



# Abstract Book

for the 5<sup>th</sup>

I F H A B

Workshop

Greetings colleagues,

The IFHAB Workshop Organizing Committee is pleased to be hosting you at the 5th Interdisciplinary Freshwater Harmful Blooms Workshop in the Université de Montréal.

Here's a reminder of our mission:

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.

Best regards,

Organizing Committee, IFHAB Workshop

Daniel Beach, National Research Council of Canada

David McMullin, Carleton University

Sebastien Sauvé, Université de Montréal

René Shahmohamadloo, Washington State University and University of Guelph

Dana F. Simon, Université de Montréal

Susan Watson, Trent University

Arthur Zastepa, Environment and Climate Change Canada

## Sponsors

We would like to thank our sponsors for their generous support,

**ThermoFisher**  
SCIENTIFIC

 **FlowCam**  
Particle Analysis with Vision  
FLUID IMAGING TECHNOLOGIES, INC.

 **SCIEX**

  
**GOLD  
STANDARD  
DIAGNOSTICS**

**Waters**  
THE SCIENCE OF WHAT'S POSSIBLE.®

 **phytoxigene**<sup>™</sup>

 **Agilent Technologies**

## Location

This year's *IFHAB Workshop* will be held in Université de Montréal - Campus MIL. The driving address is **1375 Ave. Thérèse-Lavoie-Roux, Montreal, QC**. To get there via STM, you can take the metro to station Outremont (500m. 6-minute walk to Campus MIL).

*Campus MIL is represented in blue.*

*The oral presentations will take place in block A, room **A3502.1**.  
The registration, coffee and lunch breaks, sponsors and posters session will take place in **Block B, Atrium**.*

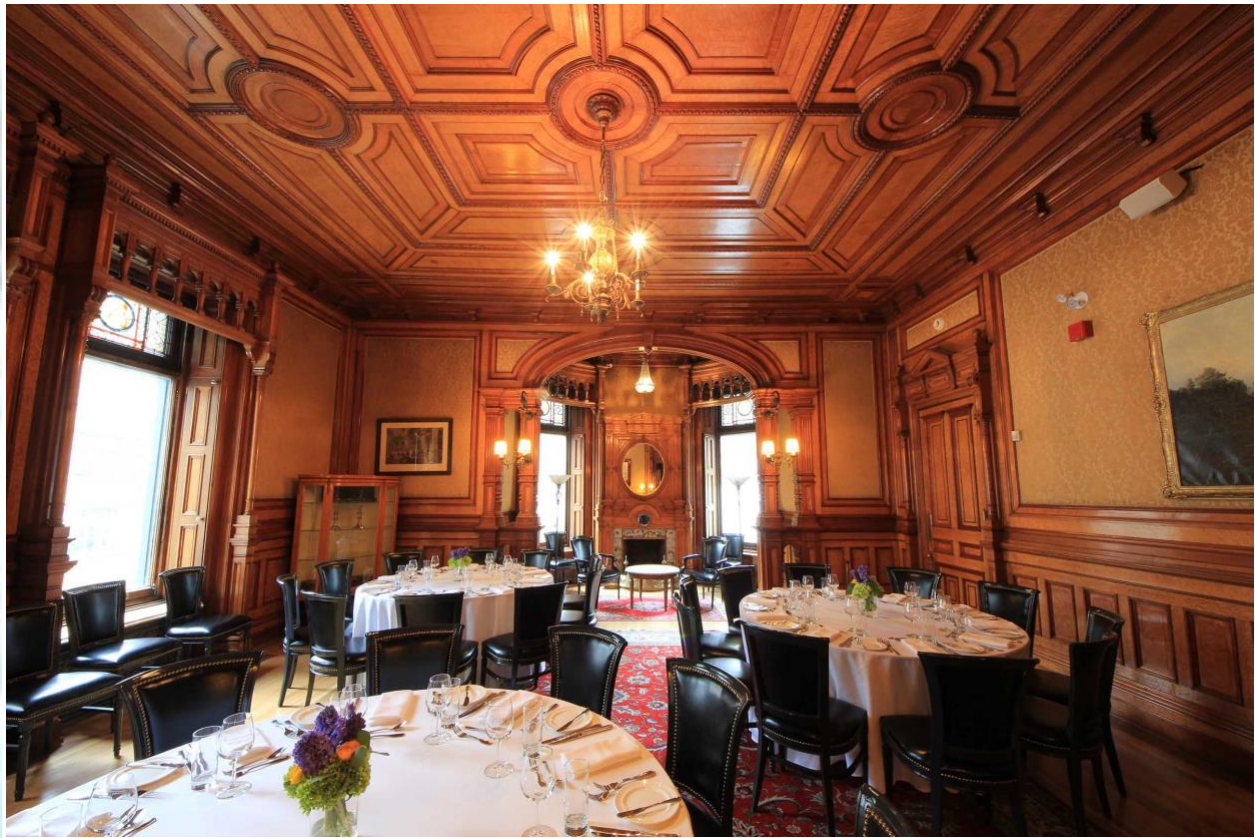
*There will be signs showing the way in and out of the auditorium.*



### Social Event

This year's social event will be held at **Bar George**,  
on Le Mount Stephen, 1440 Drummond St, Montreal, Quebec H3G 1V9.

*We will meet there for a drink and some food at 6:30 PM.*



The 5th Interdisciplinary Freshwater Harmful Algal Blooms Workshop

**Thursday, May 4.**

<b>08:50</b>	Opening Remarks	
<b>09:00 – 09:40</b>	Helen Baulch, Keynote Speaker <i>University of Saskatchewan</i>	The capacity gap: Opportunities and needs to build capacity in addressing bloom risks.
<b>09:40 – 10:00</b>	Arthur Zastepa <i>Environment and Climate Change Canada</i>	Beyond total microcystins: Toxic and bioactive metabolites produced by cyanobacteria in Lake Erie's western basin.
<b>10:00 – 10:20</b>	Krista Thomas <i>National Research Council Canada</i>	Multi-class analysis of Cyanobacterial toxins using liquid Chromatography-Mass Spectrometry.
<i>Coffee Break</i>		
<b>10:40 – 11:00</b>	Chris Gill <i>Vancouver Island University</i>	Paper Spray Mass Spectrometry for high throughput Cyanotoxin measurements.
<b>11:00 – 11:20</b>	Mark Van Asten <i>Phytotoxigene</i>	The Development and Challenges of a Molecular qPCR assay for the Detection of Anatoxin and Guanitoxin Producing Cyanobacteria in Environmental Samples.
<b>11:20 – 11:40</b>	Audrey Roy-Lachappelle <i>Environment and Climate Change Canada</i>	Determination of cyanopeptides by targeted, suspect and non-target screening in the Lake Saint-Pierre watershed.
<b>11:40 – 12:00</b>	Stuart Oehrle <i>Northern Kentucky University</i>	Targeted UPLC/MS/MS method for cyanobacterial toxins in recreational water: a comparison to metagenomics.
<i>Lunch</i>		
<b>13:00 – 13:40</b>	David Fewer, Keynote Speaker <i>University of Helsinki</i>	Genomic insights into the biosynthesis of cyanobacterial toxins and bioinformatic methods for drinking-water quality control.
<b>13:40 – 14:00</b>	Janice Lawrence <i>University of New Brunswick</i>	Detailed study of anatoxins and anaC genes detected in Microcoleus-dominated mats along the Wolastoq.
<b>14:00 – 14:20</b>	Nicolas Tromas <i>McGill University</i>	Predicting cyanotoxins concentrations in lakes and reservoirs using microbial community information.
<i>Coffee Break</i>		
<b>14:40 – 15:00</b>	Vani Mohit <i>MELCCFP</i>	Rapid on-site detection of potentially toxic cyanobacteria.
<b>15:00 – 15:20</b>	Marianne Potvin <i>Université Laval</i>	Real-time on-site cyanobacteria identification using Oxford Nanopore sequencing.
<b>15:20 – 15:40</b>	Liya Ma <i>Polytechnique Montréal</i>	Factors affecting the interpretation of online phycocyanin fluorescence to manage Cyanobacteria in drinking water sources.
<b>15:40 – 16:00</b>	Sathya Ganegoda <i>Auburn University</i>	Off flavors as a By - product of Harmful Algal Blooms; A green approach to remove geosmin and 2-MIB from water using native bacteria.
<b>16:00 – 17:20</b>	Poster Session	
<b>18:20 +</b>	Social Event	

**Friday, May 5.**

<b>08:50</b>	Welcome Back!	
<b>09:00 – 09:20</b>	Paul MacKeigan, Winner of IFHAB 2022 Best Student Paper <i>McGill University</i>	Biotic and abiotic predictors explain significant variation in cyanobacteria biomass across lakes from temperate to subarctic zones.
<b>09:20 – 09:40</b>	Sophie Crevecoeur <i>Environment and Climate Change Canada</i>	Link between microbes involved in nutrient cycling and cHABs in Lake Erie watershed.
<b>09:40 – 10:00</b>	Cecilio Valadez-Cano <i>University of New Brunswick</i>	Closely related cyanophages from geographically distant North American rivers are predicted to infect anatoxin-producing <i>Microcoleus</i> species.
<b>10:00 – 10:20</b>	Gertrud Nurnberg <i>Freshwater Research</i>	Cyanobacteria response to climate-affected internal phosphorus loading in two drinking water reservoirs.
<i>Coffee Break</i>		
<b>10:40 – 11:00</b>	Kevin Errat <i>University of Toronto</i>	Climate change amplifies the risk of potentially toxigenic cyanobacteria.
<b>11:00 – 11:20</b>	David Hatton <i>Queen's University Belfast</i>	Elucidating the drivers of shallow lake cyanobacteria bloom events from sediment core records.
<b>11:20 – 11:40</b>	Hana Trigui <i>Polytechnique Montréal</i>	Impact of oxidation and stagnation on the diversity of cyanobacteria in drinking water treatment plant sludge.
<b>11:40 – 12:00</b>	Maude Lachapelle <i>University of Saskatchewan</i>	Does lake browning and eutrophication affect cyanobacterial communities and the presence of cyanotoxins?
<i>Lunch</i>		
<b>13:00 – 13:20</b>	Akaash Bansal <i>University of Saskatchewan</i>	Impact of Cyanobacteria on the pelagic phosphorus cycle.
<b>13:20 – 13:40</b>	Emily Varga <i>Great Lakes Institute for Environmental Research</i>	Understanding the effects of environmental drivers of phytoplankton community succession and toxin production in Canadian waters of Lake St. Clair.
<b>13:40 – 14:00</b>	Rebecca Gasman <i>York University</i>	A Pan-Canadian comparison of Cyanobacteria bloom management policies, programs, and practices.
<b>14:00 – 14:40</b>	John Downing, Keynote Speaker <i>University of Minnesota</i>	Cyanobacteria in the Anthropocene: Their past is a key to our future.
<b>14:40 – 15:00</b>	Closing Remarks.	

