12TH INTERNATIONAL CONFERENCE ON

TOXIC CYANOBACTERIA

TOLEDO, OH /// MAY 22–27, 2022

Welcome to Toledo and ICTC12! After having circumnavigated the globe, ICTC finally returns to the state of Ohio, where Wayne Carmichael organized the very first meeting back in 1980. Indeed, we are fortunate to have Wayne provide our opening remarks on Sunday evening. Given that Toledo was the site of a complete water shutdown due to a cyanobacterial HAB back in 2014, our city is the ideal location for the week’s discussions. To develop the program to ICTC12, we are grateful to the Scientific Committee for their role in scheduling the platform and poster sessions, and we thank our many sponsors for financial support. Consequently, we are pleased to announce that we have over 180 presentations from 18 nations covering topics ranging from cyanobacterial ecology to toxin biochemistry and ‘omics, to management of HAB events. Regarding management and in the spirit of binational stewardship of our shared Great Lakes resource, we have partnered with our Canadian colleagues at IFHAB, who will host a session and panel on Wednesday focused on challenges to mitigating cyanobacterial HABs. Overall, we are most happy to meet with all of you in person, and we look forward to the discussions and future collaborations to emerge.

The Organizing Committee:
George Bullerjahn
Mike McKay
Tom Bridgeman
Justin Chaffin
Reagan Errera
Arthur Zastepa

Greetings colleagues,

The Interdisciplinary Freshwater Harmful Algal Blooms (IFHAB) Workshop Organizing Committee is pleased to be jointly hosting you at the 12th International Conference on Toxic Cyanobacteria (ICTC) held at the University of Toledo. We will be hosting two special sessions. Session 6 (Wednesday afternoon) is a special IFHAB session with talks covering fundamental and applied research and its potential impact on citizen science, policy, or resource management. Session 6 will begin with a keynote address by Greg Boyer from the State University of New York. Session 7 (Wednesday evening) will be an interactive panel discussion focused on the question "What is the greatest challenge facing us in the management and reduction of cHABs?" Our diverse panel includes Wayne Carmichael, Ingrid Chorus, Lori Bradford, Keith Loftin, René Shahmohamadloo and Arash Zamyadi, and will be moderated by Susan Watson. We look forward to welcoming you in Toledo, Ohio this spring.

Best regards,

Organizing Committee, IFHAB Workshop

Daniel Beach, National Research Council of Canada
Ngan Diep, Ontario Ministry of the Environment, Conservation and Parks
David McMullin, Carleton University
Sebastien Sauvé, University of Montreal
René Shahmohamadloo, Washington State University and University of Guelph
Dana F. Simon, University of Montreal
Arthur Zastepa, Environment and Climate Change Canada

IFHAB Mission Statement

The Interdisciplinary Freshwater Harmful Algal Blooms (IFHAB) Workshop is a yearly meeting of scientists focusing on the study of cyanobacterial and harmful algal species in freshwater environments. During this workshop, researchers from different disciplines present their most recent study plans and results, including on monitoring and fate of phytoplankton and their bioactive metabolites including toxins, ecology, analytical method development, drinking water treatment, risk assessment and management, and science communication. The workshop takes place in an informal environment, aiming to help researchers expand their networks and learn from the different fields and specialization focused on this complex environmental threat.

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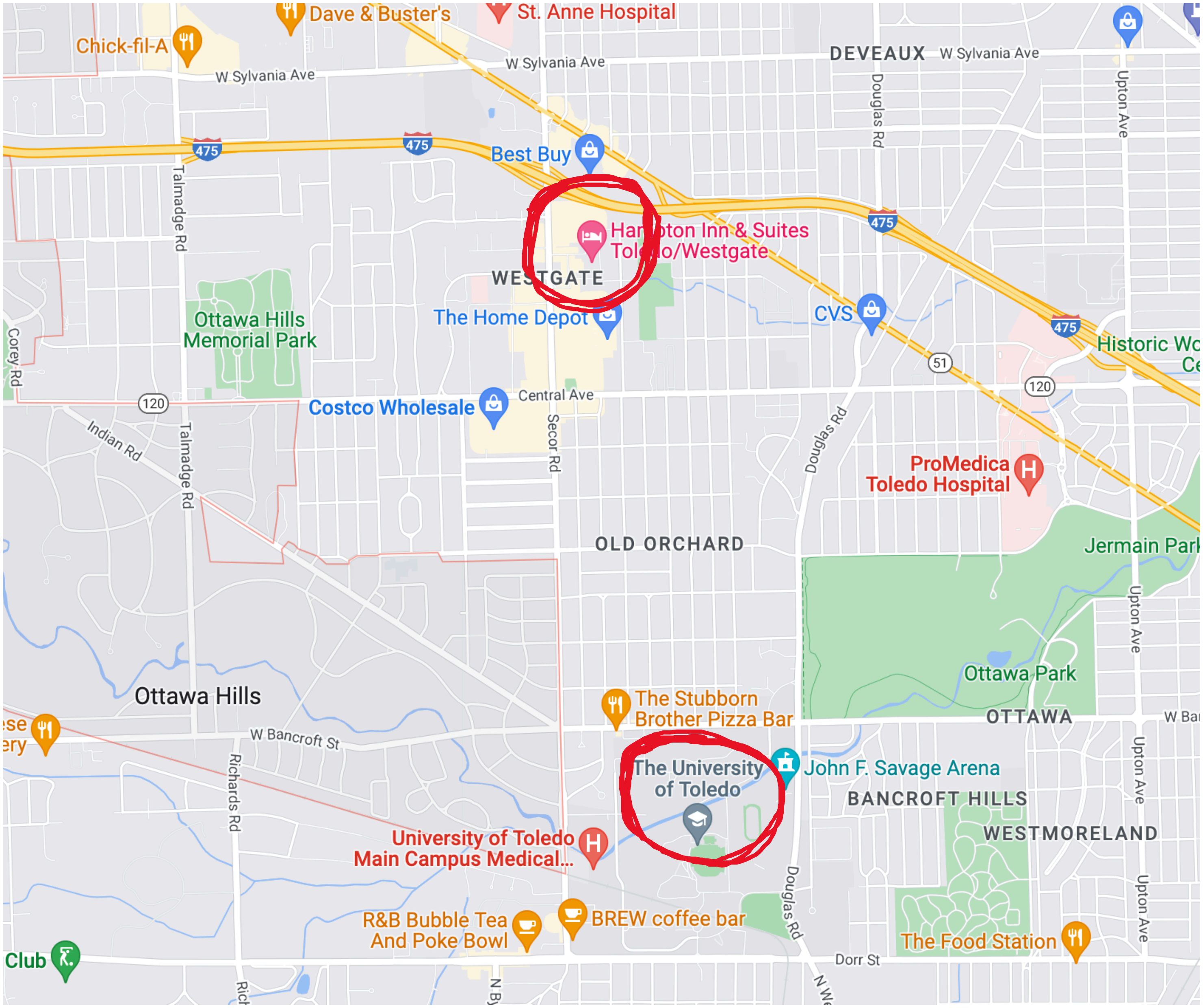
Location of hotels



Hotels are located on Secor Road, ~2.5 km north of the University of Toledo campus

Morning van pickup begins at the Holiday Inn Express at 0745 each morning. It will take several trips in the vans to fetch everyone, please be patient

Those staying on campus, see map on the next page!

THE UNIVERSITY OF TOLEDO
1872

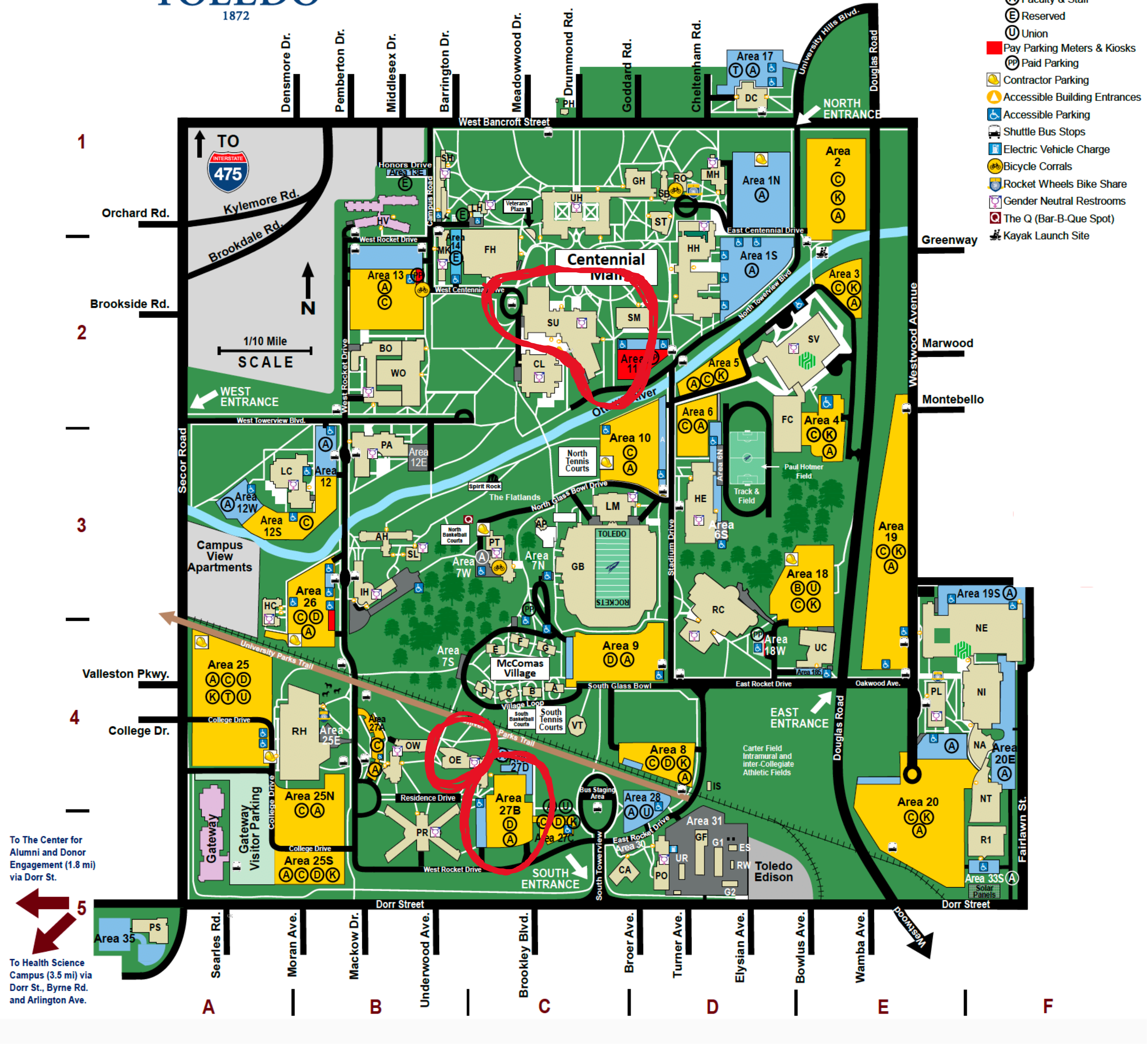
The University of Toledo

Welcome to Main Campus - www.utoledo.edu

2801 West Bancroft Street, Toledo OH 43606

MAP KEY

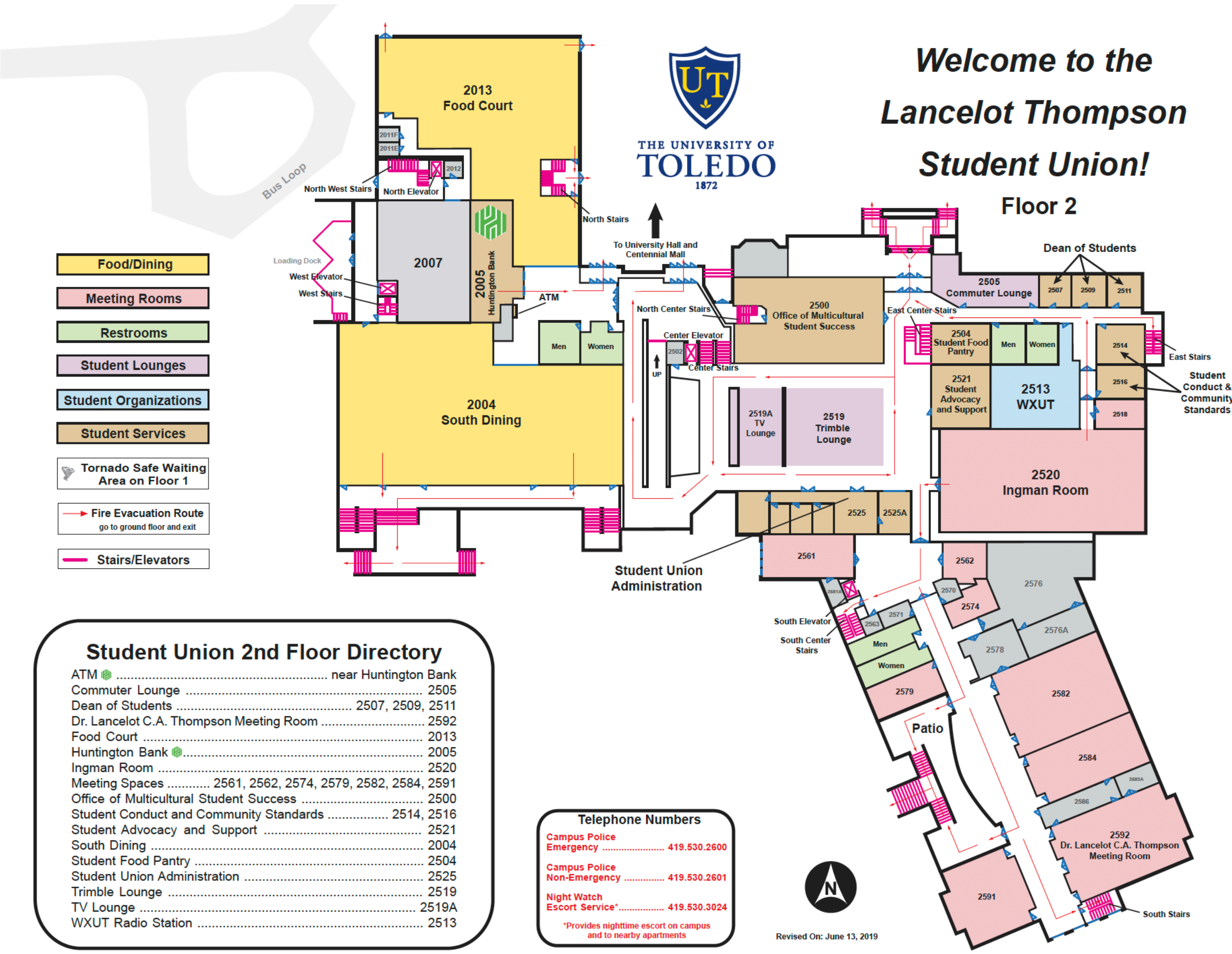
- 🟨 Student Parking
  - Ⓐ Faculty & Staff
  - Ⓒ Commuters
  - Ⓓ Residents
  - Ⓚ Commuters (first year)
  - Ⓣ Toledo Early College
- 🟦 Faculty & Staff Parking
  - Ⓐ Faculty & Staff
  - Ⓔ Reserved
  - Ⓤ Union
- 🟥 Pay Parking Meters & Kiosks
  - Ⓟ Paid Parking
- Contractor Parking
- Accessible Building Entrances
- Accessible Parking
- Shuttle Bus Stops
- Electric Vehicle Charge
- Bicycle Corrals
- Rocket Wheels Bike Share
- Gender Neutral Restrooms
- Ⓠ The Q (Bar-B-Que Spot)
- Kayak Launch Site



ICTC activities are at the Student Union (SU on the top of the map)
Parking will be in Area 27B, use South entrance on Dorr Street (circled at bottom of map)
Residence Hall for those staying on Campus is Ottawa House East (circled at bottom of map)

Welcome to the Lancelot Thompson Student Union!

Floor 2



Revised On: June 13, 2019

Student Union 2nd Floor Directory

ATM	near Huntington Bank
Commuter Lounge	2505
Dean of Students	2507, 2509, 2511
Dr. Lancelot C.A. Thompson Meeting Room	2592
Food Court	2013
Huntington Bank	2005
Ingman Room	2520
Meeting Spaces	2561, 2562, 2574, 2579, 2582, 2584, 2591
Office of Multicultural Student Success	2500
Student Conduct and Community Standards	2514, 2516
Student Advocacy and Support	2521
South Dining	2004
Student Food Pantry	2504
Student Union Administration	2525
Trimble Lounge	2519
TV Lounge	2519A
WXUT Radio Station	2513

Telephone Numbers

Campus Police Emergency	419.530.2600
Campus Police Non-Emergency	419.530.2601
Night Watch Escort Service*	419.530.3024

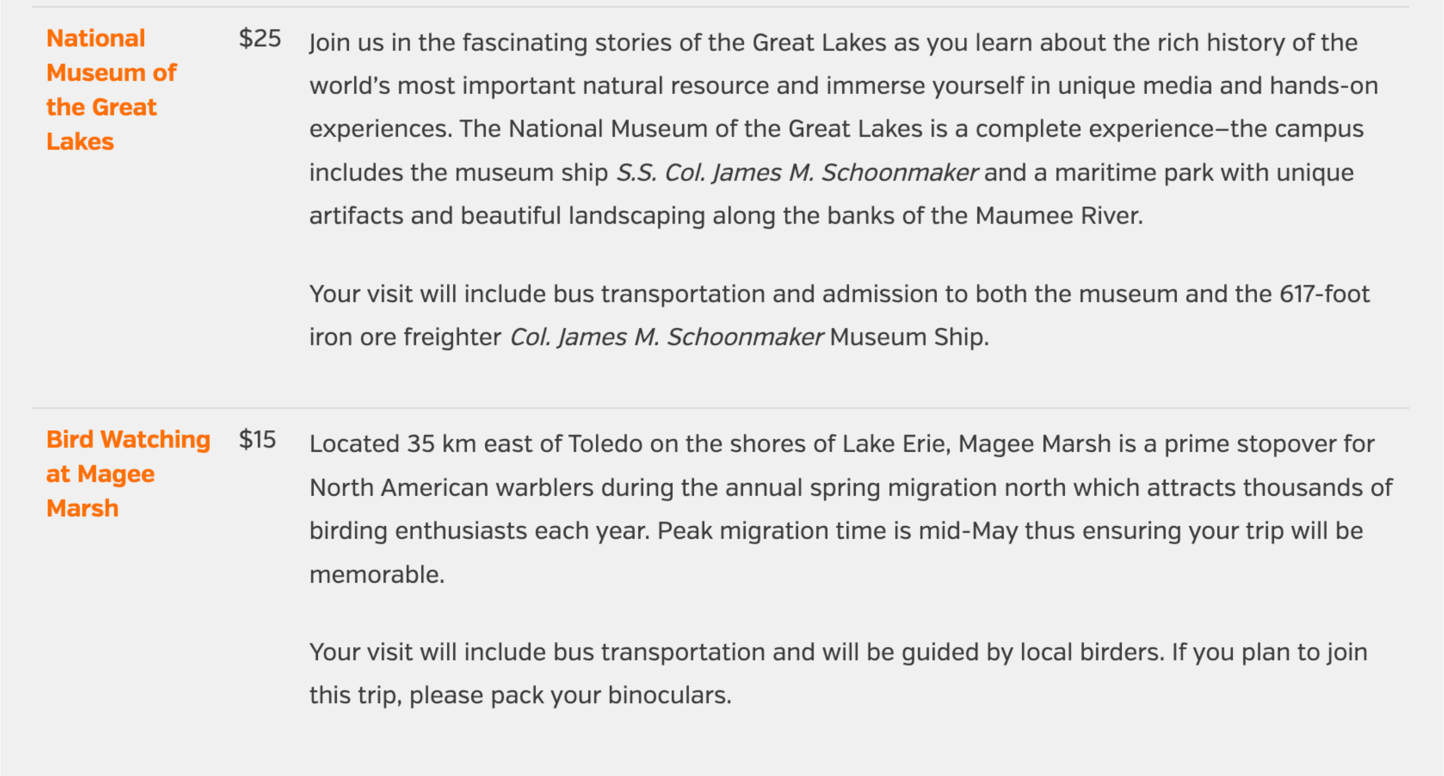
*Provides nighttime escort on campus and to nearby apartments

All activities except lunch are on the 2nd floor of the University of Toledo Student Union:

Reception area for check-in and breaks, outside of Ingman Room
Talks are in the Ingman Room (rm 2520)
Exhibits are on display in the Trimble Lounge (rm. 2519)
Speaker ready room: (rm. 2579)
Posters are in rms. 2582/2584
Looped talks in rm. 2591
Lunch is in South Dining 2004 (1st floor)

Excursions, Thursday afternoon, banquet Thursday evening

Buses leave from the UT Student Union at 1400, return by 1730 to head to the banquet at the Toledo Train Station (https://www.greatamericanstations.com/stations/toledo-oh-tol/)



National Museum of the Great Lakes	$25	Join us in the fascinating stories of the Great Lakes as you learn about the rich history of the world's most important natural resource and immerse yourself in unique media and hands-on experiences. The National Museum of the Great Lakes is a complete experience–the campus includes the museum ship *S.S. Col. James M. Schoonmaker* and a maritime park with unique artifacts and beautiful landscaping along the banks of the Maumee River. Your visit will include bus transportation and admission to both the museum and the 617-foot iron ore freighter *Col. James M. Schoonmaker* Museum Ship.
Bird Watching at Magee Marsh	$15	Located 35 km east of Toledo on the shores of Lake Erie, Magee Marsh is a prime stopover for North American warblers during the annual spring migration north which attracts thousands of birding enthusiasts each year. Peak migration time is mid-May thus ensuring your trip will be memorable. Your visit will include bus transportation and will be guided by local birders. If you plan to join this trip, please pack your binoculars.

Meeting schedule

Sunday		
1300	Check in open	
1700	Welcome Reception and opening comments by George Bullerjahn and Mike McKay	
1715		
1730		
1800		
1815	Wayne Carmichael	A Paradigm for preventing cyanoHABs: The Why and How of Moving from Monitoring and Management to Prevention and Remediation of Cyanobacteria Harmful Algae Blooms (cyanoHABs)
1830		
1845		
1900		
Monday		
730	Breakfast	
745		
800		
815	Coffee	
845	Welcome Meeting	
Detection, Identification and Diversity of CHABs (Heather Raymond, convener)		
900	Susan Wilde	Ecosystem effects of the novel "eagle killer" cyanotoxin aetokthonotoxin responsible for neuropathy and mortality in wildlife
915		
930		
945	Cody Sheik	Hiding in plain sight: Characterizing a novel cyanobacterium that packs a potent punch
1000	David Berthold	Diversity of toxin-producing benthic cyanobacterial proliferations from the Gulf Coast of Florida (USA)
1015	Ethan McCann	Deciphering the diversity and concentrations of cyanopeptides from blooms in Ontario and Quebec, Canada
1030	Coffee/Exhibits	
1045		
1100	Sabina Perkins	Comparison of imaging flow cytometry and manual counts for assessing ecological status and harmful cyanobacterial bloom monitoring
1115	Malcolm Barnard	Evaluating the FluoroProbe as a tool for rapid chlorophyll *a* and phytoplankton group identification during Western Lake Erie CyanoHAB bloom conditions
1130	Benjamin Gincley	Accessible quantitation of surface water phytoplankton with ARTiMiS
1145	Sandra Lagauzere	Genetic biosensors to measure the activity of toxigenic cyanobacteria: towards a new standardized method to forecast harmful algal blooms
1200	Stuart Oehrle	Cyanobacterial toxins in recreational and intake waters using a targeted UPLC/MS/MS method
1215	Els Faassen	Critical evaluation of commercially available rapid tests for cyanobacterial toxin detection in surface water

1230	Lunch	
1245		
1300		
1315		
1330		
New Technologies, Modeling, and Smart Lakes (Mandy Michalsen, convener)		
1345	Reagan Errera	Exploring toxic cyanobacteria blooms using emerging technologies: From space to the benthos
1400		
1415		
1430	José Risco-Martin	IA-GES-BLOOM-CM: Creating synergies between biologist and engineers to develop tools for Cyanobacteria Bloom Management
1445	Ernest Neafsey	Using Actionable Data to Achieve Successful HAB Control
1500	Ed Verhamme	Lake Erie Early Warning Network for HABs: Past, Present, Future
1515	Jessica Cicale	Field verification of Cyanobacteria Assessment Network (CyAN) derived satellite estimates of cyanobacteria harmful blooms in Ohio lakes
1530	Hoi-Ying Holman	Visualizing Cyanotoxins Behavior Using Synchrotron Infrared Spectral Microscopy
1545	Coffee/Exhibits	
1600		
1615	Franesco Pomati	Automated underwater microscopy and machine learning to understand and forecast algal blooms
1630	Emily Summers	Utilization of high frequency monitoring for an inland lake eutrophication model
1645	Karina Gin	Multi-class secondary metabolites in cyanobacterial blooms from a tropical water body: distribution patterns and real-time prediction
1700	Eva Riehle	Exploring cyanotoxin diversity in field samples – an untargeted HRMS/MS based approach
1715	Bar open	
1730		
1745	Posters (odd numbers)	
1800		
1815		
1830		
1845		
1900		

Tuesday		
730	Breakfast	
745		
800		
815	Coffee	
Ecology of Cyanobacteria - Toxic CHAB in Freshwaters and Coastal Systems (Sandra Azevedo, convener)		
830	Thad Scott	Biogeochemical constraints on ecosystem stoichiometry and its influence on cyanotoxin hazards
845		
900		
915	Hans Paerl	Tackling the global expansion of CyanoHABs along the freshwater to marine continuum: The need for a dual nutrient (N and P) control strategy
930	Leah Egan	Phenology of cyanobacteria bloom development and maintenance in nutrient-rich Minnesota lakes differing in nutrient limitation status
945	Kaela Natwora	The physiological and transcriptional response of harmful algal blooms in nitrogen limited lakes
1000	Iwona Jasser	Cyanobacteria in hot pursuit: Characterization of cyanobacteria strains from thermal springs, including novel taxa, and comparison of their thermophilic ability with the genetic background
1015	Nada Tokodi	Photosynthetic efficiency in Polish and Australian *Raphidiopsis raciborskii* strains
1030	Coffee/Exhibits	
1045		
1100	Mikolaj Kokociński	Environmental factors related to the distribution pattern of *Raphidiopsis raciborskii* and *R. mediterranea* in Central East Europe
1115	Lukasz Wejnerowski	Identification of cyanometabolites and toxicity assessment of cyanobacteria isolates from chosen Polish eutrophic waters and polar glaciers
1130	Sarah Caltabiano	Characterization of nitrate reductase in *Microcystis aeruginosa* PCC7806 wild type and non-toxic mutant strain
1145	Oloyede Adekolurejo	Low environmental microcystin concentrations affected sublethal population-level responses but not survival among freshwater keystone species
1200	Justin Chaffin	Rates of microcystin production and biodegradation in the western basin of Lake Erie
1215	Lunch	
1230		
1245		
1300		

Secondary Cyanometabolites – Structure, Biosynthesis, Physiological Function, Environmental Significance and Biotechnical Application (Wayne Carmichael, convener)

1330	Elizabeth Janssen	Increasing confidence in identifying cyanobacterial metabolites to study their occurrence and behavior
1345		
1400		
1415	John Ricca	Investigating the interaction between microcystins and cyanobacterial cells using nanoscale secondary ion mass spectrometry
1430	Ruben Moron-Asensi	Monitoring of intracellular and extracellular toxin/bioactive peptide production under physiological stress conditions through labeling by chemical modification
1445	Sevasti-Kiriaki Zervo	Analytical workflow integrating LC-HRMS untargeted analysis and CyanoMetDB for fast and extensive detection of cyanobacterial metabolites
1500	Ariel Kaminski	Impact of unidentified secondary metabolites from five new cyanobacterial species on aquatic plant
1515	Colleen Yancey	Natural *Microcystis* populations reveal the presence and abundance of truncated *mcy* operons and microcystins: a continuing source of research for water quality research
1530	Coffee/Exhibits	
1545		
1600	Elke Dittmann	Non-canonical carbon fixation in microcystin-producing cyanobacteria
1615	Manthos Panou	Linking environmental heterogeneity and chemo-diversity in cyanobacteria: A culture-dependent profile based analysis
1630	Pierre Bouteiller	Accumulation and elimination kinetics of free and total (free plus protein-bound) microcystin-LR in the common carp *Cyprinus carpio*
1645	Emani Pinto	Investigation of CyanoMetabolites in Brazilian cyanobacteria using LC-MS-QTOF: distribution in different cultured strains, bloom samples and physiological studies
1700	Bar open	
1730		
1745	Posters (even numbers)	
1800		
1815		
1830		
1845		
1900		

Wednesday		
730	Breakfast	
745		
800		
815	Coffee	
CHABs and Omics: Advances in Genetics, Transcriptomics, Metabolomics, and Proteomics to Understanding CHABs (Cody Sheik, convener)		
830	Morgan Steffen	Progressing from exploratory studies to hypothesis testing: Harnessing the power of -omics tools to understand cyanobacterial harmful algal blooms
845		
900		
915	Theo Dreher	Diverse genome sequences from recent toxic and non-toxic US Pacific Northwest freshwater HABs belong to the Nostocales ADA clade
930	Anders Kiledal	The Great Lakes Atlas of Multi-omics Research (GLAMR) Database: Facilitating the Great Lakes Research of Tomorrow
945	Robbie Martin	Sulfolipid profiles of *Microcystis aeruginosa* and cyanobacterial blooms as an indicator of P availability
1000	Xavier Mayali	Do *Microcystis* laboratory cultures hold clues to bacterial microcystin degradation?
1015	Gwendolyn Stark	Microcystin aids in photo-acclimation during prolonged cold stress treatment in *Microcystis aeruginosa* strain PCC7806
1030	Coffee/Exhibits	
1045		
1100	Cecillio Valadez Cano	Anatoxin-producing and non-toxic strains of *Microcoleus* sp. coexist in benthic cyanobacterial mats in the Wolastoq (Saint John River, Canada)
1115	Xuexiu Chang	Comparative metabolomics of exudates between toxigenic and non- toxigenic *Microcystis aeruginosa*
1130	Jason Woodhouse	Insights from the global pangenome of *Raphidiopsis raciborskii*
1145	David Fewer	Phylogenomic insights into toxic bloom formation in the Baltic Sea
1200	Katelyn McKindles	Cyanophages Infecting *Planktothrix agardhii* from a Lake Erie Embayment
1215	Darius Dziga	Cyanophage resistance on proteome level in the bloom-forming cyanobacterium *Aphanizomenon flos-aquae*
1230	Lunch	
1245		
1300		
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1330		

Special Session Hosted by the Interdisciplinary Freshwater Harmful Algal Bloom Workshop (IFHAB) - Current and Future Issues in cHAB Detection, Monitoring, Mitigation and Management

(Sébastien Sauvé, convener)

1345	Greg Boyer	Evolution of our understanding of harmful algal blooms and their monitoring
1400		
1415		
1430	Dana Simon	Using citizen science to track harmful cyanobacterial blooms through crowdsourcing and crowdfunding - Adopt a Lake project
1445	Paul MacKeigan	Quantifying microcystin concentrations, their composition and drivers across over 400 north-temperate and boreal Canadian lakes
1500	Charles Trick	Harmonizing science and management options to reduce risks of cyanobacteria blooms
1515	Akaash Bansal	Impact of Cyanobacteria on the pelagic phosphorus cycle
1530	Kevin Erratt	The missing middle – Investigating a North American metalimnetic cyanobacteria layer
1545	Coffee/Exhibits	
1600		
1615	Robert Smith	The economic costs of algal blooms: Great Lakes evidence and research priorities
1630	Sébastien Sauvé	Presence of less monitored bioactive cyanotoxins (anabaenopeptins, saxitoxins, and BMAA isomers) in surface waters
1645	Monica Samit	Understanding HABs and the Florida Department of Health Role: Notification, Response, Resources and Research in the State of Florida
1730	**Special Panel Discussion Hosted by the Interdisciplinary Freshwater Harmful Algal Bloom Workshop (IFHAB) - Experts in the Hot Seat: Challenges and Lessons with Research, Risk Communication and Community Engagement** Moderator: Susan Watson - IFHAB/Trent University Wayne Carmichael - Wright State University Keith Loftin - U.S. Geological Survey Arash Zamyady - Water Research Australia Ltd Rene Sahba Shahmohamadloo - Washington State Univ/Univ of Guelph Ingrid Chorus - German Environment Agency Lori Bradford - Univ of Saskatchewan/Safe Drinking Water Association Special reception hosted by the Interdisciplinary Freshwater Harmful Algal Bloom Workshop (IFHAB)	
1745		
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1930	Special reception hosted by the Interdisciplinary Freshwater Harmful Algal Bloom Workshop (IFHAB)	
1945		
2000		
2015		
2030		

Thursday

730	Breakfast	
745		
800		
815	Coffee	
Interactions Between CHABs and the Phycosphere (Darius Dziga, convener)		
830	Greg Dick	New dimensions of *Microcystis* diversity: the phycosphere
845		
900		
915	Vincent Denef	Trait variation in resource competition traits among *Microcystis* strains in presence and absence of their microbiomes
930	Joanna Mankiewicz-Boczek	Bacterioplankton communities affect toxigenic *Microcystis* bloom – from environmental to laboratory study
945	Wei Li	New insights on organic nitrogen assimilation in *Microcystis* phycosphere and impacts on microcystin production
1000	Adam Antosiak	Modification of the freshwater *Aphanizomenon flos-aquae* cyanobacterium proteome at cyanophage infection
1015	Christopher Ward	Association between microbiome composition and microcystin levels in the 2018 Lake Erie HABs Grab
1030	Coffee/Exhibits	
1045		
Toxic Cyanobacteria in the Context of Climate Change (Petra Visser, convener)		
1100	Dedmer van de Waal	Impacts of multiple global change factors on toxic cyanobacteria: from elements to traits and toxicity
1115		
1130		
1145	Todd Miller	Unusual toxic cyanobacterial blooms in a tannin stained lake
1200	Jerome Kok	Potential role of sulphate in cylindrospermopsin dynamics within urban freshwaters
1215	Muriel Gugger	Bloom capacity of various *Microcystis* strains, are they all the same?
1230	Spyros Gkelis	A Greek *Raphidiopsis raciborskii* strain and microcystins: A toxic relationship
1245	Lunch	
1300		
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1400	Excursions	
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1745	Buses depart for Banquet	
1800	Banquet - Toledo Port Authority (former Toledo Union Terminal) Martin Luther King Plaza 415 Emerald Ave, Toledo	
1815		
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1930		
1945		
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2015		
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2045		
2100	Buses depart from Banquet	
Friday		
730	Breakfast	
745		
800		
815	Coffee	
Risk, Prevention, Management, and Mitigation of CHABs: Applied Applications from Establishing Risk to Remediation (Dail Laughinghouse, convener)		
830	Faith Kibuye	Performance of source water control strategies for cyanobacterial blooms
845		
900		
915	Heather Raymond	Field-scale evaluation of nanobubble ozone technology for cyanobacterial harmful algal bloom control
930	Maria Antoniou	Exploring the potential of metallic peroxide granules for the in-situ mitigation of cyano-HABs
945	Carlos Pestana	A paradigm-shift in water treatment: In-reservoir UV-LED-driven TiO2 photocatalysis for the removal of cyanobacteria – a mesocosm study
1000	Sathya Ganegoda	Development of a novel & low cost domestic filter unit to treat drinking water contaminants
1015	Coffee/Exhibits	
1030		
1045	Jyotshana Gautam	Using native Lake Erie bacteria and their enzymes for degradation/removal of microcystin toxins from water
1100	Jason Dexter	Application of CyanoGate, a Cyanobacterial Synthetic Biology Molecular Cloning Suite, for Exploring Optimal Heterologous Microcystinase (MlrA) Production for Freshwater Bioremediation from Cyanotoxins
1115	Elivira Perona	Ecology of cyanotoxin-producer cyanobacterial communities in Spanish National Parks
1130	Petra Visser	Suppression of cyanobacterial blooms using hydrogen peroxide: effects on phytoplankton and bacteria
1145	Apurva Lad	Cyanotoxin Degrading Lake Bacteria Significantly Alleviate Microcystin-LR Induced Hepatotoxicity in Both In Vitro and In Vivo Models
1200	Ingrid Chorus	Toledo triggered new WHO guidance for cyanotoxin risk assessment
1245	Lunch, discuss next venue, close meeting at 1400	
1300		
1315		

Oral Presentations

Sunday evening: Welcome/Kick off presentation

Wayne Carmichael

A Paradigm for preventing cyanoHABs: The Why and How of Moving from Monitoring and Management to Prevention and Remediation of Cyanobacteria Harmful Algae Blooms (cyanoHABs)

Water pollution in the Great Lakes, linked with eutrophication and cyanobacteria was first brought to the scientific literature in the 1970s. Despite some reductions in Great Lakes waterblooms, due to selected nutrient management efforts, cyanobacteria reemerged in the 1990’s as toxic harmful algae waterblooms (cyanoHABs), once again linked to eutrophication. Over the ensuing 50-year span, toxic cyanobacteria Harmful Algae Blooms (cyanoHABs) have been shown to degrade freshwater resources worldwide. While much has been learned about their occurrence, organisms responsible, toxicology, biochemistry and genetics, most approaches dealing with the problem have been restricted to: (a) “Monitoring and Detection”: trying to improve our ability to predict when and where cyanoHABs will strike, and (b) Reactive Symptomatic Treatments that target individual symptoms of eutrophication after the fact. Despite our best efforts, the water quality in our ecological water infrastructure continues to worsen because we are failing to correctly identify the problem. This presentation aims to highlight the competitive advantages cyanobacteria gain in eutrophic conditions, and in particular, their ability to access the accumulated nutrients in the sediment of eutrophic water bodies. It then examines how current reactive interventions targeting individual symptoms of eutrophication provide short-term aesthetic relief, but only serve to entrench conditions that favor cyanobacterial dominance in the long-term. The discussion also details how we can develop an understanding of the complex dynamics of the Nutrient Cycle – as well as resilient and sustainable food webs and ecosystems – from the domains of biology and biochemistry (or Biotechnology) and the implementation of the Systems Theory paradigm. The presentation concludes by looking at how an understanding of Systems Theory, and the inevitable feedback loops they entail, enables us to implement a more thoughtful and applied scientific approach to combating the fundamental drivers of the Global Water Crisis: eutrophication and toxic cyanoHABs, through the application of proactive Prevention and Remediation strategies based on Biotechnology. This will allow us to target the root causes of the degradation of source water bodies around the world and leverage the power of Nature’s self-regulating and self-renewing biological processes to safeguard our freshwater resources for future generations.

