laborious and time-consuming especially with the long chains formed by *A. monilatum* during a bloom. qPCR is problematic for enumerating rapidly-replicating *A. monilatum* cells, often overestimating the number of cells. Last summer (2020) we introduced a new cell enumeration method to document blooms of both species in the York using flow-imaging technology with a FlowCam. A semi-automatic classification filter was created with the FlowCam and VisualSpreadsheet analysis software to fit single, double, and chains of cells of similar lengths into separate classes. This expedited the cell count process by eliminating the need to preserve cells or count each cell individually, reducing manual labor and time spent at the microscope. Cell count accuracy was compared with the manual light microscopy and qPCR cell count methods and will be shared during the presentation.

MODELING DEGRADATION OF PARALYTIC SHELLFISH TOXINS IN FRESHWATER SYSTEMS

Tryston Metz, John Ferry

University of South Carolina

Lyngbya wollei is a benthic cyanobacteria known to produce several acetylated analogues of the paralytic shellfish toxin saxitoxin. Infestations, or blooms, of *Lyngbya wollei* appear as filamentous masses attached to sediment surfaces that can persist for several years at levels of 20-30 kg/m^2. The associated saxitoxin analogues, known as the *L. wollei* toxins, or LWTs, are not as toxic as saxitoxin but typically occur in much higher concentrations. Work presented in this seminar addresses the environmental fate of the LWTs by simulating the environmental conditions of a local reservoir, Lake Wateree SC, in the laboratory. *L. wollei* samples were collected from multiple points in the reservoir and processed in the laboratory to quantify the type and amount of LWTs present in each sample. Leaching studies were performed to determine the possibility that toxins could directly effuse from live or dead algal samples into surrounding waters. Hydrolytic degradation was determined at pH 8-10; notably the LWTs appeared to experience deacetylation to yield decarbamoyl analogues for a net increase in toxicity. The temperature dependence of hydrolysis was determined over the range 10-40 Degree C. A model of the varying hydrolysis rate of the LWTs is presented based on measured diel variation in pH and T in Lake Wateree at a site immediately adjacent to a microbial mat.

ESTIMATING PSEUDO-NITZSCHIA DOMOIC ACID PRODUCTION IN A CHANGING WORLD

[1]Allison R. Moreno, [2,3]Clarissa Anderson, [4]Raphael M. Kudela, [5]Faycal Kessouri, [5]Martha Sutula, [5]Jayme Smith, and[1] Daniele Bianchi

[1]*University of California, Los Angeles*
[2]*University of California, San Diego*
[3]*Southern California Coastal Ocean Observing System*
[4]*University of California, Santa Cruz*
[5]*Southern California Coastal Water Research Project*

Pseudo-nitzschia species are one of the leading causes of harmful algal blooms (HABs) along the western coast of the United States. Approximately half of known *Pseudo-nitzschia* strains can produce domoic acid (DA), a neurotoxin which has been shown to negatively impact wildlife, fisheries, and put human life at risk through amnesic shellfish poisoning. DA is a secondary metabolite, synthesized during periods of low primary metabolism. Environmental stressors are hypothesized to stimulate DA production and accumulation. Si limitation, in diatoms, results in decreasing primary metabolism, i.e., DNA synthesis and gene expression, alleviating energy for DA production. Changes in light levels have also been shown to adjust biosynthetic properties in *Pseudo-nitzschia*. To quantify and estimate the feedbacks between DA production and environmental conditions, we designed a simple mechanistic model, which is validated against batch and chemostat experiments. Similar to observations, our model shows that as nutrients (i.e., silicon, iron, and phosphorus) become limiting DA production increases. Under Si limitation, we found an approximate doubling in DA production. Additionally, our model indicates a positive relationship between light and DA production. These results support the idea that the relationship with nutrient limitation and light is based on direct impacts on biosynthesis and biomass accumulation in *Pseudo-nitzschia*. Because it can easily be embedded in existing ecosystem models, our model represents the first step toward predicting the occurrence of *Pseudo-nitzschia* HABs across the California coast.