# Oral Presentations

*In alphabetical order.*



**Akaash Bansal**

*University of Saskatchewan*

Impact of Cyanobacteria on the pelagic phosphorus cycle.

*With Arthur Zastepa, John-Mark Davies, David Vandergucht, Noim Imtiaz and Jeff Hudson.*

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 which receives 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, zooplankton composition 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.

**Helen Baulch**

*University of Saskatchewan*

The capacity gap:  
Opportunities and needs to build capacity in addressing bloom risks.

*With Anthony Baron, Leyden Fernandez, Blair Kardash, Pat Lloyd-Smith, Kristin Painter, Danielle Spence Sébastien Sauvé, Jesse Shapiro, Dana F. Simon, Mauro de Toledo, Nicolas Tromas, Jason Venkiteswaran and Colin Whitfield.*

Science-based solutions to harmful cyanobacterial blooms feel urgent. Yet, in many cases, solutions also remain elusive. In this talk I will highlight challenges from a case study lake in Western Canada – exemplified by bloom conditions and high toxin diversity in a key water source. A long-term research partnership has helped adapt, plan, and implement solutions related to blooms and other stressors by characterizing key considerations in flow management, tools for early warning of treatment impacts, and work to characterize toxin risk. Currently, capacity for similar work across the region remains limited, while the scope of the problem is daunting. Given the myriad bloom-affected lakes nationally, a fundamental challenge lies in knowing the key areas of science and management we most urgently need to advance, and building capacity for adaptation, as well as mitigation. Importantly, public demand and willingness to pay for improved water quality is high. This talk will highlight, with a view ‘from the west’, key needs for training in risk and risk communication, for advancing agricultural extension work informed by limnology, for advancing watershed-based management and for supporting water utilities. These are lessons relevant across the nation, and will hopefully lead to further discussion about how we can better share lessons, enhance capacity, and better link science and management to help address the urgency of current and expanding issues of blooms.

**Sophie Crevecoeur**

*Environment and Climate Change Canada*

Link between microbes involved in nutrient cycling and cHABs in Lake Erie watershed.

*With Lori Phillips, Jérôme Comte, Ngan Diep, Alice Dove, Thomas Edge, Thijs Frenken, R. Mike McKay, Susan Watson and Arthur Zastepa.*

Lake Erie is heavily impacted by urban and agricultural nutrient loading and faces recurring events of cyanobacterial harmful algal blooms (cHABs). Research focusing on abiotic drivers of cHABs, such as nutrients, continues to produce inconsistent results related to bloom prediction. Yet, there is increasing evidence that cyanobacteria are influenced by biogeochemical processes rendered by the greater aquatic microbial community (i.e. aquatic microbiome), but those biotic factors and processes are rarely considered when studying cHABs. Here we used high throughput sequencing of the 16S rRNA gene and qPCR targeting genes involved in N and P cycling to assess the taxonomic and functional diversity of the aquatic microbiome associated with cHAB events along the Thames River-Lake St. Clair- Lake Erie continuum, and across different seasons. We found that, throughout the year, genes involved in P acquisition and denitrification were in high abundance in the Thames River and Lake St. Clair, but decreased in abundance in Lake Erie. There were changes in the dominant cyanobacterial genera along the water continuum from April to October, and their relative abundance successively correlated with different genes involved in P or N cycling. Our results highlight the link between the changing microbiome, the microbial processes, and the presence and establishment of cHABs, which ultimately will help better understand the factors leading to their occurrence.

**John Downing**

*Minnesota Sea Grant University*

### Cyanobacteria in the Anthropocene: Their Past is a Key to Our Future

Limnologists have been working to forecast Cyanobacteria dominance and toxicity for several decades. Although we have learned much, our progress toward management is a pretty dismal disappointment. I will describe in uncomfortable economic and population detail why we need a HABs “moonshot” and how we can get on the right track to make it. A solution lies with gestalt. Limnologists’ frames of reference are often more eukaryotic (Linnean) than prokaryotic. Prokaryotes have greater plasticity and a broader range of function that derives from their evolutionary history. The morphologically distinguishable “species” of Cyanobacteria may embody a broader range of tools they can employ in the eutrophication arms race. They should be viewed as versatile generalists, more able to change and adapt, than their specialist descendants. Solving Cyanobacteria problems in the Anthropocene has more to do with understanding the polyvalence of these long-standing competitors than seeing them as specialists that succeed under extreme conditions.

**Kevin Erratt**

*University of Toronto*

Climate change amplifies the risk of potentially toxigenic cyanobacteria.

*With Irena Creed, David Lobb and Charles Trick.*

Cyanobacteria dominance signifies a global challenge for water security and has been linked to anthropogenic forcing. However, the relative importance of different human stressors remains to be determined, as the study of climate and land-use changes is often restricted to short temporal scales. In this study, we reconstructed cyanobacteria abundance and microcystin-producing potential in temperate lakes across a human impact gradient using a paleolimnological approach. We discovered breakpoints (points of abrupt change) and investigated the influence of climate and landscape proxies on these breakpoints. Cyanobacteria abundance in high-impacted lakes (e.g., agricultural catchments) increased 40 years earlier than in low-impacted lakes (e.g., forested catchments), with land-use change as the dominant predictor. Microcystin-producing potential increased in both high- and low-impact lakes around the 1980s, with climate warming as the dominant predictor. Our findings chronicle the importance of climate change in increasing the risk of toxigenic cyanobacteria in freshwater resources.

**David Fewer**

*University of Helsinki*

Genomic insights into the biosynthesis of cyanobacterial toxins  
and bioinformatic methods for drinking-water quality control.

Cyanobacteria produce a variety of potent toxins through secondary metabolism that are linked to numerous cases of animal poisonings and occasional human fatalities. Biosynthetic pathways for all major toxin families were assigned over the last two decades. Many of the biosynthetic mechanisms employed by cyanobacteria to synthesize toxins are unique and provide a new understanding of the chemical diversity observed in nature. Comprehensive, publically accessible and curated databases of secondary metabolites from cyanobacteria now facilitate research into their occurrence, prevalence, fate and toxicological risks. Advances in genome sequence technology, new community genomic standards and new bioinformatics tools coupled with curated databases of secondary metabolites make it possible to rapidly dereplicate samples but also provide new insights into the nature of the toxin-producing cyanobacterial community. Bioinformatic analyses of toxin biosynthetic pathways from the rapidly expanding body of public microbial genomes make it possible to study the prevalence and diversification of toxin biosynthetic pathways in different strains and genera of cyanobacteria in finer detail than ever before. Advances in the understanding of toxin biosynthesis and diversification has paved the way for molecular techniques and new methods to assess the quality of drinking water and may inspire the development of new methods to manage bloom formation in the future.

**Sathya Ganegoda**  
*Auburn University*

Off flavors as a By - product of Harmful Algal Blooms;  
A Green Approach to remove Geosmin and 2-MIB from water using native bacteria.

*With Alahakoon C*

Harmful Algal blooms (HAB) produce variety of by-products during different stages, and off flavors have been a major issue related to HAB worldwide. Presence of off flavors in treated drinking water is one of the significant issues in global drinking water suppliers. Geosmin (trans-1, 10-dimethyl-trans-9-decalol) and 2- MIB (2- Methylisoborneol) are responsible for earthy and musty taste and odour (T&O) in drinking water. Despite of no recorded health hazards, water consumers reject the water if these odorants are present, due to their unpleasant aesthetic properties. According to WHO, the provision of drinking-water that is not only safe but also acceptable in appearance, taste and odour is of high priority. Therefore, removing Geosmin and 2-MIB from drinking water is a necessity for worldwide water authorities and consumers. Several physical and chemical methods are available to remove T&O compounds such as activated carbon, chlorination, membrane filtration, UV photolysis and Ozonation. These methods have their inherent drawbacks mainly as high cost and production of toxic byproducts. Biodegradation using native bacteria as an eco-friendly green approach has been proved compelling to remove Geosmin and 2- MIB. Hence, this study was designed to determine biodegradation capability of Geosmin and 2- MIB using native bacteria. Raw water and soil were collected from 18 reservoirs around Sri Lanka where drinking water T&O issues are prevailing. Isolation of Geosmin and 2- MIB degrading bacteria in water and in sediment were carried out using standard microbiological procedures following enrichment, isolation and screening of potential degraders using Biolog MT2 plate assay. Potential Geosmin and 2- MIB degraders were subjected to degradation kinetics study and identification was carried out using 16S rRNA sequencing. Morphologically different 150 bacteria colonies from Geosmin treated samples and 95 colonies from 2- MIB treated water samples were isolated. Biolog MT2 plate assay was used to identify positive Geosmin (40) and 2-MIB (23) degraders from the initially isolated bacteria. Five gram negative bacteria showed complete degradation of Geosmin (initial level 20 ppt) at 7 days of incubation and they were identified as *Myroides odoratimimus*, *Providencia rettgeri*, *Providencia rettgeri*, *Myroides odoratimimus* and *Proteus mirabilis*. *P. mirabilis* was isolated from Tissa wewa water, showed 100% degradation of Geosmin (initial level 20 ppt) at 5 days with a half-life time of 3 days when compared to control and other bacteria species. Accordingly, *P. rettgeri* isolated from Nuwara wewa soil, showed 100% degradation of 2- MIB (initial level 20 ppt) at 4 days incubation having 2 days of half-life time. Further three gram positive *Bacillus* strains (bio chemical identification) were identified as potential Geosmin and 2- MIB degraders and further studies with these strains are in progress. Degradation mechanism showed first order kinetics. *P.mirabilis* and *P.rettgeri* were previously reported antibiotics and xenobiotic degraders, and this is the first report of them, regarding degradation of Geosmin and 2- MIB.

**Rebecca Gasman**

*York University*

A Pan-Canadian Comparison of Cyanobacteria Bloom Management Policies, Programs, and Practices.

*With Lewis Molot and Dan Walters.*

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.



