

I F H A B

3RD INTERDISCIPLINARY FRESHWATER HARMFUL ALGAL BLOOMS WORKSHOP

April 24–26, 2019

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ABOUT THE IFHAB WORKSHOP

The Interdisciplinary Freshwater Harmful Algal Blooms Workshop is a yearly meeting of scientists focusing on the study of cyanobacterial and other harmful algal species in freshwater environments. During this workshop, researchers from different disciplines will present their most recent studies, including harmful algal blooms monitoring and fate, ecology, analytical method development and drinking water treatment. The workshop will take place in an informal

environment, aiming to help researchers expand their networks and learn from the different areas affecting this complex environmental threat.

The 3rd Interdisciplinary Freshwater Harmful Algal Blooms Workshop, hosted in Toronto, Ontario from April 24–26, 2019, will draw in leading experts from across North America into a synergistic forum to share their research findings and discuss innovative solutions.

WORKSHOP LOCATION:
GEORGE BROWN COLLEGE, ST. JAMES CAMPUS AUDITORIUM
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Anatoxins and *Phormidium* spp. in samples associated with dog deaths in New Brunswick

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In July 2018 three dogs died after visiting the water's edge at two different parks on the St. John River near Fredericton, New Brunswick. All showed signs of toxicosis following exposure, and necropsies on two of the dogs revealed non-specific pulmonary edema and multiple microscopic brain hemorrhages. Studies were conducted to establish whether consumption of toxic algal material was responsible for the deaths. Samples analysed included the vomitus of one dog, stomach contents of two dogs, and water and biota from the mortality sites. Targeted and untargeted analysis by liquid chromatography high-resolution mass spectrometry (LC-HRMS) confirmed the presence of anatoxins in all samples. The highest levels were measured in a dried algal mat that two of the dogs were consuming before falling ill, with concentrations of ~330 and ~980 mg/kg for anatoxin-a and dihydroanatoxin-a, respectively. LC-HRMS also showed a rich profile of additional known analogues and previously un-reported ATX conjugates. Cyanobacteria, including species of the genera *Phormidium*. were identified as the causative organism(s) by light microscopy and ribosomal RNA gene sequencing. Phylogenetic analysis revealed diverse cyanobacterial community in the benthic algal mats responsible for the poisonings including multiple species containing the anaC gene. This event highlights the need for increased awareness of risks posed by cyanobacteria in Canadian freshwater systems. Future efforts will focus on understanding factors behind regional *Phormidium* sp. occurrence and implementing a broader range of tools for monitoring based on genetic and chemical analytical techniques.

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Remote sensing algal bloom indices for enhanced monitoring of Lake Erie algal blooms

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Lake Erie has seen a dramatic increase in phytoplankton biomass, manifested in particular by the rise in the severity of cyanobacteria blooms and the prevalence of potentially toxic taxa such as *Microcystis*. Satellite remote sensing has provided a unique capacity for the synoptic detection of these blooms, enabling spatial and temporal trends in bloom conditions to be documented. We will present satellite derived indices for algal bloom intensity, spatial extent, severity, and duration, which are soon to be made available operationally by Environment and Climate Change Canada to provide objective, consistent measures of algal bloom conditions in near-real-time. Results will be presented on the derivation of these bloom indices and their validation against in situ observations. Algorithms for satellite detection of Lake Erie algal blooms often rely on a single consistent relationship between algal or cyanobacterial biomass and spectral indices such as the Maximum Chlorophyll Index (MCI) or Cyanobacteria Index (CI). Blooms, however, are known to vary significantly in community composition over space and time. A suite of phytoplankton and optical property measurements during the western Lake Erie algal bloom of 2017 showed highly diverse bloom composition with variable absorption and backscatter properties. Elevated backscattering coefficients were observed in the Maumee Bay, likely due to phytoplankton cell morphology and buoyancy regulating gas vacuoles associated with the often *Microcystis* dominated blooms compared with typically *Planktothrix* dominated blooms in Sandusky Bay. We will discuss how these and other phytoplankton assemblages impact confidence in satellite retrievals.

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Bridging the gap between catchment management and lake response

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Effectiveness of terrestrial nutrient control measures in controlling lake eutrophication and restoring lake dissolved oxygen (DO) concentrations were quantified in Lake Simcoe, Ontario through a combined high resolution monitoring and modeling program. Water quality responses to simulated nutrient load reductions varied spatially across the lake, and although lake DO concentrations were consistently improved, in many instances algal mass also increased. Importantly, simulated strategies which inadvertently affected tributary flow had greater impacts on lake recovery than nutrient control; and when multiple strategies were implemented simultaneously larger flow reductions induced warming throughout the water column, and higher algal growth rates. Negative impacts of lake warming overwhelmed the positive effects of nutrient reductions, and limited the effectiveness of restoration strategies in improving DO concentrations. The decoupling of algae and DO from nutrient control measures suggests lake recovery rates could be accelerated by coordinated management approaches which consider the potential for physical and biological feedbacks associated with interactions between management strategies.

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Polyphosphate in picoplankton in the eutrophic embayment of Lake Ontario (Hamilton Harbour): a hidden P source for HABS?

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Polyphosphate (polyP) has been found ubiquitously in living organisms including aquatic primary producers, and the dynamic processes of polyP (accumulation/liberation) as stress responses are suggested to be important. Yet such processes of polyP metabolism has not been documented and investigated in freshwater eutrophic environments. The role of polyP in P recycling on ecosystem scale in such systems remains largely unquantified. We show results of seasonal variability and spatial distributions of polyphosphate in particulate matters in the water column of Hamilton Harbor at two sites (nearshore vs offshore). Polyphosphate in the surface water ranged 0.03 - 0.33 $\mu\text{mol/L}$ and 0.03 – 0.19 $\mu\text{mol/L}$ nearshore and offshore, respectively, with 30 – 64% of polyP contributed by small size particles of 0.2 – 2 μm that likely represent picoplankton. PolyP was preferentially lost in the water column compared to total particulate P (TPP), with recycling efficiencies of $53 \pm 13\%$ nearshore and $80 \pm 13\%$ offshore. These results suggest the highly dynamic polyP metabolism in plankton responding to variable P availability and the active cycling of polyP within the water column promotes retaining bioavailable P to support primary productivity. We will discuss this finding from a perspective on harmful algal bloom.

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Seabirds alter the environment via the introduction of nutrients and metals

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Biovectors, such as seabirds and anadromous fishes, shunt significant amounts of nutrients and metals from their marine feeding grounds inland to breeding territories. The elevated nutrient biotransport can potentially eutrophy aquatic environments and restructure terrestrial vegetation. Here, we studied the extent of ecosystem impact of a large colony of seabirds on an otherwise minimally influenced environment. We utilized a paleolimnological approach (diatoms, $\delta^{15}\text{N}$, sedimentary metals, palynology, chlorophyll-a and aerial photography) to study 5 ponds impacted to varying degrees by the world's largest colony (> 2 million pairs) of Leach's Storm-petrel (*Oceanodroma leucorhoa*), breeding on Baccalieu Island, Newfoundland. The seabird's guano and refuse created a hypereutrophic water column (> 100 $\mu\text{g/L}$ phosphorus), and elevated metal concentrations, including arsenic, cadmium and zinc to potentially toxic levels. Utilizing the sedimentary geochemistry, we developed a bioenrichment factor that temporally tracks the inputs from the seabirds, and when used in conjunction with other paleolimnological proxies, reveals long-term seabird population dynamics. Using the palynological record and chlorophyll-a, we determined that the petrel colony also greatly modifies the terrestrial habitat, elevating the concentration of nutrients and acting as ecosystem engineers, increasing the amount of preferred nesting habitat and reducing the marginal treed habitat. Additionally, seabird-introduced nitrogen likely reduced the competitive advantage of N_2 -fixing plants, reducing their relative abundance. With archival aerial photography, we visually confirmed the habitat impacts of the petrel colony, as it increased the vegetation, and thereby available nesting habitat of the island, by 35% from 1940 to 2017. Our research describes the significant limnological and terrestrial impacts of seabirds, and by extension other biovectors, as well as the potential for biovectors to act as drivers of algal blooms and potential environmental contamination.

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Danger lurks in warm, murky water: microcystin production modulated by temperature and browning

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The global changes that mark the Anthropocene have rendered freshwaters susceptible to rapid change. In addition to nutrient over-enrichment, freshwaters are now confronted with additional challenges, such as warming and browning (i.e., increased terrestrially-derived organic matter (DOM) loads), reflecting the influential nature of climate change. These alterations have been linked to the recent upsurge in cyanobacteria bloom reports in northern rural and remote lakes. In this lab-based study, the synergetic effects of increased temperature and DOM were investigated to identify the resulting impact on cyanobacteria performance (growth and photosynthetic parameters) and toxin production. We hypothesized that the positive effects of increased temperature on cyanobacteria performance would be amplified by the light attenuation properties of terrestrially-derived DOM. We further hypothesized that disruptions in carbon (C) and nitrogen (N) balance—due to DOM, from clear to darker conditions—would favour the production of N-rich metabolites (e.g., microcystins). We assessed the combinatorial effects of temperature and browning (as indicted by colour) by exposing the cosmopolitan freshwater bloom-forming species, *Microcystis aeruginosa*, to three temperature (20, 24 and 28°C) and six colour treatments (0 to 800 true colour units (TCU)). Our results show that *Microcystis* growth and photosynthetic performance peak at elevated temperatures (24-28°C) and moderate colour (100-200 TCU). In addition, microcystin production mirrored this response and was paralleled with an increase in intracellular N content, suggesting that microcystin serves as a N storage compound. Our findings help disentangle the underlying physiological mechanisms by which warming and lake browning may favour cyanobacteria and toxin production in northern lakes. Ultimately, this study builds on established paradigms, by integrating new knowledge of environmental changes to render a deepened understanding of the dynamic linkages between terrestrial and aquatic landscapes.

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Inferring historical changes in cyanobacterial production from lake sediments using Visible Near-infrared Reflectance Spectroscopy (VNIRS)

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Cyanobacterial blooms are a pervasive and escalating environmental issue globally, with important consequences for potable water resources, the recreational economy, water-front property value, and ecosystem health. However, there remains a lack of long-term monitoring data available to provide ecological context, and for identifying drivers of the increasing frequency and severity of cyanobacterial blooms reported in the last few decades. We develop a model to infer historical changes in cyanobacterial production using visible and near-infrared reflectance spectroscopy (VNIRS) on lake sediments. A dilution series ($n = 15$) of a sediment matrix inoculated with increasing amounts of mixed cyanobacterial culture from 0 to 50 parts per thousand was established and algal pigments were measured using both VNIRS and high-performance liquid chromatography (HPLC). A partial least squares regression model was developed to correlate dilution series spectra to parts per thousand of cyanobacteria additions. Applications of the resulting 2-component model ($r^2 = 0.93$, RMSEP = 0.24 %) to four lakes in Ontario yielded temporal trends that were comparable with downcore HPLC determinations of cyanobacterial pigments in three out of four cases. Although preliminary in some respects, this application of VNIRS to detect cyanobacterial photosynthetic pigments in lake sediments has the potential to be an important rapid assessment tool for lake management.