Monday morning, 0900

Session: Detection, Identification, and Diversity of CHABs (Heather Raymond, convener)

Plenary: Susan Wilde, University of Georgia (swilde@uga.edu)

Ecosystem effects of the novel "eagle killer" cyanotoxin aetokthonotoxin responsible for neuropathy and mortality in wildlife

Detection, identification and Diversity of CHABs

0945 Monday

Hiding in plain sight: Characterizing a novel cyanobacterium that packs a potent punch

Cody Sheik (cssheik@d.umn.edu) University of Minnesota – Duluth; Kaela Natwora, University of Minnesota – Duluth; Robbie M. Martin, University of Tennessee, Knoxville; Steven W. Wilhelm, University of Tennessee; Todd Miller, University of Wisconsin-Milwaukee; Kurt Svoboda, University of Wisconsin-Milwaukee

Cyanobacteria are one of the most studied groups of microbes on the planet. Yet, molecular tools, like 16S rRNA gene sequencing, show us uncultured lineages lurk across the cyanobacterial tree of life. Cyanobacterial blooms are a recurring threat to global fresh and marine waters and are exacerbated by anthropogenic climate change. The proliferation of toxin producing species in many of these blooms is concerning and a risk for public health. Cyanobacteria are known to produce a plethora of secondary metabolites, some of which are detrimental to humans. However, many of these compounds remain enigmatic regarding their role in the cell and environment. There is also an absence of information for the genes that encode many of these compounds. Here we isolated a cyanobacterium from a late season bloom in Siskiwit Bay in Lake Superior. A phylogenomic reconstruction using a 5.4 MB, circular genome shows the isolate belongs to a novel genus within the *Nostocacea*. The culture does not produce or carry any genes for common toxins. However, zebrafish embryos exposed to extracts taken from the culture either died or were malformed at varying ppb concentrations. This highlights that novel toxins are lurking within dark green matter of novel cyanobacteria.

1000 Monday

Diversity of toxin-producing benthic cyanobacterial proliferations from the Gulf Coast of Florida (USA)

David E. Berthold (dberthold@ufl.edu), University of Florida; Forrest W. Lefler, University of Florida; Matthew Bertin, University of Rhode Island; Paul Zimba, Texas A&M University; Maximiliano Barbosa, University of Florida; H. Dail Laughinghouse, University of Florida.

Overgrowth of benthic cyanobacterial mats (BCMs) is prevalent in marine environments, partly driven by nutrient loading and climate change. Reports of BCMs occurring on both Florida coasts have increased, demonstrating a need to understand the diversity involved in the blooms and potential toxicity of the BCMs. As an initial survey on this environmental health threat, benthic proliferations were sampled on the Florida Gulf Coast in summer 2021. Cyanobacterial mats were collected, DNA was extracted, and the 16S rRNA of both bacteria and cyanobacteria were amplified using respective specific primers. The homocytous filamentous *Dapis*, *Okeania*, *Planktothricoides*, *Salileptolyngbya*, and *Sirenicapillaria* dominated BCMs. Additionally, the most abundant bacterial orders included Chitinophagales, Cytophagales, Flavobacteriales, and Rhodobacterales. LC-MS/MS analyses detected high concentrations of anatoxin-a, dihydroanatoxin-a and BMAA and low concentrations of cylindrospermopsin, within both the water column and mat material. Large benthic marine mats are often reported in Florida from Sarasota Bay to Charlotte Harbor and we observed similar microbial communities and toxicity among proliferations. Results from this study indicate a need to further assess the spatial and temporal aspects of these blooms, in addition to their community structure, influencing factors and the production of toxic compounds.

1015 Monday

Deciphering the diversity and concentrations of cyanopeptides from blooms in Ontario and Quebec, Canada

Ethan T. McCann (ethanmccann@cmail.carleton.ca), Carleton University; E. McCann, D. McMullin*

Cyanobacteria blooms release mixtures of biologically active compounds into freshwater systems. These poorly studied compounds pose undetermined risks to both human and ecosystem health, and negatively impact local economies that depend on freshwater resources. While microcystins are extensively studied, little is known about the chemistry, structural diversity, toxicology and environmental concentrations of less studied cyanopeptide groups such as, anabaenopeptins, cyanobactins, cyanopeptolins, microginins and aeruginosins. The application of mass spectrometry and metabolomic data processing techniques are powerful tools for deciphering the mixtures of compounds released by cyanobacteria. A Non-targeted high resolution tandem mass spectrometry-based metabolomics approach was used to detect more than one-hundred unique cyanopeptides from fifty-five bloom samples collected from fifteen watercourses near the city of Ottawa in Canada. Microcystins and select other cyanopeptides were quantified with reference materials. The concentrations of other cyanopeptide groups for example, anabaenopeptins cyanopeptolins and microginins, were determined semi-quantitatively. Sixty of the compounds detected contributed significantly to the variation in cyanopeptide profiles between watercourses. The most commonly detected cyanopeptide groups were anabaenopeptins (thirty-three congeners) and cyanopeptolins (thirty-two congeners). Microcystins were detected in forty-one of the fifty-five bloom samples, where microcystin-LR was detected most often; however, microcystin-LA amounts were consistently the highest when detected for this toxin group.

1100 Monday

Comparison of imaging flow cytometry and manual counts for assessing ecological status and harmful cyanobacterial bloom monitoring

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Numerous sampling and analytical methods exist to assess ecological status and to monitor harmful cyanobacterial blooms (HCBs) in freshwater systems. Imaging flow cytometry presents a powerful tool for assessing phytoplankton assemblages, where it reduces processing time while maintaining an intermediate level of taxonomic detail. This increased capacity and reduced processing time makes flow cytometry an effective method for HCB monitoring and large-scale ecological assessment. Here, we compare manual counts and results from the Imaging Flow Cytobot (IFCB) for a project in New York. Preserved and live samples from two lakes in the Finger Lakes region were included in the analysis, with 22 sampling events. Each sample was analyzed using both manual counts and the IFCB. Comparisons between the manual and IFCB counts were analyzed using multidimensional scaling, specifically focusing on differences between functional groups, species classes, and critical water indicators such as HCB species. The differences between live and preserved samples were also compared.

1115 Monday

Evaluating the FluoroProbe as a tool for rapid chlorophyll *a* and phytoplankton group identification during Western Lake Erie CyanoHAB bloom conditions

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There is a need for rapid detection and quantification of CyanoHAB composition to evaluate potential environmental and public health threats. FluoroProbe uses *in situ* fluorimetry to estimate phytoplankton biomass (as chlorophyll *a*, chl*a*) and community composition (as chl*a* by group). To evaluate the efficacy of a FluoroProbe during bloom conditions in nutrient addition bioassay experiments, we examined the relationship between extracted chl*a* and HPLC diagnostic photopigment analysis post-processed with CHEMTAX matrix factorization and FluoroProbe analysis from Maumee Bay/Western Lake Erie (June 2019 and August 2019 and 2021) and Sandusky Bay (June and August 2019) experiments. Across experiments, total chl*a* ranged from < 10 µg/L to > 400 µg/L. The FluoroProbe and extraction methods produced similar estimates of chl*a* ($R = 0.881$, $p < 0.0001$). The community composition fractions measured by the FluoroProbe were significantly comparable to extraction methods for cyanobacteria ($R = 0.880$, $p < 0.0001$), diatoms ($R = 0.963$, $p < 0.0001$), chlorophytes ($R = 0.758$, $p < 0.0001$), and cryptophytes ($R = 0.584$, $p < 0.0001$). We conclude the Fluoroprobe is an effective tool for rapid quantification of chl*a* concentration estimates, phytoplankton community composition and CyanoHABs compared to extraction methodologies in bioassay experiments of highly productive systems.

1130 Monday

Accessible quantitation of surface water phytoplankton with ARTiMiS

Benjamin Gincley (bgincley3@gatech.edu), Georgia Institute of Technology; Farhan Khan, Georgia Institute of Technology; Andrea Busch, Great Lakes Water Authority; John Norton, Great Lakes Water Authority, Ameet Pinto, Georgia Institute of Technology

Access to accurate real-time data on the presence of target phytoplankton can enable timely, proactive, and data-driven decision making to protect drinking water systems from harmful algal blooms (HABs). These decisions can include initiating targeted actions such issuing public health advisories or changes in raw water access/treatment strategies. Measurements of parameters such as *Chlorophyll a* provide bulk estimates of photosynthetic biomass but are unable to provide taxonomic resolution to differentiate between toxin-producing species and their benign counterparts. Taxonomic data is primarily determined by two methods: microscopic inspection and genetic sequencing. Both require extensive laboratory infrastructure, operational expertise, and considerable time investment for sample characterization. Existing automated commercial instruments are exceedingly cost prohibitive for wide-scale adoption. These barriers to access have motivated development of ARTiMiS: the Autonomous Real-Time Microbial Scope. ARTiMiS is designed for order-of-magnitude instrument cost reduction, enabling use at the point of sampling with real-time data acquisition and machine learning-based processing to enable species-level phytoplankton characterization. This talk will highlight the research team's efforts adapting ARTiMiS towards a plug-and-play algal monitoring system that can be deployed for phytoplankton monitoring in the Detroit River (water treatment intake source waters for the Detroit metropolitan area) as an early HAB detection device.

1145 Monday

Genetic biosensors to measure the activity of toxigenic cyanobacteria: towards a new standardized method to forecast harmful algal blooms

Jean-Baptiste Burnet (jeanbaptiste.burnet@list.lu), Luxembourg Institute of Science and Technology; Tasnim Anjum Mou, Université du Québec en Abitibi-Témiscamingue; Jonathan Held, Swiss Federal Institute of Aquatic Science and Technology; Miria Elias, National Research Council of Canada; Henry-Michel Cauchie, Luxembourg Institute of Science and Technology, Nathalie Fortin, National Research Council of Canada; Guillaume Grosbois, Université du Québec en Abitibi-Témiscamingue; Elisabeth M-L Janssen, Swiss Federal Institute of Aquatic Science and Technology; Sandra Lagauzere, Microbia Environnement

Efficient and proactive management of public health risk associated with cyanobacterial harmful blooms requires appropriate tools that can generate rapid and informative data on the proliferation of toxigenic cyanobacteria in freshwater environments. The present pilot study aimed at assessing the suitability of a new biosensor to follow the population dynamics of toxigenic genera *Microcystis*, *Planktothrix*, *Aphanizomenon* and *Dolichospermum* to rapidly assess the toxic risk associated with their occurrence. The genetic biosensor is adapted into a simple ELISA-type colorimetric format that has been designed to recognize and quantify ribosomal RNA to rapidly detect a population entering a growing phase. Five different reference lakes in Canada, Switzerland and Luxembourg were selected to conduct temporal series in addition to depth profiles and spatial investigations. Biosensor measurements were compared with (*in situ*) algal pigment screening, taxonomic analyses as well as microcystin quantification using both standard LC-MS workflows and rapid *in situ* strip tests. Preliminary results demonstrated a high sensitivity of the biosensor to detect the onset of blooms. The proof-of-concept will provide insights for the use of the biosensor to track toxigenic cyanobacteria and establish risk categories. A conceptual model is presented to implement this new tool into future monitoring programs and early warning systems as a complement to conventional microscopy and toxin analyses.

1200 Monday

Cyanobacterial toxins in recreational and intake waters using a targeted UPLC/MS/MS method

Stuart Oehrle (oehrle@nku.edu), Northern Kentucky University; Wendy Strangman, University of North Carolina at Wilmington; Catharina De Souza, University of North Carolina at Wilmington

Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Various cyanobacterial algae that produce toxins, microcystins being one of the more well-known, represent an emerging class of algal toxins of concern to both the recreational and drinking water industry. In this paper we investigate the use of smaller column packing (sub 2 µm particles) to monitor for many of these toxins using a generic gradient method. Specifically, microcystins, anatoxin-a, cylindrospermopsin as well as newer toxins, such as euglenophycin, anabaenopeptins and micropeptins using Ultra-Performance Liquid Chromatography (UPLC®) combined with tandem mass spectrometry will also be tested. We investigated the analysis of a wide variety of freshwater samples from throughout the United States and cultured strains from the lab. Examples showing additional toxins detected in freshwater samples will be presented including data from the recent blooms in the North Carolina, Ecuador and Ohio. Comparison of blooms from a similar source over several years will also be discussed with similarities shown in the targeted analysis over that time.

1215 Monday

Critical evaluation of commercially available rapid tests for cyanobacterial toxin detection in surface water

Els Faassen (els.faassen@wur.nl) Wageningen University and Research; Naomi Dam, Wageningen University and Research; Inger Lise Van der Graaf, Wageningen University and Research; Toine Bovee, Wageningen University and Research; Arjen Gerssen, Wagenigen University and Research

Dutch water managers are becoming more aware of the need of assessing the risk of cyanobacterial blooms by analyzing toxin concentrations in surface waters. However, they lack specialized analytical equipment to rapidly detect the major toxin groups. Therefore, reliable and rapid tests for microcystins, anatoxins, cylindrospermopsins and saxitoxins detection in the field or the lab are needed. We critically evaluated 6 commercially available field tests (Lateral Flow Devices, LFDs) and 7 lab tests (ELISAs).

Most ELISAs performed acceptably in terms of accuracy and precision. The saxitoxin ELISA had a low cross reactivity for some congeners, resulting in lower performance on these criteria. Some of the LFDs lacked the option of on site cell lysis, making it difficult to estimate the total toxin content. Also, some LFDs structurally gave false negative results during the first experiments. Finally, some had a handling time of more than one hour, which makes routine analysis in the field expensive.

In conclusion, for each of the four tested toxin groups, a suitable ELISA was commercially available, most LFDs however had some serious drawbacks. Also, testing for multiple toxin groups with ELISAs or LFDs is time consuming and expensive, a multiplex test would therefore be preferred.

Monday afternoon, 1345

Session: New technologies, modeling, and smart lakes (Mandy Michalsen, convener)

Plenary: Reagan M. Errera (reagan.errera@npaa.gov) , NOAA-GLERL, Ann Arbor, MI

Exploring toxic cyanobacteria blooms using emerging technologies: From space to the benthos

Traditionally coastal systems have been monitored using ship-based sampling methods, which provide a discrete spatial and temporal snapshot of current conditions. These discrete sampling strategies often miss key environmental shifts that lead to high variability in bloom biomass and toxin concentrations (e.g. 2014 Toledo Water Crisis). Understanding and interpreting the complex interactions between biological, chemical, and physical variables in coastal systems require versatile monitoring approaches. To enhance our spatio-temporal resolution, NOAA is developing a monitoring network in Lake Erie to study and track cyanobacteria harmful algal blooms using traditional methods and emerging technologies. Weekly monitoring at fixed sampling sites provides baseline environmental conditions. The extent of bloom biomass and phytoplankton community composition is determined through daily satellite and weekly hyperspectral imaging. Paired with physical observations and modeling, bloom biomass and toxin concentration trajectories are forecasted up to 5 days in the future. Within the water column, real-time nutrient buoys and second generation (2G) Environmental Sample Processors (ESP) provided near real-time relevant water quality data and particulate microcystin concentrations, respectively. With colleagues at the Monterey Bay Aquarium Research Institute (MBARI) we have tested a 3rd Generation (3G) ESP, an autonomous molecular diagnostic device integrated with an uncrewed underwater vehicle, capable of collecting eDNA and conducting near real-time toxin (e.g., microcystin) analysis using an embedded Surface Plasmon Resonance (SPR) module. Finally, field-based experiments evaluate the role of resuspension events, cell buoyancy, resting cells, and community dynamics on bloom development. This collection of actionable temporal and spatial environmental data is provided to the scientific community, managers, and public stakeholders to support decision making and enhance our understanding of bloom succession.

New technologies, modeling, and smart lakes

1430 Monday

IA-GES-BLOOM-CM: Creating synergies between biologist and engineers to develop tools for Cyanobacteria Bloom Management

José Luis Risco-Martín (jlrisco@ucm.es), Universidad Complutense de Madrid; Segundo Esteban, Universidad Complutense de Madrid; Samuel Cirés, Universidad Autonoma de Madrid; Antonio Quesada, Universidad Autonoma de Madrid; Eva Besada-Portas, Universidad Complutense de Madrid

IA-GES-BLOOM-CM is a synergy project for boosting the collaboration of researchers of different fields to develop disruptive solutions for Cyanobacteria Bloom (CB) management. Its aim is to bring together researchers from Biology, Economy, Automation, and Information & Communication Technologies to make them envision and develop new tools for the early detection and prediction of Cyanobacteria Blooms (CBs). The original proposal provides for using Modeling and Simulation to predict where and when CBs take place; intelligently-guiding Autonomous Surface Vehicles (boats, ASVs) to optimize the observations of relevant 3D information related to CBs; and deploying an Internet of Things infrastructure with Artificial Intelligence to bring and summarize the information (acquired by the ASVs sensors and provided by the simulations) to/for the managers of water bodies. Discussions among researchers are key to decide how to exploit the technologies and the biologist expertise to achieve a final solution that not only fulfills the current demands by the authorities but that also opens opportunities for future requirements and procedures. During this presentation we will provide an overview of the main ideas of the project and of its initial developments.

1445 Monday

Using Actionable Data to Achieve Successful HAB Control

Ernest J. Neafsey (ej.neafsey@lgsonic.com), LG Sonic US

Effective HAB control starts with knowing your water. Learn how water resource managers defined their operational challenges, established key performance indicators, and achieved success using actionable, real-time water quality data. Case studies include different applications and sites throughout the United States. Explore how continuous monitoring and prescriptive analytics support proactive lake stewardship and the "One Health" approach to HABs, enhancing environmental quality and public safety. We will review the data pipeline, as well as the site selection, model evaluation, and implementation processes.

1500 Monday

Lake Erie Early Warning Network for HABs: Past, Present, Future

Ed Verhamme (everhamme@limno.com), LimnoTech

Since 2014 the number of real-time sensors on Lake Erie has increased from zero (2014) to nearly 20 (2021). This ad-hoc sensor network provides year round observations of basic lake conditions near water intakes and provides actionable data to optimize raw water treatment for removal of algal toxins. In 2022, in collaboration with the Cleveland Water Alliance, LimnoTech is deploying more sensors closer to shore and plans to develop a robust testbed program that allows new technology to be tested at water intakes. Despite imperfect sensors, data from this network has correlated very well with algal toxin concentrations and has proven to be a highly valuable early warning network. The presentation will focus on the past, present, and future of this network.

1515 Monday

Field verification of Cyanobacteria Assessment Network (CyAN) derived satellite estimates of cyanobacteria harmful blooms in Ohio lakes

Jessica Cicale (jcicale@usgs.gov), USGS

Limited scientific understanding of cyanobacterial harmful algal blooms (cyanoHABs) frequency, spatial extent, and magnitude prevents prediction of toxic cyanobacteria blooms in lakes and reservoirs. The Cyanobacteria Assessment Network (CyAN) is a national, multi-agency project between the Environmental Protection Agency (EPA), National Aeronautics and Space Administration (NASA), National Oceanic and Atmospheric Administration (NOAA), and the U.S. Geological Survey (USGS) to develop a nationwide early warning system using satellite data to detect cyanoHABs in freshwater systems. On the ground verification is necessary to validate satellite algorithms used to detect and quantify cyanobacteria blooms, and to improve the utility of CyAN as an early-warning monitoring system for the occurrence of HABs and algal toxins in freshwater.

At inland lakes in Ohio, the presence of cyanoHABs has been increasing in recent years. During 2020 and 2021, critical *in situ* water-quality, biological, and environmental data were collected during satellite overflights from Caesar Creek and Buck Creek Lake, Ohio (cyanoHAB-impacted reservoirs) to provide data for the CyAN project. Discrete samples were analyzed for microcystin, cylindrospermopsin, saxitoxin, anatoxin-a, chlorophyll, nutrients, cyanobacterial genes, phytoplankton community composition and abundance. Additionally, site-specific statistical models to predict the probability of exceeding an advisory-level cyanotoxin concentration threshold are being developed as a potential tool for management decisions.

1530 Monday

Visualizing Cyanotoxins Behavior Using Synchrotron Infrared Spectral Microscopy

Hoi-Ying N. Holman (hyholman@lbl.gov), Lawrence Berkeley National Laboratory

Microcystis aeruginosa LE3 is one of the most common toxigenic cyanobacteria species present in freshwater globally when waters are high in nitrogen or phosphorus concentrations. During bloom conditions, it can produce harmful cyanotoxins such as microcystins (MC) that have adverse effects on fish, pets, livestock and humans Characterizing critical components of cyanotoxin production and secretion -- for example how they are produced and released from cyanotoxin-producing cells into water -- requires label-free chemical imaging at microscale of the intact cells and their immediate surrounding with minimum disturbances. Current technologies and approaches cannot adequately address these requirements. Here, we employ the non-invasive multiplexed synchrotron infrared spectromicroscopy to examine changes in cellular composition at the whole-cell level induced by the MC production with high spatial resolution and throughput. By using the bright synchrotron infrared as a light source, we can scan a 100 mm x100 mm sample area in less than 30 minutes. We demonstrate the potential of synchrotron infrared spectromicroscopy imaging by visualizing the spatial distribution of the intact LE3 cells and microcystins. This multiplexed imaging approach allows us to rapidly quantify changes in the composition of MC-producing versus non-MC-producing *Microcystis aeruginosa* cells, and to visualize how microcystins are released into water.

1615 Monday

Automated underwater microscopy and machine learning to understand and forecast algal blooms

Francesco Pomati (francesco.pomati@eawag.ch) Eawag, Department of Aquatic Ecology

We present an approach for automated in-situ monitoring of phytoplankton and zooplankton communities based on a dual magnification underwater dark-field imaging microscope: the Dual Scripps Plankton Camera (DSPC) system. A DSPC is installed permanently at 3 m depth in Lake Greifensee (Switzerland), delivering images of plankton that are automatically processed and classified using deep-learning models at hourly timescales. The DSPC is able to track the dynamics of ~100 taxa in the size range between ~10 µm to ~ 1 cm, covering virtually all the components of the planktonic food web, including potentially toxic cyanobacteria. Time series collected by the DSPC and associated sensors allow tracking plankton ecological succession patterns and algal bloom dynamics with a temporal frequency and resolution on functional traits that are unprecedented in plankton ecology. The data are robust for water quality monitoring and allow integration with machine learning models to i) study the interaction between abiotic and biotic controls of phytoplankton net growth rates, ii) test ecological hypotheses of ecological processes triggering harmful algal blooms, and iii) develop forecasting models of different types of blooms.

1630 Monday

Utilization of high frequency monitoring for an inland lake eutrophication model

Emily J. Summers (emilyjane1215@tamu.edu), Texas A&M Galveston; Jodi L. Ryder, US Army Engineer Research and Development Center

The USACE has an extensive combination of grab sample and continuously monitored water quality data available for inland reservoirs, however the use of continuously monitored data to enable modeling of water quality has not been routinely practiced. A three-dimensional nutrient water quality model (ICM-Lite) was applied to a USACE reservoir to explore the utilization of high frequency multi-parameter water quality data for short term modeling of key variables within the reservoir such as temperature, dissolved oxygen, nutrient levels, and phytoplankton. In addition, long term sample records at several sites were used to add a spatial context and aid in interpretation of model performance for simulating in-reservoir processes. This example case highlights challenges and insights for anticipating and managing HAB events within reservoirs.

1645 Monday

Multi-class secondary metabolites in cyanobacterial blooms from a tropical water body: distribution patterns and real-time prediction

Karina Yew-Hoong Gin, (ceeginyh@nus.edu.sg), National University of Singapore; Luhua You, National University of Singapore; Shu Harn Te, National University of Singapore

Cyanotoxins produced by cyanobacterial blooms contaminate freshwater bodies worldwide. Yet, the distribution patterns of these secondary metabolites in tropical regions are still not well-understood and predictive models using simple water quality indicators are rarely discussed. Here, we investigated the co-occurrence and spatiotemporal trends of 18 cyanobacterial metabolites (including 11 microcystin varints, anatoxin-a, homoanatoxin-a, cylindrospermospin, nodularin, anabaenopeptins A and B) in a tropical freshwater lake plagued with blooms. Random forest (RF) models were developed to predict microcystins and cylindrospermopsin and assess the relative importance of 22 potential predictors that determined their concentrations. The results showed that microcystins, cylindrospermopsin, anatoxin-a, homoanatoxin-a and anabaenopeptins were found at least once in the studied water body, with microcystin-RR and cylindrospermopsin being most detected. Anabaenopeptins A and B were detected for the first time in tropical freshwaters at low concentrations. The metabolite profiles were highly variable at both temporal and spatial scales. The rapid RF prediction models for microcystins and cylindrospermopsin were successfully developed (i.e., chlorophyll-a, total carbon, rainfall and ammonium for microcystins prediction; and chloride, total carbon, rainfall and nitrate for cylindrospermopsin prediction). The models can help to envisage the relationships between cyanotoxins and environmental variables and provide useful information for making policy decisions.

1700 Monday

Exploring cyanotoxin diversity in field samples – an untargeted HRMS/MS based approach

Eva Riehle (eva.riehle@uni-konstanz.de), University of Konstanz & National Research Council Canada; Elliott J. Wright, National Research Council Canada; Pearse McCarron, National Research Council Canada; Daniel R. Dietrich, University of Konstanz; Daniel G. Beach, National Research Council Canada

Robust cyanotoxin analysis is challenging because of the existence of multiple cyanotoxin classes with differing chemical properties, a large number of structural analogues, the scarcity of chemical standards and the complex matrices in which cyanotoxins occur. Studies of cyanotoxin occurrence often rely on class-specific and/or targeted analytical methods, unlikely to detect whole toxin classes or unexpected variants. Here, cyanobacterial field samples from different environments were prepared with a simple methanolic extraction and then analyzed using a comprehensive strategy including both LC-MS/MS and LC-HRMS/MS methods. A multi-class LC-MS/MS method for microcystins, anatoxins, saxitoxins and cylindrospermopsins was used for initial screening, and then untargeted LC-HRMS/MS methods designed to detect a broad range of toxin variants and other cyanobacterial metabolites were employed. The downstream untargeted data processing included metabolomics data analysis software, which has been adapted to recognize several classes of cyanobacterial secondary metabolites. With the targeted LC-MS/MS methods we identified various toxins from all major classes, while the untargeted methods provided in-depth information for the specific classes in conjunction with showing the presence of other notable cyanobacterial secondary metabolites. This comprehensive workflow will be used for ongoing analyses of cyanobacterial field samples from temperate to polar environments and assessment of cyanotoxin diversity.

Tuesday morning, 0830

Session: Ecology of Cyanobacteria - Toxic CHAB in Freshwaters and Coastal Systems (Sandra Azevedo, convener)

Plenary: Thad Scott (thad_scott@baylor.edu), Baylor University

Biogeochemical constraints on ecosystem stoichiometry and its influence on cyanotoxin hazards
Cyanobacteria growth and toxin production in lakes is often regulated by the absolute and relative availability of limiting elements such as phosphorus (P) and nitrogen (N). Yet, the relative supply of these elements to phytoplankton is governed by a complex set of biogeochemical interactions that are interdependent with physical factors and the biogeochemical cycles of other elements such as carbon (C) and micronutrients. Indeed, increased productivity caused by elevated nutrient loading to lentic ecosystems alters the relative rates of P and N recycling and exacerbates stoichiometric imbalances between the availability of these nutrients and their demand by cyanobacteria and other phytoplankton. Here I will explore recent developments in modeling the stoichiometric variation in lakes across large spatial scales by utilizing common models of P and N cycling. In particular, I will explore how stoichiometric variability in P and N availability changes with trophic state due to interactions with the C cycle that modifies nutrient burial, denitrification, and N fixation rates. By combining nutrient-phytoplankton biomass yield relationships, I will estimate the tipping point N:P ratio for lentic ecosystems that results in putatively P or N limited phytoplankton and subsequently compute the specific stoichiometric imbalance in single nutrient (P or N) currency. These estimates provide quantitative metrics for the degree of P or N deficiency (or sufficiency) relative to the other nutrient and are useful for predicting cyanotoxin hazards across large spatial scales. When combined with human use data, cyanotoxin hazard predictions may be formalized into a human health risk framework.

Ecology of Cyanobacteria - Toxic CHAB in Freshwaters and Coastal Systems

0915 Tuesday

Tackling the global expansion of CyanoHABs along the freshwater to marine continuum: The need for a dual nutrient (N and P) control strategy

Hans W. Paerl (hans_paerl@unc.edu), UNC-Chapel Hill, Institute of Marine Sciences; Nathan S. Hall, UNC-CH Institute of Marine Sciences; Malcolm A. Barnard, UNC-CH Institute of Marine Sciences, Haley E. Plaas, UNC-CH Institute of Marine Sciences, Karen L. Rossignol, UNC-CH Institute of Marine Sciences

Anthropogenic nutrient over-enrichment, coupled with rising temperatures and an increased frequency of extreme hydrologic events, has promoted the expansion of harmful cyanobacterial blooms (CyanoHABs) along the interconnected freshwater-to-marine continuum. Pressures exist to reverse this trend in ecosystems to conserve drinking and irrigation, fishery, and recreational waters. Traditionally, reducing phosphorus (P) inputs are prescribed for freshwater systems, while nitrogen (N) inputs control estuarine/coastal CyanoHAB formation. However, microcosm to whole-lake nutrient-enrichment experiments increasingly indicate that CyanoHABs are stimulated by enrichment with both P and N or sometimes N alone. The accumulation of P "legacy" loads in water bodies supports effective internal P recycling, making it difficult to stem eutrophication with P-only external reductions. In most waterbodies, biological N2 fixation does not satisfy ecosystem N needs, while N can "escape" via denitrification, leading to perpetual N limitation. Therefore, dual N and P point and non-point reductions on watershed scales are needed to protect the continuum. In addition, a more climatically extreme world will augment watershed-based nutrient management challenges. In the short-term, physical, chemical or biological manipulative controls may improve immediate beneficial uses, but they are only temporary "fixes" that should be accompanied by long-term dual nutrient management for CyanoHAB control along the continuum.

0930 Tuesday

Phenology of cyanobacteria bloom development and maintenance in nutrient-rich Minnesota lakes differing in nutrient limitation status

Leah R. Egan (egan0133@d.umn.edu), University of Minnesota - Duluth

Cyanobacteria harmful algal blooms (cHABs) represent both chronic and emerging water quality threats in lakes globally and are the consequence of complex, interacting stressors. While we know that water temperature, nutrient loading and availability, and water column mixing conditions are important drivers of cHABs, the combination of abiotic conditions leading to bloom development, maintenance, and toxicity remain poorly understood across different lake types. To better understand the correlation of biovolume and toxin production to nutrient conditions, we monitored two southern Minnesota lakes of differing nutrient statuses. This project combined limnological approaches and cutting-edge molecular methods to determine mechanisms leading to bloom formation and toxicity. Findings show that our study lakes had differing bloom phenologies, along with the nitrogen limited lake having greater cyanobacteria biovolumes. The dominant cyanobacteria taxa present switched midsummer from nitrogen fixers (*Dolichospermum sp.*) to non-nitrogen fixers (*Microcystis sp.*) which tracked with decreasing nitrogen to phosphorus ratios. Shotgun metagenomic analyses showed microcystin genes were present among various genomes throughout the sampling season. Microcystin concentrations corresponded to the cyanobacteria biovolume and toxin gene presence. Cyanotoxin production can vary at the species level, therefore, it is essential to determine abiotic drivers of cHABs of differing lake types to properly inform management and mitigation of future system specific HABs.