BORONATE AFFINITY POLYMERS FOR SELECTIVE EXTRACTION, CONCENTRATION, AND CLEANUP OF CIGUATOXINS IN FISH EXTRACTS

[1]Elizabeth M Mudge, [2]Alison Robertson, [3]Pearse McCarron, [3]Christopher O. Miles

[1]NRC, Department of Marine Sciences
[2]University of South Alabama
[3]Biotoxin Metrology, National Research Council

Ciguatoxins (CTXs) are a class of polycyclic ethers that accumulate in fish flesh from the consumption of benthic *Gambierdiscus* microalgae. Several precursor metabolites have been shown to be produced by *Gambierdiscus* and are biotransformed into ciguatoxins detected in fish flesh. Ciguatoxin concentrations as low as the ng/g range have been reported to induce ciguatera poisoning symptoms. These low concentrations and the complex sample matrix impede positive confirmation using techniques such as liquid chromatography-mass spectrometry (LC-MS). Sample preparation procedures typically used to remove matrix interferences in CTX analysis require several clean-up steps and sample transfers, increasing the potential for losses and low recoveries. Many CTXs detected in fish contain cis-1,2 diols, making them candidates for selective isolation with boronate affinity polymers. Dispersive solid-phase extraction procedures were used to evaluate several polymer-bound boronic acids for selective binding of CTXs. The CTXs are subsequently released into solution, resulting in CTX-rich extracts with reduced matrix interferences. Preliminary results indicate that polymer-bound 3-aminophenylboronic acid binds greater than 95% of CTXs, with recoveries greater than 70 % achieved so far. This technique was applied to effectively reduce matrix components in LC-MS and reduce the number of subsequent clean-up steps required for sample preparation. The use of boronate affinity polymers can be adapted to sample preparation and purification procedures to selectively extract CTXs from complex fish matrices and remove contaminants.

REPORT ON HARMFUL ALGAL BLOOM SPECIES DETECTED IN LONG ISLAND SOUND DURING THE SUMMER OF 2020

[1,2]Zabdiel A Roldan Ayala, [1,2]Georgia Humphries, [2]Silvia Anglès, [2]Jessica Espinosa, [1,2]Maximillian Brown, [2]Mariapaola Ambrosone, [3]Maria Tzortziou, [4]Joaquim Goes, [1,2]Dianne I. Greenfield

[1]*School of Earth and Environmental Sciences, Queens College, CUNY*
[2]*Advanced Science Research Center at the Graduate Center, City University of New York*
[3]*City College Center for Discovery and Innovation, CUNY*
[4]*Lamont-Doherty Earth Observatory*

Long Island Sound (LIS) receives high levels of nitrogen (N) inputs from the nearby NYC metropolitan area due to runoff, combined sewer overflow systems, and other sources. N loading is linked to western LIS (WLIS) hypoxia formation as well as harmful algal blooms (HABs) caused by multiple phytoplankton genera across LIS. This study leverages long-term, year-round LIS water quality monitoring conducted by the Connecticut Department of Energy and Environmental Protection (CTDEEP). Samples were acquired routinely from five Western LIS stations (A4, B3, 04, D3 and C2), in addition to several Central and Eastern LIS stations, all at two depths (surface and sub-chlorophyll maxima. We report initial evaluations of physical water quality, chlorophyll a and nutrients (N, P, Si) from these efforts. Phytoplankton community composition was analyzed using microscopy and molecular (sandwich hybridization assay) approaches. Microphytoplankton cell biovolume was also measured to assess the relative biomass contributions of different genera. During the summer of 2020, several HABs species were detected in the water column, *Prorocentrum triestinum* causing a significant July bloom (~1800 cells/ml) in WLS. Other key HAB species observed included *Margalefidinium polykrikoides*, *Alexandrium* spp., and *Dinophysis acuminata* . Results will refine our understanding of the spatiotemporal transitions in LIS phytoplankton assemblages and improve bloom prediction capabilities.

CYANOBACTERIAL BLOOMS AND EUTROPHICATION IN RECREATIONAL LAKES NEAR LOS ANGELES, CALIFORNIA

Dana Shultz[1], Susanna Theroux[1], Bowen Du[1], Wayne Lao[1], Emily Duncan[2] and Jayme Smith[1]

[1]*Southern California Coastal Water Research Project*
[2]*Los Angeles Regional Water Quality Control Board*