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The effect of climate change on sick cyanobacteria and consequences for the rest of the aquatic food web

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Aquatic systems are generally warming and receive an increased nutrient input. During these conditions phytoplankton can proliferate and form dense surface blooms that often consist of large-sized phytoplankton taxa, such as diatoms or filamentous cyanobacteria. In many cases these are inedible to zooplankton and thus form trophic bottlenecks, preventing efficient transfer of energy and elements to higher trophic levels. Fortunately, just as any other organism, cyanobacteria can also get infected by a wide array of pathogens, including fungal parasites. Until now, it remains unclear how warming and changes in nutrient input affect these parasites. We know that infections can kill cells and lead to fragmentation of filaments, making them more edible to zooplankton. Additionally, these parasites may provide zooplankton with a complementary food source in the form of fungal zoospores, thereby alleviating trophic constraints. We show that warming can accelerate termination of a phytoplankton bloom and can lead to a changed stoichiometry of phytoplankton, which has consequences for reproduction and stoichiometry of fungal parasites. Changes in N:P supply ratios of growth media are reflected in both phytoplankton but also chytrid stoichiometry. Additionally, chytrids seemed to be more responsive to changes in N, indicated by a high correlation between N:P and zoospore production rates. This work highlights the need to incorporate the complex dynamics between infections, stoichiometry and grazing to better understand how future aquatic food webs and their phytoplankton communities will respond to climate change. Also, we emphasize that sometimes diseases can be considered as a positive component of an ecosystem, since they might be able to actually kill the 'bad guys' and simultaneously provide high quality food to grazers.

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Microcystis, Planktothrix and few others, what do we fear when they are blooming in our freshwater bodies?

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Institut Pasteur, Collection of Cyanobacteria

Microcystis, Planktothrix and few other bloom-forming cyanobacteria have been well studied. Their ecosystem preference, their massive development dynamic, and their nutrient requirement were notoriously reported. Above all the hepatotoxin microcystin they synthesise were deeply scrutinized for diversity, toxic effect, transfer and removal. Regulations are even applied safe levels for microcystin and bloom forming cyanobacteria in freshwater bodies used for recreational and consumption. Remedial measures with restoration lake protocols are long standing but managed efficiently. Thus, what we don't know yet about these bacteria? Lately, OMICs applied to these bacteria have taught us more about their natural product potential and uncovered unexpected compounds. I will focus on Microcystis and Planktothrix that we have studied in the frame of genome sequencing of the pure strains of the collection Pasteur Culture of Cyanobacteria. The diversity of these two blooming cyanobacteria and their natural product potential will be resumed as well as what we have still to uncover to better know these bacteria dispersed globally on Earth.

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The prevalence of microcystin congeners in Alberta waters

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Cyanobacteria can produce various microcystin (MC) congeners possessing different toxicity potencies. The Alberta Cyanobacteria Monitoring Program for Public Health has been measuring total MCs as well as 9 congeners including MC-RR, -YR, -HtyR, -LR, desmethyl -LR, -LA, -LY, -LW and -LF at public recreational beaches since 2013. Data was collected between 2013 and 2018 for 1245 samples from 68 waterbodies using LC-HRMS analysis. 657 samples had detectable MCs. All nine congeners occurred multiple times at multiple locations throughout the province. MC-LR was the most prevalent found in 97% of the samples. Its concentration accounted for 65.61% of the total MC concentration. MC-LA was found in 67% of samples, accounting for 30.38% of the total MC concentration. Desmethyl MC-LR, MC-RR, MC-LY, MC-HtyR and MC-YR are known to be less toxic than MC-LR and MC-LA based on the LD50 in mice. They are less common (3.27% of the total MC) in Alberta. MC-LF and MC-LW were detected at low levels in few samples (6 and 3, respectively) in 2013, 2014, 2015 and 2017. These congeners appear relatively late in the season and in most cases co-occur with all other 7 congeners. Of the two main congeners, MC-LR was consistently predominant in the majority of the waterbodies (50 lakes); MC-LA was always predominant in 5 lakes; and a mixed pattern was observed in 13 lakes. This pattern may indicate relatively stable cyanobacteria populations in some waterbodies, or environmental conditions that favor certain cyanotoxin production profiles. There is no clear relationship between MC (total and congener) concentrations and water color, temperature, turbidity and evidence of bloom at time of sample collection. Further correlation analysis will be carried out between toxin profile, cyanobacteria species and toxin genes. In conclusion, MC-LR is not always the dominant congener present in cyanobacterial blooms. Quantification based on MC-LR only can potentially miss more than 30% of total toxicity.

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The effect of climate change and nutrients on *Anabaena*: a harmful, diazotrophic cyanobacterial genus

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Freshwater ecosystems experiencing rising global temperatures and eutrophication have been subjected to toxic cyanobacterial blooms of an expanding size and duration that threaten human and animal health. Excessive nutrient, particularly nitrogen, loading selects for certain groups of cyanobacteria, namely toxin-producing, non-diazotrophic (non-N₂-fixing) strains. Despite this, intense blooms of diazotrophs such as *Anabaena* have also been reported in eutrophic systems and are often dominated by toxin-producing strains. The underlying factors that cause these blooms are not completely understood. More specifically, a comprehensive understanding of the nutrient demands of *Anabaena*, particularly among strains that produce cyanotoxins, has yet to be attained. How rising temperatures will affect toxin production and N₂-fixation in diazotrophic cyanobacteria is also unclear, as are interactive effects between temperature and specific nitrogen species availability. Moreover, the potential for elevated carbon dioxide (CO₂) levels to affect toxin production and N₂-fixation in freshwater cyanobacterial communities and diazotrophs specifically is also poorly understood. Thus, we have investigated the individual and interactive effects of temperature, nutrients, and CO₂ on toxic strains of *Anabaena*. Preliminary results indicate that some *Anabaena*, namely the anatoxin-producing strain sp. 54, exhibit significant differential gene expression, particularly with respect to nitrogenase activity, when grown under various nutrient regimes. This strain also exhibited a negative correlation between growth rate and toxin content, suggesting that the production of this neurotoxin may be a function of stress. In contrast, the microcystin-producing *Anabaena* strain sp. 90 was less sensitive to different nutrient regimes, particularly in terms of growth and toxin production. The former strain also exhibited significantly repressed N₂-fixation with rising temperatures; sp. 54 and natural *Anabaena* populations increase nitrogenase activity under elevated CO₂ conditions, suggesting that the two main effects of climate change may counteract each other with respect to N₂ fixation in at least some members of this genus.

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Cyanobacterial blooms -- the effects of an extreme rainfall event on bloom onset

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The prevalence and increasing global distribution of cyanobacteria-dominated harmful algal blooms is strongly associated with changing climatic patterns and local biogeochemical and hydrological processes. Changes to precipitation frequency and intensity, as predicted by current climate models, are likely to alter bloom development and composition due to nutrient fluxes and water column mixing. However, few studies have directly documented the effects of precipitation events on cyanobacterial composition, biomass, and toxin production. In this study, we describe an early-initiated cyanobacterial bloom in Conestogo Lake, a eutrophic flood control reservoir located in southwestern Ontario, following heavy rainfall and subsequent catchment flooding. A surge in bioavailable phosphorus resulted in biomass increases of *Aphanizomenon flos-aquae* throughout the reservoir approximately 2 weeks post-flooding. Anabaenapeptin-A and three microcystin congeners (microcystin-LR, -YR, and -RR) were detected at varying levels across sites during the bloom period, which lasted between 3 - 5 weeks. In addition, each of the three sampled sites varied in physio-chemical properties throughout the sampling campaign suggesting different eco-zones within the reservoir. Together, these findings indicate that water column mixing and phosphorus concentrations were the key drivers for the early cyanobacterial bloom in Conestogo Lake and further highlight the complex relationship and variability within reservoir systems. Therefore, effective management goals and mitigation strategies for bloom-related water quality impairment must be both responsive and adaptive to the complexity of drivers affecting blooms.

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Identification of cyanotoxin producers in New Brunswick waterbodies using genetic tools

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Cyanotoxins are an emerging threat to freshwater resources worldwide, impacting the health of humans, wildlife, and ecosystems. Determining the potential for cyanotoxin production, including identifying potential producers, is hindered by a lack of morphological features that correlate with cyanotoxin production. However, genetic approaches permit the detection of cyanotoxin genes in environmental samples, and therefore the identification of environments that are at risk for cyanotoxicity. Genetic approaches can also identify which cyanobacteria in a complex community are responsible for toxin production by identifying the organisms that harbour toxin genes. We have been using genetic approaches to detect the genes involved in the production of the hepatotoxin microcystin, and the neurotoxin anatoxin-a, in diverse freshwater environments around New Brunswick. For microcystin, we detect the *mcyE* gene and can determine if the source is *Microcystis*, *Anabaena/Dolichospermum*, or *Planktothrix*. For anatoxin-a, a new threat to New Brunswick, we detect the presence of the *anaC* gene, and are working to determine the genera associated with toxicity using deep-DNA sequencing and phylogenetic approaches. Our preliminary studies indicate that microcystin and anatoxin-a biosynthesis genes are widespread in New Brunswick waterbodies, and that planktonic and benthic/epiphytic communities may harbour producers of both toxins. Where microcystin genes are present, they appear in summer and persist through to late fall. The dominant genus associated with microcystin is *Microcystis*, while *Planktothrix* is only a sporadic contributor in some waterbodies. Anatoxin-a genes were detected in both lentic and lotic waterbodies throughout the 2018 sampling season. Development of quantitative methods for both toxins is underway. The application of genetic approaches has provided important information regarding toxin-production potential, and has revealed that cyanobacteria armed with genes for toxin biosynthesis are part of complex and diverse microbial communities.

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The challenge of algal blooms for public health practitioners in Ontario

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Cyanobacterial blooms create a challenge for public health practitioners. Blooms often generate significant community concern despite the absence of confirmed reports of human illness. Currently, a combination of proactive and reactive monitoring programs are used to monitor the presence of cyanobacterial blooms and associated toxins. As concentrations of blooms and any associated toxins can change rapidly in time and location, their use in providing site and time specific messages to the public can be problematic. The effectiveness of messages in changing public behaviour is not known and the number of water bodies with public access potentially susceptible to blooms may number in the thousands for some local health authorities. Although a variety of response measures exist in the event a bloom event, these response measures range from risk messaging to restrictions of water body usage, and the evidence to which these responses prevent harm is unknown. To inform evidence-based decision making during these episodes, Public Health Ontario is completing scientific reviews on exposures to cyanobacterial toxins and the relationship to documented human health effects. The reviews focused on three scenarios where members of the public could potentially be exposed to cyanobacterial toxins: exposure via drinking water, during recreational water activities and via consumption of fish. The objective is to identify documented cases of human illness that have resulted from exposure to cyanobacteria or their toxins.

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Comparative analyses of cyanobacteria assemblages using microscopy and metabarcoding from the LakePulse Network

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The taxonomy of cyanobacteria has been evolving with the development of molecular tools. DNA-based techniques are increasingly being used for measuring aquatic biodiversity as it could improve the effectiveness of monitoring and basic science programs. For bacteria, the “gold standard” for metabarcoding work is the 16S rRNA gene and this has provided great insights into dynamics of cyanobacteria blooms in several ecosystems. However, methodological comparisons between molecular and traditional microscopic approaches have rarely been applied to a wide suite of lakes. In this study, we are conducting comparative analyses of cyanobacteria community composition generated by means of microscopy and high-throughput sequencing of the 16S rRNA gene. We are adopting this approach to assess epilimnetic cyanobacteria assemblages across hundreds of Canadian lakes within the LakePulse network. From snapshot sampling of eastern Canadian lakes, the dominant genera (based on biomass) identified via microscopy were *Aphanizomenon*, *Anabaena/Dolichospermum* and *Aphanocapsa* whereas dominant genera identified based on 16S rRNA gene relative abundances were *Synechococcus*, *Snowella* and *Anabaena* (but upon removal of picoplankton, the dominant genera were *Anabaena*, *Snowella* and *Microcystis*). We are applying a combination of ordination analyses and RV coefficients (i.e. a generalization of the Pearson correlation that relates two matrices with corresponding sites) to quantify the coherence in assemblage data based on traditional vs. molecular approaches. Our preliminary analysis based on the first 60 lakes shows noteworthy correspondence between the two approaches. Overall, we hope that identifying the strengths and limitations of each of the approaches will help inform future lake monitoring efforts.