0945 Tuesday

The physiological and transcriptional response of harmful algal blooms in nitrogen limited lakes

Kaela Natwora (natwo001@d.umn.edu), University of Minnesota – Duluth; Leah Egan, University of Minnesota – Duluth; Cody Sheik, University of Minnesota – Duluth; Chris Filstrup, University of Minnesota - Duluth

Nitrogen fixation (N_{Fix}), although potentially a significant source of nitrogen (N), is often overlooked or underrepresented in many ecological models. Given the cellular N-demand, and inherent N-rich toxin compounds, N_{Fix} may be an important microbial process in proliferating cyanobacterial harmful algal blooms (cHABs) in inland lakes. N_{Fix} is a lynchpin in the nitrogen cycle that converts inaccessible dinitrogen, N_2, to bioavailable ammonia, NH_3. To understand nutrient constraints, specifically N and P, and its effects on the physiological response and gene expression of cHABs we sampled a small, bloom prone lake every two weeks for five months. We monitored shifts and changes in community composition (using metagenomics), nutrients, and N_{Fix} rates. Peltier Lake, in Lino Lakes, MN routinely exhibits cHABs that include *Dolichospermum*, a cyanobacteria capable of producing toxins and fixing N. When Peltier became N-deficient, N_{Fix} rates increased from 0.089 nmol N $hr^{-1}L^{-1}$ to 2.5 nmol N $hr^{-1}L^{-1}$, roughly a 28x increase. This dramatic increase in N_{Fix} could offset the N-demand for the microbial community potentially supporting blooms and funding N to toxin production. Interestingly, *Microcystsis*, a non-N-fixer was also present in these blooms during N-limiting conditions. Together, this suggests that N_{Fix} although generally ignored may be important in cHABs.

1000 Tuesday

Cyanobacteria in hot pursuit: Characterization of cyanobacteria strains from thermal springs, including novel taxa, and comparison of their thermophilic ability with the genetic background

Iwona Dorota Jasser (i.jasser@uw.edu.pl), University of Warsaw; Matthos Panou, Aristotle University of Thessaloniki; Nataliia Khomutovska, University of Warsaw; Malgorzata Sandzewicz, University of Warsaw; Emmanuel Panteris, Aristotle University of Thessaloniki; Toirbek Niyatbekov, tohir-73@mail.ru; Lukasz Lach, University of Warsaw; Jan M. Kwiatowski, University of Warsaw; Mikolaj Kokocinski, Adam Mickiewicz University in Poznań; Spyros Gkelis, Aristotle University of Thessaloniki

Thermal spring microbial mats serve as excellent models to study microbial community ecology as well as a source of novel taxa. Cyanobacteria are integral part of such ecosystems. We sampled thermal springs, spanning from Iceland and Poland to Greece and Tajikistan. Thirteen strains of cyanobacteria were isolated and characterized with taxonomic indices and molecular markers (16S-23S rRNA region and *cpcBA* gene), and their thermotolerance was evaluated. Screening for the presence of genes encoding three heat shock proteins, non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) was performed. We described two new genera (*Hillbrichtia* and *Amphirytos*) with type species (*Hillbrichtia pamiria* and *Amphirytos necridicus*) representing Oscillatoriales and Synechococcales orders, respectively. We also found unique lineages inside the genus *Thermoleptolyngbya*, describing a novel species (*T. hindakiae*). Isolated strains differed in their thermophilic ability; with the strains best adapted to high temperatures exhibiting presence of all three investigated heat shock proteins' as well as studied PKS and NRPS genes. Gradual decrease of these genes number complemented the lower tolerance to high temperatures. Additionally, *Hillbrichtia pamiria* demonstrated production of debromoaplysiatoxin. In this work, we show novel cyanobacteria diversity from thermal springs from disparate environments and possible correlation of thermotolerance and their genetic background.

1015 Tuesday

Photosynthetic efficiency in Polish and Australian *Raphidiopsis raciborskii* strains

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Raphidiopsis raciborskii, as a potentially toxic, invasive cyanobacteria has attracted great attention. Worldwide distribution could have contributed to its phenotypic plasticity and even the formation of ecotypes adapted to the local environment. To determine mechanisms responsible for the omnipresence of *R. raciborskii*, photosynthetic efficiency in several strains differing in origin and toxicity has been investigated. Three non-toxic Polish strains and two toxic Australian strains were grown under standardized light and temperature conditions. Concentration of photosynthetic pigments, qualitative and quantitative analysis of carotenoid composition, and photosynthetic performance (chlorophyll fluorescence *in vivo*) were analysed. No significant differences between strains were observed in the photosystem II quantum efficiency (Fv/Fm), the photochemical (qP) and non-photochemical quenching (NPQ) and dark and light levels of plastoquinone reduction. Nonetheless, a positive correlation between Fv/Fm and overall NPQ was found. The phycocyanin concentration was negatively correlated with the carotenoid concentration in all five examined strains. Furthermore, in four strains roughly one third of pigments consisted of myxoxanthophylls. The ability to accumulate pigments involved both in light harvesting and photoprotection may contribute to the optimization of photosynthetic efficiency of investigated *R. raciborskii* strains in different environmental conditions. Documented parameters could not be ascribed to the difference in growth conditions, but could result from variations in their genetics.

1100 Tuesday

Environmental factors related to the distribution pattern of *Raphidiopsis raciborskii* and *R. mediterranea* in Central East Europe

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Raphidiopsis raciborskii originates from tropical and subtropical regions, however its expansion toward temperate zone during last decades has been observed. Phenotypic plasticity, occurrence of ecotypes differing in their environmental adaptations and climate changes are indicated among factors responsible for its successful expansion. Much less is known about distribution pattern of *Raphidiopsis mediterranea* native for warm areas along Mediterranean cost. Therefore, the aim of the study was to determine the current distribution of these species in Polish and Lithuanian lakes and to detect environmental factors related to their occurrence. The *R. raciborskii* was much more common and occurred in 31 out of 112 lakes, while *R. mediterranea* in 7 lakes. The contribution of these species in total phytoplankton biomass ranged from 0.1 to 31% and from 0.1 to 2.6% respectively. Similarly to previous studies *R. raciborskii* was much more common in Western Poland where it significantly increased its contribution in phytoplankton biomass however it was detected in several new lakes in Eastern Poland. In Lithuania its occurrence is still limited to one lake. *R. mediterranea* occurred in similar number of lakes in both regions of Poland only. Among environmental factors conductivity and temperature were positively correlated with *R. raciborskii* biomass.

1115 Tuesday

Identification of cyanometabolites and toxicity assessment of cyanobacteria isolates from chosen Polish eutrophic waters and polar glaciers

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Cyanobacteria have the ability to produce various types of bioactive compounds. Some of them can exert toxic effects on aquatic fauna, including daphnids, which are sensitive organisms that respond fast to toxins. Therefore, these crustaceans are useful for aquatic toxicity assessment. In this study, several dozen cyanobacterial strains isolated from different types of water bodies were analyzed for the presence of anabaenopeptins (APs), anatoxin-a (ATX-a), β-methylamino-L-alanine (BMAA), cylindrospermopsin (CYN), microcystins (MCs), nodularin (NOD), saxitoxin (STX) using immunoassay and chromatographic techniques. We also investigated the toxicity of extracts and filtrates from 40-days-old cyanobacterial cultures using biotests with *D. magna* and *D. pulicaria*.

Analytical techniques revealed the presence of the cyanometabolites studied in isolates of *Aphanizomenon* (APs, BMAA, CYN, STX), *Planktothrix* (APs, MCs), *Cuspidothrix* (ATX-a, CYN) and *Limnothrix* (BMAA). Polar strains were free of examined compounds. Biotests revealed a reduction in *Daphnia* survival in response to some extracts and filtrates. It was also found in the case of one polar strain. However, there was no clear pattern indicating that extracts/filtrates from strains with identified toxic compounds reduced *Daphnia* survival more than other strains. This suggests that other yet unknown toxic compounds are at play.

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1130 Tuesday

Characterization of nitrate reductase in *Microcystis aeruginosa* PCC7806 wild type and non-toxic mutant strain

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Nitrogen is an essential element for the growth of cyanobacteria. Runoff of nitrate fertilizers into freshwater systems can contribute to the proliferation of cHABS and their toxicity. Characterizing the enzyme activity of nitrate reductase (NR) in Microcystis aeruginosa is an important step to understanding the bloom dynamics of this organism when nitrate is present. Nitrate reductase isolated from the toxin-producing wild type (WT) strain Microcystis aeruginosa PCC7806 and in its non-toxic -mcyB mutant was characterized for suitable electron donors, pH optimum, and substrate affinity. Suitable electron donors for WT strain included ferredoxin reduced by ferredoxin-NADP+ reductase and methyl viologen. No NR activity was detected using NADH, NADPH, FADH2 or ferredoxin reduced by sodium dithionite. Using methyl viologen as the electron donor the WT NR activity showed Michaelis Menten kinetics with a Km(nitrate) of 3.4-7.40 µM. The pH optimum was 8.5 for ferredoxin reduced by ferredoxin-NADP+ reductase, and 10.5 for methyl viologen. These results indicate M. aeruginosa WT NR activity is typical of a bacterial NR and very different from purified NR obtained from Arabidopsis thaliana and Aspergillus nidulans. Differences NR activity between WT and its –mcyB mutant under laboratory conditions will be presented.

1145 Tuesday

Low environmental microcystin concentrations affected sublethal population-level responses but not survival among freshwater keystone species

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Microcystin is an emerging global environmental threat, widely associated with the increasing incidence of freshwater cyanobacterial harmful algal blooms. However, the ecological effects of environmental microcystin concentrations on survival and fitness-related traits that underpin ecosystem functions among key freshwater populations remains poorly understood. Using a suite of sublethal and chronic studies, we tested the population-level effects of purified MC-LR and crude extract of *Microcystis aeruginosa* on individual fitness-related endpoints among three ecologically important freshwater species, *Daphnia magna*, *Gammarus pulex* and *Dikerogammarus villosus*. Low microcystin concentrations had no effects on survival but altered a range of ecologically relevant sublethal responses across the three test species studied. Purified MC-LR increased the feeding rate and stimulated parameters of reproduction in *D. magna*. Non-monotonic responses were observed on the mean number of broods produced per female, mean number of neonates produced per female and the intrinsic rate of natural increase (r), resulting in the stimulation of the population growth rate. However, the somatic growth rate of daphnids was unaffected by purified MC-LR concentrations. Both treatments affected feeding and growth rates of amphipods differently, suggesting low environmental microcystin concentrations can alter energy acquisition and biological fitness, thereby influencing population size, community structure and ecosystem functioning.

1200 Tuesday

Rates of microcystin production and biodegradation in the western basin of Lake Erie

Justin Chaffin (chaffin.46@osu.edu), The Ohio State University

Here we studied microcystin (MC) production and degradation during two growth seasons (2018 and 2019) in two western Lake Erie sites, one site in Maumee Bay and the second in the center of the western basin. MC production and biodegradation rates were quantified using the microcosm approach. MC production rates were greater with elevated nutrients than under ambient conditions and were highest near shore during the initial phases of the bloom, and production rates were lower in later bloom phases. We examined biodegradation rates of MC-LR in natural waters by the addition of extracellular $^{15}N_{10}$-MC-LR (1 µg L^{-1}). Extracellular MC-LR remained stable in the abiotic treatment, and adsorption onto sediments was minimal, while extracellular MC-LR decreased in all biotic treatments, suggesting biodegradation. Greatest rates of extracellular MC-LR biodegradation (-8.76 d^{-1}) were observed during peak bloom conditions, while lower rates were observed with lower cyanobacteria biomass. Cell-specific incorporation of $^{15}N_{10}$-MC-LR measurements by NanoSIMS showed that a small percentage of the heterotrophic bacterial community actively degraded MC-LR. Our measured bulk rates of MC production and biodegradation, combined with the microscale analysis of MC incorporation by single cells, suggest that MC predictive models could be improved by incorporating MC production and biodegradation rates.

Tuesday afternoon, 1330

Session: Secondary Cyanometabolites – Structure, Biosynthesis, Physiological Function, Environmental Significance and Biotechnical Application (Wayne Carmichael, convener)

Plenary: Elisabeth Janssen (elisabeth.janssen@eawag.ch), Eawag, Dübendorf, Switzerland

Increasing confidence in identifying cyanobacterial metabolites to study their occurrence and behavior

Cyanobacteria and their unique secondary metabolites offer many opportunities for research across disciplines. Over the past decades, a legion of new cyanobacterial metabolites has been identified but we often do not know yet what their concentrations and behaviour in the environment are. We often point to the key challenge of the lack of reference standards and thus the high analytical requirements for identification and quantification before further studies of these compounds become feasible. Until recently, even a comprehensive list of all known cyanobacterial metabolites was not openly available. As a result of the 11th ICTC in Krakow (2019) we started a collaborative effort to merge, expand, and validate existing private and public datasets into one open access structural database: CyanoMetDB. The 2021 version of CyanoMetDB contains complete structural and literature information for more than 2000 secondary metabolites from cyanobacteria. We actively facilitated the integration of CyanoMetDB into other platforms (e.g., PubChem, The Natural Products Atlas, MetFrag, Norman List Exchange, CompTox). We continue updating CyanoMetDB to include information and compounds currently missing.

A structural suspect list such as CyanoMetDB is essential for dereplication work on cyano-metabolites using mass spectrometry. However, to improve our confidence of compound identification, we need to be able to compare empirical fragmentation data to available reference spectra, when no authentic standard is directly available. Currently, reference spectra are publicly available for a small fraction of the metabolites listed in CyanoMetDB. One main shortcoming is the lack of commercially available reference standards or high costs associated with these materials. Metabolites identified in extracts that have not been purified often do not meet the quality standards of spectral databases. This dilemma prevents making cyanobacterial metabolite identification more widely available. The CyanoMetDB team now takes on the next challenge to systematically record mass spectral reference data of available standards, purified bioreagents and compounds identified with high certainty in cyanobacterial extracts. We will share the reference data through open access spectral databases open to such data to enhance dereplication of known cyanobacterial metabolites. The availability of reference spectra offers many new opportunities including studies of compound- and class-specific gas phase fragmentation patterns. Without the collaborative and often selfless work of the researchers involved, these efforts would not be possible. CyanoMetDB is open to all community members to contribute to and support our efforts.

Secondary Cyanometabolites – Structure, Biosynthesis, Physiological Function, Environmental Significance and Biotechnical Application

1415 Tuesday

Investigating the interaction between Microcystins and cyanobacterial cells using nanoscale secondary ion mass spectrometry

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Secondary metabolites are thought to bring about evolutionary advantages for their producer organisms. Among these are small peptides called Microcystins (MCs), which are produced by multiple genera of cyanobacteria. Considerable energy and resources are diverged from primary metabolism for MC production; however, their physiological roles remain unclear. Cyanobacteria store large amounts of MCs, which are released upon cell lysis and dense colonies of cyanobacteria can drive MC concentrations to alarmingly high levels (>1ppm), but how released MCs interact with remaining cyanobacterial cells is not understood. We are studying this interaction between MC-producing cyanobacteria and exogenous MCs using nanoscale secondary ion mass spectrometry (nanoSIMS). In these experiments, cultures of *Microcystis aeruginosa* (PCC 7806) are grown under various conditions in media supplemented with ^{15}N,^{13}C-labeled MCs. At multiple time points, cells are harvested and the distribution of ^{15}N and ^{13}C in individual cells is resolved via nanoSIMS. Cell extracts are analyzed by high-pressure liquid chromatography paired with tandem mass spectrometry to determine if exogenous MCs remain intact and/or breakdown upon interaction with living cells. This study examines the effects of MCs on the cyanobacteria responsible for their production to better understand the fate and function of released MCs within cyanobacteria dominated blooms.

1430 Tuesday

Monitoring of intracellular and extracellular toxin/bioactive peptide production under physiological stress conditions through labeling by chemical modification.

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CyanoHAB forming cyanobacteria *Microcystis* and *Planktothrix* produce high intracellular amounts of cyanotoxins and other bioactive peptides such as microcystins (MCs) and anabaenopeptins (APs). Often the production of cyanopeptides has been related to physiological stress conditions, for example induced by low light or nutrient limitation. Both MC and AP peptides are produced via Non-Ribosomal Peptide Synthesis (NRPS). Natural mutations in adenylation domains can lead to an unspecific amino acid incorporation which can be exploited to incorporate non-natural amino acids (AA) carrying azide or alkyne moieties producing chemically modified MC/AP molecules. Modified MC/AP molecules are subsequently labeled via copper-catalyzed azide-alkyne cycloaddition (CuACC) using various fluorophores. This labeling technique has been established previously, showing a rather specific increase in fluorophore signal. High resolution imaging is subsequently used to localize and track modified MC/AP-molecules. The CuAAC based labeling which is considered as a rather specific chemical reaction is applied in parallel with conventional staining techniques of intracellular structures or other organelles. Thus by analyzing the spatial distribution of MC/AP in relation to known intracellular structures such as lipids or other organelles we hope to better understand the production, storage and release of MC/AP under the above mentioned physiological stress conditions.

1445 Tuesday

Analytical workflow integrating LC-HRMS untargeted analysis and CyanoMetDB for fast and extensive detection of cyanobacterial metabolites

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Cyanobacteria produce a large number of secondary metabolites including cyanotoxins and a variety of bioactive peptides with great structural diversity. Identification of these metabolites (cyanometabolites) is a great analytical challenge due to the extremely limited availability of analytical standards and lack of a well-documented fragmentation mass spectra database. In the present study, an analytical workflow was developed for the detection of the cyanometabolites in bloom samples from Greek lakes. Samples were extracted [1,2] and analyzed by LC-HRMS (Orbitrap Fusion Lumos Tribrid MS) in data depended acquisition (DDA) mode. Fragmentation spectra of compounds were obtained with collision-induced dissociation (CID) and higher-energy C-trap dissociation (HCD) modes. Acquired data were processed with Compound Discoverer software in combination to the recently published CyanoMetDB mass list [3] and other related tools for the annotation and structural elucidation of cyanometabolites. Verification of proposed structures was performed based on *in silico* fragmentation and fragment ion search (FISh) scoring. Application of the workflow revealed the presence of numerous congeners belonging to the cyanotoxins class microcystins and to the understudied cyanopeptides classes of cyanopeptolines, microginins, aeruginosins, anabaenopeptins and aeruginosamides. Furthermore, new congeners were annotated clearly demonstrating the suitability of the approach for the characterization of cyanobacterial chemodiversity.

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[3] M. R. Jones, E. Pinto, M. A. Torres, F. Dörr, H. Mazur-Marzec, K. Szubert, L. Tartaglione, C. Dell'Aversano, C. O. Miles, D. G. Beach, P. McCarron, K. Sivonen, D. P. Fewer, J. Jokela and E. M.-L. Janssen, Water Research 196 (2021) 117017.

1500 Tuesday

Impact of unidentified secondary metabolites from five new cyanobacterial species on aquatic plant

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Cyanobacteria are a great source of bioactive secondary metabolites, exerting harmful (cyanotoxins) or beneficial (drugs) effects on other organisms. In this work, we investigated the impact of the cyanobacteria strains *Jaaginema* sp. and *Trichormus variabilis*, and the newly described species *Komarekiella chia*, *Nodularia mediterranea*, and *Iphianassa zackieohae* with unknown metabolome on *Lemna trisulca* macrophyte. The experiments were prepared in short- (cyanobacteria extracts, up to 24h) and long-term (co-cultivation in BG11, 2 weeks) periods. Co-cultivation with *I. zackieohae* cells initially stimulated plant growth, but after 14 days the value was close to the control, while the remaining cyanobacteria slightly inhibited the accumulation of macrophyte biomass. After 14 days of co-cultivation, *K. chia*, and *N. mediterranea* significantly increased the total amount of protein in the plant [$mg \cdot g^{-1}$ dry weight], by 33% and 44%, respectively. The extracts of all analyzed cyanobacteria significantly increased the respiration process after 24-hour exposure. The tested cyanobacteria strains, except *I. zackieohae*, decreased the content of chlorophylls *a* and *b* by an average of 48% and 53%, respectively. *Jaaginema* sp. extract temporarily inhibited plant photosynthesis just within 7 minutes. The above effects on photosynthesis were confirmed by ultrastructural imaging of chloroplasts. Oxidative stress induced after treatments is also discussed.

1515 Tuesday

Natural *Microcystis* populations reveal the presence and abundance of truncated *mcy* operons and microcystins: a continuing source of research for water quality research

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CyanoHABs are often dominated by *Microcystis aeruginosa*, which produce microcystins, a class of hepatotoxins that have been studied since the 1950s and have been responsible for drinking water crises in recent years. Microcystin toxicity is influenced by distinct structural elements across related molecules encoded by variant *mcy* operons. Currently over 270 structural variants have been identified through mass spectroscopy. Here, we present findings of a novel, truncated *mcy* operon that is at times the most abundant genotype across multiple years of sampling in *Microcystis* populations of Lake Erie. The partial operon contains truncated *mcyA*, complete *mcyB-C*, and is missing *mcyD-J* genes. Metatranscriptomic analysis revealed that this truncated operon is also transcriptionally active. Furthermore, it was predicted to synthesize tetrapeptide molecules. A compound with the same mass as the predicted tetrapeptide was detected in cyanoHAB samples from western Lake Erie. Current methods fail to detect this molecule or genes responsible for its biosynthesis. The persistence and dominance of this *mcy* genotype in Lake Erie, which has been intensively studied for decades, suggests that such novel genotypes and compounds may be common in natural populations and highlights the utility of shotgun metagenomics to detect novel and potentially toxic compounds produced by cyanobacteria.

1600 Tuesday

Non-canonical carbon fixation in microcystin-producing cyanobacteria

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The success of the bloom-forming genus *Microcystis* is closely linked to its ability to produce a number of signature metabolites including the potent hepatotoxin microcystin. Recent studies on the role of microcystin have pointed to a role of the heptapeptide in inorganic carbon adaptation of *Microcystis*. In particular, microcystin binds to proteins of the Calvin-Benson cycle including the key enzyme RubisCO. Subsequent studies on the subcellular localization of RubisCO in the model strain *M. aeruginosa* PCC 7806 and its DmcyB mutant revealed not only pronounced differences between the genotypes but also a frequent non-canonical localization of RubisCO underneath the cytoplasmic membrane. To further dissect the peculiarities of *Microcystis* RubisCO and the role of microcystin, we have heterologously expressed the enzyme in the cyanobacterial model strain *Synechocystis* sp. PCC 6803. We could thereby address both *in vivo* and *in vitro* differences of the RubisCO variants and the influence of microcystin separately. Comprehensive data from enzyme assays, crystal structure analysis, immunofluorescent microscopic analyses and native mass spectrometry indicate significant differences between *Microcystis* and *Synechocystis* in the efficiency, dynamics, and orchestration of carbon fixation. We hypothesize that microcystin contributes significantly to the plasticity of carbon fixation and to the versatility of bloom-forming cyanobacteria.

1615 Tuesday

Linking environmental heterogeneity and chemo-diversity in cyanobacteria: A culture-dependent profile based analysis

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Cyanobacteria are ecologically versatile microorganisms that inhabit most environments, ranging from marine systems to arid deserts. Given their ability to survive under harsh and extreme conditions, we hypothesize that cyanobacteria could produce a wide variety of compounds in specific niches. In this context, we sampled a number of different environments, from freshwater and brackish ecosystems to terrestrial and anchialine caves, spanning from the Canary Islands and Iceland to Estonia and Greece. Forty-four (44) cyanobacteria strains were analyzed with de novo peptide fragmentation in order to detect their metabolome profile; further, their antimicrobial, cytotoxic, and enzyme inhibitory activity was investigated. Both freshwater/planktic and rock-dwelling/benthic strains exhibited different types of inhibitory activities. However, cyanopeptides were only detected in freshwater strains; microcystins, anabaenopeptins, and aeruginosins congeners from *Microcystis* spp., *Thrichormus variabilis*, and *Calothrix epiphytica* strains. Therefore, our results indicate a high degree of unknown chemo-diversity, as we could not link the presence/absence of any known cyanopeptides and inhibitory activities from strains derived from other habitats, in contrast with freshwater cyanobacteria strains. In this work we discuss the correlation between the cyanobacteria chemo- and lifestyle diversity providing a missing study material for profile-based analysis on cyanobacteria from under-explored environments.

1630 Tuesday

Accumulation and elimination kinetics of free and total (free plus protein-bound) microcystin-LR in the common carp *Cyprinus carpio*

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Microcystins (MCs) are widespread hepatotoxins produced by several genera of freshwater cyanobacteria. After ingestion of cyanobacteria or contaminated water or food, MCs accumulate in aquatic organisms and are present in their cells as a free available fraction and as a fraction covalently bound to proteins, mainly phosphatase proteins that they inhibit. MCs are toxic to humans and animals and the related intoxications can cause symptoms of varying intensity ranging from gastroenteritis to kidney and liver failure, sometimes even death. It is therefore essential to monitor these toxins both in the environment and in exposed species, including fish. In this study, both total (free plus protein-bound) and free accumulation fractions of MCs were monitored in carp orally exposed on a daily basis (by voluntary feeding) to pure microcystin-LR during a period of 7 days followed by a elimination period of 21 days. The accumulation of total toxins in carp muscles was determined using Lemieux oxidation, although free microcystins fraction were determined by methanolic extraction and both fractions were analyzed by LC-MS/MS. Here will be presented the optimization of the Lemieux oxidation procedure on fish muscle matrix and the kinetics of free and of total MCs (measured by Lemieux oxidation).

1645 Tuesday

Investigation of CyanoMetabolites in Brazilian cyanobacteria using LC-MS-QTOF: distribution in different cultured strains, bloom samples and physiological studies

Ernani Pinto (ernani@usp.br), University of Sao Paulo, Brazil; Vanessa Geraldes, University of Sao Paulo, Brazil; Fernanda Jacinavicius, University of Sao Paulo - School of Pharmaceutical Sciences

The diversity of Brazilian cyanobacterial secondary metabolites has been investigated by our group by mass spectrometry and informatic tools for structural identification. We used both cultured strains and samples of blooms from water reservoirs. This study aimed to evaluate the metabolomic profiles of potentially toxic and non-toxic cyanobacteria by high-resolution mass spectrometry. Samples were analyzed by Ultra Performance Liquid Chromatography and quadrupole time of flight mass spectrometry (UPLC-QTOF) and data were assessed to verify changes in metabolomic profiles. Matrices were used to generate principal component analysis biplots, and molecular networks were obtained using the Global Natural Products platform. Most strains showed significant changes in their metabolomic profiles after comparison. The identified compounds included aeruginosins, spumigins, cyanopeptolins, microginins, namalides, pseudospumigins, anabaenopeptins, mycosporine-like amino acids, nodularins and microcystins. Data showed that cyanobacteria display broad metabolic plasticity among species and genera, including the synthesis and differential expression of a variety of secondary metabolites. CyanoMetabolite identification and dereplication using Metaboanalyst 4.0 integrated with CyanoMetDB (available at 10.1016/j.watres.2021.117017) are a powerful tool for identification and screening of natural products in cyanobacteria. The different cyanobacterial genera from diverse Brazilian biomes and environments are prolific sources of cyanopeptides and other classes of metabolites, including cyanotoxins.

Wednesday morning, 0830

Session: CHABs and Omics: advances in genetics, transcriptomics, metabolomics, and proteomics to understanding CHABs (Cody Sheik, convener)

Plenary: Morgan Steffen (steffemm@jmu.edu), James Madison University

Progressing from exploratory studies to hypothesis testing: Harnessing the power of -omics tools to understand cyanobacterial harmful algal blooms

The -omics haven't just revolutionized our understanding of the breadth of the microbial biodiversity of our planet, but they've also pushed many fields toward a more comprehensive and holistic approach to biological questions. Rather than a traditional, reductionist approach studying a single model organism, the -omics have helped us recognize that biological interactions are the foundation of biodiversity and even ecosystem function. We've seen this shift in research on cyanobacteria in the last few years, with a renewed focus on interactions between cyanobacteria and the members of their broader microbial community that span all three domains of life (and viruses).

My talk will focus on how we can apply the various -omics tools to our research questions about cyanobacteria in the context of interactions and exchanges that occur between microbes in cyanobacterial harmful algal blooms. These exchanges leave molecular signals in the expression profiles of the participants. We can use these signals as clues in -omics style data to deconstruct the physiological mechanisms that shape community dynamics in the various cHAB communities. In my lab, we've transitioned from using an exploratory approach, using genomics and transcriptomics to generate hypotheses about bloom communities to testing those hypotheses both in the field and in the lab. I'll share examples that range from genome sequencing of individual constituents of bloom microbiomes, to co-culture expression studies, to experiments in field populations to highlight the various ways the -omics have allowed us to grow our understanding of bloom dynamics. These data can eventually be harnessed to develop a more comprehensive suite of mitigations strategies that consider both biotic and abiotic drivers of cyanobacterial harmful algal blooms.

CHABs and Omics: advances in genetics, transcriptomics, metabolomics, and proteomics to understanding CHABs

0915 Wednesday

Diverse genome sequences from recent toxic and non-toxic US Pacific Northwest freshwater HABs belong to the Nostocales ADA clade

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Combined PacBio long-read and Illumina short-read DNA sequencing has recently allowed us to determine the genome sequences (without cultivation) of the main cyanobacteria present in recent freshwater HABs from the US Pacific NW. Our studies have emphasized blooms in multiple lakes that were dominated by Nostocales cyanobacteria. The 12 *Dolichospermum* and 5 *Aphanizomenon flos-aquae* genomes that we have assembled all represent members of the Nostocales ADA clade. Non-toxigenic members are included, as well as verified producers of microcystin (MC-LR, [Dha^7]MC-HtyR), 7-epi-cylindrospermopsin and anatoxin-a. The ADA genomes are a genetically diverse group that we propose belong to 4 of 10 species that comprise an ADA genus, which includes *Dolichospermum*, *Aphanizomenon flos-aquae*, benthic *Anabaena* and *Cuspidothrix*. We have also assembled 3 *Gloeotrichia* echinulata genomes, all of which possess geosmin synthase but lack cyanotoxin genes. The relationships between geography, morphology and genotype will be described, as well as different patterns of genome evolution between ADA members and *Gloeotrichia*.

0930 Wednesday

The Great Lakes Atlas of Multi-omics Research (GLAMR) Database: Facilitating the Great Lakes Research of Tomorrow

E. Anders Kiledal (kiledal@umich.edu) The University Of Michigan; Gregory J. Dick, The University Of Michigan; Teal Furnholm, The University Of Michigan; Derek J. Smith, The University Of Michigan; Robert Hein, The University Of Michigan; Vincent J. Denef, The University Of Michigan; Melissa B. Duhaime, The University Of Michigan; Subba Rao Chianti, The University Of Michigan; Reagan M. Errera, NOAA Great Lakes Environmental Research Laboratory; Casey Godwin, The University Of Michigan; Ryan J. Newton, University of Wisconsin – Milwaukee; Cody S. Sheik, University of Minnesota – Duluth; Daniel D. Heath, University of Windsor; Henry A. Vanderploeg, NOAA Great Lakes Environmental Research Laboratory

Research and monitoring of harmful algal blooms increasingly relies on ‘Omics information obtained by characterizing and quantifying biomolecules including DNA, RNA, proteins, and metabolites. While individual studies and/or types of ‘omics data produce valuable insights on their own, additional insights can be gained by integrating the complex relationships of multi-omics data from multiple studies in a unified database with environmental data. With this in mind, we are building a database to facilitate analyses of ‘omics data collected from the Great Lakes, including analysis of complex interactions and across expanded time and geographic scales. While the database will support many types of queries, users will–for example–be able to compare across annual Cyanobacteria blooms, where toxin production and strain abundance vary considerably over time. Here we present the concept and architecture of this database, progress on loading existing datasets, use case scenarios, and example output. From the research community, we seek input on desired capabilities and use-cases, and additional high-quality Great Lakes datasets for inclusion in the database. By addressing common barriers to analysis of ‘omics data—including bioinformatics expertise and the need for high-performance computing—the database will expand public data access both for experts and inexperienced users.

0945 Wednesday

Sulfolipid profiles of *Microcystis aeruginosa* and cyanobacterial blooms as an indicator of P availability

Robbie M. Martin (rmarti49@vols.utk.edu) University of Tennessee, Knoxville; Maddie K. Denney, University of Tennessee, Knoxville; Helena L. Pound, University of Tennessee, Knoxville; Justin D. Chaffin, The Ohio State University; George S. Bullerjahn, Bowling Green State University; Robert M. McKay, University of Windsor; Katarina A. Jones, University of Tennessee, Knoxville; Hector F. Castro, University of Tennessee, Knoxville; Shawn R. Campagna, University of Tennessee, Knoxville; Steven W. Wilhelm, University of Tennessee, Knoxville

While phosphorus (P) scarcity can limit primary productivity in lakes, its oversupply can lead to the formation of cyanobacteria-dominated algal blooms. P availability influences bloom dynamics and can be seasonally limiting, even in eutrophic lakes. Marine phytoplankton alter their lipid profile by increasing sulfolipids when P is limiting. We asked whether *Microcystis* spp. respond in a similar manner. The ratio of sulfoquinovosyl diaclyglycerol (SQDG) to phosphatidylglycerol (PG) was used to examine lipid remodeling. In batch cultures of *M. aeruginosa*, the SQDG:PG ratio increased from ~0.9 to ~3.3 with decreasing initial P concentration. In P-limited Lake Erie mesocosms, SQDG:PG increased in controls from ~6 to ~11 after 48 hr, while P-addition decreased the ratio from ~6 to ~3. In non-P-limited mesocosms, the ratio was unchanged after 48 hr and P-addition treatments had no effect. In Lake Erie *in situ* measurements, SQDG:PG showed an inverse correlation with total dissolved P. There was no correlation with either soluble reactive P or N:P ratio. This study demonstrates that *Microcystis* remodels its lipid profile in response to P scarcity, providing a potential short-term, time-integrating marker of nutrient history for cyanobacterial populations during fresh water blooms.

1000 Wednesday

Do *Microcystis* laboratory cultures hold clues to bacterial microcystin degradation?

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Microcystins do not accumulate in freshwater ecosystems, and their degradation rates in freshwater suggest that both biotic and abiotic factors play critical roles in their removal. Most *Microcystis aeruginosa* laboratory cultures harbor a microbiome made up of heterotrophic bacteria that use *Microcystis*-produced organic matter for growth. We analyzed the microbiome of two *M. aeruginosa* cultures from Lake Erie with metagenomic sequencing, and found that some of their metagenome-assembled genomes included known genes for microcystin degradation. We tested whether these mixed communities can degrade microcystins by incubating them with *Microcystis* lysate (freeze-thawed cells) and monitoring dissolved microcystins by liquid chromatography mass spectrometry (LC/MS). We also tested whether these bacterial populations could incorporate carbon and nitrogen from microcystin-LR (MC-LR), the most common and most toxic of the microcystins, as well as bacteria from non-toxic *Microcystis* culture as a control. To accomplish this, we first labeled a *M. aeruginosa* culture with ^{13}C and ^{15}N stable isotopes, purified extracted MC-LR from the cell pellets, and added this labeled substrate back to *Microcystis*-bacteria co-cultures. Using nanoscale imaging mass spectrometry (NanoSIMS), we quantified the net incorporation of ^{15}N and ^{13}C into heterotrophic bacteria as well as the *Microcystis* cells to identify the fate of MC-LR C and N in this microbial ecosystem.