Chris Gill

*Vancouver Island University*

### Paper Spray Mass Spectrometry for High Throughput Cyanotoxin Measurements

*With Lucas R. Abruzzi, Daniel G. Beach and Erik T. Krogh.*

The detection and measurement of cyanotoxins across multiple classes with differing structures, polarities and modes of toxicity presents significant analytical challenges. Testing with chromatography-based methods provides the sensitivity and specificity needed to detect trace level cyanotoxins in water, but high analysis costs preclude widespread, frequent testing of recreational water bodies and drinking water reservoirs. Paper spray mass spectrometry (PS-MS), a relatively new analytical measurement approach, is seeing rapid growth in the clinical, forensic and environmental sectors. PS-MS is simple, rapid and sensitive, using disposable paper strips as direct sampling/measurement devices. Small samples (e.g. 10  $\mu\text{L}$ ) spotted on pointed paper strips are ionized directly for mass spectrometry, resolving analytes by tandem mass spectrometry. PS-MS measurements (~1 minute for up to 600 different targets) obviate complex sample preparations, and commercially available, multiplexed PS-MS can autonomously measure 240 samples/day. We demonstrate PS-MS for rapid quantitative analysis of multiple classes of cyanotoxins in water. Addition of isotopically labelled internal standards (in the samples or co-deposited on the paper strips) corrects for matrix effects and inter-run variation, allowing for rapid, reproducible measurements. Calibrations for a variety of microcystins, anatoxin, and nodularin show <10% CVs across all calibration levels for each compound, with  $R^2$  values greater than 0.99. All microcystins had lower limits of quantitation (LLOQ) below the Canada and WHO guideline values (10, and 24  $\mu\text{g/L}$ , respectively) for microcystins in recreational waters. Anatoxin-a had a LLOQ well below both WHO recreational and short-term drinking-water values of 60  $\mu\text{g/L}$  and 30  $\mu\text{g/L}$ , respectively. Comprehensive analysis of all cyanotoxin classes almost always requires multiple analyses, and PS-MS may offer the opportunity for rapid, multi-class screening or quantitation. Ongoing work focuses on expanding the method to include saxitoxins, evaluating sample matrix effects, as well as application to screening strains of cultured cyanobacteria, field samples and reference materials.

David **Hatton**

*Queen's University Belfast*

Elucidating the drivers of shallow lake cyanobacteria bloom events from sediment core records.

*With Helen Roe, Timothy Patterson, Peter Leavitt, Ethan McCann, David McMullin, Eduard Reinhardt, Nawaf Nasser and Matthew Marshall.*

Harmful cyanobacterial algal blooms (CyanoHABs) in freshwater systems have garnered increased public and scientific interest due to the potentially devastating impacts of their toxic secondary metabolites on biota. This has created significant impetus to elucidate the drivers of CyanoHAB occurrence, and to better understand the resilience of lakes to these toxic bloom events. Dated sediment cores ( $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ ,  $^{14}\text{C}$ ) from shallow lake sites in New Brunswick, Canada, a maritime region which has seen an increased frequency of lake CyanoHABs in the last decade, have been examined to explore relationships between past toxic algal bloom events and their controls. Multi-proxy sediment analyses (grain-size, Itrax-XRF; stable isotopes:  $\delta^{13}\text{C}$ ;  $\delta^{15}\text{N}$ ) were employed to explore the mechanistic links between the drivers of lake change (i.e. climatological stressors and catchment related disturbances) and past episodes of CyanoHAB development. Cyanobacterial responses were investigated using taxonomically specific sedimentary pigments: aphanizopyll (N<sub>2</sub>-fixing), echinenone (total cyanobacteria), canthaxanthin (nostocales) and myxoxanthophyll (colonial). Microcystin congeners LA, LR, [Dha7] LR and RR, cyanobacteria-specific metabolites that preserve in lake sediments, were quantified using liquid-chromatography tandem mass spectrometry. Preliminary results show that in Wheaton Lake, microcystin congeners were detected intermittently throughout the upper 10 cm of the record (representing the last ca. 100 years), before increasing significantly towards the present day. These shifts in microcystin concentration broadly correlate with changes in pigment occurrence. Notably, the appearance of aphanizopyll, which indicates water quality degradation and a shift towards nitrogen fixing cyanobacteria, mirrors increases in total microcystins, and a significant spike in microcystin RR. These initial results demonstrate the utility of applying a palaeolimnological approach to reconstructing CyanoHAB events, providing an opportunity to significantly extend the inferences which can be made from water quality monitoring data alone. This may provide invaluable insights into how lakes might respond to future stressors.

**Maude Lachapelle**

*Environment and Climate Change Canada*

Does lake browning and eutrophication affect cyanobacterial communities and the presence of cyanotoxins?

*With Susanne Kramer, Paul Mackeigan, Marie-Ève Monchamp, Marie-Pier Hébert, Marc Amyot, Irene Gregory-Eaves and Zofia E. Taranu.*

Climate warming and changes in biogeochemical cycles have already caused a notable increase in lake browning and eutrophication. While the effect of eutrophication on cyanobacteria biomass, dominance and toxin production are well studied, the effect of lake browning on these parameters remains unclear. Browning not only increases dissolved organic carbon (DOC), but also alters the concentration of nutrients, metals, and contaminants. DOC can also decrease light intensity and lower oxygen levels (increased hypolimnion decomposition), with important repercussions on community composition and bioavailability of contaminants (e.g. mercury methylation by bacteria under anoxic conditions). Studies examining the synergistic effects of browning and eutrophication on food webs remain either limited to short time frames, single drivers, or drivers with narrow ranges of variation. To address this gap, we conducted a nine-week mesocosm experiment (from summer to fall) crossing browning (DOC) and eutrophication (total phosphorus and nitrogen) treatments, and tracking responses in aquatic communities via fluoroprobe readings and high-throughput sequencing of environmental DNA (using a 16S rRNA primer). To identify the most important drivers of changes in the bacterial community (including cyanobacteria), we applied distance-based RDA and Generalized Dissimilarity Models, which showed that DOC (leading driver), pH, and temperature were key drivers of changes in microbial community structure. To then identify the species that responded most to the DOC gradient, we applied a Threshold Indicator Taxa Analysis, which showed that within the cyanobacterial community, seven decreasing ASVs (order Synechococcales) and three increasing ASVs (orders Leptolyngbyales and Pseudanabaenales) were indicative species of DOC change. Multiple Factor Analysis and variation partitioning further showed that the microbial community was correlated to changes in major phytoplankton (fluoroprobe) and zooplankton (classical microscopy and metabarcoding) groups, as well as physico-chemistry. Upcoming analyses of mercury and cyanotoxins in plankton samples will shed some light on whether browning, eutrophication, and warming have altered contaminant bioaccumulation.

**Janice Lawrence**

*University of New Brunswick*

Detailed study of anatoxins and anaC genes detected in *Microcoleus*-dominated mats along the Wolastoq.

*With Daniel Beach, Joshua Evans, Jake Stillwell, Cecilio Valadez-Cano, Adrian Reyes-Prieto, Pearse McCarron and Meghann Bruce*

Cyanobacteria and their toxins are an emerging issue in Atlantic Canada, where anatoxins (ATXs; potent neurotoxins) have been responsible for at least six dog deaths since 2018. In these cases, toxins have originated from proliferations of benthic cyanobacterial mats rather than surface blooms. In 2019, we conducted a detailed study along a 20 km stretch of the Wolastoq|Saint John River where three dogs had died in 2018. We sampled benthic microbial mats (n = 169) from 8 sites representing different hydrographic environments every two weeks from early June to late September. Mat samples were analyzed by direct analysis in real time-MS/MS to detect ATXs, and extracted DNA by droplet digital-PCR (ddPCR) to determine the abundance of the anaC gene, which is critical for anatoxin biosynthesis. Up to  $5.6 \times 10^9$  copies of anaC g<sup>-1</sup> wet and 1.7 mg ATXs g<sup>-1</sup> wet were measured. We detected ATXs in 138 samples, with a good correlation ( $R^2 = 0.67$ ) between ATX concentration and number of anaC copies measured in the 127 samples positive for anaC. ATX and anaC abundance peaked in late June and again in early September at most sites, with cellular toxin quota as high as 275 pg cell<sup>-1</sup> and decreasing throughout the sampling season. Metagenomic surveys of a subset of mats indicated they were dominated by 4 different *Microcoleus* species, including a single subspecies armed with the genetic repertoire (i.e., the ana cluster) required for ATX production. The relative abundance of ana cluster genes in the metagenomes correlated well ( $R^2 = 0.76$ ) with the ddPCR quantitation of the anaC gene. These results are helping us understand the drivers of ATX-producing benthic cyanobacterial proliferation in the Wolastoq, and will be useful in other regions as this emerging issue becomes better recognized.

Liya Ma

*Polytechnique de Montréal*

Real-time phycocyanin monitoring in water supply systems to manage cyanobacterial risks.

*With Juan Francisco Guerra Maldonado, Arash Zamyadi, Sarah Dorner and Michèle Prévost.*

Worldwide, there has been an increase in the presence of potentially toxic cyanobacterial blooms in drinking water sources and within drinking water treatment plants (DWTPs). The objective of this study is to validate the use of in situ probes for the detection and management of cyanobacterial breakthrough in high and low-risk DWTPs. In situ phycocyanin YSI EXO2 probes were devised for remote control and data logging to monitor the cyanobacteria in raw water, clarified water, filtered water, and treated water in three full-scale DWTPs. An additional probe was installed inside the sludge holding tank to measure the water quality of the surface of the sludge storage tank in a high-risk DWTP. Simultaneous grab samplings were carried out for taxonomic cell counts and toxin analysis. A total of 23, 9 and 4 field visits were conducted at the three DWTPs. Phycocyanin readings showed a 93-fold fluctuation within 24 hours in the raw water of the high cyanobacterial risk plant, with higher phycocyanin levels during the afternoon period. These data provide new information on the limitations of weekly or daily grab sampling. Also, different moving averages for the phycocyanin probe readings can be used to improve the interpretation of phycocyanin signal trends. The in situ probe successfully detected high cyanobacterial biovolumes entering the clarification process in the high-risk plant, and high cyanobacterial biovolume in the sludge for both high and low-risk plants.

**Paul MacKeigan**

*McGill University*

Biotic and abiotic predictors explain significant variation in cyanobacteria biomass across lakes from temperate to subarctic zones.