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Source-tracking a toxigenic *Microcystis* bloom in a major Great Lakes tributary

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In late September 2017, a cyanobacterial HAB (cHAB) was detected in the estuary of the Maumee River, the largest tributary to Lake Erie. A survey extending 160 km from the river mouth identified *Microcystis* spp. as the dominant taxa, with bloom biomass extending 10 km from the river mouth and accumulation of microcystins detected by ELISA exceeding Ohio's Elevated Recreational Public Health Advisory threshold (20 µg/L) at multiple locations. Screening samples by LC-MS identified MC-LR as the dominant microcystin congener in all samples. Coincident with the river cHAB, an expansive *Microcystis* cHAB was covering much of western Lake Erie. Addressing potential continuity between the blooms, samples were screened using high-throughput 16S rRNA gene sequencing to yield amplicon sequence variants (ASVs). Unique *Microcystis* ASVs identified from the Maumee estuary were found in western Lake Erie, but not in sites sampled upriver. The two most abundant river ASVs were consistent with oligotypes commonly found in Lake Erie's western basin. Several lines of evidence support the advection of cells into the Maumee estuary from Lake Erie. First, low river discharge coincident with negligible precipitation through much of September combined with seiche signals measured 10 km upriver from the river mouth suggest a strong lake influence within the estuary. Second, predominantly eastward winds experienced over the course of the cHAB may have advected surface waters into the river mouth during these low-flow conditions. These results highlight the need to broaden our understanding of biophysical coupling within freshwater estuaries. This is particularly important in places where these estuaries fall within large metropolitan areas, as is the case for Toledo, OH. By combining biological (e.g., -omics and toxin surveys) and physical (e.g., discharge, wind) datasets within these environments, we can achieve a more holistic picture of the mechanisms driving cHABs in Lake Erie and its watershed.

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Unusual microcystins revealed in *Planktothrix* by untargeted LC-HRMS, ¹⁵N-labeling and chemical derivatizations

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The microcystins are a group of more than 250 hepatotoxic heptapeptides produced by a range of cyanobacterial species. Although the 2- and 4-positions of microcystins are highly variable, microcystins containing glutamic acid at position-2 have only been reported in a single strain of *Anabaena* that produced a range of esters of MC-EE. Recently, during LC-MS analysis of a culture of *Planktothrix prolifica*, we detected a minor candidate microcystin with [M+H]⁺ m/z 997.5, in addition to the major microcystin [Asp3]MC-RR that had been reported previously in this strain. LC-HRMS/MS studies involving derivatization with mercaptoethanol, esterification with diazomethane, immunoaffinity chromatography, ¹⁵N-labeling, and periodate oxidation were combined with detailed examination of product ion spectra to tentatively elucidate the structures of the microcystins present in the culture. These studies revealed the minor m/z 997.5 compound to be the previously unreported microcystin [Asp3]MC-ER, together with trace levels of [Asp3]MC-EE. This is the first report of a microcystin containing glutamic acid and arginine at positions 2 and 4, underscoring the flexibility of microcystin biosynthesis in cyanobacteria, and the power of combining LC-MS/MS techniques with functional-group-specific derivatizations. Additional studies also revealed the presence in the culture of a conjugated form of [Asp3]MC-RR with Mwt 2116, by far the highest molecular weight microcystin identified thus far in nature. Software developed in-house for analyzing the level of ¹⁵N-incorporation and the isotopic profiles of the molecular ion envelopes of the labeled and unlabeled compounds is providing a powerful new tool to assist in the determination of the elemental composition of these microcystin congeners in the culture.

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Microcystin liver toxins at hazardous levels in algal dietary supplements revealed by a combination of bioassay, immunoassay and mass spectrometric methods

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Microcystins (MCs) are peptide liver toxins produced by cyanobacteria (blue-green algae) in aquatic environments and are potent inhibitors of protein phosphatases 1 and 2A in eukaryotic cells. Algae used to make dietary supplements and food products are harvested from natural lakes or outdoor built environments and can be contaminated with MCs. Therefore, routine monitoring of MCs in these products is necessary. Yet, testing for MCs is complicated by their structural diversity in nature where over 200 different congeners have been detected. We sought to use a combination of bioassay, immunoassay and mass spectrometric methods to fully quantify and identify the pool of MCs in algal dietary supplements. Eighteen supplements were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS), followed by a multi-hapten enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PPIA). LC-high resolution MS (LC-HRMS) with thiol derivatization was used to identify the full suite of MCs. LC-MS/MS revealed some products >40 times the maximum acceptable concentration (MAC) of 1 µg/g MCs, but ELISA and PPIA showed products > 50 and 60 times the MAC, respectively. LC-HRMS identified all congeners targeted by LC-MS/MS plus a rare MC congener not typically monitored (MC-(H4)YR). This congener was present in four products, including the two most contaminated, contributing up to 18% of total MCs. Congener profiles for these two most contaminated products were identical, suggesting these supplements were produced from the same raw material lot. Recommended dosages of products >MAC would result in daily body burdens 2.6 to 75 times higher than the tolerable daily intake, presenting considerable risk to consumers. During the period of this study, the manufacturers of the two most highly contaminated supplement products both issued voluntary recalls totaling >70,000 units worldwide. This study confirms the continued need for monitoring of these raw materials and products, and presents a monitoring framework to fully describe the total MC pool in environmental samples and algal products.

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3RD INTERDISCIPLINARY FRESHWATER
HARMFUL ALGAL BLOOMS WORKSHOP

APRIL 24 - 26, 2019
TORONTO, ON

Deterministic model for gradient-based taxis: an approach to predict algal bloom pattern

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Relationships between the algal bloom patterns and involved factors are highly complex, therefore appropriate prognostic techniques to forecast blooms and evaluate their spatio-temporal evolution are indispensable. Taxis, or directional motion, is a common collective behavior of living organisms. This motion of microorganisms is a response from transport signals through cell motion which is gradient based velocity or gradient following velocity. The gradient-based taxis is the general case which can stand for many other taxis of cells including phototaxis, oxytaxis, thermotaxis and prey-taxis, etc. Algal blooms from green or blue-green algae in the waterbodies (marine water or fresh water) seem to be an important gradient-based taxis application, specifically when they are combined with chemotaxis (nutrients for algae) or phototaxis (light intensity). Taxis in algal blooms can be understood through the fact that algae species have to adapt to their environment, i.e they migrate towards preferred stimuli, including temperature. Thermal stratification in waterbodies is increased by warmer temperatures, in which the growth of some algal species including cyanobacteria is favoured. Over the summer months, the warming of the surface water can lead to the stratification on the entire depth of waterbody. The stratified waterbody will restrict the vertical movement of oxygen and nutrients in it. The surface water is usually warmer and less dense in comparison to the colder, denser bottom water. This may increase the competitive advantage of certain species such as cyanobacteria over other kinds of algae. In the present work, we suggest a deterministic approach for gradient-based taxis, with thermotaxis as study-case. The facet of stability analysis and development of convection regimes that go further than critical thresholds will be studied. As the suggested model includes the directional motion term under the gradient stimuli, it could generalize a variety of gradient based taxis as above mentioned.

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Analytical methods for the quantification of microcystins in drinking water: a regulatory perspective.

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Microcystins are hepatotoxins produced by cyanobacteria in marine and freshwater environments such as the Great Lakes. They have a cyclic heptapeptide structure, with different possible amino acid substitutions. To date, over 250 different variants have been reported in literature. There are several guidelines regulating maximum levels of microcystins in drinking water. The Ontario Drinking Water Quality Standards is set to 1.5 µg L⁻¹ of microcystin-LR (one of the most toxic and common variants in the Great Lakes), whereas Health Canada recently included in the Canadian Drinking Water Quality Guideline a maximum concentration of 1.5 µg L⁻¹ for total microcystins (sum of all the individual variants). This leads to some analytical challenges, as it is difficult to accurately quantify total microcystin concentrations in water samples. Two of the most common techniques for microcystins analysis are Enzyme-Linked ImmunoSorbent Assay (ELISA) and liquid chromatography-mass spectrometry (LC-MS). ELISA can quantify the total amount of microcystins regardless of the variants present in the sample, but can be prone to false positives due to interferences during the bioassay. LC-MS can accurately quantify about a dozen of the most common individual variants (whose standards are commercially available) but might be prone to false negative results when calculating total microcystins by not targeting all the variants present in the sample. During this presentation, pros and cons of regulating maximum amount of microcystins in drinking water expressed as total microcystins or microcystin-LR will be discussed, with emphasis on the strengths and weaknesses of the available analytical methods. MECP's ELISA and LC-MS 2018 season results will be summarized (over 2,100 samples) and correlation between the methods will be compared.

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Not all temperate lakes respond to climate change

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Temperate lakes in relatively remote reaches of the Laurentian Great Lakes Basin are changing. Some are becoming eutrophic, but some are becoming oligotrophic, while others are remaining stable (either oligotrophic or eutrophic). This contradicts the perspective that climate change is increasing temperatures that benefit algal growth leading to potential harmful algal blooms. To understand why some lakes are becoming more eutrophic while others are not, we examined peak algal biomass using a time series of chlorophyll-a (chl-a; a proxy of algal biomass). We hypothesized that lakes become eutrophic in the face of climate change if landscape properties “allow” to respond to climate change by increasing nutrient loading into and nutrient availability within lakes. To test this hypothesis, we: (1) built a continuous 28-year (1984-2011, August-October) time series of chl-a in lakes from satellite data; (2) calculated landscape morphometrics for a representative subset of these lakes (n=281); and (3) related climate (temperature and precipitation) and landscape morphometric properties to median chl-a in a regression tree model. We found that lakes with the highest mean chl-a concentration (7.6 $\mu\text{g L}^{-1}$) that were eutrophic or eutrophying (57% and 16%, respectively) had relatively small volumes (415 $\times 10^3 \text{ m}^3$) that were more sensitive to precipitation change and were either oligotrophic and oligotrophying (deep lakes with water discharged from catchments with small wetland cover) or eutrophying lakes (shallower lakes with water discharged from catchments with larger wetlands cover). These findings indicate that (1) lakes with smaller volumes were most responsive to climate change and that this response was more “typical” (higher temperatures = higher chl-a concentration), and (2) lakes with larger volumes were less responsive to climate change, but those lakes with larger volumes that were shallow and fed by catchments with large wetland cover behaved more similarly to lakes with smaller volumes. In overall, lakes that have landscape properties beneficial for growth of algae and, therefore allowing for eutrophication, respond to climate change in a typical way, while lakes that do not have these “beneficial properties” may not respond at all (remain stable oligotrophic) or respond in an atypical way (oligotrophy).