The majority of freshwater harmful algal blooms (FHABs) are caused by cyanobacteria. Many genera of cyanobacteria have the potential to produce multiple toxins, collectively referred to as cyanotoxins. Cyanotoxins can contaminate drinking water, endanger human and animal health, and can be transported between systems as water moves throughout the environment. Eutrophication is a major driver of cyanobacterial blooms and cyanobacterial preference for warm, stagnant and nutrient rich waters suggest that climate change will exacerbate the magnitude and duration of blooms. This study sought to understand what levels of cyanotoxins (total microcystins, cylindrospermopsin and anatoxin-a) and which cyanobacterial genera were present in 17 lakes with designated recreational uses throughout Los Angeles and Ventura counties during August and September 2020. Additionally, eutrophication indicators (nutrients and dissolved oxygen) were collected to determine a FHAB risk-level for each lake based on both eutrophication markers and the cyanobacterial community present. Preliminary toxin results analyzed with ELISA have shown microcystins present in water samples at 6 out of 17 (35%) lakes, cylindrospermopsin present in 1 out of 17 lakes and anatoxin-a present in 1 out of 17 lakes. Diverse cyanobacterial communities were observed across lakes. *Microcystis* was identified as contributing to greater than 5% relative abundance of the cyanobacterial community in 4 out of 17 lakes, while *Synechococcus* was one of the most widespread genera identified among the lakes. The results of this study will have important implications for future monitoring efforts in these recreational lakes and in understanding the potential for future bloom events.

BACTERIAL ANTAGONISM IN A LAKE TAI (TAIHU) MICROCYSTIS BLOOM

Lucy K Utz, Morgan Steffen

James Madison University

Microcystis blooms are comprised of diverse consortia of heterotrophic bacteria. Some of these bacteria may provide important growth-promoting services to *Microcystis* and other bloom-forming cyanobacteria. However, these growth-promoting functions may be inhibited by competing and/or antagonistic bacteria. The aim of this study was to determine the potential for antagonistic interactions between bloom-associated bacteria isolated from a *Microcystis* bloom in Lake Tai (Taihu) in August 2018. A set of 45 bacterial isolates have been tested against each other in a plate assay to determine growth inhibition capabilities. Surprisingly, in over 2,000 interactions tested, no microbial antagonism was identified. This suggests few antagonistic ecological interactions exist in the culturable microbiome of *Microcystis* blooms in Taihu.

AUTHOR CONTACT INFORMATION

Name	Institution/Agency/Organization	Email address
Kathleen Aarvig	CDC	qph2@cdc.gov
So Hyun (Sophia) Ahn	University of Maryland Center for Environmental Science, Horn Point Laboratory	sahn@umces.edu
Taylor Armstrong	University of Maryland Center for Environmental Science-IMET	tarmstrong@umces.edu
Malcolm Barnard	University of North Carolina at Chapel Hill	malcolm.barnard@unc.edu
Angelea Belfiore	Auburn University	apb0053@auburn.edu
Clayton Bennett	University of South Alabama/ Dauphin Island Sea Lab	cbennett@disl.org
Rachel Brewton	Harbor Branch Oceanographic Institute, Florida Atlantic University	brewtonr@fau.edu
Erik Broemsen	The University of North Carolina at Charlotte	elbroems@uncc.edu
Katelyn Brown	Bowling Green State University	browkat@bgsu.edu
Seth Buchholz	Bowling Green State University	sbuchho@BGSU.edu
Claudia Carrion	University of the Virgin Islands	kayacarrionmusic@gmail.com
Rosaly Castorena	Vanguard University & Southern California Coastal Water Research Project	rosaly.castorena@vanguard.edu
Christina Chadwick	Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute	christina.chadwick@myfwc.com
Jacqueline Chrabot	Old Dominion University/Department of Ocean and Earth Sciences	jchrabot@odu.edu
Ricardo Colon	Florida Intl University	rcolo030@fiu.edu
Jordana Cutajar	Texas A&M University	jcutajar@islander.tamucc.edu
Dominique Derminio	EGET LIBER/SUNY ESF/Keuka College	dsderminio@gmail.com
Muriel Dittrich	University of Alaska Southeast	mcdittrich@alaska.edu
Michelle Edwards	Harbor Branch Oceanographic Institute, Florida Atlantic University	edwardsm2019@fau.edu
Johnathan Evanilla	Bigelow Laboratory for Ocean Sciences	jevanilla@bigelow.org
Evangeline Fachon	Woods Hole Oceanographic Institution	efachon@whoi.edu
Shiqi Fang	North Carolina State University	sfang6@ncsu.edu
Cong Fei	New York University Abu Dhabi	cf2290@nyu.edu
Edna Fernandez-Figueroa	Auburn University	egf0013@auburn.edu
James Fiorendino	Texas A&M University	fiorenja@tamu.edu
Kyra Florea	University of Southern California	kflorea@usc.edu