*With Zofia Taranu, Frances Pick, Beatrix Beisner and Irene Gregory-Eaves.*

Cyanobacteria remain a persistent and growing threat in many lakes worldwide, and as a result, there has been a concerted effort to model the drivers of their biomass and community structure. However, cyanobacteria response models from large-scale analyses are often restricted to just a few predictor variables, potentially limiting predictive power. Furthermore, there was no systematic national sampling program or complementary analyses of cyanobacteria and their drivers across Canadian lakes until just recently. To address these gaps, we developed and analyzed a pan-Canadian dataset of 640 lakes to quantify cyanobacteria biomass and their community composition, as well as identify the predictors of total cyanobacteria biomass, and the biomass of several toxin- and bloom forming genera (*Microcystis*, *Aphanizomenon* and *Dolichospermum*). We applied a series of modelling approaches that considered an exhaustive list of environmental predictors (n=55) including water chemistry, land use and zooplankton variables; all of which we collected using standardized methods. The highest biomasses of cyanobacteria were recorded in central Canada within the Prairies and Boreal plains ecozones, where *Microcystis* and *Aphanizomenon* dominated assemblages. Across all modelling approaches, total phosphorus was identified as the most important predictor of total cyanobacteria biomass, focal general and of community composition. In addition to nutrients, cyanobacteria also showed positive and non-linear associations with ions and dissolved organic carbon. Our analyses also identified positive relationships between cyanobacteria and zooplankton groups, particularly daphnids and copepods, which echoes emerging evidence from other studies. Zooplankton may be indirectly promoting cyanobacteria growth through localized top-down forces and/or evolutionary adaptation. From a management perspective, our models provide continuing support for the regulation of nutrients to control cyanobacteria growth. Moreover, due to limited regional variation in the response of cyanobacteria to these predictors, these models can be applied to lakes across the country.

Vani Mohit  
MELCCFP

Rapid on-site detection of potentially toxic cyanobacteria.

*With Marianne Potvin, Christophe Langevin, Naila Barbosa da Costa, Irena Kukavica-Ibrulj, Jeff Gauthier, Daniel Verreault, Roger C. Levesque and Jérôme Comte.*

With the increasing frequency of cyanobacterial harmful algal blooms (cHABs) across the world, it is important that environmental stakeholders have access to rapid and early diagnostic tools for the identification of toxin-producing cyanobacteria as these can have harmful effects on human and animal health. The objective of the RosHAB (Rapid on-site detection of Harmful Algal Blooms) project is to develop an in-field applicable metagenomics workflow for real-time, reliable, accessible, and cost-effective detection of potentially toxic cyanobacteria. This workflow will offer a simplified water filtration, DNA extraction, library preparation as well as sequencing and bioinformatic pipeline. As a workflow designed for non-scientific personnel, it can be easily transferred to environmental stakeholders for the monitoring of cHABs to complement traditional workflow based on microscopy. RosHAB will enable environmental stakeholders to reduce the duration of the cyanobacterial identification process, expand the geographical coverage of cHABs monitoring and increase their analytical capacity while reducing analytical cost. RosHAB represents the next generation of monitoring practices for not only cHABs monitoring in the field, but for environmental monitoring in general.

**Gertrud Nurnberg**  
*Freshwater Research*

Cyanobacteria response to climate-affected internal phosphorus loading in two drinking water reservoirs.

Phosphate released from bottom sediments can present a nutrient source for cyanobacteria growth in late summer and fall in two low-productive drinking water reservoirs of the City of Moncton, New Brunswick. The upstream reservoir (feeding the downstream reservoir with deep water withdrawal since 2014) experienced a potentially toxic cyanobacteria bloom in the fall of 2017. Both reservoirs are relatively unproductive (2018-2022 growing periods, epilimnetic upstream: meso-eutrophic, 0.012 - 0.020 mg/L total phosphorus, TP; downstream: oligotrophic, 0.007 – 0.012 mg/L TP; TN of both: <0.250 mg/L), but experience oxygen depletion (< 3.0 mg/L DO) every summer (anoxic factor of 4 - 29 d/yr and 6-11 d/yr). Phosphorus and metal depth profiles, their seasonal changes, and sediment P fractionation indicate internal P release from anoxic sediment in 6 study years (sampled 16-20 times/growing period). Since 2017, internal load was up to 28% of total TP load in the upstream and up to 12% in the downstream reservoir. Phytoplankton (measured by sensor profiles of chlorophyll and phycocyanin, Secchi transparency, and cell counts at multiple sites) responded to internal P load, but were also affected by climate conditions. As expected, cyanobacteria proliferated in dry and warm summers (low daily precipitation and high maximum air temperature in Jun-Sep) that also provided sufficient P released from sediments, but were further influenced by reservoir mixing status as controlled by reservoir elevation. In addition, major precipitation and storm events enhanced water column fertilization by early mixing, but could also shorten potential bloom periods. The observed short-term weather variability and expected long-term climate effects (warmer and more disturbed weather patterns) complicate any forecasts of cyanobacteria proliferation in these reservoirs in response to internal P sources. A physical approach to remediation involves the increase of the upstream dam height and is based on theoretical limnological considerations and TP mass balance modeling.



**Stuart Oehrle**

*Northern Kentucky University*

Targeted UPLC/MS/MS method for cyanobacterial toxins in recreational water:  
A comparison to metagenomics.

*With Joshua Cooper.*

In this paper we monitor several cyanobacterial toxins using a generic gradient method and a targeted Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) method. We analyzed various freshwater lake samples from a region in Ecuador for a variety of toxins including microcystins, anatoxin-a, cylindrospermopsin as well as newer toxins, such as euglenophycin, anabaenopeptins and micropeptins. In addition, Metagenomic sequencing and analysis of the samples identified one genomic bin identified tentatively as *Planktothrix agardhii*, and several genomic fragments of other toxin producing cyanobacteria. Using the genome we examined which genes were present and what toxins were capable of being produced. In this case both the DNA analysis and LC/MS/MS analysis gave results that agreed. Data from both techniques will be presented including the novel detection of various forms of cylindrospermopsin in this sample. Additional work from Ohio River water samples will be discussed as well showing commonalities of toxic productions over several years as well as other samples from throughout the US.

**Marianne Potvin**

*IBIS*

Real-time on-site cyanobacteria identification using Oxford Nanopore sequencing.

*With Jeff Gauthier, Christophe Langevin, Naila Barbosa da Costa, Vani Mohit, Irena Kukavica-Ibrulj, Daniel Verreault, Jérôme Comte and Roger C. Levesque.*

Rapid on-site detection of Harmful Algal Blooms (RosHAB) represents the next generation of monitoring practices, incorporating an innovative, in-the-field applicable genomics workflow for real-time, reliable, accessible and cost-effective early detection of cyanobacteria harmful algal blooms (cHABs) outbreaks. Critical strategies include on-site sample processing, sequencing and analysis on a laptop using the Oxford Nanopore Technologies (ONT) MinION portable sequencing system coupled to the International cyanobacterial toxin database (ICYATOX). In preliminary experiments, we demonstrated that this genomics-derived solution is cost-effective and rapid in diagnostics. We compared the data obtained from a full-length sequencing run to a 30-min sequencing run with ONT MinION and Flongle systems. We analysed samples with 3 different concentrations of cyanobacteria sequenced on Flongle with rapid library preparations compared with microscopy data. Results showed that RosHAB gives similar results to the species level when compared to microscopy but in a cost and time effective manner. Rapid, in real time and on-site analyses requires a representative database of cyanobacteria isolated from freshwater ecosystems in Quebec and Canada. We will build the on-line ICYATOX database of cyanobacterial strains, phenotypes, genotypes, genomes, and metadata. Comparative genomics and phenomics analyses from cyanobacteria will identify traits typical of cyanobacteria diversity detected in Quebec, Canada and abroad.

**Audrey Roy-Lachapelle**

*Environment and Climate Change Canada*

Determination of cyanopeptides by targeted, suspect and non-target screening  
in the Lake Saint-Pierre watershed.

*With Morgan Sollicec, Sébastien Sauvé and Christian Gagnon*

Cyanobacteria are going under the radar worldwide due to increased episodes of harmful algal blooms. Under favorable conditions, they can produce cyanotoxins and their toxicity is systematically linked to the appearance of microcystins. Other bioactive cyanopeptides, such as anabaenopeptins, microginins and cyanopeptolins, can be just as present as microcystins during episodes of cyanobacterial blooms. Over 500 cyanopeptides have been structurally identified, including over 279 microcystins. However, due to their complexity and the lack of available standards for identification and quantification, their production and potential impact on human health and biodiversity are little known. An analytical method based on mass spectrometry using tiered approach has been developed for the screening of 27 cyanopeptides representing seven different families including microcystins, anabaenopeptins, microginins, aeruginosins, aeruginosamide, nodularin and cyanopeptolin. Samples positively screened in for the presence of any cyanopeptides were analyzed a second time in order to identify cyanopeptides without analytical standards with a suspect screening method using the CyanoMetDB database and non-target screening strategies to characterize potential new congeners. These methods have allowed the identification of new microcystins and anabaenopeptins, as well as the detection of known congeners, but until now not analyzed by cyanotoxin monitoring programs. These results demonstrated that many microcystin congeners can fly under the radar if left unchecked. Moreover, anabaenopeptins can be abundant in toxic cyanobacteria blooms, indicating that their presence could be at play in the toxicological interpretation of harmful cyanobacteria if not followed.

**Krista Thomas**  
*Biotoxin Metrology*

Multi-class analysis of Cyanobacterial Toxins  
using Liquid Chromatography-Mass Spectrometry.

*With Elliott Wright, Daniel Beach, Cheryl Rafuse and Pearse McCarron*

Cyanobacteria can produce diverse classes of toxins including microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs), and saxitoxins (STXs). These span a wide range of mechanisms of toxicities, polarities and molecular weights, generally requiring multiple extraction and analytical techniques for evaluation. Here we present a single LC-MS/MS method for the detection and quantitation of all the aforementioned toxin classes. A comprehensive liquid-solid extraction method was developed using 75% MeCN/water (0.1% HCOOH). HILIC-MS/MS was used with gradient elution and selected reaction monitoring (SRM) on a triple quadrupole MS with positive/negative polarity switching (35 min run time). Confirmatory transitions and retention time matching with standards were used for identification. Recoveries (between 66% (CYN) and 107% ([Dha7]MC LR)) were determined by spiking freeze-dried algae samples (*Aphanizomenon* sp.). Validation included evaluation of selectivity, calibration models, precision (between 4.4% (dcSTX) and 11% ([Dha7]MC-LR)), and detection limits (between 0.2 (ATX) and 7 (GTX4) ng/mL). This method was implemented to evaluate toxin profiles of in-house cyanobacterial cultures for the development and characterization of a cyanotoxin freeze dried matrix CRM. It was also applied to dietary supplements, environmental samples of benthic and planktonic cyanobacteria, and shellfish, demonstrating suitability for screening and quantitation in a wide range of sample matrices of interest. This included application of the method for class-based screening in response to two dog deaths in NS, Canada in 2021, which quickly revealed the presence of ATXs and the absence of other cyanotoxin classes, facilitating more a more targeted follow-up study. The addition of other classes of polar fresh water and marine toxins to the method, including *Lyngbya wollei* toxins, guanitoxin, domoic acid and tetrodotoxin, offers even broader utility of this method in the future. This could include applications in estuarine environments impacted by both marine and freshwater phycotoxins.