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Use of high-resolution DNA metagenomics to identify cyanobacteria in algal blooms occurring in Ontario lakes

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Cyanobacterial algal blooms have been increasing in frequency and severity over the past few years in Ontario. Depending on the presence and species composition of toxigenic cyanobacteria, these blooms have the potential to release toxins into the water posing a public and environmental risk to humans and animals. We applied a metagenomics strategy to investigate the composition of cyanobacterial communities in Ontario lakes during algae bloom events. Samples were collected from distinct Ontario lake locations and river sites north of Toronto during the years 2014 and 2015. In addition we characterized the Lower Great Lakes microbiome and water samples were collected from six different locations around Lake Erie and Lake Ontario for 16 S ribosomal RNA sequencing. DNA was extracted and either in a shotgun metagenomic sequencing approach fragments of 250 bp size were obtained and sequenced or 16 S RNA genes amplified and sequenced. Based on a bioinformatics pipeline that includes MEGAN and MG-RAST analyses steps, initially the genotypic richness and diversity of the microbial communities was determined. A comparative analysis between the sequencing data obtained from the different lake microbiomes was performed. The metagenomic analyses provided community insights into the species composition of the samples obtained from the selected Ontario lakes, including a preliminary characterization of their metabolic uniqueness. Depending on the geographic location of the samples, up to 20% of the sequences mapped to cyanobacterial species and, within the phylum of cyanobacteria, the majority of the DNA hits resembled sequences from the orders Nostocales and Oscillatoria. Exploring and further refining the detection and characterization capability of the metagenomic approach with main focus the cyanobacterial community composition, our work indicates that metagenomic DNA analysis may be used to augment routine microbiological testing of drinking water supplies, recreational waters and environmental sites for maintaining public safety.

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Remote sensing program for cyanobacteria at the Northern Ontario School of Medicine

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The Northern Ontario School of Medicine laboratory for environment and health surveillance is a remote sensing facility focused on environmental events with the potential to affect human health. Examples of current priorities include surveillance of wildfires and cyanobacteria blooms, which are currently recognized to adversely affect the health of our communities by a number of mechanisms. Our studies are highly multidisciplinary and are conducted in collaboration with key research partners. One such partner is MAG Aerospace, one of Canada's largest providers of specialized aircraft services, including aerial imagery. Our current wildfire research has provided our group valuable operational experience into large-scale monitoring of our province's northern land mass, as part of an aircraft-based wildfire detection program used by Ontario's MNRF. In parallel, our laboratory has developed spectroscopic methods for differentiating cyanobacteria from green algae using hyperspectral sensing techniques. Hyperspectral sensors have a significant advantage in that they can be mounted on aerial platforms, allowing the characterization of very large target areas, or a large number of targets (such as lakes). During the coming season, we will use hyperspectral sensors mounted to aircraft to acquire imagery of lakes with algae blooms. Results will be compared to hyperspectral images of algae grown in a laboratory environment, with the goal of evaluating the feasibility of differentiating cyanobacteria blooms from green algae blooms from the air. When possible, we hope to align our studies with other groups evaluating aspects of blooms from ground-based studies. Should results be positive, we will be developing data transmission techniques to report bloom details to decision makers in near real-time, potentially providing information on the presence of HABs in our waterways. Studies are also planned which include an evaluation of possible spectral changes within cyanobacteria associated with toxin production. These studies are supported by the Northern Ontario Heritage Fund Corporation, FedNor, and MAG Aerospace Canada

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ATRAPP – Overview of first two year of results

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Numerous reports indicate that cyanobacterial blooms are increasing in frequency and magnitude globally. Here we are presenting the first results of a study started in 2016 spanning four years financed by Genome Quebec and Genome Canada entitled Algal Blooms, Treatment, Risk Assessment, Prediction and prevention through Genomics (ATRAPP). For two summer campaigns six lakes in Canada were intensively sampled over a few months to obtain the profile of “before, during and after” the blooms. The spatial (17 sampling sites) and temporal sampling generated close to 900 samples. The samples were tested by mass spectrometry for toxins concentrations (MCtotal and 17 specific variants [1]), nutrients (total & dissolved nitrogen and phosphorous, nitrite and nitrate, ammonium, soluble reactive phosphorous, DOC and TOC), total metal concentrations (ICP-MS). Taxonomy reports 16S genomics are also available and more advanced genomic analysis is underway. The picture emerging from these results supports the complexity of the bloom appearance and toxicity triggers.

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Use of DNA metagenomic sequencing and conserved signature sequences to characterize harmful algal blooms in Ontario Lakes

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Harmful algal blooms (HABs) may be caused by several distinct cyanobacterial and eukaryotic species. Identification of causal organisms by traditional methodologies may yield important diagnostic information but are highly dependent on user training and experience. DNA identification methodologies offer the potential to provide a comprehensive portrait of microbial communities that cause HABs including not just the causal organism but associated microorganisms that may also be important in HAB formation by providing nutrients in symbiotic associations. In association with MOECC, we explored the use of next-generation DNA sequencing to characterize seasonal bloom events in Ontario Lakes over the 2015 and 2016 sampling seasons. These results indicate that high throughput multiplex sequencing can provide important information regarding microbial community associated with HABs they can augment traditional identification methodologies. One limitation in next-generation sequencing methodologies is the need for expensive core equipment and large computing capacity. Our long-term goal is to develop inexpensive robust identification and quantitation methodologies based on conserved signature sequences (CSS) found in phylogenetically coherent taxonomic groups like the cyanobacteria. In this study, as a proof of principle, we tested CSS identification strategies to several dozen metagenomic HAB datasets to explore the possibility that this approach can form the basis for an alternative identification methodology that has low equipment and computational requirements.

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Revisiting the concept of “harmful”: when cyanobacteria don’t bloom

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Whether by producing toxins, inducing anoxia, or inhibiting light, cyanobacteria blooms can have severe consequences for ecosystem and human health. However, even without bloom formation, cyanobacteria can result in ecological imbalance, such as food web interference. Food web disruption stems from their inability to synthesize specific omega-3 essential fatty acids (EFA) that are produced by their eukaryotic aquatic counterparts and are necessary for consumers’ metabolism, growth, and reproduction. In this study, we assessed how changes in aquatic conditions driven by browning (i.e., increased terrestrial dissolved organic matter loads) benefit cyanobacteria, consequently reducing omega-3 EFA availability and transfer in 30 Ontario temperate lakes. Our results indicate that increases in refractory (i.e., aromatic, high molecular weight) dissolved organic matter concentrations (from 2 to 10 mg/L) increase water colour, benefiting cyanobacteria that can adapt to the low-light conditions, in part due to their unique pigmentation. Increasing cyanobacteria prevalence, even in the absence of blooms, reduced the availability of the omega-3 EFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) necessary for crustacean zooplankton (e.g., cladocerans and copepods). However, under low omega-3 EFA conditions, primary consumers may supplement their diet with EPA and DHA transferred through the microbial loop (i.e., the microbial pathway through which carbon is transferred from bacteria primary consumers) to meet their nutritional requirements. Our findings highlight that both blooming and non-blooming events should both be considered within the concept of ‘potentially harmful’. Non-blooming cyanobacteria episodes are especially detrimental for human communities dependent in fish as their main diet source.

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Drivers of frequent algal blooms and environmental change inferred from a sediment core archive of Stoco Lake (ON, CA)

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Stoco Lake (Tweed, ON) is a relatively shallow lake situated on the Boreal Shield-Mixedwood Plains ecotone. The lake is ~23 km downstream from the Deloro-Stellite mine site (closed in 1961), a source of minor metal contamination (primarily Ni, As, and Zn). Substantial nutrient inputs from agricultural and urban development are likely related to frequently observed cyanobacterial blooms that have been reported since the 1970's. Modern monitoring records indicate a shallow oxycline, yet long-term hypolimnetic oxygen dynamics remain poorly understood but critical to understand the potential for internal loading of phosphorus and iron. To determine how Stoco Lake has changed since the 1940's, we used sediment cores to examine trends in VRS-inferred chlorophyll a, midge assemblages and midge-inferred volume-weighted hypolimnetic oxygen (MI-VWHO). The VRS-inferred chlorophyll a profile doubled between ~1950 and ~1970. In contrast, changes in the midge assemblages were muted and MI-VWHO changed little through time, but this is likely because the lake record showed already persistent hypoxia at the beginning of our sediment archive in the 1940's ([MI-VWHO]_{mean} = 2.5 mg/L). The most striking midge change observed was a five-fold increase in head capsule density, occurring in ~1970, and gradually decline at the top of the core. Overall, the observed changes are likely related to eutrophication; however, the increased abundance of littoral taxa may also be related to increasing air temperatures, expanded littoral microhabitats, or worsening oxygen conditions via changes in thermal stratification patterns. Furthermore, the reduction of Simuliidae remains after the 1990's may indicate hydrological changes in the Moira River that could influence algal blooms. The persistence of hypoxia indicates that the risk of internal nutrient and contaminant loading has been present since the 1940's.

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Determination of total microcystins (free and protein-bound) in fish tissue using Lemieux Oxidation

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Microcystins (MCs) are cyanobacterial toxins generated from harmful algal blooms (HABs). MCs are hepatotoxins found in freshwater system worldwide and are known for being hazardous to the living organisms. Studies show that MCs are bioaccumulative and exist in two different forms in biota: free and covalently bound to proteins. These toxins can be transferred through the food web to high trophic levels and to human; therefore, MCs can have potential risk to human health. More than 200 MCs structures have been reported. All these variants exclusively contain an unusual amino acid side chain abbreviated Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid). Different approaches, such as Lemieux oxidation, have been explored for the extraction and analysis of free and bound microcystins in aquatic living organisms. Using this method, the oxidative product of Adda, 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), is analyzed. In our study, factorial design was applied to better optimize the Lemieux oxidation procedure and oxidize MCs in fish tissue (liver and fillet). Moreover, we generated calibration standard, MMPB, in situ by oxidizing four MCs standards spiked to blank fillet/liver. We also applied matrix-matched calibration approach to correct the co-elute matrix influence on ion suppression. MMPB was analyzed using UPLC-Xevo G2-XS-QToF Mass spectrometer. The optimized oxidation condition along with the optimized solid phase extraction led to the highly linear calibration curve and the recovery ranged from 50 to 2000 ng/g.

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Identifying major producers of microcystin across multiple lakes using paleo-records

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Globally, the reported number of elevated cyanotoxin concentrations in surface waters has risen over the last decades. However, it is still unclear if this represents a trend of increasing toxicity of algal blooms, increased reporting or the development of more advanced laboratory techniques. Furthermore, it is unclear whether changes in toxicity are related to changes in the abundance and dominance of specific toxin-producing taxa (e.g. *Microcystis*, *Dolichospermum*, *Planktothrix*). In this study we analyzed sediment records dating back 200 years from three Minnesota lakes that stretch from tallgrass prairie through to mixed temperate and boreal forests. We tracked changes in both cyanobacterial toxicity and community structure using a combination of ddPCR amplification (targeting and quantifying the *mcyE* gene) and high-throughput sequencing (cyanobacterial 16S rRNA gene). We then tested which cyanobacterial community member(s) best predicted microcystin gene abundance, and whether this changed over time and/ or space using an elastic net - machine learning approach (hybrid approach between LASSO and Ridge regression). Preliminary results show that in this particular lake set, the *mcyE* gene was strongly related to long-term trends in *Microcystis*.

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Fluorescence spectra predict microcystin-LR occurrence in lake water

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Disinfection byproducts (DBPs) and algal toxins can be expensive to monitor and represent significant potential risks to human health. Microcystin-LR[?] produced by cyanobacteria[?] is linked with various adverse health effects. Here we show that fluorescence spectra predict microcystin-LR occurrence in lake water. We compared models with either fluorescence spectra or a suite of water quality predictors as inputs. A regularized logistic regression model with fluorescence spectral inputs correctly classified 94% of test data with respect to microcystin-LR occurrence, with a 96% probability of correctly ranking a detect/nondetect pair. A gradient-boosted classifier with seven water quality inputs was nearly as accurate in detecting microcystin-LR (91% correct). Results highlight the potential for multiparameter monitoring via fluorescence spectroscopy, extending previous work on predicting DBPs. As a high frequency monitoring tool, this approach could be used to decide when and where to monitor and to interpolate monitoring data, supplementing mass spectrometric methods that may only be applicable at low frequency due to resource limitations.