1015 Wednesday

Microcystin aids in photo-acclimation during prolonged cold stress treatment in *Microcystis aeruginosa* strain PCC7806

Gwendolyn F. Stark (gstark2@vols.utk.edu), University of Tennessee, Knoxville; Robbie M. Martin, University of Tennessee, Knoxville; Laura Smith, University of Tennessee, Knoxville; Bofan Wei, State University of New York College of Environmental Science and Forestry; Gregory L. Boyer, State University of New York College of Environmental Science and Forestry; Steven W. Wilhelm, University of Tennessee, Knoxville

Microcystis aeruginosa PCC7806 has been shown to increase toxin production in response to prolonged cold stress. To test whether this increase in toxin production is a beneficial adaptation that aids in cold fitness, the non-toxic mutant *Microcystis aeruginosa* PCC 7806 Δ*mcy*B was grown in duplicate chemostat cultures alongside chemostats containing the wildtype isolate. Strains reached steady-state at 26°C, then were subjected to an 11-day shift to 19°C, followed by a reversion back to 26°C. Throughout the experimental period, samples were collected to measure cell abundance, excitation pressure, effective quantum yield, microcystin production, reactive oxygen species (ROS) formation, and for RNA-seq analyses. During the 19°C period, the mutant experienced elevated excitation pressure and ROS formation relative to the wildtype strain. Microcystin quota doubled in the wildtype strain by day 7 of the 19°C treatment, followed by decreased effective quantum yields 24 h later. This was not observed in the mutant strains. Both mutant and wildtype populations began to recover after 8 days at 19°C. Microcystin did not aid in growth recovery during cold stress, although it seemed to play a part in the photo-acclimatory process based on excitation pressure and quantum yield readings.

1100 Wednesday

Anatoxin-producing and non-toxic strains of *Microcoleus* sp. coexist in benthic cyanobacterial mats in the Wolastoq (Saint John River, Canada)

Cecilio Valadez-Cano[1], Adrian Reyes-Prieto[1], Cheryl Rafuse[2], Daniel G. Beach[2], Pearse McCarron[2] and Janice Lawrence[1]
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The presence of toxigenic benthic cyanobacteria in riverine ecosystems is an increasing concern around the world. In 2018, the death of three dogs along the Wolastoq in New Brunswick, Canada, was attributed to anatoxin exposure after they ingested benthic microbial mats found along the shore. Four samples of the material ingested by the dogs and from the vicinity were collected. Then, 15 non-axenic cyanobacterial isolates were obtained from the same material. Total DNA of the 19 samples was sequenced using Illumina technology. Metagenomic assemblies recovered near-complete *Microcoleus* genomes from 12 of the sequenced samples. The high average genomic sequence similarity (>95% identity at the nucleotide level) suggests that the 12 genomes are representatives of the same "genomic" *Microcoleus* species. The genetic repertoire to produce anatoxin-*a* and dihydroanatoxin-*a* was identified in 9 of these genomes. The capacity for anatoxin production was confirmed by LC-MS. The overall comparison revealed that genomes of the 9 toxigenic *Microcoleus* isolates contain a higher number of accessory genes than their 3 non-toxigenic relatives. These differences suggest that toxigenic *Microcoleus* variants from the Wolastoq would be more responsive to changing environments, nutrient limitation and/or bacteriophage infection. Our results suggest that the two *Microcoleus* strains inhabited the original benthic mats in similar relative abundances.

1115 Wednesday

Comparative metabolomics of exudates between toxigenic and non-toxigenic *Microcystis aeruginosa*

Xuexiu Chang (xchang@uwindsor.ca), College of Agronomy and Life Sciences, Kunming University (China) and Great Lakes Institute for Environmental Research, University of Windsor (Canada); Yuan Zhou, School of Ecology and Environmental Science, Yunnan University (China); Hugh MacIsaac, Great Lakes Institute for Environmental Research, University of Windsor (Canada); Michael McKay, Great Lakes Institute for Environmental Research, University of Windsor (Canada)

Cyanobacterial Harmful Algal Blooms (CHABs, dominated by *Microcystis aeruginosa*) have received high attention for decades owing to the noxious and harmful secondary metabolites and toxins that they produce and release upon into water. Research on the active components and future risk assessment of them are demanded. *M. aeruginosa* strains could be characterized as ‘toxigenic’ (genomics containing mcy gene) or ‘non-toxigenic’ (genomics not containing mcy gene), according to whether they produce microcystins or not. We employed nontargeted metabolomics technique to compare components and their abundances in exudates from toxigenic *M. aeruginosa* strain (FACHB-905) and non-toxigenic strain (FACHB-526) at exponential and stationary growth stages. In total, 409 metabolites were identified and lipids, organoheterocyclic compounds and benzenoids were found with high abundances. The two strains in the same growth stage had similar metabolites, but more metabolites were accumulated at stationary phase than exponential stage. Although FACHB-905 exudated more metabolites than FACHB-526, some known bioactive and toxic metabolites, e.g. 7-ketocholesterol, glyceraldehyde and myristoleic acid, were significantly accumulated higher in FACHB-526 exudates. Our results would be valuable for further risk prediction of cyanobacterial booms, especially for the non-toxigenic strains, and healthy water management.

1130 Wednesday

Insights from the global pangenome of *Raphidiopsis raciborskii*

Anusuya Willis, Jason Woodhouse and a global network of participants* Australian National Algae Culture Collection, CSIRO, Hobart, Tasmania, Australia.

Raphidiopsis raciborskii is a species, including both toxic and non-toxic strains. It occurs, and frequently blooms, in freshwater ecosystems across tropical and temperate environments. Local scale studies have shown high physiological and genomic diversity between strains of *R. raciborskii*, indicating possible rapid adaptation to new environments and resilience to environmental changes.

To investigate the global genomic diversity of *R. raciborskii*we sequenced the full genomes of ~85 strains from 22 countries, spanning the continents Africa, America, Asia, Australia, and Europe.

Comparative genomics show a small core genome and a large variable shell genome, suggesting a flexible genome evolution strategy. However, many gene presence/absences appear to be redundant and overall metabolic functions are similar across all genomes. There is a lack of unique features within each cluster and genome structural changes appear haphazard across the genome. These genomes are particularly rich in anti-phage systems, with over 500 CRISPR arrays, suggesting high rates of phage interaction.

Speciation is occurring through geographic isolation, and three distinct species clusters were evidenced, indicating taxonomic changes and new species descriptions are needed within *Raphidiopsis*. The *Raphidiopsis* global pangenome reveals species with a flexible genome and local adaptation without local functional differences.

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1145 Wednesday

Phylogenomic insights into toxic bloom formation in the Baltic Sea

David Fewer (david.fewer@helsinki.fi), University of Helsinki, Finland

The Baltic Sea is a unique and delicate brackish water ecosystem with high primary productivity driven by oceanic biogeochemical cycles of oxygen, iron, silicon, nitrogen and phosphorus. Elevated anthropogenic nutrient loading into the Baltic ecosystem is associated with an increase in the intensity of cyanobacterial blooms. The toxic cyanobacterium *Nodularia spumigena* forms blooms when phosphate has been depleted from surface waters. Here we analyze the genomes of 53 cyanobacteria isolated from the Baltic Sea for the presence of secondary metabolite biosynthetic pathways as well as metabolic pathways for the acquisition, transport and storage of phosphorous. Bioinformatics analysis and HR-LC-MS analysis demonstrate the production of nodularins, microcystins and cylindrospermopsins in strains of cyanobacteria isolated from planktonic and benthic habitats of the Baltic Sea. Growth experiments demonstrate that a series of phosphonates, phytic acid and phosphite all support the growth of toxic cyanobacteria as the sole source of phosphorous. These results indicate that toxic cyanobacteria can utilize several phosphorus compounds previously unknown to contribute to eutrophication in the Baltic Sea, which may aid in understanding how such organisms dominate phytoplankton blooms under phosphate-limiting conditions and help inform future efforts to mitigate eutrophication.

1200 Wednesday

Cyanophages Infecting *Planktothrix agardhii* from a Lake Erie Embayment

Katelyn M. McKindles (kmckind@bgsu.edu), University of Windsor; Michelle Neudeck, Bowling Green State University; Robert Michael McKay, University of Windsor; George S. Bullerjahn, Bowling Green State University

Planktothrix agardhii is a microcystin-producing cyanobacterium found in Sandusky Bay, an embayment of Lake Erie that is shallower and more turbid than the main body of the lake. Previous work has indicated that cyanophages are an important natural control factor of harmful algal blooms. Currently, there are few cyanophages that are known to infect *P. agardhii*, with the best-known being PaV-LD, a podoviridae isolated from Lake Donghu, China. Here we present work on the molecular characterization of *Planktothrix* specific cyanophages in Sandusky Bay. Viral sequences were identified from analyzing the spacer sequences of CRISPR-cas regions alongside metagenomic data from the bay in 2013 and 2018. Transcriptomic data from 2015, 2018, and 2019 were also employed for the further identification of cyanophages, as well as activity of select viral sequences. Finally, viral quantification was tested using qPCR in 2015-2018 for PaV-LD like cyanophages to identify the relationship between lysis events and environmental parameters.

1215 Wednesday

Toward understanding of cyanophage resistance in the bloom-forming cyanobacteria *Aphanizomenon flos-aquae*

Antonia Lobodzinska (antonia.lobodzinska@doctoral.uj.edu.pl), Jagiellonian University Cracow; Adam Antosiak, Jagiellonian University, Poland; Kinga Chlebicka, Jagiellonian University; Emilia Bonar, Jagiellonian Universit; Piotr Suder, AGH University of Science and Technology, Poland; Sigitas Šulčius, Nature Research Centre, Lithuania; Dariusz Dziga, Jagiellonian University

Interactions between cyanobacteria and cyanophages often lead to the formation of resistant sub-populations. However, the resistance mechanisms in cyanobacteria, in particular in filamentous freshwater species, remain poorly investigated. In this study, we have investigated proteome differences between cyanophage (vb_AlphaS-CL131) susceptible *Aphanizomenon flos-aquae* and its evolved resistant isolates by means of two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectroscopy identification of differentially expressed protein spots.

The growth rate and photosystem II performance of both sub-populations were similar. Despite the fact that small proteomic differences were expected, the two sub-populations differed in the expression of more than 40 proteins (a change of 2 to 10-fold). A functional analysis documented that the resistant sub-population had significantly upregulated metabolic pathways crucial for cell functioning, in particular: aminoacid and protein biosynthesis pathways and carbon metabolism (Glucose metabolism, Krebs cycle). Furthermore, enhanced expression of iron uptake porins has been found, suggesting the importance of iron import during phage infection. This first ever determination of cyanobacterial proteomes from sub-populations resistant and susceptible to a cyanophage showed, that the resistance mechanisms in freshwater filamentous cyanobacteria include large-scale modifications of the basic cellular metabolism. This may have significant ecological implications for host-phage interactions which strongly affect population and bloom dynamics.

Wednesday afternoon, 1345

Session: IFHAB Special session – Research from Temperate to Polar Regions
(Sébastien Sauvé, convener)

Plenary: Gregory L. Boyer (glboyer@esf.edu), State University of New York College of Environmental Science and Forestry

Evolution of Harmful Algal Bloom Monitoring in New York State; a 20-Year Retrospective

Harmful algal blooms (HABs) are increasing in their occurrence in New York. HAB monitoring within the state has transitioned from a purely academic research project (2002-2008), to partnership with state agencies and its incorporation in the Citizens Statewide Lake Assessment Program (2011-present), to the establishment of multiple community-based HAB monitoring programs in the Finger Lakes following the widespread blooms of 2017. Early efforts focused on techniques to detect microcystins in lakes. Current efforts have expanded to include the neurotoxic cyanotoxins anatoxins and the paralytic shellfish toxins, and to include more diverse habitats. Our ability to identify HAB events and their escalating numbers have outstripped our ability to pay for needed cell and toxicity testing. Positive aspects in the current approach to HAB monitoring include community involvement in the process. This engagement promotes community ownership of the issue and lead to greater acceptance of potential solutions. On the negative side, our focus on microcystin monitoring in lakes underestimates the diverse nature of cyanotoxins within the state. Microcystins are a poor surrogate for predicting the occurrence of cyanobacteria neurotoxins. Traditional in-water sampling may miss benthic sources of cyanotoxins and underestimate the threat they pose to animal and human health.

1430 Wednesday

Using citizen science to track harmful cyanobacterial blooms through crowdsourcing and crowdfunding - Adopt a Lake project

Dana Florina Simon (df.simon@umontreal.ca) Université de Montréal; Gabriel Munoz, University of Montreal; Tuc Quoc Dinh, University of Montreal; Nicolas Tromas, University of Montreal; Jesse Shapiro, University of Montreal; Kat Kavanagh (kat@waterrangers.ca), Water Rangers; Sébastien Sauvé, University of Montreal; Sung Vo Duy, University of Montreal

The proliferation of waterborne cyanobacterial harmful algal blooms (CyanoHABs), some of which can produce potent toxins, poses serious risks for environmental and human health. Academic and governmental monitoring efforts may be constrained by budget, time, and staff, and thus miss otherwise significant pollution events. Here, we report on the implementation of a citizen science project to track CyanoHABs in lakes and waterways across Canada. Through both crowdsourcing and crowdfunding, *Adopt a Lake* aimed to document the potential presence of cyanobacteria and toxins with the assistance of citizens, altogether improving public awareness to the issue of water quality preservation. Diverse water ancillary parameters were measured, combining in situ analyses by volunteers for basic water physico-chemical parameters. Samples were sent to the laboratory for more complex analyses including nutrients analysis, multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) using online SPE-UHPLC-HRMS and sequencing the 16S rRNA gene as a taxonomic marker for bacteria. Data analysis of four years of sampling revealed problematic lakes that could be used to further the study of HAB occurrence.

1445 Wednesday

Quantifying microcystin concentrations, their composition and drivers across over 400 north-temperate and boreal Canadian lakes

Paul MacKeigan (paul.mackeigan@mail.mcgill.ca), McGill University; Beatrix Beisner, University of Québec at Montréal; Zofia E. Taranu, Environment and Climate Change Canada; Frances Pick, University of Ottawa; Arthur Zastepa, Environment & Climate Change Canada; Irene Gregory-Eaves, McGill University

Microcystins (MCs) are the most commonly measured of the cyanotoxins. Roughly 250 MC congeners have been identified to date with noted differences in their toxicity and persistence. Regional and national scale models for different parts of the world have been instrumental in identifying the drivers of total MC concentrations including toxin-producing cyanobacteria biomass, nutrients (total nitrogen and phosphorus), agricultural development in the watershed, temperature and light. Most of the MC work in Canadian lakes has focused on total concentrations and congener-specific data are lacking. Using the first Canada-wide lake set, with standardized sampling generated through the NSERC LakePulse Network, we quantified the importance of biotic and abiotic predictors of total MCs as well as several congeners from up to 440 lakes. Overall, MCs were detected in 30% of lakes using ELISA, mostly in central Canada within the Prairies and Boreal Plains ecoregions. Total concentrations were generally low, with just 10% of lakes exceeding the WHO drinking water guideline. While considering a broad suite of variables – physiography, water quality, land use, zooplankton abundance, climate, and cyanobacteria enumerations - MCs were most associated with high nutrients, low hypolimnetic oxygen, high chlorophyll-a and the biomass of known MC producers, particularly Microcystis.

1500 Wednesday

Harmonizing science and management options to reduce risks of cyanobacteria blooms

Charles Trick, University of Saskatchewan; Kevin Erratt, University of Saskatchewan; Irena Creed, University of Toronto, Scarborough

Managing cyanobacteria blooms has become an increasingly complex venture, as lakes are changing at unprecedented rates and management targets established decades earlier are shifting. The nexus of complex management challenges and existing knowledge gaps invoke significant conflict in advising optimal bloom management strategies. Concerned communities are further disillusioned by unarticulated and often complicated scientific approaches from lake managers or scientists offering remedial measures from opposing fronts. While a universal system (i.e., one-size-fits-all management) fails to provide a management path forward due to the increased diversity of causal pathways and management options, we offer a method of triaged knowledge exchange. The bow-tie analysis and harmonizing scientific and managerial perspectives enable the design of a multi-barrier approach for localized strategies customizable to the stressors and the desired outcome. We then present harmonized management interventions that are flexible, agile, and informed by advances in scientific understanding to get ahead of the rise in cyanobacteria harmful algal blooms. Cyanobacteria bloom management that continually adjusts/adapts in response to scientific advancements and employs a calculated assortment of interventions across the upstream-downstream continuum will be essential as society pushes forward in an era of bloom uncertainty.

1515 Wednesday

Impact of Cyanobacteria on the pelagic phosphorus cycle

Akaash Bansal (akb844@usask.ca), University of Saskatchewan; Arthur Zastepa, Environment & Climate Change Canada; John-Mark Davies, Water Security Agency; David Vandergucht, Water Security Agency; Jeff Hudson, University of Saskatchewan

Research concerning cyanobacteria has increasingly focused on the health implications and causal factors promoting cyanobacterial blooms. However, there is another important aspect of cyanobacterial blooms that have received less attention. Recent studies have provided indirect evidence that pelagic food web function is affected when cyanobacteria are abundant in lakes. This may be due to a combination of factors, such as their inedibility, poor food quality, or their production of cyanotoxins. With the use of a radiotracer, we measured pelagic phosphorus cycling in a variety of lakes from the Canadian Prairie Region and beyond (i.e., Lake Erie and Lake of the Woods). These lakes provided a broad range in cyanobacterial biomass (0 to 75% cyanobacterial biomass). Phosphate turnover, planktonic regeneration, and most important, planktonic food web turnover will be presented as a function of cyanobacterial biomass to determine if food webs dominated by cyanobacteria do indeed have reduced cycling efficiencies.

1530 Wednesday

The missing middle – Investigating a North American metalimnetic cyanobacteria layer

Kevin J. Erratt (kerratt@uwo.ca), Western University; Irena Creed, University of Toronto, Scarborough; Erika Freeman, University of Cambridge; Arthur Zastepa, Environment & Climate Change Canada; Judy Westrick, Wayne State University; Charles Trick, University of Saskatchewan

While the majority of cyanobacteria research and bloom reports pertain to surficial events, research centred on subsurface cyanobacteria remains understudied. Metalimnetic cyanobacteria layers (MCL) are a subsurface phenomenon forming distinct depth stratum, often going unreported due to their inconspicuous nature, particularly in a North American context. Sunfish Lake (Ontario, Canada) represents a North American lake known for hosting an MCL. Here, we (1) reconstructed long-term cyanobacteria records to establish the changing risk of cyanobacteria blooms; and (2) investigated the spatial distribution of cyanobacteria and toxin-producing potential with real-time monitoring. The sediment record at Sunfish Lake revealed an unprecedented abundance of cyanobacteria in modern times (i.e., 1980s onwards), coinciding with increasingly warmer and wetter climatic conditions in the region. Real-time monitoring (2017) revealed an MCL and subsequent toxin analysis showed that peak toxin production (anabaenopeptin and microcystin) coincided with the MCL. Our findings provide (1) evidence for climate-driven shifts in cyanobacteria abundance and that even incremental alterations in climate signals over short temporal scales can push freshwater lakes towards cyanobacteria dominance; (2) importance of comprehensive monitoring to avoid "missing the middle" due to potential health risks at greater depths.

1615 Wednesday

The economic costs of algal blooms: Great Lakes evidence and research priorities

Robert B. Smith (rob@midsummer.ca), Midsummer Analytics

Over the past two decades there has been a re-emergence of harmful and nuisance algal blooms in Lake Erie and, to a lesser extent, Lake Ontario due mainly to increasing phosphorus loadings from non-point agricultural sources. Citizens on both sides of the Canada-U.S. border face economic costs due to these blooms. This presentation will draw upon two studies undertaken to evaluate these costs for the province of Ontario using standard economic approaches. These studies suggest that algal blooms impose considerable costs today (hundreds of millions of dollars annually) and that these costs will grow if blooms are left unchecked. The studies also consider the amount by which costs might fall if policy measures were taken to control phosphorus loadings, providing an economic basis for assessing the desirability of control. The presentation will also consider the broader questions around this kind of analysis, which is increasingly looked to by decision makers as an aid to policy development. These broader issues include the state of the socio-economic data available to undertake cost evaluations, the suitability of economic analysis as an aid to decision-making around freshwater quality and the main barriers to greater application of the approach.

1630 Wednesday

Presence of less monitored bioactive cyanotoxins (anabaenopeptins, saxitoxins, and BMAA isomers) in surface waters

Sébastien Sauvé s(ebastien.sauve@umontreal.ca), Sung Vo Duy, Gabriel Munoz, Tuc Quoc Dinh, Dana Florina Simon; Université de Montréal

The proliferation of harmful cyanobacterial algal blooms is of concern due to the associated release of toxins affecting ecosystems and human health. Among a range of cyanobacterial toxins, the hepatotoxic microcystins are the most documented and have been associated with numerous poisoning episodes. Other bioactive cyanopeptides, such as anabaenopeptins or cyanopeptolins, are rarely monitored in surface water and drinking water. Nonproteinogenic neurotoxic cyanotoxins such as saxitoxins (and analogues) and β-methylamino-l-alanine (BMAA) (and isomers) can also be produced, but there are many analytical challenges in their measurement. Here, we set out to document the occurrence of saxitoxin, neosaxitoxin, and their decarbamoyl analogues, BMAA and isomers (AEG, BAMA, DAB), and anabaenopeptins -A and -B in bloom-impacted surface waters. A large set of lake samples were collected from North America and Western Europe countries and submitted to three specific high-resolution mass spectrometry methods, with limits of detection in the range of 0.7-20 ng/L. The initiative provides much-needed data on the occurrence of infrequently monitored cyanotoxins beyond microcystins.

1645 Wednesday

Understanding HABs and the Florida Department of Health Role: Notification, Response, Resources and Research in the State of Florida

Monica Samit (Monica.Samit@flhealth.gov) and Rick Clark, Florida Department of Health

Harmful algal blooms (HABs) are a regular occurrence in Florida, and with predictions indicating that their frequency and intensity may increase over time, understanding and communicating the risks of these events are important to the protection of public health. Florida boasts a year-round climate of warmth and sunshine, which – when combined with the state's numerous marine and freshwater habitats and harmful algae species – can make for abundant and potentially toxic blooms. In 2016 and 2018, Florida experienced large-scale, concurrent episodes of cyanobacteria and *Karenia brevis* red tide. The environmental and economic consequences prompted the development of a state-wide, inter-agency collaborative network for routine waterbody surveillance, testing, and public notification of blooms. The Florida Department of Health has been tasked with disseminating the resulting information since 2019 in an effort to minimize human and animal exposures. This presentation will cover the Florida Department of Health's ongoing progress in streamlining its public notification protocol, developing trainings and resource materials for general and targeted audiences such as healthcare practitioners, and contributing to the overall body of HAB research through state funding for the discovery of long-term implications on human health.

Wednesday evening, 1730

Special Panel Discussion Hosted by the Interdisciplinary Freshwater Harmful Algal Bloom Workshop (IFHAB)

Experts in the hot seat: What is the greatest challenge facing us in the management and reduction of cHABs?

Moderator: Susan Watson

Panelists:

Wayne Carmichael, Wright State University, emeritus
Keith Loftin, United States Geological Survey
Arash Zamyady, University of Melbourne
Rene Sahba Shahmohamadloo, University of Guelph
Ingrid Chorus, Federal Environment Agency of Germany, retired

Thursday morning, 0830

Session: Interactions between CHABs and other organisms (phycosphere, trophic interactions, pro-euk interactions, viruses, fungi) (Darius Dziga, convener)

Plenary: Greg Dick (gdick@umich.edu), The University of Michigan

New dimensions of *Microcystis* diversity: the phycosphere

Interactions between bacteria and phytoplankton shape biological processes from cellular to ecosystem scales, including the development of harmful algal blooms. However, the nature of bacterial interactions with toxic cyanobacteria, and their influence on toxicity of cyanobacterial blooms, remains unclear. *Microcystis*, which forms blooms and threatens freshwater systems worldwide, provides an interesting case study for bacterial interactions. It is a single-celled organism that forms macroscopic colonies with a diverse bacterial community embedded in mucilage, thus making a well-defined microbiome and phycosphere. Analysis of individual colonies across a time series in Lake Erie shows that *Microcystis* microbiomes are distinct from whole water communities, lack 'core' taxa found across all colonies, and correlate with sampling date and *Microcystis* strain. Metagenomic and metatranscriptomic data suggest metabolic interactions in which bacteria use cyanobacterial products and exudates including organic acids, amino acids, peptides, and cobalamins. In return, they likely regenerate nitrogen for *Microcystis*. Finally, the heterotrophic bacteria dominate catalase expression in *Microcystis* blooms, suggesting that they protect *Microcystis* from oxidative stress, though susceptibility to H_2O_2 is strain-specific and independent of microcystin production. Overall, these results suggest that intimate mutualistic interactions between *Microcystis* and its microbiome likely modulate stressors, nutrient availability, and phenotypic diversity of *Microcystis*.

Interactions between CHABs and other organisms (phycosphere, trophic interactions, pro-euk interactions, viruses, fungi, etc)

0915 Thursday

Trait variation in resource competition traits among *Microcystis* strains in presence and absence of their microbiomes

Dylan Baker (bakerdyl@umich.edu), The University Of Michigan; Casey M. Godwin, The University Of Michigan; Gregory J. Dick, The University Of Michigan; Vincent J. Denef, The University Of Michigan

Freshwater harmful algal blooms are often dominated by Microcystis, a phylogenetically cohesive group of cyanobacteria marked by extensive genetic and physiological diversity. We have previously shown that this genetic diversity and the presence of a microbiome of heterotrophic bacteria influences competitive interactions with eukaryotic phytoplankton. In this study, we sought to explain these observations by characterizing Monod equation parameters for resource usage (maximum growth rate µmax, half saturation value for growth Ks) as a function of N (25-30,000 µg/L) and P (1-3,000 µg/L) levels for 4 different strains (NIES-843, PCC 9701, PCC 7806, PCC 7806 ΔmcyB) in presence and absence of a microbiome derived from Microcystis isolated from Lake Erie. Results indicated significant differences in maximum growth rates and half saturation values among isolates, and variable effects of the microbiome depending on the Microcystis strain, either increasing, decreasing, or not affecting growth rates and/or the half saturation values. As these parameters are commonly used in predictive models for harmful algal blooms, our data suggest that models can be improved by incorporating genotype dependencies of growth kinetic parameters. Additionally, our data highlights the importance of the microbiome in altering Microcystis-intrinsic traits and thus bloom dynamics.

0930 Thursday

Bacterioplankton communities affect toxigenic *Microcystis* bloom – from environmental to laboratory study

Joanna Mankiewicz-Boczek, (j.mankiewicz@erce.unesco.lodz.pl), European Regional Centre for Ecohydrology of the Polish Academy of Sciences; Arnoldo Font-Nájera, European Regional Centre for Ecohydrology of the Polish Academy of Sciences; Jesus Morón-López, European Regional Centre for Ecohydrology of the Polish Academy of Sciences; Liliana Serwecińska, European Regional Centre for Ecohydrology of the Polish Academy of Sciences; Tomasz Jurczas, UNESCO Chair on Ecohydrology and Applied Ecology, University of Lodz; Mikołaj Kokociński, Department of Hydrobiology, Adam Mickiewicz University; Ilona Gągała-Borowska, European Regional Centre for Ecohydrology of the Polish Academy of Sciences

Particle-attached bacteria are key biotic factors influencing the development of bloom-forming cyanobacteria dominated with toxigenic *Microcystis*. Therefore, knowledge about their ecological interactions (synergistic or antagonistic), niche occupancy, and co-occurrence patterns during different bloom developmental stages, and the looking for of autochthonous strains for cyanobacterial regulation, are essential. The aim was to describe bacterioplankton dynamics and their functional potential, including their influences on cyanobacteria and water quality, at the Sulejów Reservoir, Poland (Central Europe). Subsequently, bacteria with algicidal properties against *Microcystis* were isolated and characterized. Nitrogen-transforming proteobacterial taxa performing nitrogen fixation, nitrification, and denitrification, dominated at the beginning of summer. Then, in mid-summer, during mixed cyanobacterial bloom of *Snowella*, *Aphanizomenon*, and *Microcystis*, bacterial diversity significantly decreased, with higher domination of parasites/predators that fed on cyanobacteria. The post-summer was significantly dominated by toxigenic microcystin-producing *Microcystis*, which drove the highest negative impact on particle-attached bacteria. Despite this, *Microcystis* was associated with taxa feeding on their cells - Vampirovibrionales, and decaying exudates, including microcystin - Sphingobacteriales_env.OPS17, Sutterellaceae, and *Anhiella*. Parallelly, strains belonging to Bacillales, Exiguobacterales, Pseudomonadales, Enterobacteriales, Rhizobiales, and Xanthomonadales were isolated from the monitored reservoir, capable to lyse toxigenic *M. aeruginosa* cells in laboratory studies.

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0945 Thursday

New insights on organic nitrogen assimilation in *Microcystis* phycosphere and impacts on microcystin production

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Microcystis is one of the most globally abundant bloom-forming cyanobacteria, comprising species that produce nitrogen-rich hepatotoxic compounds known as microcystins (MCs). Although dissolved organic nitrogen can exceed 50% of the nitrogen pool in aquatic ecosystems, organic nitrogen compounds are often overlooked in terms of their impact on community dynamics and *Microcystis* bloom development. Further, cyanobacteria are constantly interacting with other microorganisms in their surrounding environment, but nitrogen cycling in the phycosphere is poorly understood. In the present study, we monitored the growth and MC production of several *Microcystis* strains on various organic nitrogen sources including amino acids and proteins, investigated the impacts of organic nitrogen on *Microcystis* microbiome via amplicon and metagenomic sequencing, and traced the nitrogen assimilation within the phycosphere at the single-cell level by measuring isotopic tracer incorporation via secondary ion mass spectrometer imaging (NanoSIMS). We demonstrate that 1) organic nitrogen species shape the microbiome community structure in the *Microcystis*phycosphere, and 2) competition for and/or transport of nitrogen between heterotrophic bacteria and cyanobacteria potentially play important roles for cyanobacterial succession especially under inorganic nitrogen scarcity.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344

1000 Thursday

Modification of the freshwater *Aphanizomenon flos-aquae* cyanobacterium proteome at cyanophage infection

Adam Antosiak (adam.antosiak@doctoral.uj.edu.pl), Jagiellonian University, Poland; Antonia Łobodzińska, Jagiellonian University; Kinga Chlebicka, Jagiellonian University; Bożena Skupień-Rabian, Jagiellonian University; Piotr Suder, AGH University of Science and Technology, Poland; Emilia Bonar, Jagiellonian University; Sigitas Šulčius, Nature Research Centre, Lithuania; Dariusz Dziga, Jagiellonian University

Marine cyanophages can maintain the metabolism of infected cyanobacterial cells and switch metabolic processes to multiply progeny viruses efficiently but there is no sufficient knowledge on whether freshwater cyanophages can control biochemical pathways of the host and if so, how this is done. Preliminary studies of *Aphanizomenon flos-aquae* phage infection suggested a modification of carbon metabolism by the impact on gene expression and the level of ATP and NADPH. In the current work the proteomes of *A. flos-aquae* cells infected and uninfected with vb-AphaS-CL131 virus were compared using 2D-DIGE and LS-MS^n to investigate proteomic alterations caused by the infection.

The level of proteins involved in carbon dioxide assimilation has been significantly reduced in the infected *A. flos-aquae* population in favour of the stimulation of the pentose-phosphate pathway. The expression of proteins related to glycolysis as well as the synthesis of nucleotides, amino acids, pigments and chaperones was also stimulated compared to the uninfected control cultures. Therefore, these alterations indicate infection-induced translational alterations of the host cells, most likely to enhance the production of new virions. This suggests that freshwater cyanophages may modify the host's metabolism.

1015 Thursday

Associations between microbiome composition and microcystin levels in the 2018 Lake Erie HABs Grab

Christopher S. Ward (chrward@bgsu.edu), Jennifer Harper, Kaitlin Plate, Margaret Rettig, Bowling Green State University; Justin Chaffin, The Ohio State University; Thomas Bridgeman, University of Toledo; Edward Verhamme, LimnoTech; Jorge Santodomingo, Environmental Protection Agency

Annual cyanobacterial harmful algal blooms (cHABs) threaten the well-being of western Lake Erie and the surrounding region. To resolve an entire bloom at unprecedently high spatial resolution, researchers coordinated a single-day survey of the Western Lake Erie cHAB ('HABs Grab'; August 9, 2018). Amplicon sequencing of the V4 region of the 16S rRNA gene was performed on 100 samples to determine how the microbial community varied spatially and in relation to environmental conditions within the bloom. While wide variation in community alpha diversity was observed in and out of the bloom, out-of-bloom communities had higher average evenness. Despite Microcystaceae being the dominant cyanobacterial family, Nostocaceae, Synechococcaceae and Pseudanabaenaceae were present in the bloom as well, while diatoms (mainly *Aulacoseira*) and cryptomonads (*Teleaulax*) were abundant both in and out of bloom. Common bloom-associated heterotrophic bacterial groups Flavobacteriia (Cryomorphaceae, Flavobacteriaceae), Planctomycetia (Planctomycetaceae), and Sphingobacteriia (Chitinophagaceae, Saprospiraceae) vary throughout the dataset, in association with nutrient concentrations, algal groups and toxin detections. Environmental and biotic drivers of toxin-producing cyanobacteria are explored to improve our understanding of how the interplay between algae and their microbiomes contributes to bloom composition and toxin production in Lake Erie.

Thursday morning, 1100

Session: Toxic Cyanobacteria in the Context of Climate Change (Petra Visser, convener)

1100

Plenary: Dedmer van de Waal (D.vandeWaal@nioo.knaw.nl), Netherlands Institute of Ecology

Impacts of multiple global change factors on toxic cyanobacteria: from elements to traits and toxicity

Global environmental change involves shifts in a wide range of environmental factors including elevated CO_2 levels, warming and changes in nutrient availabilities. This interplay of environmental stressors complicates our understanding on the impacts of global change for harmful cyanobacteria blooms. Many cellular processes rely on the availabilities of nutrients, while temperature is a major driver for metabolic rates, and CO_2 for photosynthesis and carbon acquisition. Together, these environmental factors determine a wide range of cellular traits. Inspired by trait-based approaches and ecological stoichiometry, this talk will explore the combined effects of CO_2, temperature, and nutrient availabilities on various traits, notably growth, nutrient acquisition, and toxin synthesis in harmful cyanobacteria. Results demonstrate that both CO_2 and temperature enhance nutrient acquisition in diazotrophic and non-diazotrophic cyanobacteria. Some responses are largely consistent across genotypes while other vary, suggesting for the potential for selection. Moreover, CO_2, temperature, and nutrient mediated changes in carbon and nutrient acquisition affected cellular elemental ratios, with largely predictable patterns in a range of cyanobacterial competitive traits, including colony formation, gas vesicle synthesis, as well as toxin quota and composition. Together, these findings contribute to a mechanistic understanding on the impacts of combined global change stressors on harmful cyanobacterial physiology and, ultimately, bloom toxicity.

Toxic Cyanobacteria in the Context of Climate Change

1145 Thursday

Unusual toxic cyanobacterial blooms in a tannin stained lake

Todd R. Miller (millertr@uwm.edu) University of Wisconsin - Milwaukee

Cyanobacterial harmful algal blooms (cyanoHABs) are increasingly detected in new environments not expected to have them. CyanoHABs are generally not expected to occur in acidic tannin - stained lakes. Tannins (polyphenols) have been shown to inhibit the growth of cyanoHAB species and used as a mitigation measure for cyanoHABs. Here we describe unusual toxic cyanoHABs caused by *Microcystis* and *Dolichospermum* in a tannin - stained acidic lake (pH 6.8) in Wisconsin. In collaboration with the Tug Lake Community Association a study was undertaken to describe the toxicity of these blooms. Microcystins (MC), anabaenopeptins (AP) and cyanopeptolins (CP) were measured by liquid chromatography tandem mass spectrometry in samples collected throughout the open water season. MCs occurred in all but two samples at an average concentration of 26.8 µg/L at the deep hole whereas nearshore scum samples contained a maximum of nearly 4000 µg/L. The MC congener MC-LA was most abundant followed by MC-LR > MC-LY > MC-LF > [Dha^7]MC-LR. AP's and CP's occurred less frequently with an average of 10.2 and 8.5 µg/L. High frequency in situ chlorophyll and phycocyanin measurements detected two major bloom events. These bloom events coincided with fall mixis and entrainment of soluble reactive phosphorus in the photic zone. Watershed land usage is primarily forested which may suggest these cyanoHABs are driven by internal loading. Further work is needed to understand the existence of these blooms in an acidic tannic environment.