**Hana Trigui**

*Polytechnique de Montréal*

Impact of oxidation and stagnation on the diversity of cyanobacteria  
in drinking water treatment plant sludge.

*With Farhad Jalili, Juan F. Guerra Maldonado, B. Jesse Shapiro, Yves Terrat, Nathalie Fortin, Sébastien Sauvé and Michèle Prévost.*

Health-related concerns about cyanobacteria-laden sludge of drinking water treatment plants (DWTPs) have been raised in the past few years. The objectives of this study were to (i) investigate the impact of oxidation on cyanobacteria and cyanotoxins in both lab- and full-scales, (ii) determine the efficiency of oxidation during sludge storage and, (iii) evaluate the impact of oxidation and stagnation on microbial and cyanobacterial communities. Methodology. Microscopic taxonomy, shotgun metagenomic sequencing, microcystin (MC) measurement and MC synthesis genes quantification were applied to study the fate of cyanobacteria and cyanotoxins after sludge oxidation and storage (stagnation) under controlled steady-state conditions and in a full-scale drinking water treatment plant (DWTP). For controlled laboratory trials, two common oxidants, KMnO<sub>4</sub> (5 and 10 mg/L) and H<sub>2</sub>O<sub>2</sub> (10 and 20 mg/L) were applied, and non-oxidized and oxidized sludge were left to stagnate in the dark for 7 to 38 days. To simulate a shock treatment in the sludge holding tank, on-site oxidation was performed by adding a single dose of 10 mg/L KMnO<sub>4</sub>. Results. Controlled laboratory oxidation showed that KMnO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> decreased total taxonomic counts, as well as potential MC producers. However, stagnation caused cell growth up to 2.6-fold in 8 out of 22 oxidized samples. MC producing orders as Chroococcales and Synechococcales were persistent while Nostocales was sensitive to combined oxidation and stagnation. On-site shock oxidation decreased taxonomic cell counts by up to 43% within 24 h. Stagnation preceded by on-site shock oxidation could increase total cell counts by up to 55% as compared to oxidation alone. The increase of cell counts and mcyD gene copy numbers during stagnation revealed the impact of oxidation/stagnation on cyanobacterial cell growth. Conclusions. These findings show the limitations of sludge oxidation as a strategy to manage cyanobacteria and cyanotoxins in sludge and suggest that alternative approaches.

Nicolas **Tromas**  
*McGill University*

Predicting cyanotoxins concentrations in lakes and reservoirs  
using microbial community information.

*With Zofia E. Taranu, Dana F Simon, Barry Husk, Helen M Baulch, Nathalie Fortin, Charles W Greer, Megan L Larsen, Jason J Venkiteswaran, Felipe Fernando Martínez Jerónimo, Alessandra Giani, Chris D Lowe, Sébastien Sauvé and B. Jesse Shapiro.*

Cyanobacteria harmful algal blooms are a worldwide problem and are increasing in frequency due to agricultural intensification and climate change. Under certain conditions, cyanobacteria rapidly form high-density blooms and produce a high diversity of toxins (including hepatoxins and neurotoxins), which can cause severe human illness (e.g. gastroenteritis, liver failure) or even death when consumed in high amounts via drinking water. Eutrophication and warmer temperatures both contribute to blooms, but it is still difficult to predict precisely when and where toxic blooms will occur. One reason that prediction is difficult is that blooms can be comprised of different species or genera that differential respond to biotic (e.g interactions with bacteria) and abiotic (environmental) factors. Cyanotoxin prediction based on biotic factors is attractive because the composition of the microbial community can be thoroughly measured through culture-independent, high-throughput sequencing, whereas it is not always clear which are the relevant (or most predictive) abiotic factors that should be measured. Moreover, the microbial community composition may contain information about both measured and unmeasured abiotic variables, insofar as these variables impact the community. To examine this, the objective of our study was to predict toxin concentrations and diversity in drinking water sources (i.e., lakes, reservoirs, and water treatment plants) using microbial community information (composition of taxa, genes and allele frequencies) and environmental variables. We used data generated from the Genome Canada-funded ATRAPP project, focusing on eight lakes with ten or more samples per lake: Lake Champlain (Canada), Petit Lac Saint-François (Canada), Buffalo Pound Lake (Canada), Lake Saint-Charles (Canada), Conestogo (Canada), Valle de Bravo (Mexico), Argal (UK), and Woolwich reservoir (Canada), for a total of 834 metagenomes. Our first set of analyses, Joint Species Distribution Modeling (JSDM), aimed at improving our understanding of how toxins respond to environmental variables, and how this may vary across the season. Additionally, this approach allowed us to track what remained unexplained by the model (latent variables) and interpret these as responses to unmeasured environmental variables and/or biotic interactions. The JSDMs showed that seasonality was a key variable in explaining the presence-absence and continued rise in cyanotoxins, followed by ammonia and total phosphorus concentrations. To further examine the seasonal succession of toxins, and identify which toxins co-occurred and in what proportions, we ran a Latent Dirichlet Allocation (topic model). This showed that certain

toxins appeared earlier in the season in many lakes (microcystin-LR and -LY), and were replaced by a different community of toxins by summer (microcystin-LA, anabaenopeptin-A and -B).

To examine in detail the biotic interactions suggested by the JSDMs, we then used a network modeling approach (Spiec-easi) and found that *Microcystis* was associated with the presence of most, but not all, forms of microcystins in lakes. In contrast, *Planktothrix* co-occurred with anabaenopeptin-B. Given these strong biotic relationships, our final analysis made use of Bayesian Additive Regression Trees (BART) to predict total microcystin concentrations and the time until toxicity reached the WHO guideline of  $>1$   $\mu\text{g/L}$  from cyanobacteria community data. This approach showed that we predicted total microcystin concentrations well using either all observed species of cyanobacteria (Spearman correlation of observed vs. predicted values = 0.84) or the top 500 non-cyanobacterial species (correlation = 0.83). The model trained on all lakes and tested on held-out data from individual lakes provided good predictions for six lakes (obs. vs pred. correlations ranging from 0.57 to 0.85), but poor predictions for the remaining two lakes (Valle de Bravo and Conestogo). Predictions of the time until the WHO guideline was reached were more uncertain (correlation = 0.7 using cyanobacterial taxa or 0.67 using non-cyanobacteria), but still significant.

**Cecilio Valadez-Cano**

*University of New Brunswick*

Closely related cyanophages from geographically distant North American rivers are predicted to infect anatoxin-producing *Microcoleus* species.

*With Adrian Reyes-Prieto and Janice Lawrence*

The toxigenic potential of *Microcoleus*-dominated mats in rivers is an emerging issue in North America. Among the environmental and biological factors that may play a role in regulating mat proliferation, phages are an unexplored possibility. Here, we examine phage diversity in *Microcoleus*-dominated mats from the Wolastoq (New Brunswick, Canada) and the Eel (California, USA) rivers. Our bioinformatic analysis of shotgun-sequenced mat samples from both rivers allowed us to recover multiple virus-like sequences associated with different bacterial hosts. Predicted phages include two cyanophages with different replication strategies that putatively infect *Microcoleus* species. A lysogenic cyanophage genome (predicted as a prophage), phylogenetically related to previously described Phormidium phages, was found integrated in the chromosome of a non-toxic *Microcoleus* species from the Eel River. The prophage is predicted to encode genes that may influence host population dynamics, such as a protein that facilitates the proteolytic degradation of phycobilisomes during nitrogen starvation. In addition, a lytic cyanophage with a jumbo genome was found in mats dominated by toxic *Microcoleus* species from the Wolastoq and Eel rivers. Despite the geographical distance between the two rivers, the lytic *Microcoleus* phage genomes share similar genes with high sequence identity. Phylogenetic analysis suggests that these lytic *Microcoleus* phages are distant from any other cyanophage previously described and are predicted to encode genes for structure, nucleotide metabolism, and enzymes required for host lysis. These results reveal the unexplored diversity of phages associated with the microbial community of benthic mats in rivers and their potential role shaping the abundance of anatoxin-producing *Microcoleus* species.



**Mark Van Asten**

*Phytoxigene*

The Development and Challenges of a Molecular qPCR assay for the Detection of Anatoxin and Guanitoxin Producing Cyanobacteria in Environmental Samples.

*With Bakir Al-Sinawi, Diagnostic Technology and Greg Ford.*

North American lakes and reservoirs have seen an increase in the number and severity of harmful algal blooms (HAB) caused by cyanobacteria (blue-green algae). The cyanobacteria present in the HABs can potentially produce toxins capable of causing illness and/or death. Timely and accurate identification and reporting of these toxins is critical for issuing water quality advisories. The methods used to analyze toxin are very expensive and selecting the correct one can be difficult. Another challenge is the necessity for a skilled analyst to be available to identify the microscopic algae. Furthermore, some cyanobacteria can produce multiple toxins (e.g., the genus *Anabaena* can produce anatoxin-a, saxitoxin, and microcystin; the genus *Aphanizomenon* can produce saxitoxin, anatoxin-a, and cylindrospermopsin). The current Phytoxigene Quantitative PCR (qPCR) assay has been in use by states and water agencies to for screen for potential risk of the toxins Microcystin/Nodularin, Cylindrospermopsin and Saxitoxin, plus total cyanobacteria by the 16SrRNA gene since 2016. Testing with toxin analysis has previously demonstrate that there is a very high correlation between toxin gene detection and toxin production. The results from these studies indicate that this method can predict HAB risk and simplify the process of toxin analysis. Anatoxin- is a secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity and can cause death in all mammals, with dog deaths attracting the most attention. The toxin is produced by multiple cyano-bacteria genera throughout the world, most commonly by benthic organisims including *Microcoleus*, *Anabaena*, *Oscillatoria* and *Aphanizomenon*. Guanotoxin, formerly known as Anatoxin-S, is a nueurotoxin most commonly produced by the benthic cyanobacteria, *Anabeneae*. A lesser understood toxin as it is more labile, genetic screening for a gene responsible for its's production will provide water quality professionals a better understanding of its scope and subsequent risk. Phytoxigene is developing a multiplex qPCR assay that will detect the presence of an Anatoxin and Guanitoxin gene required for Anatoxin and Guanitoxin synthesis, along with an Internal Amplification Control to check for environmental inhibition. The presentation will highlight the development process and challenges related to the design of this assay.

**Emily Varga**

*Great Lakes Institute for Environmental Research*

Understanding the effects of environmental drivers of phytoplankton community succession and toxin production in Canadian waters of Lake St. Clair.