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Cyanobacterial toxins in the UK – can they be a food safety threat?

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In recent years, blooms of cyanobacteria have on rare occasion proliferated in freshwater bodies located directly upstream from commercial shellfishery zones in estuaries. Relatively little is known from UK studies, however, regarding the potential impact on food safety of the shellfish consumers, given the potential for cyanobacteria to become a food source for molluscs such as mussels, oysters and clams. Consequently over the past few years, a cyanotoxin programme of work has been developed to try and address this issue. Approaches utilising modern chemical detection methods such as LC-MS/MS have been developed, validated and accredited to provide simple, accurate and rapid quantitative determination of cyanotoxins such as the microcystins and nodularins in water, algae and shellfish tissues. Alongside this development, studies have been conducted using laboratory-based tank studies to assess the potential uptake of cyanotoxins into mussel tissue. Results have demonstrated the rapid uptake of toxins into tissues during blooms, followed by lengthy depuration, inferring risks may be significant if high density blooms impact upon mussel beds. In tandem with these studies, and to aid the ISO17025 accreditation programme for these methods, we developed a set of reference materials (RM) for use in Quality Control. Mussel tissue RMs have been created which will enable repeatability and reproducibility assessments to be made in the future. These have been assessed for homogeneity and stability over the long term. Work is ongoing to characterise our RMs, to optimise the method for additional cyanotoxins of potential importance and to ultimately develop a routine approach for ongoing assessment of food safety threats during cyanobacterial blooms situated close to organisms destined for human consumption.

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Constant nutrient loads yield changing annual cyanobacteria bloom dynamics

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Nuisance and harmful algal blooms dominated by cyanobacteria impair water quality and threaten our use of lakes for recreation, fisheries, and drinking water. Managing and mitigating the effects of blooms requires understanding their drivers to develop mitigation site-specific strategies. We used the environmental and climatic forcings as external drivers that change bloom dynamics in Lake 227, a small, soft-water, experimentally eutrophied lake at the IISD-Experimental Lakes Area. Since 1990, weekly phosphorus additions during the open-water season have created a multidecadal, replicated experiment. We observed a gradual transition from a single, long mid-season bloom of the nitrogen-fixing cyanobacteria, *Aphanizomenon*, into two shorter blooms in the epilimnetic layer. The early bloom is heavily dominated by *Aphanizomenon* whereas the later bloom is more diverse. Nevertheless, the total seasonal phytoplankton biomass has remained relatively consistent over the last 27 years. This pattern may be partially driven by earlier water column stratification or a nearly two-week increase in the number of ice-free days which thereby increased the length of the stratified season. Together, the results demonstrate that even within a strictly controlled nutrient-addition experiment, bloom topology and composition will change. This means that managing freshwaters with varying external nutrient loads requires recognizing that recovery targets are constantly moving.

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Relationship of sulfate, sediment biogeochemistry, and hypolimnetic iron levels in the promotion of cyanobacterial blooms

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In order to further define the sediment-iron-sulfide relationships which inhibit release of ferrous ions from anoxic sediments, sediment incubation experiments were conducted. Surficial sediments from Hamilton Harbour and Lake Erie were collected, sieved, and mixed into aerated slurries, and portions of sediment were anaerobically incubated in core tubes to simulate the biogeochemical processes leading to anoxia and internal loading with varying levels of sulfate and organic matter. Levels of dissolved oxygen, redox, conductivity, soluble iron, dissolved phosphorous, sulfate, and nitrate were monitored throughout each incubation. Preliminary results indicate that biogeochemical changes take place very slowly at 5° C, which suggests that most perceptible activity occurs when hypolimnetic temperatures are elevated during the summer months. Redox zones and solutes become significantly stratified in the aqueous layer within two weeks at elevated temperatures. The organic matter content of sediments has a very significant role on the rate of internal loading and solute composition, as does the sulfate level concentration of the overlying water column. High organic matter was associated with highly accelerated biogeochemical activity in accordance with the redox state, which quickly lowered oxygen levels and released elevated levels of soluble phosphate and iron, the latter of which was quickly precipitated as insoluble sulfides, provided sulfate levels were sufficient. These results suggest that the sequence of biogeochemical release and moderation is dependent upon several factors, which likely determine the phytoplankton succession in each system.

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Developing preventative bioremediation strategies to curb harmful algal blooms in freshwater systems in Canada and China

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Cyanobacterial Harmful Algal Blooms (CHABs), one of the major threats to freshwater quality, are now increasing in severity worldwide. Nutrient-source control strategy, especially controlling the limiting factor of CHABs formation - phosphorus, has been considered as the best and ultimate solution to deal with CHABs. However, this strategy may not be feasible for areas because of the increasing population pressure and pollution which are further complicated other nutrients loading such as nitrogen, elevated CO₂ influx into aquatic systems and climate change. In the last decade, we have carried out series of restoration projects to curb CHABs in China, the most eutrophicated area where nutrient-source control is poorly implemented and cyanobacteria infested regularly. In these projects, we have not only successfully controlled CHABs when the eutrophication condition was not reduced, but also managed to maintain a long-term effect. We propose a proactive strategy, an effective preventative proactive biological control method. The implementation of those projects itself is an adaptive learning process, through which we tried to develop and demonstrate a successful integrative strategy to control CHABs in representatives of water bodies that are most susceptible to algal blooms. Our work indicates that successful implantation needs to combine early pre-bloom detection (using UAV), hydrological methods with the consideration of temporal and spatial dynamics of the food-web, and should be tailor designed with profound understanding of science, biology, available technology and as well as management system (monitoring, regulatory). This strategy is not just temporarily preventing the incoming blooms, but eliminating CHABs thus providing a chance for the ecosystem to restore itself, which makes it an important supplement or even alternative of current strategy.

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HABs in drinking water supply systems: Expression of toxicity, treatment process issues, management tools and public health

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A wide range of harmful algal/cyanobacterial species are increasingly detected in water bodies in Canada, exacerbated by climate change and human activities. Harmful metabolites including cyanotoxins and taste and odour (T&O) compounds were associated with these bloom events. A number of commonly occurring cyanobacterial species have the ability to produce potent toxins with irreversible human health effects and even mortality. Cyanobacteria have also been hypothesised to be a possible cause of neurodegenerative illness, including Alzheimer's disease, and their cell walls contain compounds (lipopolysaccharides) that can act as contact irritants. On the other hand, T&O compounds produced by these blooms, including geosmin and 2-Methylisoborneol (MIB), are not harmful to human health but they are one of the main sources of consumer complaints and dissatisfaction, and consequently harmful to the utilities reputation and public trust. These bloom events and their undesirable effects demonstrate the ongoing challenges of algal/cyanobacterial related issues for Canadian water utilities, health authorities, and regulatory agencies. Understanding the fate of algal/cyanobacterial cells and their associated metabolites in water supply systems is necessary for their successful removal, protection of public health and customer satisfaction. The objectives of this presentation are (1) to review results of recent studies exploring the fate of algal/cyanobacterial cells and their metabolites (including toxins, T&O and organic matter) in Canadian full scale water treatment plants, and (2) to demonstrate benefits of using smart real time management strategies to prevent the breakthrough events. These results help to define the scale of algal/cyanobacterial and their harmful metabolites monitoring and treatment challenges in Canadian water treatment plants. Furthermore, this presentation will outline the knowledge gaps in removal of algal/cyanobacterial cells and their metabolites from water. Introduction of a real time treatment adjustment strategy would enhance removal of algae/cyanobacteria and their harmful metabolites from water and reduce the associated costs.

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What lurks below, toxic secondary metabolite production in deep-living layers dominated by the cyanobacteria *Planktothrix isothrix*

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Our perception of deep-living phytoplankton in Canada is largely based on studies of the offshore waters of the Great Lakes, where dominance by phycoerythrin-rich picocyanobacteria and Heterokontophyta and Pyrrophyta has been observed. A few meters from shore in the waters of Georgian Bay, Lake Huron dominance by larger, filamentous, bloom-forming, and toxic species of cyanobacteria lurk in the depths. *Planktothrix isothrix* was the dominant species during the height of thermal stratification in each of the two deep chlorophyll layers under investigation (65% of total biomass at ~7.5 m in South Bay, 40% at ~9.5 m in Twelve Mile Bay). Although both embayments had elevated concentrations of microcystins and anabaenopeptins as well as corresponding genes, only in Twelve Mile Bay were there vertical peaks coincident with *Planktothrix isothrix*. Anoxic and low redox conditions just below drove the release, from sediments, of not only phosphate but also key trace metals iron, manganese, and cobalt. While this relieved the deep-living phytoplankton in South Bay of the phosphorus limitation experienced by their epilimnetic counterparts, the community in Twelve Mile Bay was not as fortunate. The magnitude and toxin concentration of a hypothetical surface bloom derived from these deep chlorophyll layers was estimated using a Gaussian distribution, predicting a more than two-fold greater peak concentration of chlorophyll when entrained at the surface. Although this phenomenon has been documented under both natural and artificial disruption of deep-living biomass, the role of this mechanism in toxic surface bloom formation has had little attention.

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Research and development of reference materials for cyanobacterial toxins at NRCC

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Cyanobacteria and their associated cyanotoxins pose a serious threat to wildlife, pets, livestock and humans exposed to contaminated water, algal dietary supplements and fish. As new regulations and monitoring programs are adopted worldwide there is an increased need for accredited analytical testing of cyanotoxins. A major impediment to the development of accredited test methods for cyanotoxins has been the limited availability of cyanotoxin certified reference materials (CRMs) that offer traceability in compliance with laboratory testing quality standards (e.g. ISO 17025). These materials are needed for instrument calibration as well as method development and validation to ensure comparability between measurements carried out at different times or in different laboratories. The development of CRMs for cyanotoxins is particularly challenging because of the large number of toxin variants that must be monitored. The National Research Council of Canada (NRCC) has produced a number of publicly-available cyanotoxin CRMs for instrument calibration including microcystins (MC-LR, dmMC-LR, MC-RR), cylindrospermopsin, anatoxin-a, nodularin, lyngbyatoxin and saxitoxins. Feasibility studies have been conducted in support of matrix reference materials for cyanotoxins in dietary supplements. This presentation will provide an overview of the R&D that has gone into cyanotoxin CRMs at NRCC, outlining key steps in their preparation including toxin production and isolation, method development, purity testing, stability testing, and accurate quantitation. Metrological traceability to The International System of Units (SI) and uncertainty are key features of NRCC CRMs. National and international collaborations will be highlighted along with future objectives.