1200 Thursday

Potential role of sulphate in cylindrospermopsin dynamics within urban freshwaters

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Cylindrospermopsin is a cyanotoxin that poses severe human health threats and is a major concern in freshwater systems around the world. It is mainly produced by *Raphidiopsis* sp., although pico-cyanobacteria have recently also been discovered to be causative. Urban freshwaters are especially vulnerable because due to human activity within its watershed that drives eutrophication and pollution. Understanding the urban drivers of cylindrospermopsin dynamics is crucial for managing these waters. We therefore sampled a tropical urban freshwater system at a bi-weekly interval over a year and at three-hour intervals over three days. Multivariate analyses from both sampling periods indicated poor correlation between *Raphidiopsis* sp. and cylindrospermopsin, but pico-cyanobacteria density correlated with cylindrospermopsin ($p < 0.05$; $r^2 = 0.16$) over the one-year sampling. During the three-day sampling, pico-cyanobacteria densities were very low and not significantly correlated with cylindropermopsin. Across both sampling periods, nitrogen also provided a poor explanatory factor for the cylindrospermopsin patterns. However, sulphate was significant. Within eutrophic urban freshwaters, where macronutrients tend to be in excess, this study therefore suggests a need to focus instead on micronutrients such as sulphate which may be closely associated with pollution regimes. Such information is useful in identifying important targets for freshwater management efforts.

1215 Thursday

Bloom capacity of various *Microcystis* strains, are they all the same?

Muriel Gugger (muriel.gugger@pasteur.fr), Institut Pasteur; Boullié Anne, Institut Pasteur; Thierry Laurent, Institut Pasteur; Marwa Melik, Lycée Galilée, Genevilliers; Emilie Cantin, Université de Brest; Caroline Haddad, Université de Paris; Stella Perez, Muséum national d'Histoire naturelle; Elisa Lemaitre, Université de Paris

Microcystis is a well-known toxic cyanobacterium that blooms in freshwater ecosystems around the word. At the PCC collection (France), we maintain about 30 pure *Microcystis* strains originating from various blooms on all continents isolated from the 1970s to this century and kept alive since by successive transfer at 22-25°C.

Using a high-density culture system (CellDEG, Germany), we tested these *Microcystis* to form rapid bloom-like cell development within a week. Light, CO2 and nutrients were provided to 10-mL stirred cultures to allow the cell development over 80 h at 30°C.

We were surprised to observe so many different behaviors within *Microcystis* strains that resemble each other in low-density culture (DO~2). Depending on the light and shade, they all formed more abundant mucilage. Some challenged strains reached DO >10 and presented high metabolism while other never bloomed in such conditions. For certain, the blooming capacity was in function of age inoculum or ability to form colonial aggregation. We concluded that *Microcystis* is far from fully understood, and that it is best to characterize the growth of the *Microcystis* model in your laboratory before undertaking experiments based on what you think of its behavior.

1230 Thursday

A Greek *Raphidiopsis raciborskii* strain and microcystins: A toxic relationship

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The cyanobacterium *Raphidiopsis raciborskii* is extensively studied for its toxicity and invasive behavior, which is presumably enhanced by global warming. The widening of its geographic distribution and the isolation of strains showing high optimum growth temperature underline its ecological heterogeneity, suggesting the existence of different ecotypes. In this study, we investigate the ecotoxicology of *Raphidiopsis raciborskii* TAU-MAC 1414 strain, isolated from Lake Karla, Greece. The effect of different growth conditions (temperature, light intensity, phosphorus concentration, co-culture with toxic and non-toxic *Microcystis* spp. extracts) on microcystin production by *R. raciborskii* was examined. MC-LR and MC-HilR were detected with LC-MS/MS, mainly during the cultivation of *R. raciborskii* with toxic or non-toxic *Microcystis* spp. strains' extracts. Further, the subcellular phytotoxic effects of *R. raciborskii* on *Oryza sativa* (rice) are discussed. Our research demonstrated unambiguously for the first time that *R. raciborskii* is able to produce microcystins under certain conditions, shedding new light in the ecotoxicology of the species, whereas the triggering of microcystin production remains to be further investigated.

Friday morning, 0830

Session: Risk, prevention, management, and mitigation of CHABs: applied applications from establishing risk to remediation (Dail Laughinghouse, convener)

Plenary: Faith Kibuye, Carollo Engineers, Boise, ID, USA; Southern Nevada Water Authority

Performance of source water control strategies for cyanobacterial blooms
Cyanobacterial blooms produce nuisance metabolites (e.g., cyanotoxins and T&O compounds) thereby posing water quality management issues for aquatic sources used for potable water production, aquaculture, and recreation. A variety of in-lake/reservoir control measures are implemented to reduce the abundance of nuisance cyanobacteria biomass or decrease the amount of available phosphorus (P). This presentation will provide a review of the performance of chemical, physical, and biological control strategies implemented for in-lake/reservoir management of cyanobacterial blooms. Findings from a survey of drinking water utilities is further analyzed to elucidate if current control strategies for cyanobacterial blooms meet utility needs. Research gaps in successfully implementing control programs in drinking water sources are further highlighted.

Risk, prevention, management, and mitigation of CHABs: applied applications from establishing risk to remediation

0915 Friday

Field-scale evaluation of nanobubble ozone technology for cyanobacterial harmful algal bloom control

Heather Raymond (raymond.54@osu.edu) The Ohio State University, David E. Berthold, University of Florida; Eugene Braig, The Ohio State University; Justin Chaffin, The Ohio State University; Billy Fagan, The Ohio State University; Rachel Gabor, The Ohio State University; Jing Hu, University of Florida; Dail Laughinghouse, University of Florida; Forrest W. Lefler, University of Florida; Keara Stanislawczyk, The Ohio State University; Holly Stanley, The Ohio State University; Autumn Taylor, The Ohio State University; Linda Weavers, The Ohio State University; Matthew Romanko, The Ohio State University

Effective Cyanobacterial Harmful Algal Bloom (CHAB) control strategies are needed to address recreational and drinking water impacts. Nanobubble ozone technology (NBOT) is an emerging treatment, but field-scale studies are limited. An 18-week NBOT treatment trial was conducted on a 42-acre CHAB impacted Ohio recreational lake in 2021. The trial was split into low ozone dose (60 g/h), higher dose (180 g/h), and pre- and post-treatment periods. Two multi-parameter sondes recorded continuous data and water samples and profiles were collected weekly at six sites and analyzed for nutrients, cyanotoxins, phytoplankton diversity and abundance, and additional parameters. Multi-spectral imagery was captured on seven drone flights and sediment cores were collected. Cyanobacteria chlorophyll concentrations sharply declined and remained low for four weeks after the ozone dose was increased, but concentrations increased in response to a 4.5" rain event. Microcystins and saxitoxins peaked at 2.5 and 4.0 µg/L during the low dose treatment period, then declined after increased ozone dose. Recreational CHAB advisories were not posted, in contrast to past ozone concentrations increased in held samples demonstrating potential for nanobubbles to release ozone over time. Additional NBOT trials are planned for 2022.

0930 Friday

Exploring the potential of metallic peroxide granules for the in-situ mitigation of cyano-HABs

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Several chemical treatments have been tested over the years for the mitigation of blue-green algae including harmful pesticides and algaecides. The latter ones are prohibited to be used in many US states and the European Union, hence alternative treatments that are more environmentally friendly are currently explored. Hydrogen peroxide (HP) is characterized as an environmentally friendly approach as it does not produce harmful residues and an increased sensitivity of cyanobacteria to HP treatment compared with green algae was reported. However, dense blooms require high doses of HP that can disrupt the remaining ecosystem. To address this problem, slow releasing HP metallic granules were used as an alternative to liquid HP solution. Specifically, calcium peroxide granules were applied in surface water from Kouris Reservoir, Cyprus. This study examined the HP releasing properties with varying pH values, their mitigation efficiency on *Microcystis sp.* bloom in comparison with HP liquid application and their toxicity on *Echinogammarus veneris sp.* in a range of concentrations. Results showed that treatment with granules was efficient even at the low concentration of 0,5 g/L while the equivalent liquid HP concentration was not. Toxicity studies on zooplankton indicated that the low concentrations of the granules applied were not toxic.

0945 Friday

A paradigm-shift in water treatment: In-reservoir UV-LED-driven TiO2 photocatalysis for the removal of cyanobacteria – a mesocosm study

Carlos J. Pestana (c.pestana@rgu.ac.uk), Robert Gordon University; Jose Capelo-Neto, Federal University of Ceara; Jianing Hui, University of St Andrews; Peter KJ Robertson, Queen's University Belfast Company for the Management of Hydrological Resources Ceara (COGERH); Samylla Oliveira, Federal University of Ceara; Ricardo Rogers, Federal University of Rio de Janeiro; Allan Santos, Federal University of Rio de Janeiro; Sandra MFO Azevedo, Federal University of Rio de Janeiro; Christine Edwards, Robert Gordon University; John TS Irvine, University of St Andrews; Linda A. Lawton, Robert Gordon University

Potentially harmful cyanobacteria challenge potable water treatment with high biomass events and dissolved toxic and nuisance metabolites globally. Retrofitting existing water treatment infrastructure is often impractical, if not impossible, and often prohibitively expensive. In a paradigm-shifting move we propose in-reservoir pre-treatment of cyanobacteria-contaminated raw waters to ease the burden on existing water treatment infrastructure.

In an iterative design approach over three years, treatment modules have been designed, refined, and optimised in bench and pilot-scale studies for in-reservoir deployment. TiO_2-coated beads made from recycled glass are employed in conjunction with UV-light emitting diodes (LEDs) to create highly reactive hydroxyl radicals that preferably remove cyanobacteria and subsequently released cyanotoxins from raw water. In a mesocosm study in a drinking water reservoir in Brazil water quality parameters were markedly improved within 72h of deployment and cyanobacterial presence was decreased by over 90% without affecting other phytoplankton communities. The treatment system is virtually plastic-free, low cost, utilises recycled materials and could ultimately be powered by renewable energies, thus providing a true green treatment option.

We have conclusively demonstrated that a paradigm-shift towards in-reservoir treatment is not only possible but feasible and can provide a valuable addition to conventional water treatment methods.

1000 Friday

Development of a novel & low cost domestic filter unit to treat drinking water contaminants

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The provision of adequate volumes of safe, clean drinking water to the world's growing population is a continual and increasing challenge for water authorities. In Sri Lankan context, cyanotoxin in water has been hypothesized as one of the root causes for Chronic Kidney Disease of unknown etiology (CKDu). Unpleasant Taste & Odour (T&O), pesticides and antibiotic contamination of water are other burning concerns. The aim of designing this filter was to provide a solution to these four intense and urgent issues. Water was treated by incorporating three different filter units namely membrane filter, natural rutile sand combined with UV light filter and finally activated carbon filter. Natural Rutile sand found in Srilankan coastal belt was used as a photo catalyst material which contains more than 90% of Titanium dioxide. Laboratory produced wood based activated carbon was used to increase the effectiveness and efficiency of the water treatment, by adsorbing by products of pollutants. The model filter showed complete removal of cyanotoxin, T&O forming Geosmin and 2- MIB, antibiotics and Carbofuran pesticides. Hence, in the commercial production cycle, this product will help to provide safe, clean water to population who consumed contaminated water from dug wells.

1045 Friday

Using native Lake Erie bacteria and their enzymes for degradation/removal of microcystin toxins from water

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Microcystin-LR (MC-LR) is one of the most toxic and common cyanotoxins released by freshwater harmful algal blooms (HABs). While conventional water treatment practices can reduce/remove MC-LR from drinking water, bioremediation (i.e., biofilters) has additional advantages of being cost-effective and environmentally friendly. We previously isolated Lake Erie bacteria that degraded MC-LR into non-toxic fragments but the MC-LR degradation enzymes remain unknown. As such, the goal of this study was to use genomic sequencing and transcriptomics to identify MC-LR degrading genes/enzymes for water treatment. First, whole genome sequencing was performed on five MC-LR degrading bacteria, designated as 'Group N,' to predict potential MC-LR degrading genes/enzymes. Next, Group N bacteria were grown in sterile-filtered lake water, with or without MC-LR, and samples were collected every 3-4 days for either transcriptomic (RNA sequencing) or mass spectrometry analyses (MC-LR quantitation and fragment analysis). Bioinformatic analysis of genome sequences identified potential amidohydrolase, dipeptidase, leucyl aminopeptidase, peptidase, serine hydrolase, metallopeptidase, and serine hydrolase enzymes that could cleave MC-LR. Mass spectrometry analysis confirmed that Group N bacteria degraded cyclic MC-LR into non-toxic linear and tetrapeptide fragments. Finally, transcriptomic analysis is currently underway to identify genes upregulated in MC-LR containing cultures (compared to cultures without MC-LR). Putative MC-LR degrading enzymes will be recombinantly expressed/purified and MC-LR degradation confirmed by in vitro assays. These studies have the potential to reveal new methods to efficiently, safely, and cost-effectively remove MC-LR from water sources.

1100 Friday

Application of CyanoGate, a Cyanobacterial Synthetic Biology Molecular Cloning Suite, for Exploring Optimal Heterologous Microcystinase (MlrA) Production for Freshwater Bioremediation from Cyanotoxins

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Microcystinase (MlrA) is currently the most specific catalyst and the most efficient enzyme of all known microcystins (MCs) detoxification pathways. Direct MlrA application has been demonstrated to degrade MCs under modeled environmental conditions, potentially informing sustainable methodologies for in situ MCs remediation. Heterologous MlrA expression in cyanobacteria offers a unique perspective, linking harmful cyanobacterial MCs remediation with the state of the art in cyanobacterial biotechnology. Here, we report application of the most recent cyanobacterial synthetic biology molecular cloning suite, CyanoGate, for peptide engineering of the MlrA N-terminus. Utilizing CyanoGate allowed for generation of broad-host range plasmid vectors for application of the following N-terminal sequences to MlrA in various combinations: (1) 23 amino acid (AA) secretion peptide from *Synechocystis sp.* PCC 6803 PilA (*sll1694*), (2) 38 AA secretion peptide from *Thermobifida fusca* lytic polysaccharide monooxygenase (*Tf*AA10A), (3) full length *Tf*AA10A, and (4) USTB-05 MlrA native 26 AA signal peptide. Resultant plasmids were transformed into *Synechocystis sp.* PCC 6803 and the industrially-relevant *Synechococcus elongatus* UTEX 2973. Successful transformants were verified via PCR assay, followed by analysis of both cytosolic and extracellular MlrA activity. Application of (1) and (2) (and combination) demonstrated cytosolic MlrA activity, offering novel, synthetic MlrA towards industrial applications.

1115 Friday

Ecology of cyanotoxin-producer cyanobacterial communities in Spanish National Parks

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Keywords: freshwater ecosystems, National Parks, anatoxin-a, microcystins, Management

Waterbodies in National Parks, despite their typical pristine condition, are also subjected to the presence of potentially toxic cyanobacteria. The main objective of this work is to characterize ecologically, taxonomically and toxicologically the cyanobacterial communities from rivers and reservoirs within these protected ecosystems to evaluate the potential risk to visitors, workers and protected fauna. Results obtained in reservoirs, located within a rivers network with different physicochemical characteristics, showed extensive and persistent blooms formed by different planktonic communities dominated by cyanotoxin-producer genera such as *Aphanizomenom*, *Microcystis* and/or *Planktothrix*. The results in rivers indicate an alarming increase in the persistence and abundance of benthic mats, dominated by *Phormidium* (mainly *Ph. Autumnale* also called *Microcoleus autumnalis*), showing a high toxicity risk (due to their anatoxin-a content) even at places with a low coverage (less than 10 %). This work describes these communities and discusses the presence of some cyanotoxins (microcystins, anatoxins, cylindrospermopsins and saxitoxins) measured by different methods (genetic, quantitative, etc) and their relationship with environmental parameters. The massive presence of cyanotoxin-producers cyanobacteria in Spanish National Parks is demonstrated and are the first steps for the creation of protocols to manage risks potentially usable at other protected areas.

This work has been supported by two Grants: MITERD-OAPN- 2593-2020 and CYTED 2019-P919PTE0047

1130 Friday

Suppression of cyanobacterial blooms using hydrogen peroxide: effects on phytoplankton and bacteria

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Applying low concentrations of hydrogen peroxide (H_2O_2) to lakes is an emerging method to mitigate harmful cyanobacterial blooms. While cyanobacteria are very sensitive to H_2O_2, little is known about the impacts of these H_2O_2 treatments on other members of the microbial community. In this study, we investigated changes in microbial community composition during two lake treatments with low H_2O_2 concentrations (target: 2.5 mg L^{-1}) and in two series of controlled lake incubations. The results show that the H_2O_2 treatments effectively suppressed the dominant cyanobacteria *Aphanizomenon klebahnii*, *Dolichospermum* sp. and, to a lesser extent, *Planktothrix agardhii*. Microbial community analysis revealed that several Proteobacteria (e.g., Alteromonadales, Pseudomonadales, Rhodobacterales) profited from the treatments, whereas some bacterial taxa declined (e.g., Verrucomicrobia). In particular, the taxa known to be resistant to oxidative stress (e.g., *Rheinheimera*) strongly increased in relative abundance during the first 24h after H_2O_2 addition, but subsequently declined again. Alpha and beta diversity showed a temporary decline but recovered within a few days, demonstrating resilience of the microbial community. We conclude that the use of low concentrations of H_2O_2 to suppress cyanobacterial blooms provides a short-term pulse disturbance, but is not detrimental to lake microbial communities and their ecosystem functioning.

1145 Friday

Cyanotoxin Degrading Lake Bacteria Significantly Alleviate Microcystin-LR Induced Hepatotoxicity in Both In Vitro and In Vivo Models

Apurva Lad (Apurva.Lad@rockets.utoledo.edu) The University of Toledo; Jyotshana Gautam, Andrew L. Kleinhenz, Sanduni H. Premathilaka, Prabhatchandra Dube, Shungang Zhang, Travis Stevens, Steuart Besly, Dragan Isailovic, Jason F. Huntley, David J. Kennedy and Steven T. Haller, University of Toledo

Our recent reports have shown that exposure to microcystin-LR (MC-LR) exacerbates the development of pre-existing liver and inflammatory bowel disease as well as alters gut microbiota that may significantly impact development of hepatotoxicity. We have isolated naturally occurring novel MC-LR degrading bacteria from Lake Erie, OH and hypothesized that they may alleviate MC-LR toxicity. qPCR analysis for markers of hepatotoxicity and inflammation in both *in vivo* and *in vitro* (using human Hep3B hepatocytes) settings showed significant downregulation in their expression in presence of MC degrading bacteria compared to the untreated groups. LC-MS analysis of the 24-hour urine samples in an *in vivo* setting with age matched Balb/c female mice that were pre-treated with the bacteria prior to 500 µg/kg MC-LR exposure for 24 hrs revealed significant reduction in urine MC-LR levels of mice pre-treated with MC-LR degrading bacteria as compared to the control group. Analysis of genes related to MC-LR induced apoptosis, DNA damage, ER stress, and fatty acid metabolism were also significantly downregulated in mice treated with MC degrading bacteria compared to control mice exposed to the toxin alone. These results suggest a potential novel therapeutic approach that can be developed for MC-LR induced toxicity.

1200 Friday

Toledo triggered new WHO guidance for cyanotoxin risk assessment

Ingrid M. Chorus (ingrid.chorus@gmail.com) Consultant; retired since 2019 from the German Environment Agency

In 2014, the Toledo "do not drink advisory" spotlighted the need for short-term cyanotoxin guideline values. These are particularly relevant for cyanotoxins because concentrations may fluctuate widely as blooms wax and wane, with concentrations above the values for safe lifetime daily consumption of 2 L of drinking water often being short-lived events. Allowing slightly higher concentrations for up to 2 weeks enables focusing investments on remediation (rather than on supplying bottled water). In 2016 a second independent chronic exposure study with cylindrospermopsin (conducted at US EPA) finally provided the data that the World Health Organisation needed for deriving a CYN guideline value. Thus, WHO now provides a far more comprehensive set of guideline values for short-term and lifetime exposure through drinking water as well as for recreational exposure, including all 4 major groups of cyanotoxins (MCs, CYNs, STXs, ATXs). The presentation will explain their derivation in the context of other hazardous substances in water to which people may be exposed because among these, cyanotoxins are probably the most widely occurring. It will also show how these values serve as guidance for short-term responses to blooms in the context of Alert Levels Frameworks for drinking water and for recreation.

Posters

Odd numbered posters – Monday session

Even numbered posters – Tuesday session

Posters to remain in place all week

Detection, Identification and Diversity of CHABs

Poster 1

Biosynthetic gene clusters identified in cultured and *in situ* strains of *Microcystis* reveal high biosynthetic potential for diverse compound production

Lauren Hart (lnhart@umich.edu), Colleen Yancy, Osama Mohamed, Ashootosh Tripathi, Davin Sherman and Greg Dick, University of Michigan

Microcystis aeruginosa, a bloom-forming cyanobacterium, dominates cyanobacteria harmful algal blooms (cyanoHABs) in freshwater around the world, posing threats to water quality, wildlife, and stability of freshwater ecosystems. *M. aeruginosa* is known to produce the nitrogen-rich hepatotoxin, microcystin, as well as a variety of other toxic secondary metabolites. Here, we use genomic sequencing of our western Lake Erie culture collection and metagenomic sequencing of whole microbial communities from the 2014 western Lake Erie bloom to assess biosynthetic potential through biosynthetic gene cluster (BGC) evaluation and compound production in cultured *M. aeruginosa* strains.

Poster 2

Developing a real time algae detection platform using deep learning

Jamal Ansary (jansary@rockets.utoledo.edu), The University of Toledo; Brian Trease, The University of Toledo Mechanical, Industrial and Manufacturing Engineering, Toledo, Ohio, USA

Early detection of algal buildup by continuous monitoring of water bodies is a key component in combating this environmental hazard. Recent development in deep learning and object detection has open a new chapter in developing environmental monitoring methods. The field of machine learning have been used in numerous applications and in recent years and deep learning has become the leader in this domain. One of the primary differences between this model and typical machine learning model is the use of several layers to develop computational models. Traditional machine learning methods, on the other hand, necessitate manually designing features, which places a significant hardship on users. Deep learning can be defined as a machine learning representation learning algorithm based on large-scale data. This study, therefore, aims to explore implementation of object detection algorithms using deep learning for detection of algae. Developed models can be implemented on unmanned air vehicles (UAVs) and unmanned surface vehicles (USVs) and will perform the monitoring task. This model is cheap, easy to implement, and the results of the field experiment indicates that this method is reliable, and easy to implement.

Poster 3

The impact of multispecies biofilm on the bioaugmentation of a cyanotoxin-degrader in drinking water biological filters for MC-LR biodegradation.

Parul Baranwal (pbaranw@rockets.utoledo.edu) and Youngwoo Seo
(youngwoo.seo@utoledo.edu), The University of Toledo

Recently, biological filtration systems (BFS) have received much attention for safe drinking water production, as they can potentially treat many emerging contaminants like harmful cyanotoxins including microcystin-LR (MC-LR). In order to enhance the performance of BFS for emerging contaminant removal, bioaugmentation (injection of contaminant-degrading microorganisms) has been evaluated. However, it is not well-understood how the presence of multispecies bacterial biofilms (MSB) might affect the bioaugmentation and subsequent biodegradation of MC-LR in BFS. Accordingly, the main goal of this study is to evaluate the effect of endogenous MSB in BFS on bioaugmentation and MC-LR biodegradation. Lab-scale column reactors were constructed and initially operated with MSB from biofilters at a local water treatment plant to simulate BFS conditions. Then, a MC-LR degrader was bioaugmented to these reactors. The performance of BFS (natural organic matter and MC-LR removals) was monitored. Biofilm formation and MC-LR degradation activity of the bioaugmented degrader were also monitored using Adenosine triphosphate analyses and quantitative polymerase chain reaction (qPCR) for bacterial gene expression associated with MC-LR degradation. Our results indicated that there was enhanced MC-LR removal by the MC-LR degrader in presence of MSB, which might be due to attachment of bioaugmented MC-LR degrader to preformed multispecies biofilms.

Keywords: Bioaugmentation, multispecies bacterial community, biological sand filters, Adenosine triphosphate analyses

Poster 4

Understanding the impact of natural organic matter on microcystin-LR biodegradation and their characterization using fluorescence PARAFAC analysis

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(youngwoo.seo@utoledo.edu), The University of Toledo

The successful application of bioaugmentation (injection of contaminant-degrading microorganisms) can remove harmful cyanotoxins like microcystin-LR (MC-LR) from drinking water sources. However, in natural and engineered water systems, the natural organic matter (NOM) is present in varying concentrations and might affect the bacterial biodegradation of MC-LR. In addition, changes in biomolecular characteristics of NOM during bioaugmentation can affect the treated water quality in drinking water treatment plants (DWTPs). Therefore, for application of bioaugmentation for MC-LR removal in DWTPs, it is important to investigate the impact of NOM on MC-LR biodegradation and corresponding NOM composition changes. In this study, the impact of NOM [algal organic matter (AOM) and humic substances (HS)] on MC-LR biodegradation was evaluated monitoring MC-LR biodegradation kinetics. The changes in NOM composition during MC-LR biodegradation was also characterized using a five component Parallel factor (PARAFAC) model built by 336 Excitation-emission matrix (EEMs) spectra collected at different sampling time. Our results show decrease in MC-LR biodegradation rate by 2.85 and 3.82-fold in the presence of AOM and HS, respectively. EEM-PARAFAC analyses showed a relatively greater production of terrestrial humic-like components (57%) in comparison to control conditions (27%), likely due to the microbial metabolism.

Keywords: Bioaugmentation, Cyanotoxins, Microcystin-LR, EEM-PARAFAC analyses

Poster 5

Rapid, Portable, Multiplexed Detection of Harmful Algal Toxins in Lake Erie

Katelyn Barker (katbark@bgsu.edu), Bowling Green State University; Seth Buchholz, Ohio EPA; Sarah Bickman, Cassandra Petrou, Brooks Macdonald, Gregory Lewis, LightDeck Technologies, Boulder, CO; Justin Chaffin, The Ohio State University; Max Herzog, Cleveland Water Alliance; George S. Bullerjahn, Bowling Green State University

Cyanobacterial blooms are an issue in waters around the globe and have enormous environmental, economic, and human health impacts. These blooms potentially produce various hepatotoxins (microcystin, cylindrospermopsin) and neurotoxins (saxitoxin, anatoxin). The standard method for testing affected waters for toxins is by Enzyme Linked Immunosorbent Assay (ELISA). This method can be time consuming, expensive, and require training and equipment to perform. Recently, a rapid, portable multiplexed assay has been developed by LightDeck Diagnostics to test for toxins quickly and easily in the field. We conducted a study to compare cyanotoxin detection between LightDeck and ELISA methods, utilizing a network of researchers and community science groups to sample widely throughout Lake Erie, with sampling locations covering the southern half of the lake. Two varying versions of the LightDeck assay were evaluated. Overall, we found the current formulation had fewer false positives and was more consistent when compared to ELISA. We continue to evaluate performance of LightDeck technology in detecting and quantifying cyanotoxin levels, including the effects of lysis method. While the ELISA assay remains the gold standard, the advantages of speed, expense and limited user expertise suggest that the LightDeck assay could play an increasing role in detection of toxic blooms.

Poster 6

An Integrated and High-Speed Approach to Monitoring Cyanobacteria in Lakes, Reservoirs and Finished Drinking Water

Polly Barrowman (polly.barrowman@fluidimaging.com) Yokogawa Fluid Imaging Technologies; Hunter Adams, City of Wichita Falls - Cypress Environmental Laboratory

The way in which utilities and agencies monitor and control harmful algal blooms is changing. New technology and multifaceted approaches are becoming a routine part of HAB monitoring programs Traditional microscopy methods are slow and not always ideal when trying to perform rapid sample analysis for real time lake or reservoir treatment decisions. The FlowCam® Cyano is a high throughput, semi-automated alternative to manual microscopy and has been successfully implemented into many HAB monitoring programs by utilities across the United States and worldwide. It provides imaging technology that is beneficial to Water Managers, Health Officials and Researchers who can use it as part of a comprehensive monitoring program to study community composition and make rapid real time decisions on reservoir treatment options. Here we will present a brief introduction to the FlowCam Cyano technology, detailing how this rapid identification and enumeration method can help utilities see and react to algal blooms in their nascent stages. Highlighting the City of Wichita in Texas, we will show how the FlowCam is integrated into this city's monitoring strategy in conjunction with other rapid techniques such as qPCR and how this approach informs their treatment decisions.

Poster 7

Monitoring Cyanotoxin Production in the Western Basin of Lake Erie Using SPATT

Amber A. Beecher (Amber.beecher@rockets.utoledo.edu); Thomas Bridgeman, University of Toledo; Gregory Doucette, NOAA NCCOS; Judy Westrick, Wayne State University

The array of toxins and toxin concentrations produced by cyanobacteria in lakes may change on time scales that are too short to be adequately characterized by typical weekly water samples. We explored the use of Solid Phase Adsorption Toxin Tracking (SPATT) to provide a continuous profile of cyanobacterial toxin production in western Lake Erie. SPATT devices contain porous resin that passively adsorbs toxins over days or weeks potentially useful for detecting short-lived spikes in toxin levels, and unusual toxins and congeners. SPATT bags were deployed at two locations for durations of one and two weeks June-October, 2020. Grab samples for microcystin (MC) concentrations via ELISA and samples for qPCR analysis were collected weekly. SPATT bags were analyzed for 12 MC congeners, anatoxin-a (<0.11 ppb), and cylindrospermopsin.

Poster 8

Development of multiplexed sandwich hybridization assay for in situ detection of freshwater harmful bloom-forming cyanobacterial genera

Katelyn Brown (browkat@bgsu.edu) Bowling Green State University; Emily Cooley, Bennett Aerospace, Inc.; Anna Antrim, Oak Ridge Institute for Science and Education; Jessica Ji, Bennett Aerospace; Tyler Tunnell, Oak Ridge Institute for Science and Education; Seung Ho Chung, Bennett Aerospace, Inc; Ping Gong (Ping.Gong@usace.army.mil), US Army Engineer Research and Development Center; George S. Bullerjahn, Bowling Green State University

Freshwater cyanobacteria harmful algal blooms (CHABs) are being observed more frequently due to eutrophication, more robust monitoring programs and a warmer climate at the global level. Oftentimes, CHABs result in the production of toxins such as microcystins, anatoxins, cylindrospermopsins, and saxitoxins, that are harmful or fatal to humans and other animals. Quick and informed decisions are needed for better management of CHABs. To reduce the time between a suspected bloom and subsequent management decisions, a field-deployable and multiplexed sandwich hybridization assay (SHA) was developed to detect the prominent bloom-forming and potentially toxin-producing cyanobacterial genera. The initial targeted genera were *Microcystis*, *Anabaena/Dolichospermum*, *Aphanizomenon*, *Planktothrix*, *Cylindrospermopsis*, *Lyngbya/Microseira*, and *Phormidium/Microcoleus*. The key components of a typical SHA included a target-specific capture probe, a universal signal probe and a location-specific "zip-code" tag for multiplexing. We designed oligonucleotide capture probes based on the phylogenetic analysis of a curated database of more than 1400 sequences of cyanobacterial 16S rRNA genes to target their hypervariable regions. Initial testing of capture probe specificity utilized fluorescent in-situ hybridization (FISH) and probes with the best results were further tested in combination with a signal probe using streptavidin-coated plates. All testing results will be presented, and lessons learned will be discussed.

Poster 9

Using continuous monitors to expand awareness of algae in ecosystems

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Water quality conditions in many regions are evolving over time and so, too, are the technologies that are used to measure these changes. Historically, algae concentration data has been collected manually from a site and processed in the laboratory. Today, continuous water quality monitoring allows for an expanded temporal view of that same site. Multiparameter platforms have sensors available for the measurement of algae and some of the parameters of interest that drive or are affected by blooms. Algae-specific sensor technologies include fluorescence-based sensors designed to monitor chlorophyll-a, phycocyanin, and phycoerythrin. Continuous relative fluorescence measurements from these sensors can be correlated to concentrations based on the relationship between continuous and discrete data. By using these sensor technologies and telemetering data to a cloud-based data hosting platform, real-time data-driven decisions have been made intelligently and in a cost-effective manner across entire lake systems.

Poster 10

A pan-Canadian comparison of cyanobacteria bloom management policies, programs, and practices

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Across the globe, reports of cyanobacteria blooms are on the rise. The increasing occurrence of cyanobacteria blooms and cyanotoxins is attributed to phosphorous (P) loading, climate change, among a mix of other factors. While eutrophic lakes have a higher risk of blooms, oligotrophic and mesotrophic lakes are also experiencing blooms. This means governments' need to develop a robust cyanobacteria management strategy (prevent, control, and mitigate) to protect public health. In Canada, water management is a shared responsibility among the federal, provincial, and local governments; however, cyanobacteria management is mainly a provincial and local government responsibility. This research compares and contrasts five provincial cyanobacteria management strategies from Alberta, Manitoba, Nova Scotia, Ontario, and Saskatchewan. Using a policy analysis framework, the methods of data collection include a review of grey and academic literature, legislation/regulations, and interviews with actors involved in cyanobacteria bloom management in each province. Also, three case studies – Lake Erie, Ontario; Lake Winnipeg, Manitoba; and Pigeon Lake, Alberta – were selected to analyze the policies and programs in practice. A robust cyanobacteria management strategy involves prevention, control, and mitigation to avoid public health risks. All jurisdictions in Canada have initiatives to manage cyanobacteria blooms. Nutrient management continues to be the cornerstone of bloom prevention by controlling point and diffuse sources of P runoff control. Nutrient management mostly relies on voluntary participation, so reductions in nutrient loading are heavily dependent on financial incentives, and education and outreach programs; however, there is little to no understanding or tracking of implementation. Also, P control will not reduce the risk of blooms in low P lakes. Monitoring programs and targets should include dissolved oxygen. Public health risks associated with cyanotoxins are mitigated through public reporting or monitoring drinking water sources and recreational waters. The monitoring and reporting programs vary by province. For instance, certain drinking water sources and recreational waterbodies are routinely monitored, whereas in other provinces sampling is driven by public reporting.

Poster 11

Optimization of a Microcystin Extraction Protocol for Blue-Green Algae-Based Dietary Supplements

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Blue-green algae as dietary supplements are used for their reported health benefits. Outdoor harvested algae can be contaminated by cyanobacteria producing harmful secondary metabolites, including microcystins (MCs). MCs are a group of hepatotoxic cyclooligopeptides that inhibit the function of protein phosphatases in eukaryotes. Development of a routine monitoring protocol is necessary due to the concern for human health. MC quantification in dietary supplement are subject to interferences from compounds present in the supplement. Herein, algal tablets and capsules were used to evaluate cleanup and analytical protocols to improve the accuracy of MC quantification. Two solid-phase extraction (SPE) cartridges (i.e., Bond-Elute and Oasis HLB) and two types of dispersive SPE media were tested for cleanup. Eight MCs (i.e., MC-LA, -LF, -LR, -LW, -LY, -RR, -YR, and nodularin-R) were spiked into pre- and post-cleanup processes. Samples were loaded onto two C18-based liquid chromatography columns for comparison on column specificity. MC quantification were determined using a QTrap mass spectrometer. The eight MCs can be grouped by the retention time into R-group (i.e., MC-LR, MC-RR, MC-YR and nodularin-R) and L-group (MC-LA, -LF, -LW, and -LY). Overall, L-group MCs were more susceptible to interfering compounds than R-group MCs even with these cleanup processes.