*With Michael McKay*

In recent decades, the Lake Erie watershed has experienced accelerated eutrophication due to environmental pollution and intensive agricultural activity. Consistent with eutrophication, seasonal cyanoHABs recur annually in upstream Lake St. Clair. Beyond the summer and fall cyanoHABs, the phytoplankton assemblage in Lake St. Clair undergoes seasonal succession and our research seeks to investigate environmental drivers of this succession. Beginning in August 2020, samples were collected from the Stoney Point water treatment plant (SPWTP) intake well at bi-weekly to monthly intervals. Water quality parameters including, phycocyanin as a proxy for cyanobacterial biomass, were recorded through real-time monitoring to inform of potential bloom events. Samples were analyzed for nutrient, chlorophyll and toxin (microcystin) concentrations, taxonomic composition and qPCR for detection of genes involved in the production of microcystins and saxitoxins. Preliminary results show seasonal oscillations of nutrient concentrations, with a significant difference in NO<sub>3</sub> concentrations between the growing seasons of 2020 and 2021. Microcystin and chlorophyll a concentrations both varied between 2020 and 2021, with the highest values occurring in early to mid September. Microscopic analyses show the expected seasonal succession of community assemblage from late August 2020 to mid-September 2021, with mixed colonial cyanobacteria dominating in summer and fall, followed by diatoms in winter and early spring. Peak concentrations of microcystins in the water column positively correlated to the shift in cyanobacterial dominance, specifically *Microcystis*. Results of this multi-year study will advance understanding of the environmental drivers of phytoplankton community succession in Lake St. Clair and help to build predictive models of when environmental conditions will promote the shift to a community dominance of potential toxin-producing cyanobacteria.

Arthur **Zastepa**

*Environment and Climate Change Canada*

Beyond total microcystins: Toxic and bioactive metabolites produced by cyanobacteria in Lake Erie's western basin.

*With Judy A. Westrick, Todd R. Miller, Anqi Liang and Justin D. Chaffin.*

Cyanobacteria produce toxic/bioactive metabolites that can inhibit proteases, carboxypeptidases, or phosphatases involved in metabolic processes relevant to human and ecosystem health. From surface water samples collected in 2017 (n = 44), 2018 (n=100), and 2019 (n = 171), we report concentrations of more than 20 such compounds in the western basin of Lake Erie, a large lake of multinational jurisdiction. The most frequently found compounds included microcystins LA and LR as well as anabaenopeptin B and F (>90%). Microcystins RR and YR as well as anabaenopeptin A were also detected at relatively high frequency (>80% in 2019). There was strong correlation among arginine-containing MC variants (RR, YR, HtyR, LR, HilR, WR, D-Asp3-LR, Pearson's  $r > 0.80$ ) but not with arginine-lacking LY, LW, LF. Anabaenopeptin F also correlated with these same arginine-containing MC variants (Pearson's  $r > 0.70$ ). In 2019, only 4% of lake water samples exceeded the recreational water guideline for total microcystins (10  $\mu\text{g/L}$ ) set by Health Canada with the maximum concentration  $\sim 26 \mu\text{g/L}$ . The drinking water guideline (1.5  $\mu\text{g/L}$ ) was exceeded in 34% of lake water samples but treated water was not tested. The maximum total anabaenopeptins concentration was almost four times higher (almost 100  $\mu\text{g/L}$ ) but no guideline values have been derived for this group of metabolites as their impact is currently considered to be ecological rather than on humans/animals. These results can be used to evaluate risk from cyanobacterial toxins/bioactive metabolites in Lake Erie and aid binational lake management and policy development in the Great Lakes basin.

# Posters

**Poster 1**

Jordan **Balson**  
*Queen's University*

Assessing the potential of *Caenorhabditis elegans*  
in the bioremediation of *Microcystis aeruginosa*.

*With Ian Chin-Sang, Yuxiang Wang and Daniel Lefebvre.*

Cyanobacteria dominance signifies a global challenge for water security and has been linked to anthropogenic forcing. However, the relative importance of different human stressors remains to be determined, as the study of climate and land-use changes is often restricted to short temporal scales. In this study, we reconstructed cyanobacteria abundance and microcystin-producing potential in temperate lakes across a human impact gradient using a paleolimnological approach. We discovered breakpoints (points of abrupt change) and investigated the influence of climate and landscape proxies on these breakpoints. Cyanobacteria abundance in high-impacted lakes (e.g., agricultural catchments) increased 40 years earlier than in low-impacted lakes (e.g., forested catchments), with land-use change as the dominant predictor. Microcystin-producing potential increased in both high- and low-impact lakes around the 1980s, with climate warming as the dominant predictor. Our findings chronicle the importance of climate change in increasing the risk of toxigenic cyanobacteria in freshwater resources.

**Poster 2**

**Naíla Barbosa de Costa**

*Institut National de la Recherche Scientifique.*

Overview of the project

RosHAB (rapid on-site detection of harmful algal blooms) in Quebec.

*With Marianne Potvin, Jeff Gauthier, Christophe Langevin, Vani Mohit, Irena Kukavica-Ibrulj, Daniel Verreault, Roger C. Levesque and Jérôme Comte.*

Yearly, hundreds of lakes are affected by cyanobacterial harmful algal blooms (cHABs) in Quebec. With the increasing frequency of cHABs worldwide led by multiple environmental factors, we urge the implementation of surveillance tools for early and accurate detection of blooms in Quebec waterbodies which are sources of recreational or drinking water in addition to playing important ecological services in natural landscapes. In this context, we are currently developing RosHAB (Rapid on-site detection of Harmful Algal Blooms), a pipeline that implements Nanopore Sequencing for monitoring cHABs and that will be made available for non-scientific personnel use. Beyond using metagenomic sequencing to survey blooms in Quebec, we plan to isolate cyanobacterial strains for whole-genome sequencing and therefore create a database with important phenotypic and genotypic features of the main bloom-forming species. The International Cyanobacterial toxin (ICYATOX) database will be made available online for further comparison with cyanobacterial strains causing blooms across the world. This database will provide valuable information to help elucidate associations between cyanobacterial blooms and their toxicity with genomic features and environmental conditions. The RosHAB strategy aims to improve the efficiency of cHABs surveillance and to identify prognostic markers for evidence-based decisions in the monitoring of blooms by developing standardized on-site field and genomics procedures for the early detection of cHABs outbreaks.

**Poster 3**

**Daniel Beach**

*National Research Council Canada*

Development of a Cyanobacterial dietary supplement reference material  
for multiple classes of Cyanotoxins.

*With Kelley L. Reeves, Cheryl Rafuse, Krista M. Thomas, Elliott J. Wright, Nancy I. Lewis, Christopher O. Miles, Michael A. Quilliam and Pearse McCarron.*

Some cyanobacterial dietary supplements are prepared using biomass harvested from lakes and ponds where multiple species co-occur. This can result in the contamination of products with cyanotoxins that present a risk for the consumer. Reference materials are therefore required to assist in the development, validation and quality control of analytical methods used to assess raw material and for product testing in both research and regulatory environments. Here, we report the development and preliminary characterization of a dietary supplement matrix certified reference material (CRM) for multiple classes of cyanotoxins. The CRM was prepared by blending biomass from a variety of toxic cyanobacterial strains with a typically-consumed non-toxic strain (*Aphanizomenon* sp.). The freeze-dried, homogenized material was aliquoted into over 2000 identical units and will be characterized for toxin concentration, homogeneity and stability using a novel multi-class extraction and LC-MS/MS method. A suite of class-specific methods will also be used to assign certified values to toxins for which certified reference material calibration solutions are available, using a combination of isotope-dilution and standard-addition calibration. These include five microcystins, several saxitoxins, nodularin-R, anatoxin-a and cylindrospermopsin, with preliminary concentrations ranging from 1 to 100 µg/g dry weight. Finally, untargeted high-resolution mass spectrometry methods will be used to characterize the rich profile of over 50 non-certified cyanotoxins and other cyanobacterial secondary metabolites present.

**Poster 4**

**Ann-Kristin Bergstrom**

*Umeå University*

Phytoplankton biomass does not show a unidirectional trend in its response to global change in Fennoscandian lakes

*With Aleksey Paltsev, Irena F. Creed, Dag Olav Hessen, Stina Drakare and Kristiina Vuorio.*

Global change may introduce fundamental alterations in phytoplankton community structure that can reduce the productivity of northern lakes. We hypothesized that phytoplankton biomass (FB) in Fennoscandian lakes is increasing due to warming and decreasing due to decreasing TP, TN that are essential for phytoplankton growth. To test this hypothesis, we compiled water chemistry (TP, TN, DOC, pH and Fe) and phytoplankton data in 110 Fennoscandian lakes that represent broad water chemical gradients and performed trend analysis on the lakes for the 1995-2019 period. We found that 60 lakes had an increase in FB, and 50 lakes had a decrease in FB. In the northern boreal zone, significant trends in FB were mostly positive (47 positive % vs 3% negative), while these were mixed in the southern boreo-nemoral zone (23% positive vs 27% negative). However, the rate of change in lakes with negative trends was higher than in lakes with positive trends (mean Sen's slope:  $-2,2 \mu\text{gL}^{-1} \text{y}^{-1}$  vs  $0.3 \mu\text{gL}^{-1} \text{y}^{-1}$ ). Among phytoplankton, *Gonyostomum semen* showed the strongest decline, while cyanobacteria showed the strongest incline. The decline in *Gonyostomum* was related to increasing pH (Kendal tau:  $-0.5$ ,  $p < 0.1$ ), while rising cyanobacteria was related to increasing pH (Kendal tau:  $0.34$ ,  $p < 0.1$ ) in the northern boreal zone and decreasing TP and Fe in the southern boreo-nemoral zone. Therefore, increasing pH appears to contribute to collapses of *Gonyostomum* while triggering shifts to cyanobacteria.



**Poster 5**

**Lisa Cleckner**

*Finger Lakes Institute*

Comprehensive monitoring of HABs via molecular tools and weekly sampling  
in two Finger Lakes, NY.