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Quantification of Emerging Cyanotoxins in the Freshwater of Nova Scotia Southwestern Region

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Monitoring of Canadian drinking water sources has revealed cyanotoxins such as emerging pollutants of concern. Cyanobacteria pose a worldwide human health due to their increasing presence in both drinking and recreational waters. Freshwater cyanobacteria accumulate in surface water as “blooms” and are increasing frequency in fresh, brackish and marine water. Nova Scotia doesn’t have data for total cyanotoxins and the guideline values (GV) for drinking water is relative to Microcystins (MC). GV suggested by Health Canada is 1.5 µg/L and 1 µg/L by WHO. EPA suggested 0.3 µg/L (infants) and 1.6 µg/L (adults). Our study is important for the Acadian Community of Nova Scotia since it will be the first to quantify cyanotoxins impacting the drinking water quality in the southwestern region, St. Marys Bay, where Université Sainte-Anne is located. This Region is surrounded by agricultural and maritime areas such as mink farming and seafood related industries. Their inhabitants get their drinking water from wells that can be connected to lakes such as Ogden lake (Yarmouth County) which is contaminated by relevant emerging cyanobacteria like *Anabaena flos-aquae* (Anatoxin-a) and *Microcystis aeruginosa* (MC-LR). To Anatoxin-a and MC-LR quantification we have used ELISA. We have plan 3 harvest periods of water samples/year, in Ogden Lake. We have analysed the 1st related to the development of blooms (Mai-August 2017). Our preliminary results carried out at the 1st period show presence of 0.6 µg/L of Anatoxin-a (n=3) and 0.3 µg/L of MC-LR (n=3). We have find significant quantities of cyanotoxins close to EPA- and WHO- GV. These encourage us to investigate the other periods. We aim to contribute to cyanotoxins risk assessment and management.

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Semi-automated method for detecting and counting cells of cyanobacterial colonies and filaments

Frances Buerkens

Fluid Imaging Technologies

Harmful algae blooms are increasing in frequency and intensity. Public safety and conservation agencies demand a replicable and scalable method to rapidly detect and enumerate cells comprising cyanobacterial colonies and filaments. The FlowCam is a proven technology that identifies taxa to the genus level and provides an estimate of the abundance of individual cells. It combines digital imaging, flow cytometry, and microscopy to calculate the dimensions, biovolume and abundance of cells. The FlowCam Cyano leverages recent technological developments – a 633 nm laser – enabling the instrument to distinguish cyanobacteria from other algae in a water sample. The abundance of cells within colonies and filaments are counted using a simple Excel based formula, enabling monitoring agencies and researchers to rapidly enumerate cells in large sample volumes. The FlowCam system facilitates an accurate measurement of cell abundance for large folded colonies because the colonies flatten within the unique flow cell chamber. Here we present an overview of the technology along with HAB field data from freshwater systems that affect drinking water and recreational lakes across North America.

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Characterization of spatial and temporal variability of the aquatic microbiome along the Thames River-Lake St. Clair-Lake Erie continuum

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Lake Erie is heavily impacted by urban and agricultural nutrient loading, which makes it in the poorest condition of all the Great Lakes. Despite the implementation of action plans to reduce phosphorus loads from its watershed, Lake Erie continues to face recurring events of cyanobacterial and harmful algal blooms (cHAB), which have important ecological, societal and economic implications. This in turn highlights the need to better understand the underlying mechanisms of cHABs, in order to forecast and prevent these events. Measures of nutrients and total phytoplankton biomass seem to be poor predictors of cHABs when taken individually. Therefore, a more integrated approach at the watershed scale may improve our understanding of the conditions that lead to bloom formation by assessing the physico-chemical and biological factors that influence the lake microbiome and identifying the linkages between Lake Erie and the surrounding watershed microbiomes. Here we used high-throughput sequencing of the 16S rDNA to characterize the spatial and temporal variability of the aquatic microbiome in the Thames River - Lake St-Clair - Lake Erie aquatic continuum. We found that the aquatic microbiome composition was structured along the flow path, and that the community shifted from the Thames River to Lake Erie. This shift was particularly reflected in the cyanobacterial community composition, which changed from being dominated by *Planktothrix* in the Thames River to *Microcystis* and *Synechococcus* in Lake St. Clair and Lake Erie. In future research, we will assess the connectivity between the soil and the aquatic microbiome of this agricultural watershed in order to see how different land management practices can affect the aquatic microbial community and cyanobacteria in particular. Ultimately, we anticipate a better comprehension of the origin and composition of the aquatic microbiome in the Thames River and Lake Erie basin will provide new perspectives on how to better predict the occurrence of cHABs and improve Lake Erie health.

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Optimizing satellite-based models for prediction of phytoplankton biomass across broad geographic gradients

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The frequency, intensity, and geographical distribution of harmful phytoplankton blooms are on the rise globally. Current evidence is predominately anecdotal, in which in situ monitoring efforts are not extensive enough to provide sufficient evidence, resulting in a scientific need for estimates of increased historical and current phytoplankton data. This research develops mathematical algorithms for accurate assessment of surface chlorophyll-a (chl-a), a proxy for phytoplankton biomass, within freshwater lakes using remotely sensed images. Variations and levels of chl-a are then used as a basis for improved scientific understanding of phytoplankton blooms. Landsat satellite images (4-5 TM, 7 ETM and 8 OLI) were used to create a ± 30 -year predictive model (1984 to 2017) for seven ecoregions (ranging from the tropics to arctic). Correlation tests for 82 algorithms were conducted to establish the best fit models (linear, exponential, logarithmic, power) for chl-a and environmental parameters that interfere with the chl-a assessment (true colour, TSS, and turbidity). Three band algorithms involving absorbent and reflective bands multiplied by the near infrared band using power regression provided a robust predictive model across all regions (R^2 ranges from 0.42 – 0.81)

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Anti-cyanobacterial proprieties and Chemical Characterization of essential oils obtained from the endemic Moroccan mint *Mentha suaveolens* subsp. *timija* (mint timija)

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Over the last two decades, there has been a growing concern about the impact of cyanobacteria blooms due to increasing pollution and eutrophication in aquatic ecosystems. *Microcystis* species, as producer of various microcystins congeners, are among the most important worldwide freshwater bloom-forming cyanobacteria. Due to their undesirable effects, various strategies, including mechanical, physical, chemical, and biological methods have been used to prevent and control the blooms of cyanobacteria. Through this work, we highlighted and evaluated the anti-cyanobacterial activity of the essential oil (EO) extracted from the endemic Moroccan mint *Mentha suaveolens* subsp. *timija* EO against unicellular toxic cyanobacteria *Microcystis aeruginosa*. The yield of *Mentha suaveolens* subsp. *timija* EO obtained was 0.63 ± 0.09 %. Gas chromatography–mass spectrometry (GC–MS) analysis revealed 34 compounds, representing 97.8 % of the total oil. The major constituents identified were pulegone (23.4 %), piperitenone oxide (22.6 %), menthone (10.1%) and piperitone epoxide (9.1 %). The anti-cyanobacterial activity was examined and their potency assessed both qualitatively and quantitatively by the application of paper disk diffusion and broth microdilution methods. Initial screening using the paper disk diffusion assay showed that *Mentha suaveolens* subsp. *timija* EO have an important activity against *M. aeruginosa* with inhibition zone diameter (ZI) of 29.3 ± 0.06 mm. While, the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) confirmed the results obtained by the paper disk diffusion method. The MBC value was equivalent to the MIC value (12.5 mg/mL). The obtained results suggest that *Mentha suaveolens* subsp. *timija* EO can be new biological promising source for novel bioactive compounds that can be used for the selective biocontrol of harmful cyanobacterial blooms.

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Assessment of the potentialities of *Vicia faba* seeds and *Opuntia ficus indica* as natural coagulants to reduce cyanobacterial proliferation

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Cyanobacterial Harmful Algal Blooms (CyanoHABs) have recently increased in drinking-water treatment plants that pose a serious problem for water utilities worldwide. Consequently, several physicochemical processes are used to reduce effects of cyanobacterial proliferation. Chemical coagulation -flocculation was used in potable water treatment to destabilize and remove algal blooms. However, the use of chemical coagulants induce serious problems for human health and high operation costs in treatment. To overpass those problems, ecofriendly alternative methods based on naturel coagulants are highly desirable. They have both the efficiency in application and the advantage of being biodegradable; without risk to public health and environment. In this study, decontamination of polluted water with *Microcystis aeruginosa* by the application of *Vicia faba* seeds and *Opuntia ficus indica* cladode extracts, as natural coagulants, was investigated. Optimization assays were conducted by the standard jar test method. Thus, effect of different parameters such as speed of agitation, type and dose of coagulant / flocculant and pH was studied. For this reason, coagulation-flocculation activity was performed by a screening study based in an experimental research methodology to achieve the optimal response level. The results revealed that natural coagulant extracted from bean and cladode cactus have a high abatement of turbidity that reach more than 90%, with a similar reduce in others parameters such as optical density, chlorophyll a and carotenoids The obtained results provide that these two natural coagulants can be used as an ecofriendly alternative to decontaminate polluted water with cyanobacteria.

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Iron and molybdenum influence growth and microcystin production of a freshwater cyanobacterium, *Microcystis aeruginosa*

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The cyanobacterium *Microcystis aeruginosa* is an important bloom forming species in freshwater environments. As this species produces microcystin, a hepatotoxin that can contaminate drinking water supplies, understanding the environmental factors that regulate toxicity is important as they can become a significant public health concern. We investigated the photosynthesis, growth, and toxin content of one toxic strain of *M. aeruginosa*, CCCC 300, under a combination of nitrogen supply, nitrate (NO₃⁻) or ammonium (NH₄⁺) and trace elements iron (Fe) and molybdenum (Mo) concentrations. When Fe was supplied at replete conditions, *M. aeruginosa* grew equally well on NO₃⁻ and NH₄⁺. Reducing Fe dramatically reduced growth efficiency when the cells were supplied with NO₃⁻, but not with NH₄⁺. In contrast, removal of Mo did not impair growth efficiency regardless of the form of nitrogen available. Cells preconditioned under a gradient of Fe concentrations in the absence of Mo were spiked with replete levels of Mo, Fe or in combination to assess recovery of growth, photosynthetic potential, and microcystin production. Replenishing Fe enhanced growth, photosynthetic parameters, and toxin production. However, when both trace metals were supplemented, toxin production was further enhanced in cells previously grown in deplete concentrations of Fe, showcasing the synergistic effect of these two trace metals. Our findings suggest that macronutrients are important for biomass accumulation, but micronutrients, such as Fe and Mo, regulate the effective processing of macronutrients (especially NO₃⁻), indirectly influencing C allocation towards biomass accumulation or in situations where N levels are high within the cell, allocating the excess N towards synthesis of N rich secondary metabolites like microcystin.

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Microplastic particles act as a medium to concentrate waterborne microcystin

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Both the potent liver toxin microcystin (MC) and microplastic particles (MPs) are emerging environmental contaminants now recognized as being widely distributed across the globe. MCs are a diverse group of monocyclic heptapeptide hepatotoxins produced by several genera of freshwater cyanobacteria. MPs are defined as small particles of plastic less than 5 mm in diameter. MPs may impact ecosystems by adhering to and/or being ingested by organisms, which may cause harmful physical effects. Or, MPs may be a source of hazardous chemicals via their additive ingredients and/or sorption of environmental contaminants. Recent literature has investigated the possibility that MPs act as a medium to concentrate hydrophobic organic contaminants in aquatic systems, driven by their characteristic surface hydrophobicity. This research has not been extended to MCs, which are relatively hydrophilic molecules implying that their affinity for MPs is negligible. However, some MC variants contain hydrophobic amino acid residues. Inherent in this lies the question: Do microplastic particles act as a medium to concentrate waterborne microcystin? To address this question, we conducted a 7 day laboratory-based experiment. In a 1 L glass bottle filled with 500 mL of water, we exposed a mixture of four MC congeners (20 mg L⁻¹), MC-LA, MC-LR, MC-YR and MC-RR, to one of two types of plastic pellets (n = 500), low-density polyethylene or polystyrene, each in replicates of six. Both water samples and MP pellet extracts were analysed by LC-MS/MS. Preliminary results indicate that MPs do act as a medium to concentrate waterborne MC, dependent on MC congener and type of plastic. Future work will look at furthering method development, the effect of biofilm-covered MPs, and include an analysis of field-collected samples.