Poster 12

Developing an UAV method of monitoring of cyanobacterial blooms in freshwater ecosystems

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Cyanobacterial blooms are widespread phenomenon naturally occurring in different types of water bodies. Characteristic indicator of the bloom is a change of water color to blue-green, due to pigments contained by growing cyanobacterial biomass. Recently observed climatic changes and increase of Earth’s surface temperature may lead to intensification and proliferation of cyanobacterial blooms, severe reduction of water quality and changes in functioning of freshwater ecosystems. Monitoring of cyanobacterial blooms appears to be crucial in order to undertake remediation policy, such as biomass harvesting. The aim of our study was to develop an index for cyanobacterial blooms detection with use of unmanned aerial vehicles (UAV). The data was collected from 30 frames floating on the surface of blooming waterbody. First, the reflectances inside the frames were measured by UAV equipped with multispectral camera, then immediately the and phycocyanin concentrations inside frames were measured *in situ* with use of multiparameter probe. Further statistical analyses allowed to create an index based on Red Edge 740 nm and 717 nm and NIR 842 nm spectral bands. The correlation of the index with phycocyanine concentration measured *in situ* was estimated as +70%.

Poster 13

CyanoSeq: a new curated reference database of cyanobacterial 16S rRNA sequences

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Since the advent of next generation sequencing methods (NGS), large scale metagenomic studies using targeted genes are the most common approach for studying microbial community diversity. NGS methods provide insights not captured by traditional methods, allowing for better understanding of microbial communities. However, diversity assessments require reliable reference databases for accurate assignment of taxonomic composition. While curated databases exist for bacteria, the Cyanobacteria phylum remains poorly curated within them. The taxonomic rankings of Cyanobacteria provided within bacterial databases can be incorrect as the Cyanobacterial taxonomy within these databases are: not comprehensive, deficient in many well-described cyanobacteria, and do not resolve polyphyletic genera. The combination of uncomprehensive and incorrect taxonomy within current databases can lead to incorrect taxonomic assignment of metagenomic reads causing misinterpretations of the cyanobacterial community. To ameliorate these issues, we propose "CyanoSeq", a curated database of cyanobacterial 16S rRNA sequences for taxonomic assignment of metagenomic reads. CyanoSeq is assembled from 16S rRNA sequences found within NCBI, with their taxonomies curated from cyanobacterial taxonomic literature as well as a systematic assessment of uncharacterized cyanobacterial sequences. In addition to curated cyanobacterial sequences, CyanoSeq is composed of plastid, bacterial, and Melainabacteria sequences for more robust phylogenetic interpretations.

Poster 14

Near-Real-Time Cyanobacterial Identification and Cell Counts Using Artificial Intelligence and Digital Microscopy

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Current methods of cyanobacteria monitoring rely heavily on laboratory analysis or sensor and/or fluoroprobe systems. These methods can prohibit frequent and efficient monitoring due to expense and logistical challenges (sample shipment to lab, analysis by expert, need for microscopy to ID cyanobacteria). Here, we present a cellphone-based microscopy method to rapidly identify and enumerate cyanobacterial concentrations. In this study, digital imaging microscopes were deployed to eight (8) volunteers across Wisconsin and New York. Volunteers collected cyanobacterial samples and images using the provided digital microscope. Images were emailed to a trained scientist, for cyanobacterial cell ID and processing of cyanobacterial counts. A model using colony volumes and average cell volumes was used to calculate cell densities (cells/ml). Generated reports showed great agreement with fluorometer analysis ($R^2 > 0.80$) and provided cell identification and counts in approximately 1-2 hours. We found the method to be easy-to-use across our volunteer network and that further development and replacement of scientist involvement with an artificial intelligence program will further reduce sample processing time, generating reports in 10 mins or less. Application of this method provides cost-effective, near-real-time monitoring of HABs to a wide range of users, requiring only an inexpensive field microscope and smart phone.

Poster 15

Phylogenetic analysis of cyanobacteria based on two novel molecular markers, implicated in the nitrogenase biosynthesis

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The characterization of bloom cyanobacteria communities remains challenging, as taxonomy of several cyanobacterial genera is still unresolved, especially within Nostocales taxa. Nostocales cyanobacteria are capable of nitrogen fixation; nitrogenase genes are grouped into operons and are located in the same genetic locus. Previous studies demonstrated, that the divergence rate of nitrogenase structural gene (*nif*H) in cyanobacteria appear to be in congruence with that of 16S rRNA. However, there is no available information regarding the phylogeny of structural genes of the nitrogenase cluster. In this context, we developed a set of degenerate PCR primers for the amplification of *nif*E and *nif*N, two understudied structural nitrogenase genes. Successful phylogenetic analyses of *nif*E and *nif*N genes were carried out on thirty (30) Nostocales cyanobacteria strains, representing the genera *Anabaena*, *Anabaenopsis*, *Calothrix*, *Chlorogloeopsis*, *Desmonostoc*, *Dolichospermum*, *Komarekiella*, *Nodularia*, *Nostoc*, *Raphidiopsis*, *Sphaerospermopsis*, and *Trichormus*. A similar phylogenetic pattern between *nif*E/*nif*N, *nif*H, and 16S rRNA was observed, while phylogeny based on the nitrogenase structural genes *nif*E/*nif*N showed a higher resolution between Nostocales cyanobacteria and their lifestyle. In this study, we propose the use of two novel molecular markers, which could provide better insights in the taxonomy of Nostocales.

Poster 16

CANCELLED

Poster 17

Toxin production by *Microcystis* cultures isolated from Lake Erie

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Harmful algal blooms dominated by *Microcystis*, a cyanobacterial genus, have significant public health and economic ramifications worldwide, including a $65 million economic impact from a single Lake Erie bloom. We isolated and sequenced 24 *Microcystis* cultures collected from Lake Erie between 2017-2019. Genomic data shows these cultures represent a breadth of known *Microcystis* diversity. Some cultures, isolated years apart, are genetically similar to each other (Average Nucleotide Identity >99%). The complete *mcy* operon, responsible for producing the hepatotoxin microcystin, was found in 6 cultures. A previously described partial *mcy* operon was identified in 2 cultures. The remaining 18 cultures lacked any *mcy* genes. Previous studies in Lake Erie highlighted intra-annual shifts from microcystin-producing to non-producing strains. Microcystin concentration, assessed using Enzyme-Linked Immunosorbent Assay (ELISA), supported genomic results with high concentrations (up to 900 μg L^{-1}) in cultures with the full *mcy* operon and no or low toxin detected otherwise. Current work is determining how strains interact with co-occurring bacteria, how microcystin production and congeners vary with growth, and how these traits relate to their genetic properties. Overall, these results highlight phenotypic diversity among *Microcystis* strains and support a need for a nuanced understanding of microcystin production dynamics within ecosystems.

Poster 18

CANCELLED

Poster 19

Identifying Microbial Biomarkers for Early Detection and Health Diagnosis of Harmful Algal Blooms in Freshwater Lakes

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Harmful algal blooms (HABs) have been a very common phenomenon in the last few decades, intensified by global warming, eutrophication, and increased CO_2 levels. We hypothesized that biological and chemical parameters will be available allowing early detection of HABs in coherence with microbial signatures, where the health status of the HABs could be indicated by lysis of cyanobacterial cells and release of intracellular materials. Using multi-omics and quantitative real-time PCR, we investigated applicability of microbial signatures as a prognostic tool towards determining bloom health in two Ohio lakes, Lake Erie (LE) and Grand Lake St. Marys (SM), by assessing with chemical data. Different cyanobacterial species dominated algal blooms at LE (*Synechococcus* and *Microcystis*) and SM (*Dolichospermum* and *Planktothrix*) in the year 2021. Unbound phycocyanin levels were positively correlated with the quantity of cyanobacterial 16S rRNA genes in both LE and SM, with varied strength of correlations, indicating unhealthy cyanobacterial cells being present during the bloom. Genera *Pseudomonas* and *Sediminibacterium* as well as the phylum Verrucomicrobia appeared as potential microbial signatures when investigated with relative abundance of cyanobacteria. These signatures are promising to be established and validated as biomarkers for early detection of HABs while integrated with functional gene quantification.

Poster 20

Refinements of the qPCR and RT-qPCR detection assays for detecting microcystin producers: An early warning system for microcystin production

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The frequent occurrence of CHABs is a threat to human and ecological health by their toxin products, particularly Microcystin. Microcystin is the most common cyanotoxin produced, with the major toxin-producing genera during CHABs being *Microcystis* and *Planktothrix*. Previously, we've demonstrated that qPCR and RT-qPCR can be used as an early warning detection system for microcystin production in fresh surface waters. However, to form a more robust detection system, certain PCR methods need to be defined and validated. For example, target nucleic acid concentrations and biomass derived from environmental samples can range from low levels (undetectable) to extremely high levels (up to 10^{10} L^{-1}) and thus can impact the overall assays' RT and PCR reaction steps. To create a more robust qPCR and RT-qPCR-based early warning system, two commonly used quantitative PCR systems (TaqMan qPCR and SYBR Green qPCR) were evaluated for their specificity, sensitivity, efficiency, and accuracy. Three potential inhibitory scenarios for these PCR assays and corresponding relief strategies were evaluated for their effectiveness. The resulting qPCR procedures will be evaluated to monitor the occurrences of microcystin producers during the early bloom stages of HABs. Preliminary results from this study will be presented and a proposed protocol will be discussed.

Poster 21

Evidence for Unusual Paralytic Shellfish Poisoning Toxin-like Compounds in Cayuga Lake, New York

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Cayuga Lake (part of the finger lakes in central New York (NY), USA provides drinking water for several cities and is surrounded by a community of approximately 120,000. Cyanobacterial blooms were identified widespread throughout the lake and targeted samples were collected by citizen scientists in July-August (summer) and later in September-October (fall) in 2017 and 2018. Samples (*n* = 30) in Summer were dominated by *Dolichospermum*, while *Microcystis* dominated in the fall. In periods of *Dolichospermum* dominance paralytic shellfish poisoning toxins (PSTs) were identified by HPLC post-column chemical oxidation (PCOX), with concentrations ranging from ~25-50 µg/L. While PSTs were identified from fluorescent interferents through the oxidation of the central PST ring, the structures of the PSTs were unclear as retention times did not match those of reference materials. To confirm that these were PSTs, a subset of four samples were analyzed by the receptor binding assay (RBA), saxitoxin ELISA, and LC-MS/MS. PSTs were identified by each method. Higher concentrations of toxin were identified by ELISA than PCOX, which was unexpected as generally PSTs other than saxitoxin have poor cross-reactivity. Only saxitoxin was detected by LC-MS/MS at ~1 µg/L. While some PSTs are non-toxic, some of the PSTs appear to be active against RBA receptors, with one sample at concentrations in excess ~80 µg/L. PST profiles in freshwater environments are poorly understood. As PSTs have a range of toxicities, profile information is critical to better understand health risks. PSTs are likely present in Cayuga Lake, but the structures were unclear.

Poster 22

Assessing Efficacy of Solid Phase Adsorption Toxin Tracking (SPATT) as an Indicator of the Presence of Cyanotoxins in the New York Finger Lakes

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Solid Phase Adsorption Toxin Tracking (SPATT) uses passive samplers designed to concentrate dissolved cyanotoxins over time, thereby providing relative concentrations of cyanotoxins in water bodies across known deployment periods. SPATT can confirm the presence of cyanotoxins in natural systems where the temporal and spatial variability of cyanotoxin concentrations are below the detection threshold of common analytical techniques (e.g., ELISA, LC/MS/MS). In 2019, ninety-one SPATT samplers were deployed at U.S. Geological Survey surface water monitoring platforms located on three of New York's Finger Lakes: Owasco, Seneca, and Skaneateles. SPATT samplers were deployed at near-surface, mid-depth, and near-bottom depths, with deployment periods ranging between five and twenty-two days. Extracts from samplers were analyzed for four classes of cyanotoxins using ELISA with confirmatory analyses by LC/MS/MS. Discrete water quality samples for cyanotoxins, cyanotoxin synthetase genes, and phytoplankton community identification and enumeration were collected during sampler deployment and retrieval. Multi-parameter sondes deployed at the same depths as the SPATT samplers collected continuous water quality data during each deployment. A summary of preliminary data will be presented alongside complementary data to assess the efficacy of SPATT technologies and their potential utility to resource managers as early detection tools for the presence of cyanotoxins.

Poster 23

Identification and Quantification of Degradation Products of Microcystins using High-Resolution UHPLC-Orbitrap-MS

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Microcystins (MCs) are heptapeptides produced by freshwater harmful algal blooms (HABs). Exposure to hepatotoxic MCs is a threat to humans and animals. Although conventional municipal water treatment processes can treat MC contaminated water, biodegradation of cyanotoxins using indigenous bacteria is cost-effective and environmentally friendly. We previously isolated and characterized five bacterial isolates from Lake Erie that degraded MC-LR into non-toxic fragments (Thees et al. 2018). Herein, degradation of MCs that are abundant in Lake Erie HABs, MC-LR and MC-RR, is investigated qualitatively and quantitatively using high-resolution LC-MS. UHPLC-Selected ion monitoring (SIM)-Orbitrap-MS analysis revealed two peaks at different retention times corresponding to *m/z* of a tetrapeptide degradation product. Fragmentation spectra of both peaks showed characteristic ADDA fragment with *m/z* 135.08. From the MS/MS spectrum, it was concluded that the peak with the shorter retention time than the substrate is linear tetrapeptide. These results indicate that the MC biodegradation mechanisms in Lake Erie bacteria may be distinct from those in other MC degrading bacteria. The enzymatic pathways and MC breakdown products are being investigated further.

Poster 24

A Multi-Class Cyanobacterial Toxin Screening Method using Hydrophilic Interaction Liquid Chromatography with Tandem Mass Spectrometry (HILIC-MS/MS)

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Cyanobacteria can produce diverse classes of toxins including microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs), and saxitoxins (STXs). These range in polarities and molecular weights, generally requiring multiple preparation and chromatographic techniques for evaluation. Here we present the development and validation of a HILIC-MS/MS screening method for the detection and quantitation of all the aforementioned toxin classes.

Various solvents and sample-to-solvent ratios were investigated with an in-house blend of toxic cyanobacteria to develop a universal extraction method using 75% acetonitrile-water (0.1% formic acid). HILIC-MS/MS was used with gradient elution (35 min run time) with selected reaction monitoring (SRM) settings optimized on a triple quadrupole MS using positive/negative polarity switching. Retention time matching with standards and product ion ratios were assessed for identification. Validation included evaluation of the calibration models, precision, and detection limits (between 0.2 (ATX) and 7 (GTX4) ng/mL). Excellent recoveries (> 99%) were obtained using spiked algal extracts and in-house reference materials.

This method was applied to cultures, cyanobacterial dietary supplements, Canadian bloom and benthic algal samples, water, and shellfish samples, demonstrating suitability for screening and quantitation of all major cyanobacterial toxin classes in a range of sample types. Future applications include the characterization of cyanotoxin matrix reference materials.

Poster 25

CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria

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We created a comprehensive, publically accessible database of cyanobacterial secondary metabolites following the last ICTC in Krakow (2019). CyanoMetDB is a highly curated, flat-file, openly accessible database of cyanobacterial secondary metabolites collated from more than 850 peer-reviewed articles published since 1967. The database contains structural codes of more than 2000 cyanobacterial metabolites and facilitates the detection and dereplication of known cyanobacterial toxins and secondary metabolites as well as the identification of novel natural products from cyanobacteria. Research on biosynthesis of cyanobacterial secondary metabolite as well as investigations of their abundance, persistence, and toxicity in natural environments also benefit greatly from this comprehensive suspect list. We continue curating CyanoMetDB and integrating it into existing open access platforms. We are also generating mass spectrometry reference spectra for many entries to further facilitate the level confidence with which one can identify these metabolites when no reference materials are available.

Poster 26

Unbound phycocyanin as an indicator of cell lysis and dissolved toxin release in cyanobacteria

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Rapid cell lysis events during cyanobacterial blooms may result in the release of large amounts of dissolved toxins, which, if occurring near a drinking water plant intake, may pose serious treatment challenges for plant managers. Catastrophic cell lysis can occur naturally, through the activity of cyanophages – as documented in Lake Erie during the Toledo Water Crisis of 2014, or through the overapplication of algicides and water treatment chemicals. Cell lysis is indicated by the release of 'unbound phycocyanin' which can be detected fluorometrically by instruments recently developed by bbe-moldaenke. In the present study, unbound phycocyanin in laboratory cultures was continuously monitored following three chemical treatments. *Microcystis aeruginosa* UTEX 2385 (70 µg/L) was exposed to copper sulfate, PAK-27 (hydrogen peroxide-based algaecide), and potassium permanganate for 96 hours. In a separate experiment *Synechococcus* sp. cultures were infected with cyanophages and monitored for 144 hours. For PAK-27, copper sulfate, and the phage infection, decline in cell number was preceded by or coincided with the appearance of unbound phycocyanin, suggesting that this real-time fluorescent measurement may be a good indicator of cell wall condition. Results obtained from the other treatments will be further collected and discussed.

Poster 27

The Comparison of Two ELISA Kit for the Detection of Microcystin Proficiency Testing Samples

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There are more than 90 related compounds produced by cyanobacteria that are highly toxic hepatotoxins to humans and animals. Algal contamination of drinking water sources from Lake Erie caused potential health hazards in the tap water of Toledo, Ohio in 2014. Water from Lake Erie contained 60-80% Microcystin-LR, 10-25% Microcystin-RR, and 5-15% Microcystin-YR. Microcystin-LR is the most toxic, RR is half as toxic as the LR variant, and YR is between the two. Immunoassays used to detect these toxins should detect most of the toxic congeners. Beacon has released an improved BX test kit which demonstrates the broader cross reactivity (CR) profile to the various Microcystin congeners. The following cross reactivity are compared with our LR kit, BX (Cat# 20-0300) / LR kits (Cat# 20-0068); Microcystin LR (100% / 100%), RR (86% / 73%), LA (41% / 2%), LF (34% / 3%), LW (29% / 4%), LY (30% / 6%), YR (53% / 58%), and Nodularin (58% / 126%). Sixteen Microcystins proficiency testing (PT) samples from 2020 to 2021 were compared with our two kits with R-square comparison 0.9956 vs 0.9894. Improved kit demonstrates broader CR profile than the previous LR kit, which is confirmed by 16 PT samples.

New technologies, modeling, and smart lakes

Poster 28

High Resolution Mass Spectrometry Methods for High Throughput and Untargeted Analysis of Anatoxins in Cyanobacteria

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Anatoxins (ATXs) are increasingly linked to animal fatalities worldwide, but their analysis is challenging due to their polarity, instability, sample complexity and the scarcity of standards for most analogues. Conventional methods often don’t detect or differentiate analogues or are prone to significant matrix interferences in quantitation or detection. Here, we describe two recently developed methods for ATX analysis and demonstrate their application to cyanobacterial field samples from Atlantic Canada. A direct analysis in real time-high resolution tandem mass spectrometry (DART-HRMS/MS) method with limits of detection (LOD) of approximately 5 µg/kg and a total run time of under 2 min per triplicate analysis was suitable for rapid quantitation of anatoxin-a, homoanatoxin-a and dihydroanatoxin-a. Sample preparation was simplified to only require cell lysis, homogenization and centrifugation. An untargeted LC-HRMS/MS is also presented that offered lower LODs (0.1 µg/kg) and the opportunity to differentiate isomeric species and detect unknown ATX conjugates. Both methods used isotope dilution calibration with $^{13}C_4$-anatoxin-a to correct for the significant and variable matrix effects and showed excellent quantitative performance. They are broadly applicable to other quantitative or screening applications and will allow for more comprehensive study of ATX occurrence including the typically undetected fraction of conjugated ATXs.

Poster 29

High-throughput microscope counting of cyanobacteria using “cellcount”, a newly developed analysis package in the R programming language

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Molecular approaches and novel method validations require the precise enumeration of cyanobacteria to validate cyanobacteria density, typically done via microscopic counts which are considered time consuming and technically challenging. Cell counting software tools, such as ImageJ, can help decrease enumeration time, but may offer little flexibility in software modifications and may incorrectly quantify different morphotypes. Here we provide an overview of the development and uses of the draft package cellcount, from the programming language R. We used previously published code described in Pokrzywinski et al. 2019 as a blueprint for the development of new functions and overall organization. The result is an open-source package capable of being expanded and modified by novice and experienced R users. Here, we analyzed concentrations of several species to demonstrate cellcount versatility and potential limitations. In addition, we compared cellcount against standard enumeration practices and in vivo pigment fluorescence to demonstrate ease of use and rapid analysis while maintaining the same accuracy. With the formation of this high-throughput approach, researchers can utilize cellcount for many applications, such as qPCR standard curve development, the development of biomass standard curves, and validation of emerging quantification techniques.

Poster 30

Development of a Dietary Supplement Reference Material for Multiple Classes of Cyanobacterial Toxins

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Some algal dietary supplements are prepared using cyanobacterial biomass harvested from lakes and ponds, where multiple algal species can co-occur. This can result in supplement products containing cyanotoxins that present a risk for human health. Reference materials are therefore required to assist in the development, validation and quality control of analytical methods used for raw material and product testing for both research and regulatory purposes.

A dietary supplement certified reference material (CRM), representative in composition of an *Aphanizomenon* dietary supplement, was prepared by blending biomass of a variety of toxic cyanobacterial species with a typically-consumed non-toxic species (*Aphanizomenon* sp.). The toxin profile of this material includes microcystins, nodularins, anatoxins, cylindrospermopsins and saxitoxins. An exhaustive extraction method with a multi-analyte LC–MS/MS method was optimized and validated for homogeneity and stability evaluation. Methods for specific toxin classes will be used to assign certified values for analytes where equivalent calibration solution CRMs are available. Strategies for matrix effect compensation in LC–MS include isotope dilution and standard addition. Finally, untargeted high resolution mass spectrometry methods will be used to characterize the rich profile of non-certified cyanotoxins and other cyanobacterial secondary metabolites present.

Poster 31

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Poster 32

Evaluation of the stability of hyperspectral signatures in *Microcystis aeruginosa* and *Dolichospermum* sp. under nutrient stress

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The early detection of cyanobacteria harmful algal blooms (cyanoHABs) is important to deploy effective rapid response and mitigation strategies. However, monitoring cyanoHAB-prone water bodies remains a challenge, in part due to the limitation of spatial, temporal, and spectral resolutions of current image-based remote sensing technologies. Remote sensing platforms and sensors are rapidly evolving. Before these technologies can be transitioned to operational programs for early warning and routine monitoring, they need to be evaluated and validated in controlled environments. Therefore, in this study, the stability of hyperspectral signatures from two cyanobacteria: *Microcystis aeruginosa* and *Dolichospermum* sp. were evaluated under nutrient stress. Cultures were exposed to various nitrate (0.15-1.5 $g \bullet L^{-1}$) and phosphate (0.01-0.1 $g \bullet L^{-1}$) concentrations and ratios. Assessments were made on overall growth via in vivo phycocyanin fluorescence, toxin production via ELISA, and changes in hyperspectral signatures using a Resonon hyperspectral camera (400-1000nm). Laboratory experiments showed a change in spectral signatures under all of the nutrient stressors examined. This largely coincided with culture senescence, which also corresponded with higher cyanotoxin levels. Currently, this information is being compiled into a database for the identification of cyanobacteria that will be used to validate this technology as it transitions to field-based platforms.

Poster 33

Dredged Material from Harmful Algal Bloom Affected Lake Erie harbors: Microbiome, Resistome, and Cyanotoxins

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Dredging is a common practice for the maintenance of harbors by removing the built-up sediments. Land application has been proposed as an environmental and economic-friendly way to use dredged material (DM). However, DM can be a reservoir of various pollutants, among which antibiotic resistance bacteria (ARB) and cyanotoxin are understudied. The discharge of human and agricultural wastewater can spur both algal bloom and ARB in surface water. Given this, DM must be better understood before land application.

This study characterized DMs from six harbors located in the southwest shore of Lake Erie. The profile of cyanobacteria communities and resistome was depicted with Nanopore sequencing. Dominated toxic cyanobacteria genera in DM are *Microcystis*, *Planktothrix*, *Aphanizomenon*, and *Synechococcus*. Microcystin (MC)-producing *Microcystis* is the major toxin producer in most DMs, which significantly correlated with the total cyanobacteria and MC concentration ($p < 0.000$). In line with the bloom spatial distribution in Lake Erie, from west to east, MC and toxic cyanobacteria concentration in DM decreased significantly but remained. Toledo DM has the highest MC concentration while saxitoxin and anatoxin producers were also detected. Moreover, clinically relevant AR genes like fluoroquinolone, aminoglycoside, and tetracycline resistance genes were identified with various pathogenic hosts.

Poster 34

Human Health Impact of Environmental Exposures to Cyanotoxins: Evidence from the Diversity and Innovation in Screening and Prevention of Exposure over the Long-term (DISPEL) Cohort Study

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Toxic cyanobacterial blooms are increasingly more frequent and widespread in Florida, raising the critical need to understand the short- and long-term human health impacts of ingestion, dermal, and inhalation exposure to the toxins. Since 2019, the DISPEL to HABs prospective cohort study conducts periodic surveillance of Florida workers, residents, and visitors' health status and exposure to cyanotoxins produced from blue-green algae. In the present study, we examine cohort participant baseline data on self-collected water samples, oral and stool microbiomes, pulmonary function tests (PFT), and socio-demographic characteristics. Among the 37 participants, comprised of 28.9% residents, 68.4% workers, and 2.6% visitors to Florida, microbiome samples were collected before and after exposure to HABs. Water samples, collected by participants from their home tap water and surface samples from nearby outdoor waterbodies, were analyzed by HPLC-MS for microcystin, of which four water samples had measurable microcystin with one exceeding levels of the World Health Organization drinking water guidelines. Preliminary 16S analysis identified Actinobacteria followed by Firmicutes as the dominant phylum in participant oral samples, while Firmicutes followed by Bacteroidetes dominated in the stool samples.

Poster 35

Using genetic markers to monitor toxic cyanobacterial communities at the volcanic-origin lakes of Amatitlán and Atitlán, Guatemala

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The lakes Amatitlán and Atitlán are two of the biggest freshwater ecosystems in Guatemala. Over 1000 years, lake Amatitlán has experienced cultural eutrophication, and within the last 50, toxigenic *Microcystis spp* are responsible for permanent cyanobacterial blooms. Also, eutrophication has increased in the oligotrophic lake Atitlán, and since 2010, with the appearance of *Limnoraphis robusta* blooms. Similar environmental conditions derived from their volcanic origin, could imply the accelerated deterioration of Atitlán, and thus, reliable detection methods should be implemented to better understand cyanotoxin production in both lakes. Here, we used molecular markers for the detection of genes involved in the synthesis of cyanotoxins in mentioned lakes. Sequencing analysis showed that in Amatitlán, *Microcystis* (16S rRNA) dominated and presented toxigenic genotypes with potential production of microcystins (*mcy*E) and anatoxins (*ana*C), and the concentration of microcystins reached 4.05 mg L^{-1} (HPLC analysis). In the case of Atitlán, the *mcy*E gene was detected for the first time with homology to *Microcystis*, during a bloom dominated by *L. robusta*. Our results evidence that the use of cyanotoxin gene markers can be incorporated in ongoing monitoring efforts for early detection of toxic cyanobacteria, and the importance to include prevention solutions focused on reducing nutrient loading.

Poster 36

Cyanobacteria community adaptation in response to diversion operations in coastal Louisiana

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Estuaries within the Gulf of Mexico are exhibiting profound alterations due to climatic changes and the implementation of coastal restoration activities. These changes are attributed to the increasing frequency of cyanoHABs within Lake Pontchartrain, an estuary located in southeastern Louisiana. Many cubic km of nutrient rich freshwater from the Mississippi River are diverted into Lake Pontchartrain via the opening of the Bonnet Carré Spillway (BCS). In 2019, the region saw two openings of the spillway for the longest duration on record with a total of 123 days. As a result, a massive cyanoHAB was documented across the estuary. In 2021, the BCS remained closed but there continued to be localized blooms of cyanobacteria indicating alternative sources that may fuel the growth of cyanoHABs. To determine the effects of the timing and duration of the BCS openings on cyanoHAB prevalence and toxicity, data on water quality, phytoplankton community composition, and toxin production was collected throughout 2019 and 2021. By understanding the effects of nutrient loading and physical dynamics on cyanoHAB formation and proliferation, we can more accurately predict the future impacts of the BCS and other freshwater sources on the Louisiana coastal health.

Poster 37

The Heidelberg Tributary Loading Program: Nutrients, Sediment, and cHABs- Oh My!

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The National Center for Water Quality Research has been monitoring major tributaries to Lake Erie for nearly 50 years as a part of its Heidelberg Tributary Loading Program (HTLP). Up to three samples a day are analyzed for all major nutrients and suspended sediment from six major tributaries to Lake Erie (Maumee, Sandusky, Portage, Huron, Raisin, and Cuyahoga). Long-term trends in loads and concentrations indicate that total phosphorus (TP) has decreased since the mid-1970s in the agricultural watersheds, whereas dissolved reactive P (DRP) has been increasing drastically since the mid-1990s corresponding to the recurrence of cyanobacterial Harmful Algal Blooms (cHABs) in Lake Erie. Increased DRP and cHABs appear to be associated with increased discharge in the past decade as well as recent patterns in agriculture, specifically the build-up of P at the soil surface combined with preferential flow of water to tile drainage. This high frequency water quality monitoring has allowed us to detect dominant sources and dynamics of nutrient runoff from watersheds. Further, we recently have begun using a Fluoroprobe to determine spatial and temporal dynamics of potamoplankton in the Sandusky River and have conducted investigations into the effect of sample holding time on algal community composition.

Poster 38

Diversity of cyanobacteria and associated microbial community structure within Lake Okeechobee and the St. Lucie River, Florida, USA

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Lake Okeechobee is a large eutrophic subtropical lake in the southern Florida peninsula which frequently experiences cyanoHABs. These blooms are re-occurring and can cover >90% of the lake's surface, thus it is imperative to understand the community structure during bloom and non-bloom events to identify potential drivers of these microbial communities. From August 2019 to September 2020, Lake Okeechobee and the connected St. Lucie River and estuary were sampled for 16S rRNA metagenomic analysis and limnological parameters. Results revealed community structure varying spatially and temporally within Lake Okeechobee and the St. Lucie River and estuary. The most abundant cyanobacterial families within Lake Okeechobee over the course of this study included Aphanizomenonaceae, Microcystaceae and Prochlorococcaceae and the most abundant bacterial families included Chitinophagaceae, Pirellulaceae, and Sporichthyaceae. While the majority the cyanobacterial ASVs corresponded to Prochlorococcaceae, several toxigenic genera were detected within the lake, with *Dolichospermum* and *Microcystis* occurring frequently. Additionally, cyanotoxins also varied spatially and temporally, with several different microcystin congeners detected as well as anatoxin-a and nodularin throughout both the lake and river. This study provides insights into drivers of the cyanobacterial and associated microbial communities within Lake Okeechobee, and highlights potential drivers of bloom forming taxa and their toxins.

Poster 39

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Poster 40

Microcystin-LR, -LW, and -LF on microplastics: toxic compounds adsorbed by six plastics

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Water quality is an increasing global environmental concern. An indicator of poor water quality is the presence of cyanobacterial blooms. The hepatotoxin microcystins (MC) are the most common group of cyanotoxins reported in freshwater. There are over 200 variants, often detected as a mixture in the environment. Other pollutants, e.g., microplastics, are also commonly detected in aquatic systems. There is an emerging concern that microplastics can act as a vector for micropollutants when they co-exist in the same environment, including microcystins.

This study evaluated a mixture of three microcystins (1 μg mL^{-1} each) with six microplastic types (polypropylene, polyethylene terephthalate, polystyrene, polyamide, polyethylene, and polyvinyl chloride) with average particle sizes of 5-45 μm. The effect of microcystin hydrophobicity on the adsorption by microplastics was evaluated. Polypropylene, polystyrene, polyvinyl chloride, and polyethylene adsorbed all variants, while polyethylene terephthalate only adsorbed MC-LW, and no adsorption was observed by polyamide. Polypropylene showed the greatest adsorption of the variants, adsorbing from 80% (MC-LR) to 100% (MC-LW/LF). The largest concentration adsorbed onto microplastics was observed for MC-LW, followed by -LF, and finally MC-LR.

Therefore, microplastics can act as a vector for microcystins in the aquatic environment, with possible implications when they enter the food chain.

Poster 41

Why do toxic cyanobacteria bloom? A gene to ecosystem approach

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This project aims to understand the ecological and evolutionary mechanisms triggering harmful cyanobacterial blooms (cyanoHABs) in lake ecosystems, and develop predictive models. We focus on bloom forming cyanobacteria (*Oscillatoriales*, *Nostocales* and *Chroococcales*) in temperate lakes and the production of known toxins and other bioactive metabolites. We are an interdisciplinary team of researchers in ecology, microbiology, environmental chemistry, and dynamical systems theory and together we focus on central questions about the mechanisms triggering cyanoHABs. We study the plankton seasonal progressions in a model lake (Greifensee, Switzerland) that has shown yearly occurrences of cyanoHABs. We test the relative importance and timing of different hypotheses of cyanoHAB drivers (water chemistry and physics, abundances of natural enemies and facilitative species). In parallel, we conduct laboratory experiments to estimate toxin production dynamics and fitness of cyanoHAB taxa relative to competitors in synthetically assembled communities, crossing community composition with environmental factors (e.g., temperature, nutrients, light, grazing). The range of important mechanisms will be constrained by data-analysis and modelling, and tested empirically by mesocosm experiments using natural lake plankton communities. The information acquired will be assimilated into data-driven models to forecast cyanoHABs, and integrated with previous knowledge to develop mechanistic models to predict the probability of cyanoHABs and associated communities based on fitness components and evolutionary strategies, under different environmental conditions (abiotic and biotic).

Poster 42

Cylindrospermopsin is regulated by resource stoichiometry in the cyanobacterium, *Aphanizomenon*

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While all the drivers of HAB formation are not well understood, eutrophic conditions increase the likelihood of HABs. Cyanotoxins are classified as nitrogen (N)-rich or carbon (C)-rich, with the Redfield ratio (C:N 6.6) separating the two classes. The stoichiometric hypothesis of toxin production states that conditions that cause N to be in excess [e.g., high N:phosphorus (N:P)] will cause increased N-rich cyanotoxin production. This hypothesis is supported in *Microcystis* with microcystin production; however, other cyanotoxins and cyanobacteria remain poorly understood. Here, we explored how a gradient of N:P affects the biomass, elemental stoichiometry, light-harvesting pigments, and cylindrospermopsin (CYN) concentration and cell quotas in an N-fixing cyanobacterium, *Aphanizomenon*. We found that cultures grown in low N:P conditions produced the same biomass, implying a high N-fixation efficiency that did not cause an N-fixation growth tradeoff. Even with no changes in biomass CYN concentrations increased over the N:P 1 to 100 gradient. The proportion of dissolved CYN increased with resource N:P that ranged between 0 and 75% of the CYN concentration. Our results enforce the need to control both N and P to lower the N:P in receiving waters that may decrease the potential for cyanobacteria to produce intracellular and extracellular toxins.