*With Trevor Massey, Nan Wang and Ruth Richardson*

A multi-year study of nearshore harmful algal blooms (HABs) in two different Finger Lakes in NY shows different timing of the development of blooms, toxin concentrations, and dominant cyanobacteria across summer to late fall. Blooms in mesotrophic Cayuga Lake begin in early July with *Dolichospermum* dominating early blooms in much of the lake and changing to *Microcystis* by early August into the fall. For oligotrophic Canandaigua Lake, HABs dominated by *Microcystis* generally appear in early August with nearshore blooms seen throughout the lake by early September. Weekly 0.5m composite water samples were collected dockside from four locations on each lake and analyzed for Microcystin concentrations using EPA Method 546 and Chlorophyll-a using a bbe FluoroProbe. Results reveal a change in Microcystin toxin concentration per chlorophyll-a concentration of the composite samples that mirrored changes in the toxin quota observed for HAB samples collected from Canandaigua Lake over time, but not for Cayuga Lake. Preliminary 16-s analysis of HAB samples show that Cyanobacteria are the dominant phylum, followed by Bacteroidetes, Proteobacteria and Actinobacteriota. In contrast, non-bloom samples had much more diverse communities, with these four phyla constituting less than 50% of all sequences combined. A comparison of HAB and composite water data from Cayuga and Canandaigua Lakes will be presented.

**Poster 6**

**Catrina Earnshaw**  
*Carleton University*

The elucidation of Planktothrix Cyanopeptide profiles  
using Mass-Spectrometry based Metabolomics.

*With David McMullin.*

The magnitude and frequency of cyanobacteria blooms are increasing due to environmental changes including increased temperatures, eutrophication and anthropogenic inputs. Cyanobacteria are a source of diverse natural products that can negatively impact ecosystem and human health. The most commonly studied cyanopeptides are microcystins, a group of potent liver toxins and possible human carcinogens (IARC Group 2B). Together with microcystins, common bloom forming cyanobacteria co-produce numerous other cyanopeptide groups such as aeruginosins, anabaenopeptins, cyanobactins, cyanopeptolins, microginins and microviridins. There is a lack of toxicological and environmental concentration data for these lesser studied cyanopeptide groups. Advances in analytical techniques allow further comprehension of the complex metabolomes of cyanobacteria. Mass spectrometry-based metabolomic techniques can be applied to decipher the cyanopeptide profiles of common cyanobacterial strains. In this study, the cyanopeptide profiles of six Planktothrix agardhii/rubescens strains isolated from lakes within the Ontario and Quebec area, were characterized using mass spectrometry-based metabolomics. Cyanopeptide chemical classes were determined based on shared structural features identified by characteristic product ions within their tandem mass spectra. Metabolomic strategies including multivariate analysis, diagnostic fragmentation filtering and global natural product society (GNPS) molecular networking were implemented to rapidly decipher strain-specific cyanopeptide profiles from data dependant data sets. Each strain produced different mixtures of microcystins, aeruginosins, anabaenopeptins, cyanopeptolins and microviridins. However, microginin and cyanobactin groups were not identified from studied strains. The most diverse cyanopeptide groups were cyanopeptolins and anabaenopeptins. This work provides critical information regarding the metabolite composition present in Planktothrix extracts, consequently aiding in the prioritization of novel natural products for isolation and risk characterizations. Furthermore, these complex mixtures of cyanopeptides produced by environmentally relevant strains indicate that the current monitoring strategies should be adapted to account for these lesser studied cyanopeptide classes.

**Poster 7**

Ella Joy **Kantor**  
*McGill University*

The effect of cyanobacterial blooms on antibiotic resistance genes.

With Nicolas Tromas, B. Jesse Shapiro, Sébastien Sauvé, Dana F. Simon, Barry Husk, Helen Baulch, Jason Venkiteswaran, Felipe Fernando Martínez Jerónimo, Nathalie Fortin, Charles W. Greer and Chris D. Lowe.

To improve our knowledge of how antimicrobial resistance is disseminated worldwide, it is essential to evaluate the role of aquatic ecosystems as a potential reservoir. Antimicrobial resistance (AMR) occurs when microbes evolve or acquire genes that protect them from antimicrobial medicines. The composition and variation of antibiotic resistance genes (ARGs) – a subset of AMR – in the bacterial populations of aquatic environments is a topic of interest because they are in contact with humans or animals in drinking water and recreation. Cyanobacterial blooms in freshwater ecosystems are becoming increasingly prevalent due to global warming and increased eutrophication. When this intense cyanobacterial population growth happens, it changes the structure of aquatic bacterial communities, thereby also shifting the relative abundances of genes encoded in bacterial genomes. Here, we aim to characterize the impact of cyanobacterial bloom on ARG dynamics and if different lakes around the world experiencing cyanobacterial bloom would have a similar ARG signature despite having different bloom composition. We analyzed the dynamics of ARGs from early spring to fall in Lake Champlain using shotgun metagenomic sequencing and the PathoFact pipeline to identify ARGs and MGEs. We found that ARG abundance increased in the summer months when blooms were occurring. In addition, we found that the diversity and composition of cyanobacteria and ARGs seem similar during the spring and summer, and an increase in plasmid and phage abundance in these same months. These results will inform the subsequent analysis which will be done on a much larger data set of lakes from the ATRAPP dataset. Once we understand these large-scale patterns, we can use them to inform decisions about lake management. If cyanobacterial blooms are found to be significantly increasing ARG abundance, it would lead to increased urgency to manage and track blooms that pose a great threat to global medicine.

**Poster 8**

**Rob Jamieson**

*Dalhousie University*

Temporal and spatial dynamics of toxic benthic cyanobacteria proliferation along a lentic-lotic gradient.

*With Yannan Huang, Lindsay Johnston, Tessa Bermarija, Cheryl Rafuse, Lydia Zamlynny, Andre Comeau, Meghann Bruce, Catherine Graham, Janice Lawrence and Daniel Beach.*

Proliferations of benthic cyanobacteria have been increasingly reported worldwide. The presence of toxin producing mats is particularly alarming to the public when the affected water course is used for drinking water or recreation. In this study, we monitored the growth of toxic benthic cyanobacteria species (*Microcoleus* spp.) along a stream-lake continuum by microscopic identification and PacBio bacterial 16S rRNA full-length sequencing techniques. The previous year, two dog deaths had been attributed to benthic cyanobacteria mats in the area. We found that the species were able to proliferate in both lentic and lotic environments, but temporal growth dynamics and subspecies dominance were influenced by habitat type. This in turn impacted toxin production, which was greatest in benthic cyanobacteria mats growing in a large oligotrophic lakeshore environment. Our results may influence how water resource managers assess risk associated with benthic proliferations in relatively low nutrient lakeshore environments.

## Poster 9

Elisabeth Janssen

*Swiss Federal Institute of Aquatic Science and Technology*

Update on CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria

*Ernani Pinto, Dörr Fabiane, Torres A. Mariana, Hanna Mazur-Marzec, Karolina Szubert, Robert Konkel, Luciana Tartaglione, Carmela Dell'Aversano, Antonella Miglione, Pearse McCarron, Daniel G. Beach, Christopher O. Miles, Martin R. Jones, David P. Fewer, Kaarina Sivonen, Jouni Jukela, Matti Wahlsten, Tania K. Shishido, Timo Niedermeyer, Franziska Schanbacher, Pedro Leão, Marco Petro, Pavel Hrouzek, Paul D'Agostino and Martin Baunach.*

CyanoMetDB is a comprehensive, publicly accessible database of cyanobacterial secondary metabolites. Version\_01 included 2000 entries from 850 peer-reviewed articles published between 1967-2020 (Jones et al, Water Research, 2021). The database contains structural codes to facilitates the detection and dereplication of known cyanobacterial toxins and secondary metabolites as well as the identification of novel natural products from cyanobacteria. Since being developed, CyanoMetDB has been widely used as a comprehensive suspect list in the scientific community directly and through other larger platforms where it has been integrated. Here we present two major initiatives in the ongoing development of CyanoMetDB: First, the new Version\_02 contains hundreds of new metabolites and correction of previous entries. Second, we are generating mass spectrometry reference spectra from available reference standards and (semi)purified extracts where identification with confidence level 1-2 was previously achieved by one of the CyanoMetDB Teams. The spectra are systematically recorded for a range of collision energies typically used in suspect-screening by HRMS/MS and the data undergoes a clean-up and quality control step before uploading them to public spectral databases. Open-access reference spectra will further facilitate automated data processing and enable to reach a higher the level confidence for the identification of metabolites when no in-house reference materials are available.

**Poster 10**

Kim Thien Nguyen Le  
*Polytechnique Montréal*

Effect of nitrogen on cyanobacteria and cyanotoxins.

*With Kim Thien Nguyen Le, Juan Francisco Guerra Maldonado, Thanh-Luan Nguyen, Eyerusalem Goitom, Hana Trigui, Ndèye Adiara Ndiaye, Yves Terrat, B. Jesse Shapiro, Barry Husk, Sébastien Sauvé, Michèle Prévost and Sarah Dorner*

Toxic cyanobacterial blooms are a worldwide concern. Nutrients are among the numerous factors that trigger cyanobacterial blooms and the production of cyanotoxins. Many jurisdictions regulate phosphorus loads to control eutrophication, while nitrogen regulations are often based on ammonia toxicity. The aim of this study was to assess the effect of nitrogen on cyanobacteria and cyanotoxins through a short term mesocosm experiment. To achieve this, mesocosms were installed in situ in two lakes (Missisquoi Bay and Petit-Lac-St-François) and received 700 µg/L of NH<sub>4</sub><sup>+</sup> or 500 µg/L of NO<sub>3</sub><sup>-</sup>. Total taxonomic cell counts, Dolichospermum and Aphanizomenon's cell counts significantly decreased in the mesocosms containing NH<sub>4</sub><sup>+</sup> compared to the control mesocosms, while total taxonomic cell counts, and genus cell counts in NO<sub>3</sub><sup>-</sup> mesocosms did not change significantly compared to control mesocosms two days following nitrogen addition. A shift to Microcystis in the mesocosms with nitrogen addition was observed two days after the addition in Missisquoi Bay, where nitrogen was more limited as compared to Petit Lac St. François based on nutrient ratios. There were no significant changes in intracellular toxins of total microcystins between control mesocosms and mesocosms with the addition of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> after two days of exposure at both sites. However, there was a significant increase of total extracellular MCs and MC-LR in mesocosms with addition of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> after 48 hours compared to control mesocosms. Intracellular and extracellular microcystin concentrations were associated with Microcystis. Microcystis presence was associated with NH<sub>4</sub><sup>+</sup> 48 hours after the addition of N at both sites. Therefore, toxin production following sudden nitrogen addition can occur on short time scales relevant to drinking water treatment plant operations. This information will help treatment plant operators better anticipate the arrival of potentially higher concentrations of cyanotoxins.

**Poster 11**

**Keri Malanchuk**  
*Carleton University*

Metabolomic approach to characterize the seasonal production of cyanopeptides  
in two shallow lakes.