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Cyanotoxins in Lake Torment (Nova Scotia, Canada) during summer–fall 2017–18

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Lake Torment (Kings County, NS, Canada) has been experiencing cyanobacterial blooms since 2014. There was no previous research providing information about species responsible for blooms and toxins present in this lake. The lake is dystrophic with brown water, low pH, low carbonate level and high organic contents. Our monitoring results confirmed the presence of cyanobacterium *Dolichospermum flos-aquae* responsible for bloom formations from May until November for both 2017-2018. The bloom apparition is different from year to year, but it could be summarized into five scenarios for 2017-2018: May and/or early June, late June, August, September; and October blooms. The latter can persist until the middle of November. Cyanotoxins in Lake Torment were monitored in 2017-18. ELISA kits from Abraxis (Warminster, USA) were first used for microcystins and anatoxin-a in 2017. Two additional methods, liquid chromatography-high resolutions tandem mass spectrometry and the microcystin multi-hapten ELISA were then also introduced in 2018. The following results showed a wide range of cyanotoxin concentrations with a relatively consistent for this lake in 2017-18. Two microcystins variants were detected: MC-LR in the late August – November and MC-LY in September–November for both years. The range of MC-LR concentrations was from lower than the detection limit to a maximum concentration of 680 µg/L (November 2017) while MC-LY was found with concentrations from 0.89 to 77 µg/L. Anatoxin-a was also detected in samples from November 2017 by LC-MS/MS with low concentrations (0.12-0.21 µg/L) and along with trace levels of dihydroanatoxin-a. Monitoring results from 2017–18 showed the need of further research the relationship between toxins concentration/profile with cyanobacterial species detected in different seasons and how these relate to environmental conditions in Lake Torment.

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Evidence for and quantification of anoxia and internal phosphorus loading in Callander Bay, Central Ontario

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The effect of internal phosphorus loading in polymictic systems can be significant to the overall phosphorus load. This loading can fuel algal blooms and cyanobacteria dominated blooms throughout the summer. Due to repeated mixing events in polymictic systems it can be difficult to quantify the amount of phosphorus being loaded. Callander Bay, located in Lake Nipissing in central Ontario, is classified as a meso-eutrophic polymictic system. Concerns exist over the bay's water quality including cyanobacterial blooms and eutrophication. Five summers of high-frequency data (ten minute weather, water temperature and dissolved oxygen (DO) profiles), including three years with water chemistry (surface, composite, and above lake bed), and one year with spatial DO and temperature profiles, were analyzed to confirm the occurrence of anoxia (defined here as DO concentrations).

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Tracking eutrophication using the sediment archives of lakes in southwest Nova Scotia in relation to the advent and escalation of the local mink fur farming industry

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The recent increased occurrence of algal blooms in southwest Nova Scotian lakes has prompted concern from local residents and environmental organizations. These algal blooms have been attributed by some local stakeholders to the large number of mink fur farms in the area, which produce high volumes of phosphorus-rich wastes. Mink fur farming has been present in the region since ~1930, and production intensified in the late-1980s. Structured water chemistry sampling regimes for regional freshwaters were only initiated in 2008, decades after the escalation of the industry. These initiatives indicated eutrophic to hypereutrophic conditions in lakes with mink farms in their catchment (e.g., epilimnetic total phosphorus values reaching ~0.4 mg/L). Here, we address the need for documentation of the timing of eutrophication and whether it coincides with the establishment of mink fur farming operations. We reconstruct changes in algal indicators of eutrophication preserved in the lake sediment archives and compare sites with mink farms on their catchments to reference sites. Changes in whole-lake primary production are approximated using visible reflectance spectroscopy (VRS) inferred chlorophyll-a, a technique that reconstructs past concentrations of sedimentary chlorophyll-a and its diagenetic products. Sedimentary diatom and chrysophyte microfossil assemblages will also serve as indicators of nutrient status through time. Our preliminary results indicate that farm establishment on the catchment of impacted lakes is followed by marked increases in VRS-inferred chlorophyll-a and eutrophic diatom taxa, suggesting eutrophication may be linked to farm presence. These preliminary results will contribute to scientifically informed decisions regarding protection of regional freshwaters impacted by this industry.

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Using chironomid assemblages to assess the long run changes of external nutrient inputs in tropical lakes

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Nutrient loading is a key factor contributing to deep-water oxygen depletion in lakes, and is becoming widespread in freshwater ecosystems because of the slow rate of ecosystem recovery from stressors like eutrophication, climate warming and land-use change. In the Philippines, nutrient pollution from aquaculture has degraded the water quality of many lakes which local livelihoods depend on. Changes in deep-water oxygen concentrations in water bodies result in shifts in benthic limnological communities. Chironomids are useful paleolimnological indicators of benthic habitats because of their abundance in freshwater systems, tolerance to various chemical and physical limnological conditions, and well-preserved chitinous head capsules. Chironomid assemblages have been used to infer deep-water oxygen conditions and lake productivity, as oxygen concentrations primarily regulate chironomid assemblages. In this study, I will use chironomid assemblages to assess the impact of external nutrient inputs on two lakes with contrasting histories of nutrient loading and changes in algal production in the Philippines. Chironomid assemblages will be analysed from ^{210}Pb and ^{14}C -dated sediment cores to infer deep-water oxygen conditions over the past ~200 years, as well as fossil pigment analyses to track past changes in algal abundances. I will also be taking water quality measurements (i.e. pH, conductivity, Secchi depth and total phosphorus) from each lake to put paleolimnological trends into the context of current limnological conditions. I will investigate if the timing of changes in deep-water oxygen conditions coincide with known anthropogenic disturbances in the lakes' catchment. Understanding the baseline conditions of lakes and their responses to external nutrient loading can provide insights on the tolerance of lakes to disturbance effects. This information may aid the management and conservation of lakes in the Philippines by setting restoration goals and identifying changes in deep-water oxygen conditions attributed to anthropogenic activities.

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Drivers of water quality changes in North American lakes over the past 40 years

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Freshwater lakes in North America are continuously subjected to multiple stressors which may impair water quality, including climate change, nutrient loading, lake productivity, land use development, and brownification. This study incorporates time series data (1976-2017) for 79 lakes across North America. We evaluated trends in physical (water temperature and secchi depth), chemical (phosphorus, nitrogen, dissolved organic carbon, and chloride), biological (chlorophyll), and climatic (temperature, precipitation, and cloud cover) variables using Sen's slope models for each lake. Generally, across lakes over the past 40 years, lake phosphorus has significantly declined, whereas dissolved organic carbon has increased, and chlorophyll has become increasingly variable year to year. In addition, lake water temperatures have become significantly warmer, potentially reducing the amount of cooler water habitat for sensitive aquatic life. Future work will include regression tree analyses to examine the potential drivers of chlorophyll concentrations (a proxy for water quality). As freshwater resources are becoming increasingly threatened worldwide, maintaining their integrity has emerged as a key global issue with far-reaching consequences for human and ecosystem health. Identifying the drivers that are most damaging to the water quality of North American lakes is an important first step in developing meaningful targets for future management strategies.

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Untargeted LC-MS/MS approaches to investigate *Microcystis* oligopeptide production

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Over the last hundred years, freshwater ecosystems in Canada have undergone widespread environmental change resulting from nutrient inputs and warming. A highly visible consequence of this change is the increase in frequency and magnitude of cyanobacteria blooms. While the factors that stimulate the proliferation of blooms are broadly understood, the hazards associated with cyanotoxin exposures remains to be fully elucidated. This deficiency limits the ability of regulatory officials to properly assess the risks to human and ecosystem health, as well as local economies. The objective of this study was to build capacity to investigate the diversity of chemicals produced by cyanobacteria populations. Culture based experiments examined how abiotic factors modulated the biosynthesis of different natural product classes for five strains of *Microcystis aeruginosa* and one *M. flos-aquae* obtained from the Canadian Phycological Culture Centre. The biosynthesis of different oligopeptide classes including microcystins, micropeptins, microginins and aeruginosins were monitored by an untargeted LC-MS/MS approach. Post-acquisition filtering strategies exploiting characteristic fragment ions and amino acid immonium ions were developed to detect all related structures within a class. These efforts will enable the rapid dereplication of oligopeptides produced by newly collected isolates from smaller lakes surrounding Ottawa. This investigation also provides the basis for larger scale cultivations to purify and structurally characterize the dominate oligopeptides for reference materials and biological assays. Developing knowledge of cyanotoxin identities and their biosynthesis will help support water, conservation, and public health authorities managing cyanobacteria blooms.

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Untargeted high resolution mass spectrometry approaches for identifying novel microcystins

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

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Determination of Microcystins in Water – MECP interlaboratory studies

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Herein we present the results of the interlaboratory studies (ILS) conducted by the Ministry of Environment, Conservation and Parks (MECP) from 2013 to 2017 on the determination of microcystins (MC) in drinking water by Enzyme-linked immunosorbent assay (ELISA). Every year private and government laboratories from across Canada are invited to participate in this proficiency test free of charge. However, laboratories licensed to test drinking water in Ontario are required to submit the results of proficiency tests to the MECP Performance Evaluation Coordinator as a condition for holding their license as per O. Reg. 248/03, s. 8. Toronto tap water spiked with various microcystins congeners e.g. MC-LR, MC-LA and mixtures at various concentrations were dispatched to laboratories via courier. Laboratories were required to use their routine methods to conduct the test. The evaluation was performed using z-score for samples containing LR only; A $|z| = 2$ – Satisfactory; 2 From 2013 to 2017, a total of 114 MC-LR results were submitted to the program, of which 6% (7/114) were “Unsatisfactory”. A higher rate of “Unsatisfactory” results was recorded for MC-LA, 24%, mixture – Mix A, 14% and Supplemental, 21%. The most common kits used in the province were Abraxis (ADDA/DM) and Envirologix QuantiPlate / QualiTube. Both performed equally well for MC-LR. Their performance however differed for other congeners e.g. MC-LA and mixtures. Overall for the past 5 years the performance of participating laboratories was very good related to the determination of microcystins MC-LR in drinking water.

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Salinity tolerance and toxin concentration in *Microcystis aeruginosa* in the Chesapeake Bay region

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The cyanobacteria *Microcystis aeruginosa* tends to form harmful blooms in the late summer and thrives at higher temperatures, typically above 25°C. *M. aeruginosa* produces the toxin microcystin, a hepatotoxin known to have detrimental effects on mammals, but also have been associated with deaths of birds, turtles, and other vertebrates that live in or near aquatic habitats. As such, there is keen interest in the occurrence of these blooms in terms of both human and ecosystem health globally, and management of these blooms is a growing concern. Blooms in the Chesapeake Bay have been occurring with increasing frequency and at increased locations. This study investigated toxin concentration and salinity tolerance of *M. aeruginosa* in laboratory studies on cyanobacteria sampled from a bloom that occurred in “ponds” within a restored wetland on Poplar Island in the center of Chesapeake Bay. The halotolerant strain sampled, occurred in the field at salinities from 8-12. During salinity experiments, *M. aeruginosa* showed increasing cell abundance in salinity treatments from 0-15 with a sharp decrease in abundance at a salinity of 18, and zero abundance above 22. Microcystin concentration, determined by a phosphatase inhibition assay (PP1), was highest at salinities between 6-14. Dropping off dramatically at salinities above 18. Toxin was detectable in water samples for approximately 30-34 days even after *M. aeruginosa* cells were no longer present. This suggests that *M. aeruginosa* has the ability to occur throughout the oligohaline and mesohaline portions of the Chesapeake Bay. Additionally, given that the toxin was present in laboratory experiments for ~30 days after the presence of cyanobacterial cells, toxin transport both up and down estuary may be possible.