Poster 43

Abiotic factors affecting chytrid (Chytridiomycota) infection rates on its host *Planktothrix agardhii*

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Sandusky Bay, Lake Erie (USA) is home to recreational fishing, tourism, boating, and swimming, and is a drinking water source to about 50,000 residents. It is also plagued by annual cyanobacterial blooms. *Planktothrix agardhii*, which dominates Sandusky Bay, can produce microcystin toxins that are known to be harmful to humans and animals. It is important that we understand the mechanisms driving and influencing these blooms. In this study, we examine Chytridiomycota (chytrids) and how these aquatic fungi can infect *P. agardhii* blooms. *Rhizophydium* sp. are known obligate parasites to *Planktothrix* and these species have been found and isolated from the waters of Sandusky Bay (McKindles et al. 2021). We investigated the spatial distribution of infections along with the effect turbulence has on infectivity. In lake mesocosms, coupled with lab experiments, suggest that small amounts of water turbulence can significantly reduce the infectivity of chytrids. Additionally, there was no statistical difference in spatial distribution found in the lab experiments. This is likely due to the low water volume and position of light. Understanding different environmental conditions and the effect they have on infectivity provides valuable insights into controlling factors that may be limiting chytrid pathogenesis.

McKindles, K.M., A.N. Jorge, R.M. McKay, T.W. Davis and G.S. Bullerjahn. 2021. Isolation and characterization of *Rhizophydiales* sp. (Chytridiomycota), an obligate parasite of *Planktothrix agardhii* in a Laurentian Great Lakes embayment. Appl. Environ. Microbiol. 87(4): e02308-20.

Poster 44

The influence of climate factors on the presence of microcystins in freshwaters – a case study from Poland

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Climate changes are expressed as increase of temperature and the frequency and intensity of severe weather conditions. Furthermore, climate change promotes development of bloom-forming cyanobacteria and the increase the intensity and toxicity of the blooms. According to European Environmental Agency (EEA), Europe is the fastest warming continent and particularly high warming is found over eastern Europe. The last six years were recognized as the warmest on record. However, the greatest changes will be possible to identify locally. We conducted the short-term surveys of concentrations of microcystins, which are recognised as the most often produced cyanotoxins, in two shallow and small water bodies in southern Poland. Data was collected during four years of monitoring: 2017, 2019-2021. We hypothesised that microcystin concentration is related to weather conditions. The pattern of microcystins distribution during the seasons were similar in both of them: toxins were present from July to October. Statistical analyses showed that significant factor correlated with concentration of microcystins was rainfall higher than 2.2 mm. Besides, stepwise regression demonstrated that rainfalls and air temperatures were significant contributors for dimensions PCAI and PCAII, which cumulatively explained 53% of variance

Poster 45

Comparing Cyanobacterial Bloom Phenology in Green Bay and Lake Winnebago with western Lake Erie and Saginaw Bay

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Cyanobacteria blooms are increasingly monitored around the world, owing to their potential to cause economic, environmental, and human health risks. One approach to monitor and model cyanobacterial biomass is to use satellite imagery to obtain long-term data sets. This presentation uses an existing algorithm developed for MERIS for cyanobacterial biomass validated with cyanobacterial biovolume estimates in Green Bay. The resulting satellite data set was used to determine the phenology of the cyanobacterial biomass and blooms in Green Bay and adjoining Lake Winnebago from 2002-2011. The relative size and timing of cyanobacteria blooms in Green Bay will be investigated with those in Lake Winnebago. Climatological data on several atmospheric and water variables obtained from the NASA Giovanni dataset was used to separate the upper 50% of bloom years and lower 50% of bloom years in Green Bay. The Giovanni dataset was then used to compare blooms from two other cyanobacterial bloom hotspots in the Great Lakes: western Lake Erie and Saginaw Bay. These data showed that separation was possible using as few as three variables from ancillary datasets.

Poster 46

Antibacterial activity of cyanobacterial extracts against Legionella spp.

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Cyanobacteria are recognized sources of natural compounds with pharmaceutical interest, namely antimicrobial activity. Several studies have shown the inhibitory effect of cyanobacteria against the most common bacterial pathogens. However, the bioactivity against Legionella pneumophila was never reported. L. pneumophila is ubiquitous in water environments and causes respiratory infections through water-air transmission. A rise in Legionella outbreaks might be expected, considering that climate changes will ex-acerbate water-borne diseases. In this work, we evaluated the antibacterial potential of 25 freshwater cyanobacteria from ESSACC collection, against clinical/environmental L. pneumophila strains isolated from outbreaks (cooling towers systems). Cyanobacterial biomass was extracted with n-hexane, dicloromethane:methanol (2:1), methanol 70%, and water and extracts were dried and dissolved in DMSO (25%). Disk Diffusion method was adapted to *Legionella* growth using 1 McFarland suspensions in BCYE plates. Levofloxacin (10 µg) and DMSO (25%) were used as positive/negative controls, respectively. Methanolic extracts from *Dolichospermum flos-aquae*, *Limnothrix redekei*, *Microcystis aeruginosa* and *Planktothrix agardhii* (LMECYA 165, 145, 257 and 127) induced inhibition zones ≥10 mm, demonstrating their antibacterial activity against *L. pneumophila*. These results encourage us to further investigate the potentiality of cyanobacteria as natural sources of antibiotics and/or water disinfectants, to overcome the occurrence of pathogenic *Legionella* in water environments.

Poster 47

Diel Variations of Extracellular Microcystin Influence the Subcellular Dynamics of RubisCO in Microcystis aeruginosa PCC 7806

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The cyanobacterium *Microcystis* is widely known for the production of the hepatotoxin microcystin. While the aspects regarding its toxicity have been studied extensively, only little is known about the natural function of this compound. Here we show our latest findings on how microcystin interferes with the inorganic carbon metabolism in the model strain *M. aeruginosa* PCC 7806. Both intra- and extracellular functions as a signaling molecule are discussed, as microcystin can interact with proteins of the photosynthetic apparatus, especially with RubisCO. Diel experiments showed a direct link between microcystin and its intracellular targets, as fluctuations in the extracellular microcystin content correlated with changes in the microcystin binding pattern to intracellular proteins. Concomitantly, alterations in the accumulation of RubisCO products are occurring. Interestingly, we also observed changes in the subcellular localization of RubisCO associated with high levels of extracellular microcystin. Microcystin addition experiments demonstrated that effects of externally added microcystin appear strongest at high cell densities and high light intensities. This gives further insight into how microcystin could be part of a possible fast response mechanism to environmental changes like high light and high cell density and thus contribute to the incomparable success of *Microcystis* in the field.

Poster 48

Effects of exogenous amino acids on the growth and microcystins production of *Microcystis aeruginosa*

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The effects of four amino acids (glutamic acid, phenylalanine, leucine, and arginine) and two inorganic nitrogen (nitrate and ammonium) on regulating the growth and microcystins (MCs) production of *Microcystis aeruginosa* were studied in batch culture. Algal cell density, the concentrations of five variants of MCs (MC-LR, RR, YR, LW, LF) and free amino acid were determined. Results indicated that the growth of *M. aeruginosa* enhanced with increasing of N concentrations, but decreased obviously in high concentration (8 mg-N/L) of ammonium, phenylalanine and leucine compared with other nitrogen source. Leucine, phenylalanine and glutamic acid can increase the quota of free amino acids in cells, and promote the synthesis of MCs with the corresponding amino acid in the variant's structures, respectively. The total concentrations and quotas of MCs cultured in glutamic acid and arginine was similar to that in inorganic nitrogen medium, but lower than that cultured in phenylalanine and leucine. Among the four amino acids, the glutamic acid medium has the highest extracellular MCs levels. In conclusion, exogenous amino acids had different effects on synthesis of free amino acids and MCs in cells, resulting in different MCs productions and variants.

Poster 49

Removal of Microcystins and Nodularin-R from Water by Corncobs Studied Using LC-MS Hasaruwani

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Microcystins (MCs) and nodularins are released during harmful algal blooms (HABs) in fresh and brackish water. Consumption of water contaminated with MCs or nodularins can promote health risks for humans and animals. In this study, The Andersons corncobs were acid refluxed and heat treated to remove MCs and nodularin-R from water. Simultaneous sorption of six common MC congeners (MC-RR, MC-YR, MC-LR, MC-LA, MC-LW, and MC-LF) and nodularin-R onto treated corncobs was determined in suspension and filter mode. An LC-Orbitrap-MS system was used to measure the percent removal of MCs and NOD-R in water. Results from preliminary sorption experiments showed that a sorbent (S-5), which was acid refluxed and heat treated, has the highest removal efficiency. When a mixture containing six MCs and nodularin-R (10 µg/L each) was incubated with 50 mg of sorbent, all analytes were completely removed. Even after increasing the concentration of analytes ten times, removal for all analytes by sorbent was ≥ 98.1%. When S-5 was incubated with Lake Erie water collected during the 2020 HAB, it was able to remove most of the MC variants completely. LC-MS was used to show that treated corncobs are promising sorbent materials to remove MCs and nodularin-R from drinking water.

Poster 50

Bioaccumulation of secondary metabolites from cyanobacterial blooms in tilapia (*Oreochromis niloticus*)

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Freshwater cyanobacteria can produce toxic and non-toxic secondary metabolites that accumulate in other organisms. These can negatively impact aquaculture, as different secondary metabolites can alter the quality of fish. The aim of this study was to evaluate the bioaccumulative effects of secondary metabolites from cyanobacterial blooms in tilapia fingerlings (*Oreochromis niloticus*). The experiments were carried out with 80 fish per aquarium of 58L, divided into negative control and different concentrations of the cyanobacteria biomass extract for 30 days. Cyanobacteria biomass and fish tissues were analyzed by LC/MS to detect secondary metabolites. The analysis showed different variants of microcystins, such as MC- LR (*m/z* 995) and MC-YR (*m/z* 1045) and mycosporines: palythine (m/z 245), shinorine (m/z 333), porphyra-334 (*m/z* 347) and mycosporine-glycine-alanine (*m/z* 317) in the cyanobacterial biomass. Microcystin was not found to accumulate in fish during the studied exposure time. Overall, the biomass concentrations used in the bioaccumulation experiment were not lethal to fish, as they remained alive until the end of the experiment. We found that mycosporines accumulated in muscle (palythine, shinorine and mycosporine-glycine-alanine) and liver (palythine and shinorine) of those fish exposed to higher concentrations of cyanobacterial biomass. Thus, these metabolites are capable of bioaccumulating in fish.

KEYWORDS: MICROCYSTIN; MYCOSPORINE; LC/MS

Poster 51

Bioaccumulation of methylisoborneol and geosmin on some freshwater organisms

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We investigated the bioaccumulation of 2-methylisoborneol (MIB) and geosmin (GEO) during cyanobacterial bloom episodes at Billings Reservoir in São Paulo city for a period of two years (covering all seasons in between 2017 and 2018). Cyanobacterial VOCs were monitored by a GC-MS method based on solid phase microextraction (SPME) and headspace (HS), and samples of water, cyanobacteria, copepods and fish (tilapia) were monitored. Three different methodologies to identify and quantify VOCs were tested, and the results suggested that HS-SPME-GC-MS was the most efficient among them. The highest levels of GEO and MIB were detected in fish tissue during the summer, coinciding with the higher temperatures and lower water levels in the reservoir, which lead to a more predominant growth of cyanobacteria. The presence of MIB and GEO increased higher up the food chain (water < cyanobacteria < copepods < fish). These results indicate that MIB and GEO can bioaccumulate and biomagnify in the environment and might be a concern for public health because water taste and odor can be affected as well as species used in aquaculture.

Poster 52

Microcystins modify cell wall composition and pectin methylesterase activity in plant cells

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Microcystins (MCs) inhibit protein phosphatases 1 and 2A, resulting in disruption of the cytoskeleton (i.e. microtubules and F-actin). Since microtubules play a role in plant cell wall deposition and structure, MCs could affect cell wall as well, which is among the less studied putative targets of MCs. This study focused on cell wall alterations in differentiated *Oryza sativa* (rice) root cells, induced by either pure microcystin-LR (MC-LR) or the extracts of MC- and non-MC-producing *Microcystis* strains. Immunolabeling of various cell wall components, including pectins, arabinogalactan-proteins (AGPs) and hemicelluloses, was performed on semi-thin transverse sections of LR-White embedded roots (control and affected), observed by fluorescence microscopy. The distribution patterns of pectic epitopes, such as homogalacturonans (HGs) and arabinans, were altered in affected roots, while pectin methylesterase (PME) activity assays revealed that PMEs were also affected, by both pure MC-LR and cyanobacterial extracts, in a time-dependent manner. Elevated intracellular calcium levels, along with increased callose deposition, were induced after treatments. This is the first report of cyanobacterial metabolites affecting PME activity and pectin distribution in plant cell walls. The exact mechanism of toxicity (direct effect on PMEs or indirectly, possibly via the production of reactive oxygen species) is to be further investigated.

Poster 53

Discovery and Structural Elucidation of Novel Microcystins Using MS and MSn with Python Code

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Microcystins (MCs) are cyclic heptapeptide hepatotoxins with a vast structural diversity (>300 congeners). However, many more congeners are theoretically possible, and a workflow was developed for putative identification of novel MCs using liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) and MS^n with Python code. Water samples collected from Lake Erie were sonicated and filtered, and solid-phase extraction was performed. A portion of the water sample was reacted with mercaptoethanol using the method developed by Miles *et al*. for structural elucidation. Extracted MCs were analyzed using UHPLC coupled to an Orbitrap Fusion instrument for HRMS. Collision-induced dissociation (CID) and higher-energy CID (HCD) were used for MS^n analyses. A total of 33 MCs were found in lake water samples, including two unknown MCs. Two Python codes were developed to elucidate the structures of MCs. Code 1 was written to generate a list of masses of theoretically possible MC congeners. Code 2 was written to compare the experimentally obtained masses of MC fragment ions to the theoretical fragment masses. Using Codes 1 and 2, the two unknown MCs with m/z values of 526.7980 (z=+2) and 1025.5302 (z=+1) were putatively identified as MC-HarR and MC-E(OMe)R, respectively.

Poster 54

The Occurrence of Microcystin-LR Photoisomerization Products in a New York State Local Lake

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The hepatotoxin, Microcystin-LR (MC-LR) is common in New York State lakes. Microcystins undergoes a photochemical isomerization to form several photoisomerization products. These photoisomerization products were generated in the laboratory, characterized by high-field NMR spectroscopy, and an in-house LC-MS microcystin method modified to allow for their detection in natural waters. Lake Neatahwanta (Fulton County, NY) experiences persistent cyanobacteria blooms. Samples were taken weekly from the Lake Neatahwanta during the 2016 and 2017 bloom season to measure the occurrence and concentration of these photoisomerization products in the lake. The products were detected in ~95% of samples and had a strong positive correlation with the in-situ concentration of MC-LR. The proportion of photoisomerization products were higher in dissolved phase than in the particulate phase. There was no significant daily variation observed in a diurnal experiment conducted in both 2016 and 2017. Different analytical method (LC-MS, LC-MSMS and ELISA) were evaluated for their ability to detect these photoisomerization products. This study provides important information on the fate of microcystins in natural waters and the impact of these products on different microcystin detection methods.

CHABs and Omics: advances in genetics, transcriptomics, metabolomics, and proteomics to understanding CHABs

Poster 56

Plasmid-mediated microcystin production in *Fischerella* CENA161

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The strain *Fischerella* sp. CENA161 is a microcystin producing cyanobacterium isolated from spring water in Brazil. The complete biosynthetic *mcy* gene cluster and potential microcystin congeners were published in 2018. In this work, bioinformatic analysis of whole-genome sequence and hybrid assembled data from *Fischerella* sp. CENA161 was performed. For detection of putative plasmids in this strain, the plasmid assembly mode in SPAdes was used on reads sequenced with the MiSeq and PacBio plataforms, followed by annotation of resulting contigs using PlasmidFinder. The complete genome size of CENA161 is 5.9 Mb and three plasmids were annotated with sizes of 728, 242 and 192 kb. The complete *mcy* gene cluster was found in the larger plasmid. Genome mining of available microcystin-producing cyanobacteria was also carried out to detect the partitioning of microcystin biosynthetic pathways in chromosomes and plasmids and will be presented. Plasmid-mediated microcystin production is a significant public health concern given its potential to easily spread among aquatic cyanobacteria.

Poster 57

Mobilomics of toxic/bioactive peptide production in the bloom-forming cyanobacterium *Planktothrix*

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It has been hypothesized that mobile elements induce genomic rearrangements and influence the distribution and functionality of toxic/bioactive peptide synthesis pathways. We performed an in depth genomic analysis by completing the genomes of 13 phylogenetically classified strains of the bloom-forming cyanobacterium *Planktothrix* spp. to investigate the role of IS elements for seven of such pathways. Though the variation in genome size was positively related to the proportion of IS elements (1.1-3.7% on chromosome), quantitatively IS elements as well as paralogs only had a minor share in genome size variation. Six of seven toxic/ bioactive peptide synthesis gene clusters were found located on the chromosome (i.e. non-ribosomal peptide synthesis (NRPS) of microcystin, anabaenopeptin, aeruginosin, cyanopeptolin, and ribosomal and post-translational peptide synthesis (RiPP) of microviridin, prenylagaramide). For NRPS a high share of SM synthesis gene cluster functionality was observed, while the two RiPP gene clusters were found frequently inactive. Unexpectedly the strains did not show an increased IS element frequency of occurrence in the vicinity of SM synthesis gene clusters. In contrast IS elements occurred with higher proportion in the ten breaking regions related to chromosomal rearrangements related to a localization of toxic/ bioactive peptide synthesis gene clusters on the chromosome.

Poster 58

The neurotoxin 2,4-diaminobutanoic acid (2,4-DAB): genomic insights into how and why it is biosynthesised in some cyanobacteria

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Cyanobacteria are an ancient clade of photosynthetic prokaryotes, whose worldwide occurrence presents health hazards to humans and animals due to the production of a range of toxins (cyanotoxins). These include the non-encoded diaminoacid neurotoxin 2,4-diaminobutanoic acid (2,4-diaminobutyric acid; 2,4-DAB), which can be lethal to aquatic biota and mammals. Knowledge of the biosynthetic pathway(s) to this cyanotoxin, and its role(s) in cyanobacteria, is lacking. Here, we use bioinformatics analyses to investigate hypotheses concerning neurotoxin biosynthesis in 130 cyanobacterial genomes. The presence of enzymes potentially involved in toxin production was assessed via profile hidden Markov models, sequence alignment, substrate specificity/active site identification and gene tree reconstruction. The co-localisation of these enzymes within specialised metabolite clusters known to be involved in toxin production was also considered. We show that, in cyanobacteria, the biosynthesis of 2,4-DAB appears to be either restricted to some species, or there may be multiple and additional routes, and roles, for its biosynthesis. For species where this neurotoxin can be biosynthesised enzymatically, an association with siderophore and polyamine production appears to be present in some species. We extend our comparative genomics analyses to some other toxins.

Poster 59

Molecular insights into toxic bloom development through metaproteomics

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Metaproteomics is an emerging field enabling insights into the complex interplay between microorganisms and their influence on biogeochemical processes. The field is uniquely relevant to the study of complex cyanobacterial bloom systems, with the potential to map bloom initiation, maintenance and senescence at the molecular level. Metaproteomics was applied to profile the seasonal regulation of a cyanobacterial bloom in an Australian wetland. The site was home to a transient flying fox colony contributing as a major nutrient source. 16s and shotgun metagenomics were applied to understand taxonomic distributions over the course of 1 year. Amplification of *mcyE* within the community was used to infer microorganisms containing the microcystin biosynthetic gene cluster. Protein and peptide-level identifications were used to infer the contribution of major taxonomic groups to nutrient flux based on the expression of metabolic enzymes and transporter families. Communities were investigated in the sediment and upper pelagic space to enable insights into species recruitment and potential contributions to bloom initiation. While this study is ongoing, it offers initial molecular insights into the drivers of toxic cyanobacterial blooms, and potential triggers for bloom development.

Poster 60

Metatranscriptomics of a cyanobacterial bloom in a freshwater estuary: Analysis of a community shift from a *Planktothrix* dominated community

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Harmful algal blooms in Sandusky Bay of Lake Erie (U.S.) have been documented since the early 2000's. The blooms, typically dominated by *Planktothrix agardhii*, are persistent from May through October. Biweekly sampling of the inner and outer portions of the bay was conducted from June through August of 2015. Heavy rainfalls occurred well into July throughout the region. This resulted in nitrogen loading from the surrounding agricultural areas until the rains subsided late July. Nitrate levels decreased as biomass increased during bloom formation, with N depletion also attributed to high rates of sedimentary denitrification.[1] Nitrogen fixation was also attributed to *Anabaena* spp. present as a minor component of the bloom community.[1] Metatranscriptomes prepared from these samples showed a shift from the dominance of *Planktothrix sp*. in early June in the inner bay to a transient abundance of N-fixing *Anabaena* spp. in July, then shifted back to *Planktothrix* dominance by August. The *Planktothrix* bloom persisted in the outer bay from June through August despite similar shifts in nitrogen availability. Understanding the dynamics of cyanobacterial blooms during shifts in N and P availability will be important in management decisions to prevent shifts in bloom taxa from *Planktothrix* to diazotrophic species.

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Poster 61

Comparative Genomic Analysis of Microcystis Strain Diversity Using Conserved Marker Genes

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Microcystis-dominated cyanobacterial harmful algal blooms (cHABs) have a global impact on aquatic ecosystems and human health. Diverse *Microcystis* strains have been observed to have different ecophysiological functions, including toxin production. While shotgun sequencing methods enable tracking of strain diversity and functions in the field, they are costly and require intensive data analysis. In contrast, amplicon sequencing of single genes is high-throughput and efficient but to date has been hindered by a lack of understanding of relationships between single gene sequences, whole genome content, and phenotype of *Microcystis* strains. Here we evaluated how phylogenies based on single conserved *Microcystis* genes compare to multi-gene phylogenies and the extent to which they represent the broader gene content of 159 publicly available *Microcystis* genomes as of June 1, 2020. Phylogenetic trees of 10 individual conserved marker genes did not accurately reflect phylogenies constructed from multiple genes. Regression analysis of phylogenetic distance of individual marker genes versus genes shared between genomes showed weak or no relationships between marker gene similarity and genome content. The results suggest that whole genome or multi-gene approaches are required to accurately assess genome content. Our current analysis is focused on assessing whether single gene markers provide information about gene content.

Poster 62

Metagenomic characterisation of cyanobacterial bloom development within wastewater treatment ponds

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Projected increases in the global population requires a corresponding increase in the securing and provisioning of water resources. Treated wastewater is a suitable supplementary source of both potable and non-potable water that many countries employ to alleviate pressure on freshwater reserves. The wastewater reclamation process is often hindered by the development of recurrent and persistent proliferations of toxigenic cyanobacteria that thrive in the eutrophic conditions at wastewater treatment plants (WWTPs). Whole metagenome sequencing (mWGS) enables for the comprehensive taxonomic and functional characterisation of cyanobacterial bloom events. To analyse the pervasiveness of cyanobacterial bloom formation in WWTPs the microbiome of both surface water and sediment from three treatment ponds was characterised over a five year period (2016-2020). Taxonomic identification on unassembled reads was conducted using KRAKEN2. The metaWRAP pipeline was used for metagenome-assembled genome construction, binning and taxonomic identification. mWGS analysis revealed that the recurrent cyanobacterial blooms were cosmopolitan, comprised of Oscillatoriales (17%), Synechococcales (21%) and Nostocales (39%). This differs considerably from previous amplicon analysis from the same time period which indicated that blooms were predominantly *Microcystis*. Mapping of the metagenomic reads against the COG database was used to assess which cellular processes are enriched across seasons and during bloom events.

Poster 63

Harmful algal blooms of the cyanobacterium Microcystis cause deleterious effects in aquatic organisms: Implications for freshwater biodiversity conservation

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Anthropogenically driven harmful algal blooms (HABs) continue to increase in frequency and intensity in the Great Lakes. Risks to human health from HABs drives much of the concern that motivates efforts to manage their causes. However, emerging evidence indicates that HAB-associated toxins, such as microcystins, produced by the cyanobacterium *Microcystis*, can be toxic to aquatic organisms, especially at sublethal concentrations. Whether this poses a threat to aquatic food webs and freshwater biodiversity is unclear, but understanding the risks associated with sublethal exposures requires toxicological assessment at concentrations commensurate with those typically detected in the field. Herein, we present the results of some of our recent studies assessing the toxicity of microcystins produced by a toxigenic strain of *Microcystis aeruginosa* on key functional traits (survival, growth, reproduction) of zooplankton (*Daphnia magna*) and fish (Rainbow Trout, and Lake Trout). We further investigated molecular-level impacts using nontargeted proteomics to link sublethal toxicity with adverse outcomes occurring within these organisms. Our results demonstrate that microcystins at naturally occurring concentrations induced sublethal toxicity in all three organisms, including growth and reproductive impairment, oxidative stress, and carcinogenesis. Taken together, our results demonstrate that *Microcystis* blooms have the potential to cause deleterious health effects in wildlife.

Session 6: IFHAB Special Session – Research from Temperate to Polar Regions

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Seasonal dynamics of cyanobacteria and cyanotoxins during 24 h diurnal cycle sampling in a eutrophic lake in Northeastern Brazil

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Due to its semiarid climate the Ceara state (Northwestern Brazil) presents optimal conditions for cyanobacteria growth. Since a fatal incident caused by microcystin exposure (Caruaru Tragedy), studies on environmental mechanisms that promote cyanotoxin production have been carried out. This study was performed at Castanhao reservoir, the largest multi-use reservoir of Latin America. Samples were collected regularly each 6 hours (S1, S2, S3 and S4) in a 24 h diurnal cycle sampling during the dry season close to the dam (CTN-20), and a fish-rearing site (CTN-100). Cell counts, classifications, and Elisa tests were carried out. Previous results show cyanobacteria predominance, mainly *Raphidiopsis raciborskii* at S1 (9:30 am) with poor biological diversity at both points, although at CTN-100 cyanobacteria concentrations were around 24 times higher than at CTN-20, microcystins and saxitoxins were not observed at either point for S1. However, a greater cyanobacterial biodiversity was observed microcystins concentrations increased as well. Previous results imply that an increase in cyanobacteria diversity promotes cyanotoxins production, which suggests that allelopathy should be explored. Moreover, environmental patterns can potentially favor *Raphidiopsis raciborskii* displacement to deeper layers, in terms of water management; it means the best moment to pumping from intake point to the water treatment plants.

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Wisconsin's Harmful Algal Bloom Program: Public Health & Environmental Management Partnership

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Wisconsin's Harmful Algal Bloom (HAB) Program is a partnership between the Wisconsin Department of Health Services (WDHS) and the Wisconsin Department of Natural Resources (WDNR). This interagency effort will be illustrated, with a focus on collaborative response to HAB-related illnesses, tracking bloom reports, and outreach efforts. WDHS leads capacity building and outreach to tribal and local health agencies responding to HAB issues within their jurisdictions. WDNR leads HAB tracking and documentation efforts using reports from the public to WDNR and WDHS, and remotely sensed data from the Cyanobacteria Assessment Network. Together, the agencies investigate human and animal illnesses that are potentially related to cyanobacterial exposure. WDHS receives illness complaints, administers a standardized questionnaire, and evaluates reported symptoms and health history. WDNR evaluates reported environmental conditions and satellite imagery and collects samples if possible. Testing results are jointly evaluated and conveyed to tribal and local authorities with recommendations and resources such as standardized signage for beach closures. Additional collaborative outreach efforts, such as presentations at state lake conferences, joint news releases, and HAB season webinars will be highlighted.

Interactions between CHABs and other organisms (phycosphere, trophic interactions, pro-euk interactions, viruses, fungi)

Poster 66

Insight into functional and toxicological diversity of cyanobacterial mat communities in the Eel River

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Toxic benthic cyanobacteria have started to attract attention after long neglect due to increased reports of their potential for secondary metabolites production. Particularly, this includes potent neurotoxins in the anatoxin class. Cyanobacteria commonly grow on the surfaces on riverbeds and these biofilms consist of assemblages of cyanobacteria and other microorganisms. So far, the vast majority of studies have focused solely on the cyanobacterial component, neglecting the diversity of the whole microbial assemblage and their interactions. We sampled cyanobacterial mats from the Eel River and resolved their community composition using genome-resolved metagenomics. The analyses revealed the regular presence of a range of heterotopic and viral components alongside dominant cyanobacterial taxa. Cyanobacterial genomes often encoded biosynthetic gene clusters of known toxins, as well as other metabolites belonging to different secondary metabolite classes. Several biosynthetic gene clusters were found across examined cyanobacterial genomes, and the presence of specific heterotrophic components. Our findings raise the importance of examining and characterizing the full microbial community that exists within cyanobacterial mats to understand when cyanobacteria possess toxic gene clusters and how that is correlated with the presence of surrounding microbial assemblage.

Poster 67

Genome sequences of bacteria closely associated with the dominant Nostocales cyanobacteria of recent HABs in the US Pacific Northwest

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We have assembled over a dozen genomes of bacteria that remained attached after filtration and/or washing to cyanobacterial material derived from recent blooms in freshwater lakes in the US Pacific NW. PacBio and Illumina metagenomes were generated from samples, and when sufficient sequencing reads from heterotrophic bacteria were present to allow genome assembly, those reads typically represented one or at most three distinct bacteria, whose reads were present at levels lower than those of the cyanobacterium. We interpret these genomes as representing phycosphere bacteria. The taxonomic groups represented were diverse, including alphaproteobacteria, betaproteobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Gemmatimonadetes and Vampirovibrionales. These were associated with a range of Nostocales ADA clade cyanobacteria. Typically, there were no close relatives of the new genomes in the genome or 16S rRNA database. In addition to these, a dominant cyanobacterium genome, *Dolichospermum* sp. DET69, was assembled from 5 samples from Detroit Reservoir, Oregon, taken across 4 years. From each sample, a near-identical *Opitutus* sp. genome was assembled. This suggests a recurring and specific interaction, perhaps one that survives the boom/bust cycle of over-wintering, after which an emerging bloom arises from a few surviving filaments or from newly germinated akinetes.

Poster 68

Impact of seasonality on the phycosphere response to reactive oxygen stress during cyanobacterial blooms

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Hydrogen peroxide is constantly produced by photo-oxidation in freshwater systems plagued by *Microcystis* blooms. However, unlike other cyanobacteria and aquatic microorganisms, *Microcystis* genomes do not contain catalase. Other cyanobacteria without catalase, such as *Prochlorococcus*, are known to benefit from the hydrogen peroxide degrading enzymes produced by heterotrophic bacteria. It has been shown previously that *Microcystis* may also benefit similarly from its phycosphere, improving the ability to tolerate hydrogen peroxide in the environment. Seasonality is known to shape the makeup of the phycosphere across marine and freshwater systems, and therefore its functional impact on the *Microcystis* host. In this study, we examined the effect of seasonality and bloom stage on the functional response of both *Microcystis* and the phycosphere when treated with 1 µM and 10 µM concentrations of hydrogen peroxide in field and synthetic bloom communities. Metatranscriptomic analysis was paired with growth metrics to determine the community composition of the phycosphere as well as the changes in gene expression for both the *Microcystis* and the bacterial community at different stages of bloom development. Analyzing the expression of genes related to hydrogen peroxide degradation continues to refine the interactome between *Microcystis* and its microbiome in the phycosphere.

Poster 69 – Moved to a platform presentation, 10:15 Thursday morning

Associations between microbiome composition and microcystin levels in the 2018 Lake Erie HABs Grab

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Annual cyanobacterial harmful algal blooms (cHABs) threaten the well-being of western Lake Erie and the surrounding region. To resolve an entire bloom at unprecedently high spatial resolution, researchers coordinated a single-day survey of the Western Lake Erie cHAB ('HABs Grab'; August 9, 2018). Amplicon sequencing of the V4 region of the 16S rRNA gene was performed on 100 samples to determine how the microbial community varied spatially and in relation to environmental conditions within the bloom. While wide variation in community alpha diversity was observed in and out of the bloom, out-of-bloom communities had higher average evenness. Despite Microcystaceae being the dominant cyanobacterial family, Nostocaceae, Synechococcaceae and Pseudanabaenaceae were present in the bloom as well, while diatoms (mainly *Aulacoseira*) and cryptomonads (*Teleaulax*) were abundant both in and out of bloom. Common bloom-associated heterotrophic bacterial groups Flavobacteriia (Cryomorphaceae, Flavobacteriaceae), Planctomycetia (Planctomycetaceae), and Sphingobacteriia (Chitinophagaceae, Saprospiraceae) vary throughout the dataset, in association with nutrient concentrations, algal groups and toxin detections. Environmental and biotic drivers of toxin-producing cyanobacteria are explored to improve our understanding of how the interplay between algae and their microbiomes contributes to bloom composition and toxin production in Lake Erie.

Toxic Cyanobacteria in the Context of Climate Change

Poster 70

Environmental factors influencing CyanoHABS in climate change scenario: spatio-temporal analysis in Brazilian semi-arid reservoirs

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Several environmental factors have been shown to promote cyanobacteria in semi-arid regions, including high temperature, long photoperiods, alkaline pH, and nutrient pollution. Furthermore, global warming trends are particularly intensified in these regions, with droughts becoming longer and more severe due to higher evaporation and lower precipitation volume. The goal of this study was through canonical correspondence analysis that incorporated environmental parameters (biotic and abiotic components) to prognosticate blooms of cyanobacteria and cyanotoxins in 20 drinking water reservoirs (2,489 samples) in the semi-arid region of Ceará, Brazil. Across four years, 114 different phytoplankton taxa were identified; however these reservoirs were dominated by cyanobacteria (24 taxa), interestingly, specific cyanobacterial taxa were influenced by different biotic and abiotic factors. For example, nitrogen-to-phosphorus (N:P) and evaporation were positively related to saxitoxin-producing taxa, especially *Raphidiopsis raciborskii*, while temperature, electrical conductivity, total phosphorus, and transparency were positively associated with microcystin-producing taxa, such as *Microcystis aeruginosa*. Climate forecasts predict higher evaporation and temperatures in the semi-arid Ceará region, which will likely magnify droughts and water scarcity as well as promote toxic cyanobacterial blooms in reservoirs in the future. Therefore, understanding the factors associated with algal blooms dominated by specific taxa is paramount for water resource management.

Poster 71

Effects of temperature variation on the plasticity of carbon fixation in Microcystis

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Microcystis aeruginosa PCC7806 is a bloom forming cyanobacteria, distributed globally in inland water. M. aeruginosa produces potent hepatotoxin microcystin. In eukaryotes, microcystin inhibits a class of enzymes known as protein phosphatases, especially PP-1 and PP-2A, leading to liver toxicity and tumour formation. Earlier studies have suggested both in-vivo and in-vitro binding of microcystin to specific proteins from *M. aeruginosa* such as the key enzyme of the Calvin-Benson cycle, RubisCO. High cell density, high light conditions as well as oxidative stress were shown to promote binding of the toxin to proteins. However, the effect of temperature on microcystin binding to proteins and carbon metabolism is still unknown. Recently *M. aeruginosa* cells were adjusted to low light conditions and subsequently exposed to varying temperature ranging from 20°C-35°C. We have observed striking differences in the dynamics of microcystin protein binding as well as secretion of microcystin. Furthermore, immunofluorescence microscopic analysis of subcellular localization of RubisCO and the carboxysome shell protein CcmK revealed pronounced variations at different temperatures and enhanced bio-condensate formation of carboxysomes at higher temperature when compared to its normal distribution in cytosol at low temperature. Our experiments suggest a substantial influence of elevated temperature on microcystin dynamics and carbon fixation and provide insights into the possible role of microcystin in adaptation to changing climatic conditions.