*With David McMullin and Frances Pick.*

Toxic cyanobacterial blooms can produce a diverse range of secondary metabolites known as cyanopeptides that threaten human and ecosystem health. The most studied class of cyanopeptides are microcystins, a hepatotoxin and potential carcinogen. Recent advancements in analytical techniques have allowed for the identification of hundreds of biologically active cyanopeptides beyond microcystins including aeruginosins, anabaenopeptides, cyanopeptolins, microginins, and microvirdins. Various classes of cyanopeptides can be co-produced with microcystins at similar concentrations, yet their toxicity remains largely unknown. We utilized mass-spectrometry based metabolomic techniques to quantify and characterize the seasonal variation of cyanopeptides in two small mesotrophic lakes in Ontario and Quebec through the course of the off-ice season. From surface water samples collected bi-weekly both intracellular and extracellular compounds can be extracted. Diagnostic product ions can be identified from LC-(HR)MS/MS data using software (MZmine2 and Xcalibur) to identify various cyanopeptides and molecular networks (GNPS and CyanoMetDB) used to construct metabolite profiles. Metabolomic profiles were then compared to taxonomic data and abiotic variables that typically promote cyanobacterial growth (water temperature, nutrients, and pH). Analysis of the seasonality of cyanopeptide composition and quantification will allow us to decipher environmental drivers of variation in metabolomic profiles at the lake scale and lead to stronger modelling of toxin potential and risk.

**Poster 12**

**Callie McAulay**

*National Research Council Canada*

Isolation of homoanatoxin-a from a large-scale culture of *Kamptonema formosum* for certified reference material production.

*With Cheryl Rafuse, Pearse McCarron, Krista Thomas, Christopher O. Miles and Daniel G. Beach.*

Anatoxins are a class of potent neurotoxins produced by cyanobacteria that are increasingly linked to animal fatalities in Canada and worldwide, and are a risk to human health due to occurrence in drinking water reservoirs. Analysis of ATXs remains challenging due to their polarity, chemical reactivity, the complexity of the samples in which they occur and the scarcity of analytical standards for most analogues. Towards addressing this need, we are developing a new certified reference material (CRM) calibration solution for homoanatoxin-a (hATX). The highly toxic *Kamptonema formosum* strain NIVA-CYA 92 was simultaneously produced in four 300 L Brite-Box algal bioreactors. The scale-up was monitored by optical density measurements and hATX production was assessed using direct analysis in real time–high resolution mass spectrometry (DART-HRMS), revealing a high proportion (> 90%) of toxin to be extracellular in late-stage culture. After centrifugal harvest of 650 g wet biomass, a novel approach using 2.4 kg of Diaion HP20 resin was used to harvest extracellular toxin (> 100 mg) from the medium. This resin was extracted using 200:1 acetonitrile:water with 0.1% formic acid, which was then purified using a combination of liquid-liquid partitioning, re-crystallization and preparative chromatography, including pilot-scale optimization experiments of most steps. Minor analogues including dihydrohomoanatoxin-a, were monitored in side fractions throughout the process by liquid chromatography–MS (LC–MS) for use in future reference material projects. The resulting hATX material will undergo comprehensive purity assessment using a combination of techniques including LC with HRMS and chemiluminescent nitrogen detection and quantitative <sup>1</sup>H nuclear magnetic resonance spectroscopy. The resulting CRM will be publicly distributed to enable accuracy and metrological traceability in research and regulatory testing.



**Poster 13**

**Aleksy Paltsev**

*University of Toronto*

What is driving the loss of resilience in temperate lakes?

*With Irena F. Creed*

There is a global rise of potentially harmful phytoplankton blooms. We hypothesized this rise is due to a loss of resilience and a shift from oligotrophic to eutrophic states. To test this hypothesis on lakes in the temperate forest of Canada, we: (a) monitored phytoplankton biomass (using [chlorophyll-a] as a proxy) for a 30-year period using optical remote sensing, (b) removed trends and cycles from the time series, (c) analyzed the standard deviation (SD) of the residuals as an indicator of change in resilience, and (d) explored the effects of climate (temperature and precipitation) and landscape on the gain (or loss) of ecological resilience in representative lakes. We classified lakes into one of four classes: stable lakes (SD was consistently low, 45% of all lakes); unstable (SD is consistently high, 14% of all lakes); stabilizing lakes indicating a gain in resilience (SD decreases over time, 23% of all lakes); and destabilizing lakes indicating a loss in resilience (SD increases over time, 19% of all lakes). Most stable lakes were in an oligotrophic state, with only a few stable lakes in a eutrophic state. Most destabilizing lakes were shifting from oligotrophic to a higher trophic status, while most stabilizing lakes were shifting from eutrophic to a lower trophic status. Machine learning models revealed climate had a stabilizing effect, while landscape properties had a destabilizing effect, where small lakes with significant wetlands and littoral zone were most susceptible to the loss of resilience toward a state with a greater likelihood of phytoplankton blooms.

**Poster 14**

Autumn **Pereira**  
*McGill University*

An investigation into the dynamics of toxic and non-toxic strains of *Microcystis*,  
and implications for toxin release.

*With Nicolas Tromas, B. Jesse Shapiro, Irene Gregory-Eaves, Sébastien Sauvé, Dana F. Simon,  
Felipe Fernando Martínez Jerónimo, Nathalie Fortin and Charles W Greer.*

Bacteria of the genus *Microcystis* are often associated with the release of microcystin toxins from cyanobacterial blooms, yet current research has shown that non-toxin-producing strains may be dominant during periods of significant toxin release. Despite their potential importance in mediating the release of microcystin from cyanobacterial blooms, few studies have directly investigated the role of non-toxic strains of *Microcystis* and the dynamics between potential microcystin producers and non-producers in affecting microcystin release from these communities. Here, we answer this question using samples collected monthly from a highly eutrophic, *Microcystis*-dominated lake near Mexico City, Mexico throughout 2019. Specifically, the aim of this study was to (1) characterize the strain diversity of *Microcystis* in this lake, (2) identify environmental factors which may affect the proportion of potential microcystin producers to non-producers, and (3) determine whether a relationship exists between the proportion of potential producers to non-producers and microcystin release from these communities. We obtained a strain-level characterization of our samples from shotgun metagenomic reads using StrainGE, which identified 16 strains of *Microcystis* present in our samples. Further analyses indicated that only 5 of these had the ability to produce microcystin, and that these strains were often present only at low abundance and diversity. This suggests that few toxin-producing strains of *Microcystis* in low abundance may still be able to produce and release microcystin toxins. Lastly, we conducted multiple factor analysis to determine which environmental factors may be important in shaping the relative abundances of potential-producer and non-producers, and whether microcystin concentration is related to these community dynamics. This work serves to increase our understanding of the factors affecting bloom-associated cyanotoxin release, and may allow for improved characterization and prediction of the formation of harmful cyanobacterial blooms.

**Poster 15**

Dana F. **Simon**

*Université de Montréal*

Adopt a Lake - Monitoring harmful cyanobacterial blooms through citizen science.

*With Gabriel Munoz, Paul Julian II, Nicolas Tromas, Tuc Quoc Dinh, Kat Kavanagh, Barry Husk, Jesse Shapiro and Sébastien Sauvé.*

The proliferation of cyanobacteria which can produce toxins harms the environment and human health. Monitoring efforts can be limited by budget, time, and staff, increasing the risk of missing important bloom events. Adopt a Lake is a citizen science project managed at the Université de Montréal, which encourages monitoring focused on cyanobacterial blooms in lakes in Canada. Combining analyzes done in situ by volunteers and analyses done in the laboratory for nutrients, cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) and genomics by 16S rRNA, we will present a resume of the program between 2018 and 2021. The data show identifies affected sites that can be used to advance research on cyanobacteria. This project is also a way to raise awareness and inform and engage citizens about the preservation and quality of water.

**Poster 16**

Jason **Venkiteswaran**

*Wilfrid Laurier University*

The trade-off between water quality and bloom risk in prairie drinking water.

*With Kristin Painter and Helen Baulch.*

Sometimes managing water flows has unintended consequences for lakes. Shallow lakes and small reservoirs are susceptible to changes in flow and source water quality. This can have large impacts on how and when cyanobacterial blooms form. Over seven years, inflows to a eutrophic drinking water reservoir were switched from natural runoff (2014-2015) to river flows from a larger upstream mesotrophic reservoir (Lake Diefenbaker; 2016-2020). Several changes in water chemistry and cyanobacterial blooms (as high-frequency phycocyanin measurements) occurred. Phycocyanin was greatest in years with inflow from Lake Diefenbaker but dissolved organic matter (DOM) and specific conductivity, important determinants of drinking water quality and treatability, were greatest in years when landscape runoff dominated lake water source. Despite a rapid change in source water, it took several years for water chemistry, including DOM, to return to a consistent level underlining that water quality resilience may be hindered by weak resistance to change and slow recovery. Here the trade-off was between poorer water quality via elevated conductivity and DOM, and higher bloom risk.

**Poster 17**

**Lydia Zamlynnny**

*National Research Council Canada*

Identification of new anatoxin-a analogues and conjugates.

*With Christopher O. Miles and Daniel G. Beach.*

Compared with other classes of cyanotoxins such as saxitoxins and microcystins, for which tens or hundreds of analogues have been reported, respectively, only a relatively small number of anatoxins (ATXs) have been identified. These include anatoxin-a (ATX), homoanatoxin (hATX), their dihydro analogues, their carboxylated precursors and a few degradation products. During an investigation into recent dog fatalities linked to anatoxin-producing benthic cyanobacteria in Atlantic Canada, several new putative ATXs were detected. Here we describe the optimization of a liquid chromatography–high resolution tandem mass spectrometry method for the comprehensive detection of ATXs and report the characterization of several new analogues. These include conjugates arising from chemical reactions of ATX and hATX with water, ammonia, methanol, glutathione,  $\gamma$ -glutamylcystine, and methanethiol, some of which were found to be formed primarily during sample preparation. Tetrahydro-analogues of ATX and hATX were also identified and shown, along with dihydro-analogues, to result from enzymatic conversion of parent toxins in culture. In all cases, identities of new ATXs were supported by semi-synthesis using simple and selective chemical reactions beginning from available standards. Finally, the relative proportions of conjugates and tetrahydroanatoxins were estimated in a set of benthic cyanobacterial field samples and laboratory cultures, showing conjugates to be present at relatively low levels (0.5–7% peak area, relative to ATX) while tetrahydro-analogues, on the other hand, were present at higher levels, in one case up to 130-fold higher peak area than ATX. This work demonstrates the reactive nature of ATXs in the environment and the laboratory, something that can lead to significant underestimation of total anatoxin concentrations. It also highlights the need for a broader suite of analytical standards for ATXs as well as further research into their toxicity, and the potential for deconjugation to form parent ATXs.

**Thank you  
for being part of this year's  
IFHAB Workshop!**