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A wicked problem: Internal phosphorus loading in Canadian freshwaters

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Many physical, chemical, and biological processes in freshwater ecosystems mobilize the nutrient phosphorus (P) from sediments, which in turn may contribute to the formation of harmful algal blooms. Here, we critically reviewed internal P loading in Canadian fresh waters to understand the geographic patterns and environmental drivers of this important process. From 43 publications, we consolidated 618 estimates of internal P loading from Canadian freshwater ponds, lakes, reservoirs, and coastal wetlands ($n = 70$). Expressed in terms of total P, short-term gross rates in sediment samples (L_{gross}) ranged from -27 to $54 \text{ mg m}^{-2} \text{ day}^{-1}$ ($n = 461$), while long-term net rates in whole ecosystems (L_{net}) ranged from -1694 to $10\,640 \text{ mg m}^{-2} \text{ year}^{-1}$ ($n = 157$). The main environmental drivers of this variation were oxygen, pH, geology, and trophic state. Internal P loading tended to be higher during the open-water season and most prominent in small prairie lakes. Priorities for future research on internal P loading should include resolving methodological problems, assessing the relative importance of different mechanisms, examining the influence of anthropogenic activities, and quantifying rates in understudied ecosystems.

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Advanced QToF MS acquisition modes for non-targeted analysis of microcystins

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Microcystins are cyclic heptapeptide hepatotoxins produced by cyanobacteria found in freshwater and marine environments. They are toxic to higher organisms, causing human sickness or even death. Due to the different possible amino acid configuration in their structure, over 250 variants have been reported to date. Traditional targeted methods for microcystins analysis based on liquid chromatography coupled to mass spectrometry (LC-MS) tend to underestimate the quantification of total microcystins by focusing on the most common variants only. Therefore, novel non-targeted analytical approaches must be developed to detect and identify less common and previously unknown microcystin variants. In the present study 3 different quadrupole time of flight mass spectrometry (QToF MS) advanced acquisition modes are compared for the non-targeted analysis of microcystins: data dependent acquisition (DDA), data independent acquisition (DIA), and quadrupole isolated DIA (SONAR-DIA). Despite the orthogonality of the methods, same potential previously unknown microcystin structures were identified during the analysis of a complex cyanobacteria algal bloom sample. These structures were elucidated based on their molecular fragmentation, accurate mass measurements and a custom-built exact mass amino acid calculator.

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Effects of Cobalt on Heterocyst Formation of *Anabaena flos-aquae*

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Cyanobacteria harmful algal blooms are an increasing occurrence and nuisance across Canada and much of the world. Controlling external phosphorus loading into lakes and reservoirs have led to the emergence and dominance of cyanobacterial blooms composed of nitrogen-fixing cyanobacteria such as *Anabaena*. *Anabaena* is a heterocystous cyanobacteria with specialized cells that are made to fix atmospheric N₂. Understanding the environmental factors that play a role in the dominance of N-fixing cyanobacteria is important to better manage these blooms. This study aims to investigate the impact of cobalt on the formation of heterocysts and the growth rates of *Anabaena flos-aquae*. We hypothesize that increasing Co concentrations will lead to larger growth rates and a higher heterocysts to cell ratio, thus highlighting the need to better understand and manage Co.

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Microcystis aeruginosa adversely impacts Daphnia spp.: implications on food webs in the Great Lakes

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Harmful algal blooms dominated by *Microcystis* are a troublesome nuisance to freshwater ecosystems used for drinking, irrigation, fishing, and recreational purposes. Many *Microcystis* blooms produce strains of the hepatotoxin microcystin that is capable of inflicting harm to zooplankton. Research on the effect of microcystins on zooplankton remains elusive; from one perspective, studies suggest *Daphnia* can be used to suppress phytoplankton, including cyanobacteria, and coexist in toxic blooms; however, studies have also indicated toxic effects on *Daphnia* that consume cell-bound microcystin. This dual perspective may be attributable to an evolutionary adaptation in *Daphnia* that ensures survival and reproduction in toxic cyanobacterial blooms. We examined reproduction and survival of laboratory-cultured *Ceriodaphnia dubia* and *Daphnia magna* in microcystin-producing *Microcystis aeruginosa* through a series of life-cycle bioassays. Test organisms were exposed to a concentration gradient ranging from 0.5 µg/L to 100 µg/L microcystin-LR which corresponds to values typically found in the Great Lakes during bloom season. Mortality was observed in *C. dubia* (LC50 = 2 µg/L) and *D. magna* (LC50 = 37 µg/L) exposed to microcystin-LR, and reproductive effects at concentrations as low as 2.5 µg/L. This information will improve our understanding of the risks posed to food webs in the Great Lakes.

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Getting your phyll of data: Assembling a freshwater lake water chemistry database

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Measures of chlorophyll in freshwater lakes can be used as a proxy for primary production and an indicator of water quality. However, the concentration of chlorophyll in lakes is dependent on many interacting factors including spatial heterogeneity in nutrients, climate drivers, and anthropogenic disturbances. Water chemistry sampling protocols frequently include measures of chlorophyll, and these data are readily available in published manuscripts and online repositories. Thus, we have capitalized on the opportunity to synthesize a global database on lake water chemistry with a focus on chlorophyll for freshwater lakes around the globe. We are conducting a systematic review examining over 4000 published manuscripts dating from 2000 to 2018 that measured lake chlorophyll and supplementing the published manuscripts with data available from online repositories, such as KnB, Dryad, and Pangaea. To date, we have developed a database of geographic locations, morphology, and water chemistry for over 12,744 lakes in 93 countries across 7 continents. In addition, we have time series data (>5 years) for over 352 lakes. Using the geospatial coordinates of the lakes, we can relate measures of chlorophyll to climate drivers, human population growth, nutrient input levels, and land use changes across broad spatial and temporal scales. This database will facilitate our current research on drivers influencing chlorophyll level changes at a global scale as well as future research to improve our understanding of how water quality responds to global environmental change.

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Characterizing the early development of cyanobacterial algal blooms in small fresh water bodies through high temporal frequency eDNA and photogrammetric analysis with UAV

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The environmental and socioeconomic impact of cyanobacterial harmful algal blooms are increasing on a worldwide basis. A particular species of note is *Microcystis aeruginosa*, which can produce harmful hepatotoxic microcystins and dominate freshwater ecosystems. Effectively predicting, detecting and monitoring CHABs is crucial to their proactive management and low cost, efficacious remediation. However, traditional approaches in remote sensing are limited in cost-effectiveness, temporal specificity and spatial resolution. Current predictive models of CHABs are often whole-lake and based off summative nutrient loading, and thus difficult to apply to targeted early remediation efforts. There has also yet to be a consensus on methodology for the detection of *Microcystis* and microcystins. Recent advances in consumer grade unmanned aerial vehicles (UAV) have made them viable tools for scientific research. Their application in photogrammetry and water sampling makes detecting, tracking and quantifying smaller, early CHABs or CHABs on an ad hoc basis in smaller waterbodies an unprecedented possibility. Similarly, UAV water sampling requires much lighter equipment and less training than using a boat, causes less water disturbance and mixing, and can be faster. Acquired water samples can then be tested for water chemistry and the presence of potentially toxigenic *Microcystis* through eDNA qPCR, offering the ability to detect early blooms that are under the threshold for photogrammetric detection. Through the use of UAV, we propose to investigate the topographic and location specific water physiochemical correlates of early CHAB development in small freshwater lakes in south-eastern Ontario.

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Effects of road salt application on sedimentary Cladocera assemblages in shallow softwater lakes in the Muskoka River Watershed, Ontario

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To promote safe road conditions, road salt is commonly used as a de-icing agent for winter road maintenance. Of the several compounds that can be used for this purpose, sodium chloride (NaCl) is the most commonly used in Canada. In North America, the use of road salt as a de-icing agent began in 1940 and was in widespread use by the 1950s. While it is undeniable that road salt reduces road accidents it may also have adverse effects on surface water. Because of their conservative nature and relatively high solubility, almost all chloride ions from road salts eventually find their way into waterways downstream. Jevins Lake, located in the Muskoka River Watershed and within sight of a major twinned highway, has the highest recorded chloride concentration of lakes in the Muskoka region of south-central Ontario 90.1 mg/L). To assess whether biological changes have occurred in this “worst-case” lake, coincident with historical road salt applications, high-resolution cladoceran records from ²¹⁰Pb-dated sediment cores were examined from the lake. Biological changes in Jevins Lake, including a notable shift in PCA Axis 1 scores of cladoceran assemblages in the late 1950s, suggesting that road salt application may have been a trigger for recent changes. Changes in zooplankton and Cladocera abundances can induce major changes in the trophic structure of aquatic ecosystems, potentially leading to increases in HABs.

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A paleolimnological assessment of anthropogenic impact on the lakes of Algonquin Provincial Park

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Since first leasing the land for cottage use in 1905, there has been considerable shoreline development on certain lakes within Algonquin Provincial Park, a semi-protected park in south-central Ontario. It is possible that the cottages (and especially the associated septic systems) have been a source of nutrients into the lakes. This may subsequently lead to nuisance and harmful algal blooms via eutrophication. To determine whether lakes in Algonquin Park have been impacted by the presence of cottages requires an understanding of pre-disturbance conditions. However, long-term, continuous records of historical water quality data such as nutrient concentrations do not exist for these lakes. To address this question, we will use paleoindicators preserved in ²¹⁰Pb-dated lake sediment cores including diatoms to reconstruct past lake water conditions, as well as sedimentary chlorophyll a concentration to estimate trends in primary production. Diatoms are abundant and preserve well in most lake sediments and are particularly strong indicators of lake water nutrients and climate-induced water column changes. Cores were collected from the deepest portion of five Algonquin Park lakes; three are cottage lakes, and two are uninhabited (reference) lakes. We aim to compare changes in diatom assemblage composition and spectrally-inferred chlorophyll a concentration in cottage and reference lakes over the past ~200 years to determine whether anthropogenic activities and recent climate change have affected these lakes. The results of this study can help lake managers and Park authorities make informed policies, and we will be able to support or suggest modifications to current legislation surrounding continued commercial leasing.

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Exploring Implementation of Lake Simcoe's Integrated Watershed Management Model in Callander Bay and Eagle Lake

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Cyanobacteria blooms and related water quality issues have emerged as significant threats to Ontario inland lakes. Ontario endorses the use of an integrated watershed management (IWM) approach. IWM is a complex approach, which is often limited by local capacities. There is a dearth of literature investigating local capacities to implement IWM successfully. The Lake Simcoe region employs a holistic ecosystem approach with an emphasis on IWM, which is supported by a legal framework provided through the Lake Simcoe Protection Act (LSPA). This IWM governance structure provides a basis for exploring how an IWM approach can be implemented in other regions. Callander Bay and Eagle Lake represent regions with varying local capacities for IWM. This research aims to investigate the capacity of regional authorities, using Callander Bay and Eagle Lake as case studies. Specifically, to ask whether Lake Simcoe's IWM model can be used without modification in smaller watersheds? If not, how should it be modified? A review of local capacity to implement IWM will be conducted in each region as part of a gap analysis, exploring how varying local conditions impact IWM implementation. Interviews with key stakeholders will assist in contextual analysis. For each region, data requirements for IWM implementation will be explored, and suggestions for successful adaptation of elements of the Lake Simcoe model will be made. This research will assist in addressing the capability of regional authorities in implementing IWM, something that has been particularly difficult in regions with limited resources. Additionally, it will work towards developing minimum data and capacity requirements for successful adaptation.

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