Poster 72

Acceleration of Phosphorus Flux from Anoxic Sediments in a Warming Lake Erie

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Harmful Algal Blooms (HABs) have been detrimental to the health of the Western Basin of Lake Erie (WBLE) and the safety of drinking water for surrounding communities for over a decade. Nutrient inputs have exacerbated these problems by promoting HAB growth. While external nutrient loading is being addressed, more needs to be discovered about the potential for internal loading from lake sediments. Anoxic sediments release phosphorus into overlying waters at increasing rates that correlate with increasing temperature, particularly between 20 °C and 30 °C. (Gibbons and Bridgeman, 2020). However, the trajectory of the increase in release is undetermined. In this study, sediment cores were collected and incubated in anoxic conditions at varying temperatures within the range of 20 °C and 30 °C. Results from these incubations showed the largest increase in phosphorus flux for site 4P occurred between the temperatures of 26 °C and 29 °C (average concentration increase of 100.9 µg/L). However, site 7M showed the largest increase in phosphorus flux between 23 °C and 26 °C (average concentration increase of 252.4 µg/L). Such a large flux in this temperature range is alarming as lake bottom temperatures already exceed 23 °C and are likely to increase in the future.

Poster 73

Increasing diversity of *Planktothrix rubescens* populations as a consequence of eutrophication and climate change

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Planktothrix rubescens forms dense blooms in the metalimnion of oligo- and mesotrophic lakes. This vertical position represents a compromise between the limited light penetrating through the epilimnion and the high nutrient concentrations associated with the hypolimnion. In recent years, phosphorous release from sediments has strongly fertilized Lake Stechlin internally, to a state where light in the metalimnion is extremely limited. Rather than decreasing, *Planktothrix* blooms have intensified and taken advantage of milder winters, dominating the nutrient enriched upper mixed water column. We sought to understand through metagenome sequencing how *Planktothrix* populations in Lake Stechlin have developed over the last decade. We compared monthly samples taken from the epilimnion and hypolimnion, commencing in 2011, with fortnightly epilimnic and metalimnic samples during the 2020 *Planktothrix* bloom. We identified a single *Planktothrix* species within the lake over the 10-year-period, however, detected temporally variable sub-populations. Interestingly, initial diversity of the *Planktothrix* populations in the epi- and hypolimnion was low and increased during sustained following bloom events in 2016 and 2020. These findings show a link between environmental change, (increased stratification and reduced ice cover) and the diversity of cyanobacterial populations suggesting that cyanobacteria will continue to expand their spatio-temporal range as global change intensifies.

Risk, prevention, management, and mitigation of CHABs: applied applications from establishing risk to remediation

Poster 74

Calcium peroxide (CaO2) granules enclosed in textile materials as H2O2 delivery systems to mitigate Microcystis sp. blooms

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The past years, hydrogen peroxide (H_2O_2) has been introduced as an environmentally friendly method to combat *in situ* toxic cyanobacteria blooms because of its selective oxidation and zero waste production. Calcium peroxide (CaO_2) granules are an alternative to liquid H_2O_2 due to their slow H_2O_2 release properties. Herein, concentrations of 0.5, 1.0, and 2.0 g/L CaO_2 granules were (a) added into a surface water matrix to investigate their H_2O_2 releasing properties, (b) enclosed in four types of textile materials as delivery systems to evaluate their overall oxidant releasing capacity and (c) the best ones were applied on a dense *Microcystis* sp. bloom to investigate their potential to combat cyanobacteria. No difference was observed between the maximum H_2O_2 concentrations of the direct application of granules and the fabric delivery systems of types A – C, (released up to 12 mg/L H_2O_2 for 2.0 g/L CaO_2). Fabric system type D had the lowest H_2O_2 release (2.0 mg/L). Treatment experiments showed that delivery system B with 2 g/L granules and type C of 1 g/L and 2g/L were sufficient to significantly reduce the photosynthetic activity of *Microcystis*, proving that these delivery systems have the potential to become a more environmentally friendly alternative to H_2O_2.

Poster 75

Aerosolized microcystin-LR exacerbates chemokines and other inflammatory mediators of asthma in asthmatic primary human airway epithelium

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Microcystin-LR, one of the most abundant and toxic HAB-derived cyanotoxins, has recently been detected in aerosols from HAB water. We previously reported that aerosol MC-LR exposure has a pro-inflammatory influence on the airways. Asthma, which is an extremely prevalent airway disease afflicting approximately 8% of the U.S. population, is largely driven by inflammation. However, the impact of MC-LR aerosol exposure on this at-risk population is unknown. In this study, a 3D primary human airway epithelium model was utilized, in which cells were isolated from healthy and asthmatic donors. An environmentally relevant concentration of MC-LR (1 μM) was aerosolized and delivered to the cell surface, before the cells were harvested for transcriptome analysis. Strikingly, 10% of the genes upregulated ($\log_2 FC > 0.25$) by asthma alone, were further upregulated by MC-LR exposure including inflammation mediators, such as *CXCL11* ($\log_2 FC = 0.63$); and *TLR4* ($\log_2 FC = 0.31$). These genes had significant associations with pathways, such as “immune cytokine binding” (FDR = 0.015). This study showed that aerosolized MC-LR amplifies the transcriptional differences between asthmatic and healthy donor airway epithelial cells, leading to the exacerbation of inflammatory mediators of asthma, such as chemokines, suggesting a potential for MC-LR exposure to worsen asthma severity.

Poster 76

Identifying Bacterial-Derived Compounds with Efficacy for Controlling Cyanobacteria

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Cyanobacterial harmful algal blooms (cyanoHABs) can negatively impact freshwater systems by producing taste and odor compounds, reducing dissolved oxygen leading to anoxia, and releasing cyanotoxins. Thus, there is a need to develop and improve management techniques for controlling cyanoHABs. Both short- and long-term mitigation strategies are needed for management and exploiting bacterial-derived compounds for targeted chemical control is promising. In this study we screened commercially available, biologically derived compounds tyrptoline, tryptamine, isatin, sedoheptulose, and ginsenoside against *Microcystis aeruginosa* UTEX 2385 and *M. aeruginosa* BLCC-F188 in a cyanobacteria lawn plate and a liquid assay study. The compounds that inhibited *Microcystis* growth most effectively were then subjected to screening of higher trophic level species such as *Ceriodaphnia dubia* to see how these chemicals affect non-target species. Additionally, 70 bacteria were isolated from bloom events to evaluate cyanocidal activity, and two isolates (*Pseudomonas* sp. BLCC-B42 and *Sphingomonas* sp. BLCC-B43) showed at least a 30% reduction in algal biomass in co-culture experiments, while the supernatant of *Sphingomonas* sp. BLCC-B43 led to a 37% reduction of biomass after 72 hours. Future work will be focused on characterizing and isolating secondary compounds which are secreted by bacteria via genome mining for potential clusters and mass spectrometry.

Poster 77

Improving conditions for nutrient-transforming microbial communities in multi-zoned biofilters to help prevent eutrophication of downstream reservoirs

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Sequential sedimentation-biofiltration systems (SSBSs) are nature-based solutions that aid in the removal of pollutants carried by urban stormwater runoff. They are multi-zoned biofilters constructed within the structure of urban rivers, providing water treatment, and preventing anthropogenic eutrophication with the appearance of CyanoHABs in downstream waterbodies. Microbial communities play an important role in the cycling of nutrients in natural environments. Therefore, we investigated the dynamics of microbial metabolic activity, the abundance of nitrifying (gene *amo*A), and denitrifying bacteria (*nos*Z) in SSBSs. Furthermore, bacterial populations were characterized in zones containing biofilm (16S rRNA), and bacteria were isolated from sediments and tested for their metabolic properties. Results indicated that microbial metabolic activity was higher in summer. The geochemical zone -containing limestone- showed the highest abundance of nitrifiers in spring. The denitrifying zone -with brown coal- presented the highest abundance of denitrifiers in summer. Two isolated strains -*Citrobacter freundii* Bzr02 and *Pseudomonas mandelii* Str21- showed biotechnological potential in removal of nitrogen compounds during laboratory assays. These results suggested future modifications in the construction of SSBSs that could enhance the abundance and activity of microbial communities, to improve their efficiency in nutrient removal and mitigate CyanoHABs.

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Poster 78

Management of cyanobacterial blooms in a shallow recreational reservoir

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This case represents a unique opportunity to combine efforts from academia, environmental companies, and public water managers to address a case of massive cyanobacterial blooms. Foix Reservoir (NE of Spain) is a hypereutrophic and shallow (Z_{max}=7.5 m) reservoir where chlorophyll-*a* concentrations reach up to 140 µg/L, dominated by potentially toxic species such as *Microcystis aeruginosa* and *Planktothrix agardhii*. A detailed study and management proposal were developed based on a modelling effort including meteorological, physicochemical, hydrological, and biological variables and their influences on cyanobacterial blooms (field data 2009-2020), along with an in-depth review of management experiences where similar systems have been restored.

Results indicate that, besides the hypereutrophic conditions, high water temperatures and residence times enhance cyanobacterial dominance. The most cost-effective preventive measures were found to be the wastewater treatment plant upgrading, or the diversion of treated wastewater, combined with reservoir dredging. The management of *Lemna* to reduce the internal nutrient load and light availability is also suggested. The application of hydrogen peroxide is proposed as a mitigation measure to quickly alleviate a developing bloom. This work, which is expected to be applied in the coming years, could serve as a roadmap for water managers when facing this increasingly severe issue.

Poster 79

Harmful Algal Bloom Impacts on Human Health: An Analysis of National Emergency Department Data in the U.S. from 2016 to 2018

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Numerous harmful algal bloom (HAB) cyanobacterial species produce toxins that disrupt ecosystems and are harmful to both human and animal health. We sought to determine trends and patterns in diagnostic codes relating to HAB exposures from the Healthcare Cost and Utilization Project's (HCUP) Nationwide Emergency Department Sample (NEDS). We analyzed years 2016 to 2018 as these represented the years in which complete data was available using the World Health Organization (WHO) International Classification of Diseases-10 diagnosis codes for HAB exposure. For each year's grouping, statistical analysis was performed to uncover patterns and trends. Each patient occurrence was screened for the most prevalent comorbidities associated with HAB exposures. Over the 3-year period studied, there were 118 reported patient admissions to the Emergency Department. Respiratory related illness accounted for the majority of comorbidities and were present in 53% of patients, including 30% as the primary diagnostic code. These data represent one of the first attempts to analyze HAB exposure related illness presenting to Emergency Departments in the United States. The predominance of respiratory related diagnostic codes in these patients suggests greater attention to these conditions in the risk characterization of HAB exposure in the development of evidence-based prevention and treatment strategies. Harmful Algal Bloom Impacts on Human Health: An Analysis of National Emergency Department Data in the U.S. from 2016 to 2018.

Poster 80

Investigation of novel chemical in-situ treatment methods to mitigate cyanobacteria harmful algal blooms in surface waters

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As water scarcity is becoming a universal problem to address, it is essential to maintain a high surface water quality and protect the biodiversity of current aquatic biotopes worldwide. Surface water contamination events caused by high nutrient and organic matter loads lead to the formation of cyano-HABs, which are mostly unpredictable. Therefore, highly efficient methods are required for the *in-situ* restoration of water quality. Several methods have been applied over the years to mitigate cyano-HABs with varying efficiencies. Current trends ask for more environmentally friendly approaches to be implemented in order to safeguard the overall aquatic ecosystems and reduce treatment costs. Hydrogen peroxide is currently being used as an emerging chemical alternative to copper algicides. Even though its broad application showed high efficiency on mitigating dense blooms, the required H_2O_2 doses for each case and treatment application differ, and in some instances high doses were applied with undesirable side effects on the entire lake ecosystem. Herein, calcium peroxide granules were utilized due to their slow H_2O_2 releasing properties, which simulate multiple liquid H_2O_2 doses. Specifically, granules were used in surface waters spiked with *Microcystis*, *Aphanizomenon*, and *Cylindrospermopsis* sp. and in actual water samples during blooming.

Poster 81

Monitoring of Athalassa’s Lake: Fish kills event, cyano-HABs bloom and treatment with hydrogen peroxide and hydrogen peroxide releasing granules

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Surface water monitoring is essential for maintaining a healthy status and protect the biodiversity of aquatic biotopes. Unfortunate events of high nutrient load, organic matter input, and swage run-offs that lead to the formation of cyano-HABs are mostly unpredictable and thus highly efficient methods are required to be applied for the *in-situ* restoration. During the monitoring (2020-2021), a massive fish kills event was occurred, which followed by a blooming period, that raised many questions regarding the source of toxicity in the lake. Water samples were analyzed for cyanobacteria and toxin producing genes presence, as well as for their physicochemical water characteristics (nutrients, COD, pH, conductivity, dissolved solids, instantaneous fluorescence and quantum yield). In order to find effective, cost efficient, and environmentally friendly mitigation techniques, water was collected during the blooming periods and utilized in treatment experiments with liquid hydrogen peroxide, (single and multiple H_2O_2 doses), and calcium peroxide granules (CaO_2). Multiple doses of liquid H_2O_2 proved to be more efficient and had long-lasting effects in contrast with the single dose of liquid H_2O_2, which was consumed rapidly after the addition. CaO_2 granules were efficient to treat the bloom by gradually releasing into the water H_2O_2, simulating multiple additions of H_2O_2.

Poster 82

Water Color: Inspiring Conservation of Freshwater Ecosystems by Painting With Algae

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Science is one of the best ways to explore and understand the natural world, but many significant findings remain too intimidating for people without background knowledge to access. One way to get data out to the public is by utilizing the emotive strengths of art. This project aims to use watercolor painting as the mode of communication for the science behind the causes, effects, and solutions of harmful algal blooms in freshwater ecosystems. A novel aspect of the project is the development and use of algae paint to clearly depict relationships between what people do (even when those actions are far removed from visible water), how that impacts the environment and human populations, and how those actions can be altered to promote a better future.

Poster 83

Beyond toxic cyanobacteria: Increased Legionella, antibiotic resistance genes and disinfection-byproducts in western Lake Erie water

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Western Lake Erie has chronic cyanobacterial blooms and the need to understand emerging public health issues is paramount. This study focused on disinfection byproducts, and dynamics of *Legionella* and antibiotic resistance genes (ARGs) and their associations with *Microcystis* blooms. For this, weekly source water and finished water were obtained from two drinking water plants in western (bloom) and central Lake Erie (control) for two years. The bloom site showed significantly higher levels of microcystin (MC)-producing *Microcystis* and MCs in the source water than the control site ($p<0.05$). In the finished water, the mean total trihalomethanes concentrations from the bloom site (22.4±12.6 µg/L) was significantly higher than the control site (14.0±3.5 µg/L) ($p<0.05$). Interestingly, *Legionella* spp. concentrations were significantly higher from the bloom site than the control site ($p<0.05$) and a significant relationship between total cyanobacteria and *Legionella* spp. was observed in the bloom site ($F=89.82$, $p=0.001$), but not in the control site ($F=0.31$, $p=0.5814$). The abundance ARGs and mobile genetic elements were significantly higher from the bloom sites. Our results provide new insights into emerging environmental and health impact, beyond the bloom toxicity. Co-exposure of cyanotoxin together with *Legionella* as well as ARG-related risks should be considered in future studies.

Poster 84

Novel approach to controlling toxic cyanobacteria: effect of 365 nm UV-A LED irradiation on six Microcystis aeruginosa strains and their eleven associated microcystins

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Cyanobacterial blooms pose a threat to human and animal health due to the presence of cyanotoxins and challenge potable water treatment worldwide. Retrofitting water treatment plants is impractical, thus, alternative mitigation strategies such as in-reservoir treatment are needed.

Traditionally, UV-C (250 nm) is considered germicidal, however, UV-C treatment cannot be applied *in situ* because it is non-selective and would harm other biota. In a previous study (https://doi.org/10.1016/j.jenvman.2021.113519), UV-A 365 nm irradiation successfully removed *Microcystis aeruginosa* PCC 7813 cells and its four associated microcystins. To explore the suitability of this approach for *in situ* treatment of cyanobacteria and toxins, six geographically diverse *Microcystis aeruginosa* strains (SCIENTO, NIES 1099, B2666, PCC 7820, 7813 and 7806) and their eleven associated microcystins were exposed to 365 nm UV-A LED irradiation for seven days. UV-A 365 nm LED irradiation significantly decreased photosynthetic activity after only 24 hours of irradiation. Intra- and extracellular microcystin concentrations were markedly decreased in UV-A treated samples with a combined microcystin removal of 86%. Microcystins removal was analogue dependent and varied from 9% removal for MC-YR to complete degradation of MC-WR. UV-A irradiation was demonstrated to be an effective approach in the control of *Microcystis aeruginosa* and associated toxins.

Poster 85

U.S. Army Corps of Engineers Freshwater Harmful Algal Bloom Research & Development Initiative

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Freshwater Harmful Algal Blooms (HABs) are particularly impactful to the U.S. Army Corps of Engineers (USACE), which manages vast freshwater resources and waterways that provide a variety of services including navigation, flood risk reduction, recreation, fish and wildlife management, as well as potable water supply. The Water Resources Development Act of 2018 (WRDA 2018) authorized the U.S. Army Engineer Research Development Center (USACE-ERDC) to implement a 5-year technology demonstration program to deliver scalable technologies for HAB prevention, detection and management that will reduce HAB frequency and effects to our nation’s freshwater resources across scales (e.g. small waterbodies to river reaches), ecoregions (e.g. subtropical Florida to temperate Ohio and New York), and system types (e.g. reservoirs, riverine, lakes). The USACE-ERDC HAB Research & Development (R&D) portfolio features a range of HAB-combatting methods, models, and technologies that may be used alone or in combination to effectively reduce HAB frequency and impacts to water resource development projects across the nation. An overview of USACE-ERDC sponsored HAB R&D projects will highlight the range of HAB methods, models, and technologies in development, and will provide an opportunity to engage with federal, state, local, and university partners.

Poster 86

Algicidal effects of *Morganella morganii* on different bloom-forming cyanobacteria in freshwater environments

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Cyanobacterial harmful algal blooms (cHABs) are a serious problem at a global scale. Among the different mitigation strategies, biological control by algicidal bacteria is receiving increasing attention. In this study we isolated a bacterial strain with algicidal properties from a toxigenic *Microcystis* spp. bloom occurred in a lowland reservoir in Poland (Central Europe). Analysis of the 16S rRNA gene identified the strain as *Morganella morganii* (99.51% similarity). The ability of this bacterium to lyse different bloom-forming cyanobacterial strains including, among others, the genera *Microcystis* and *Aphanizomenon*, both axenic and environmental, as well as toxigenic and non-toxigenic, was characterized. Microscopic cell counting and qPCR results showed that *M. morganii* killed the tested cyanobacterial strains after three days of co-culture. Interestingly, the ultra-high-performance liquid chromatography-tandem mass spectrometer (UHPLC-MS/MS) analyses unravelled that this bacterium was capable of producing several compounds with algicidal potential. This is the first study that demonstrates the algicidal properties of *M. morganii*, providing new insights into how these ubiquitous bacteria interact with bloom-forming cyanobacteria in nature. Therefore, this knowledge is the basis for further development of nature-based solutions that include algicidal microorganisms to regulate the presence of cHABs in freshwater.

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Poster 87

The role of cyanobacteria in aquatic resistome

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The aquatic environment is a reservoir of antibiotics, pathogenic and native microorganisms, and antibiotic resistance (AR) genes, constituting a key compartment in the One Health approach. Thus, the problem of AR is no longer restricted to bacterial pathogens, but is a consequence of the interplay between distinct clinical and environmental drivers. In the project "ExplorAR – Exploring the Aquatic Resistome", we have been investigating the putative role of cyanobacteria (CB) in freshwater resistome, using a multidisciplinary approach, based on phenotypic/genotypic/genomic tools. In this presentation we disclose the main project outputs: i) development of a microdilution-based antibiotic susceptibility assay for CB; ii) establishment of antibiotic susceptibility profile of CB genus/species; iii) identification of CB strains with reduced susceptibility to multiple antibiotic classes; iv) identification of AR genes in CB genomes. Presently, we are profiling AR of CB-associated bacteria and mapping the occurrence of CB and AR genes in surface freshwater reservoirs by HTS approaches. Overall, ExplorAR strongly supports the hypothesis that CB are environmental players in AR emergence and dissemination in water environments, contributing to the AR problematic.

Work supported by the project "ExplorAR_Exploring the Aquatic Resistome" (PTDC/BIABMA/31451/2017), funded by Portuguese Foundation for Science and Technology (FCT/MCTES), through national funds (PIDDAC).

Poster 88 – Moved to a talk, 1645 WEDNESDAY

Understanding HABs and the Florida Department of Health Role: Notification, Response, Resources and Research in the State of Florida

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Harmful algal blooms (HABs) are a regular occurrence in Florida, and with predictions indicating that their frequency and intensity may increase over time, understanding and communicating the risks of these events are important to the protection of public health. Florida boasts a year-round climate of warmth and sunshine, which – when combined with the state's numerous marine and freshwater habitats and harmful algae species – can make for abundant and potentially toxic blooms. In 2016 and 2018, Florida experienced large-scale, concurrent episodes of cyanobacteria and *Karenia brevis* red tide. The environmental and economic consequences prompted the development of a state-wide, inter-agency collaborative network for routine waterbody surveillance, testing, and public notification of blooms. The Florida Department of Health has been tasked with disseminating the resulting information since 2019 in an effort to minimize human and animal exposures. This presentation will cover the Florida Department of Health's ongoing progress in streamlining its public notification protocol, developing trainings and resource materials for general and targeted audiences such as healthcare practitioners, and contributing to the overall body of HAB research through state funding for the discovery of long-term implications on human health.

Poster 89

Degradation of Multiple Peptides by Microcystin-Degrader Paucibacter toxinivorans (2C20)

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Since conventional drinking water treatments applied in different countries are inefficient at eliminating potentially toxic cyanobacterial peptides, a number of bacteria have been studied as an alternative to biological filters for the removal of microcystins (MCs). Here, we evaluated the degradation of not only MCs variants (-LR/DM-LR/-RR/-LF/-YR), but also non-MCs peptides (anabaenopeptins A/B, aerucyclamides A/D) by Paucibactertoxinivorans over 7 days. We also evaluated the degradation rate of MC-LR in a peptide mix, with all peptides tested, and in the presence of M. aeruginosa crude extract. Furthermore, biodegradation was assessed for non-cyanobacterial peptides with different chemical structures, such as cyclosporin A, (Glu1)-fibrinopeptide-B, leucine-enkephalin, and oxytocin. When cyanopeptides were individually added, P. toxinivorans degraded them (99%) over 7 days, except for MC-LR and -RR, which decreased by about 85 and 90%, respectively. The degradation rate of MC-LR decreased in the peptide mix compared to an individual compound, however, in the presence of the Microcystis extract, it was degraded considerably faster (3 days). It was noted that biodegradation rates decreased in the mix for all MCs while non-MCs peptides were immediately degraded. UPLC–QTOF–MS/MS allowed us to identify two linear biodegradation products for MC-LR and MC-YR, and one for MC-LF. Furthermore, P. toxinivorans demonstrated complete degradation of non-cyanobacterial peptides, with the exception of oxytocin, where around 50% remained after 7 days. Thus, although P. toxinivorans was previously identified as a MC-degrader, it also degrades a wide range of peptides under a range of conditions, which could be optimized as a potential biological tool for water treatment.

Poster 90

Effect of hydrogen peroxide on a natural phytoplankton community focusing on cyanobacteria, evaluating its recovery and resistance

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As cyanobacteria are established into the water, different strategies are used for mitigating that, such as oxidation processes mediated by H_2O_2. Several authors have described them as more sensitive than Green Algae (GA) to this treatment but few studies have assessed the recovery of planktonic communities and the potential mechanisms of resistance after H_2O_2 exposure. Here, we tested the effect of different concentrations of H_2O_2 (1, 10 and 100mg.L^{-1}) on the survival of a phytoplankton community from a freshwater reservoir co-dominated by *M. aeruginosa* and *R. raciborskii*, as well as both in isolated cultures. Before H_2O_2, cyanobacteria chlorophyll(Chl) was 29 µg.L^{-1} and it contributed to most of the Chl compared to GA (14 µg.L^{-1}). After 48h, total Chl decreased in all conditions, although cyanobacteria dominated the 10mg.L^{-1} treatment (59%). In the 100mg.L^{-1} treatment, Chl decreased to 1.9 µg.L^{-1} with dominance of GA (86%). After 7 days, only GA were detected in the conditions, except 100mg.L^{-1}. Evaluating the community recovery, after the 7th-day, ASM-1 was added and only GA were able to grow in all the conditions, except 100 mg.L^{-1}. We are evaluating the expression of antioxidant enzymes as well as the influence of environmental contaminants on the efficiency of H_2O_2 for suppressing cyanobacteria.

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Keywords:

Cyanobacteria, Green algae, Oxidation, Mitigation, Environmental Contamination

Poster 91

Invoking systems theory to drive system regime change away from cyanobacteria dominance and prevent HABs

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Ecosystem behavior and regime change are governed by Systems Theory principles and processes. Cottingham et al have demonstrated and modeled this.

Cottingham, K. L., Ewing, H. A., Greer, M. L., Carey, C. C., & Weathers, K. C. (2015). Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. Ecosphere, 6(1), 1-19.)

Consistent, holistic and sustainable prevention of cyanobacteria dominance and HAB events and reversal of eutrophication has been demonstrated in a variety of water bodies ranging from 60 acre lakes averaging 15 feet deep to 850 acre reservoirs over 170 feet deep, using interventions developed in alignment with Systems Theory.

Poster 92

Managing Sediment Nutrient Recycling by Digesting and Reducing Organic Sediment

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Recycling of sediment nutrient stockpiles drive system regime change, facilitating cyanobacteria domination and succession

(Cottingham, K. L., Ewing, H. A., Greer, M. L., Carey, C. C., & Weathers, K. C. (2015). Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. Ecosphere, 6(1), 1-19.)

Digestion and elimination of organic sediments at the rate of over 1 000m3 per acre per annum have proven to be a key factor in reversing the system regime change and preventing cyanobacteria HABs

High resolution bathymetric scans quantify this sediment reduction. Phycological profiling and Dept Health monitoring shows prevention of cyanobacteria HABs.

Poster 93

Evaluation of pre-oxidation drinking water treatments for the removal of cyanotoxins produced by Microcystis aeruginosa

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Occurrence of cyanobacterial blooms in freshwaters is increasing worldwide, affecting their water quality and ecosystems. Since these blooming events may be associated with the presence of cyanotoxins, their occurrence in freshwater reservoirs intended for drinking purpose may pose a serious threat for human health. In Italy, pre-oxidation of raw water with oxidants containing chlorine is a common practice within drinking plants to inactivate harmful organisms as Cyanobacteria, nevertheless their efficiency towards cyanotoxins is still debated. Here, two chlorine-based oxidants commonly used in Italian drinking water plants were tested on cultured *Microcystis aeruginosa*, and their effectiveness in removing cells and cyanotoxins was evaluated at increasing concentrations and exposure time. Both treatments were found to efficiently remove intracellular toxins from cyanobacterial biomass, especially at higher doses, whereas no major differences were observed when increasing exposure time. Nonetheless, a simultaneous increase of cyanotoxins in the extracellular fraction was observed, suggesting their possible release from cells to water. According to the treatment applied, different fate for toxins and unknown secondary metabolites was observed. Considerations for improvements of drinking water treatment to ensure safety and high quality standards of the final product are suggested, and innovative oxidant treatments to degrade cyanotoxins are discussed.

Poster 94

Nanobubble ozone treatment effects on cyanobacterial biomass and cyanotoxins in a small eutrophic lake

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Cyanobacterial harmful algal blooms (CHABs) fueled by excessive nutrient runoff are becoming an increasing problem worldwide. Nanobubble ozone technology (NBOT) is an emerging treatment to remediate lakes from CHABs. NBOT was tested in Lake Sylvan, a 42-acre manmade eutrophic lake that experiences annual CHABs. NBOTs were deployed from 7 July to 17 September 2021, and dosage was tripled on 9 August. CHAB chlorophyll, microcystins, and saxitoxins (among other parameters) were measured June to October 2021, spanning pre-NBOT, NBOT deployment, and post NBOT treatment. CHAB chlorophyll increased throughout July (peaking at 100 µg/L) then sharply declined following the increased dose (25-35 µg/L) but increased again 3 weeks (51-78 µg/L) after increased dose. CHAB chlorophyll spiked (80-95 µg/L) in early October after NBOT treatment. Microcystins steadily increased from <0.15 to 2.5 µg/L throughout June and July and remained between 0.4 to 1.0 µg/L August – October. No contact advisories were posted during 2021 unlike previous years. Total saxitoxins rapidly increased in mid-July peaking at 4 µg/L, and then declined. Several large rainstorms (10 cm/d) occurred during NBOT treatment which may contributed nutrients. These storms complicated the evaluation of NBOT efficacy. Lake Sylvan will be monitored in 2022 without NBOT treatment for baseline conditions.

Poster 95

Laboratory-scale evaluation of microcystin persistence following treatment of five USEPA-registered algaecides at different temperatures

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An increasingly warmer climate and unsustainable land-use practices have exacerbated harmful algal blooms in many water resources. Algaecides are a proven treatment to mitigate blooms, but different species and limnological variables can impact efficacy. Water temperature is a variable that affects algaecide efficacy and toxin persistence. In this experiment, microcystin-producing *Planktothrix* was collected from Grand Lake St. Mary's in March 2021. Samples were exposed to five different copper and peroxide-based USEPA-registered algaecides at varying concentrations within label rate and incubated at 10°C, 15°C, and 20°C. Total and extracellular microcystins were measured 96 hours after treatment. At 10°C and lowest dosage, PAK 27® reduced total microcystins by 75% compared to the control, followed by GreenClean®Liquid (66%), Cutrine® (63%), SeClear® (55%), and Aligimycin® (40%). In most trials, the amount of total microcystins reduced decreased as temperature increased. However, GreenClean® resulted in a shift to nearly 100% extracellular microcystin at all temperatures, while PAK 27® resulted in 20% extracellular microcystins at 10° and less at higher temperatures. The other three algaecides also shifted microcystins to extracellular. In general, temperature had a greater impact on total microcystin reduction than dosage. This study provides important baseline data for the development of temperature-specific management decisions.

Poster 96

Molecular physiology of antagonistic bacterial interactions with Microcystis

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Microcystis aeruginosa is a cyanobacterium with the ability to cause cyanobacterial harmful algal blooms (cHABs), which are a global threat to public health and freshwater ecosystem function. *Microcystis* is especially problematic as it has the ability to produce a secondary metabolite called microcystin, a hepatotoxin which can cause liver damage if ingested. Currently there are no solutions to *Microcystis* blooms that do not involve the use of toxic chemicals, which will further damage the aquatic ecosystem. Previous studies have identified a possible biological solution to *Microcystis* blooms through the use of algicidal bacteria, which can be naturally found within the blooms. However, the genes involved in these algicidal mechanisms have currently not been identified. We have isolated algicidal bacteria cHABs in Lake Anna (Virginia, USA) and Lake Tai (China). We have conducted co-culture experiments and generated a series of physiological data to determine the impact of these algicidal bacteria on the gene expression of *Microcystis*. Co-culture transcriptomes and growth data can be used in conjunction to identify the genetic pathways responsible for its algicidal mechanisms, with potential applications for bloom mitigation strategies.

Poster 97

Microcystin exposure of the Belgian population: feasibility of unifying fragmented exposure data

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Microcystin (MC) congeners are hepatotoxins produced by aquatic cyanobacteria. Uncovering potential exposure routes in Belgium requires assessing the impact of these toxins on the population. Various approaches evaluate exposure separately for different intoxication routes (environmental and food consumption). However, the aggregated exposure from various stressors is not commonly done. With access to various collection sites and a national consumption database, a holistic approach to microcystins exposure should be feasible.

Eight MCs were quantified in different matrices representing multiple intoxication pathways using UHPLC-MS/MS. Overall, 79 samples from algal blooms, 75 drinking water samples, 35 algal-based food supplements, and 103 fruits and vegetables from supermarkets were analyzed.

MCs were found in the bloom samples (86.08%) and algal food supplements (22.86%) but not in drinking water, fruit and vegetables. Daily estimated exposure was calculated for the food supplements, and the ratio of bloom samples above the proposed guidance values for recreational waters (24 µg/L) was determined.

Aggregated exposure assessment needs additional data: consumption data for algal supplements, dietary exposure from foods, proper modelling to episodic environmental exposures. Specific exposure scenarios resulting from collaborative research show the feasibility of unifying the exposure prediction for a part of the Belgian population.

Poster 98

The impacts of different types of commercial algaecide products on cyanobacteria control and intracellular organic matter releases

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Cyanobacterial harmful algal blooms (cHABs) pose great challenges to public water systems when producing safe drinking water. Applying algaecide is one of the most common methods to control the cHABs in source waters, however, it can cause cell lyses and release the intracellular organic matter (IOM) and the toxins, which are difficult to remove in public drinking water systems with conventional drinking water treatment processes. In this study, we evaluated four commercial algaecide products (two copper-based and two peroxide-based) and assessed the impact of algaecide treatment on cyanobacteria cell removal and the release of IOM and toxins. The optimal dose and the treatment time of each product were determined using both granular (*Microcystis aeruginosa*) and filamentous (*Planktotrix agardhii*) cyanobacteria. A Parallel Factor Analysis (PARAFAC) model was built using collected Excitation-Emission Matrix (EEM) spectra from collected samples under different algaecide treatment time and doses to characterize and quantify the release of IOM during the treatment. The obtained results may help public water systems better control HABs without causing adverse impacts on finished water quality.

Poster 99

Understating of cyanobacteria associated bacterium in surface water and their impact on treatment

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Despite the large impact of environmental conditions on cyanobacterial blooms, their dynamics cannot be explained only by abiotic factors, so biotic drivers are considered to be critical. Cyanobacteria produce an extracellular mucilage or more commonly known as extracellular polymeric substances (EPS) creating a region known as the phycosphere. Various interactions and associations occur between cyanobacteria and specific other bacteria in the phycosphere. It is hypothesized that cyanobacteria-associated bacteria or epibionts have a fundamental impact on cyanobacterial growth. In addition, because of the wide range of cyanobacterial metabolic properties, including the synthesis of many secondary metabolites, various heterotrophic microorganisms benefit from association with cyanobacteria. Many studies have also reported antagonistic interactions between cyanobacteria and other bacteria. With these substantial interactions among cyanobacteria and associated bacteria, it is crucial to identify the diversity and metabolism of the entire microbial population within a water body in order to better understand the ecological processes that lead to the formation of cyanobacterial blooms in freshwater systems and their impact on water treatment processes. Hence, the this study explored the fundamental interactions between bloom-forming cyanobacteria and their associated bacteria in water and their impact on treatment processes (focus oxidation and BAC).