Byran Fuhrmann	SePRO	byranf@sepro.com
Malia Gardner	James Madison University	gardnemi@dukes.jmu.edu
Matthew Gladfelter	Auburn University	mfg0017@auburn.edu
Jennifer Harper	Bowling Green State University	jnharpe@bgsu.edu
Courtney Hart	University of Alaska Fairbanks, Juneau Fisheries Center	Chart16@alaska.edu
Emily Healey	University of Delaware	ehealey@udel.edu
Alicia Hoeglund	Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute	alicia.hoeglund@myfwc.com
Daniel Hoffman	Wright State University	drdkhoffman@gmail.com
Andrea Jaegge	University of Louisiana at Lafayette	andrea.jaegge1@louisiana.edu
Gretchen Johnson	University of Delaware	tabbag@udel.edu
Miranda Judd	Institute of Marine and Environmental Technology	mjudd@umces.edu
Kyla Kelly	University of Southern California	kylakell@usc.edu
Benjamin Kramer	Stony Brook University	benjamin.j.kramer@stonybrook.edu
Megan Ladds	Stony Brook University	megan.ladds@stonybrook.edu
Ewaldo Leitao	University of Connecticut	ewaldo.leitao@uconn.edu
Dominique Lockwood	James Madison University	lockwodj@dukes.jmu.edu
Daniela Maizel	University of Miami - RSMAS	dxm1457@rsmas.miami.edu
Savannah Mapes	Virginia Institute of Marine Science	samapes@vims.edu
Edith Martinez	Army Engineer Research and Development Center	edith.l.martinez-guerra@usace.army.mil
Tryston Metz	University of South Carolina	tmetz@email.sc.edu
Allison Moreno	UC Los Angeles	allimoreno@atmos.ucla.edu
Elizabeth Mudge	NRC	elizabeth.mudge@nrc-cnrc.gc.ca
Amanda Muni-Morgan	University of Florida	a.munimorgan@ufl.edu
Isadora Nogueira	Public Health Institute	isadora.nogueira@trackingcalifornia.org
Gihong Park	University of Connecticut	gihong.park@uconn.edu
Morgan Petrovich	Exponent	mpetrovich@exponent.com
Tabitha Phillips	University of Georgia	orcaluvr@uga.edu
Haley Plaas	UNC Institute of Marine Sciences	hplaas@live.unc.edu
Samuel Putnam	University of South Carolina	spputnam@email.sc.edu
Victoria Roberts	University of Central Florida	v.roberts446@gmail.com
Zabdiel Roldan Ayala	School of Earth and Environmental Sciences, Queens College, CUNY & Advanced Science Research Center at the Graduate Center, City University of New York (CUNY)	zabdiel.roldanayala11@qmail.cuny.edu

Dana Shultz	Southern California Coastal Water Research Project	danas@sccwrp.org
Nora Straquadine	Stony Brook University	nora.straquadine@stonybrook.edu
Peter Sylvers	Stony Brook University	peter.sylvers@stonybrook.edu
Muhammad Thuneibat	ORAU (CDC)	qov9@cdc.gov
Aubrey Trapp	UC Santa Cruz	ajtrapp@ucsc.edu
Brendan Turley	Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School for Marine and Atmospheric Science, University of Miami	brendan.turley@noaa.gov
Kevin Tyre	FAU Harbor Branch Oceanographic Institute	ktyre@fau.edu
Lucy Utz	James Madison University	lucyutz7@gmail.com
Wannes Van Hassel	Sciensano/uLi√®ge	wannes.vanhassel@sciensano.be
Yanfei Wang	University of Delaware	yfwang@udel.edu
Kai Wang	Kent State University	kwang12@kent.edu
Ernest Williams	University of Maryland Center for Environmental Science	williamse@umces.edu
Jens Wira	IMET	Jwira@umces.edu
Colleen Yancey	University of Michigan	cyancey@umich.edu
Sarah Zetterholm	US Army ERDC	sarah.g.zetterholm@usace.army.mil