

A microscopic image showing several long, parallel chains of green, oval-shaped algal cells. The chains are arranged in a somewhat haphazard manner, with some appearing more densely packed than others. The background is a light, slightly grainy white.

# Harmful Algal Blooms Conference

## Research Summary 2024

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## Introduction

The Great Lakes HABs Collaborative seeks to improve communication among scientists, and between scientists and decision-makers, on issues related to harmful algal blooms (HABs) in the Great Lakes. As part of this work, the collaborative organizes sessions at research conferences across the region. In 2024, the collaborative helped to facilitate sessions at two conferences related to freshwater research.

The International Association for Great Lakes Research (IAGLR) conference on Great Lakes research held its 67th annual meeting between May 20-24, 2024 in Windsor, Ontario. The HABs Collaborative facilitated the session titled “Great Lakes HABs: One Water, One Health, Many Questions,” chaired by Mary Anne Evans (U.S. Geological Survey), Chris Winslow (Ohio Sea Grant), and Nicole Zacharda (Great Lakes Commission). Nine presentations covered a diversity of topics across many research locations and four additional presentations from other HAB-related sessions were also shared earlier during the week. Presenters shared findings using genomic characteristics to trace bloom-forming species throughout the Laurentian Great Lakes and comparing HABs species between Lake Erie to Lake Victoria in Kenya. Other talks examined the socioeconomic consequences of HABs in Lake Victoria, compared the nutrient loading between tributaries in southern Ontario, and examined several factors of bloom formation in coastal Lake Superior. Other speakers presented on cyano-induced lake basification, innovative HABs mitigation techniques, and an ArcGIS StoryMap highlighting the effects of the benthic HAB *Microseira wollei*.

The Association for the Sciences of Limnology and Oceanography (ASLO) held its annual meeting between June 2-7, 2024 in Madison, Wisconsin. Great Lakes HABs Collaborative representative Mary Anne Evans (U.S. Geological Survey) and two partners, Elena Litchman (Carnegie Institution for Science) and Rebecca Gorney (USGS – New York Water Science Center), facilitated a session called “cHABs as a Response to Ecosystem Disturbance” arising from a September 2023 workshop on disturbance ecology co-hosted by the collaborative, the Cooperative Institute for Great Lakes Research, and the Ohio State University. Throughout this all-day session, 16 presenters shared their work on the interplay between HABs and disturbances to lake ecosystems. Additionally, three posters were shared under this session during the conference. The diversity of topics included HABs in oligotrophic lakes, HAB response to nutrient and temperature increase, reconstructing HABs historical communities, HABs response to specific environmental factors, HABs vertical migration within lakes, and benthic algae response to surface HABs.

The remainder of this document serves to share the 32 abstracts and contact information from these research sessions, as modified for a broader audience (including non-scientists). More information on the Great Lakes HABs Collaborative and a map of existing HABs-related research in the region, including much of the work discussed below, are available at <https://www.glc.org/work/habs>.

# IAGLR 2024: Great Lakes HABs:

## One Water, One Health, Many Questions

Chairs: Mary Anne Evans, Chris Winslow, Nicole Zacharda

### Comparing Cyanobacterial Biosynthetic Potential Between Winam Gulf, Lake Victoria, Kenya and Western Lake Erie, USA

Cyanobacterial harmful algal blooms (cHABs) are a growing global threat to potable water, human health, and wildlife. Excess runoff of nutrients fuels the overgrowth of cyanobacteria which can produce deadly cyanotoxins. *Dolichospermum* and *Microcystis* are two types of cHABs which dominate blooms in the Western Lake Erie Basin, USA (WLEB) and the Winam Gulf of Lake Victoria, Kenya. These shallow basins are similar in their bathymetry, proximity to human populations, and human-caused inputs of nitrogen and phosphorus, fueling dangerous cHABs. Here, we use genetic analysis to identify the genetic similarity of the main cyanobacterial bloom-formers in these two basins over time and space. We identified similar genetic trends across the two basins, displaying a diverse and potentially hazardous array of specialized biologically produced compounds. Additionally, evolutionarily based historical analyses indicate genetic similarity between representative *Microcystis* and *Dolichospermum* species in the two great lakes. These results are vital and the genetic insights into cHABs in the Winam Gulf can provide comparisons to a hydrologically similar yet distinct basin, WLEB. Furthermore, the Winam Gulf serves as a unique glimpse into how rising global temperatures may alter cHAB communities in a warming (temperate) WLEB. Future work is needed to better understand the complex dynamics of cHABs in the Winam Gulf and how they compare to cHABs around the world. Such global comparisons will improve understanding of ecological and genetic aspects of cHABs enabling better predictions and management.

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## **Socioeconomic consequences of cyanobacteria harmful algal blooms in small-scale fishing communities of Winam Gulf, Lake Victoria**

Lake Victoria has experienced progressive eutrophication which has increased the occurrence of cyanobacterial harmful algal blooms (cHABs). Fueled by human-caused nutrient loadings and climate change, these cHABs are increasing in distribution, duration, and frequency throughout the lake, particularly in areas such as the Winam Gulf. With limited resources and infrastructure, local communities have been left vulnerable as they rely on the lake for water, food, and income. Our study presents the results of a localized survey on how small-scale fishing communities perceive and respond to the threat of cHABs in the Winam Gulf of Lake Victoria, Kenya using a mixed-methods approach. Results demonstrate most (93.67%) respondents were aware of cHABs in the lake but were not knowledgeable of cHAB threats to human and animal health. Respondents noted cHABs decreased fish catches, with this economic consequence serving as a primary concern of communities. Notably, respondents altered their use of lake water during perceived cHAB events and relied on various means of water treatment or alternative water sources. Overall, cHAB information was self-sourced or passed on from community elders, with no public mechanism for adequate cHAB risk communication. Lake Victoria serves as a critical resource to the Eastern African continent and requires a concerted cHAB response effort. Therefore, we recommend the development of a public awareness program for risk and hazard communication to reduce cHAB exposure in these at-risk communities.

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## **A Comparison of Nutrient Load Estimates between the Leamington Tributaries, Thames River, and Sydenham River (2018-2022)**

Harmful algal blooms are an annual occurrence in Lake St. Clair and the western basin of Lake Erie. In 2018, Domestic Action Plans were released to aid in the 40% reduction of phosphorus to Lake Erie, focusing on priority watersheds including the Leamington Tributaries. These relatively small watersheds in the municipalities of Leamington and Kingsville (Essex County, Ontario) drain into Lake Erie's western basin, and are heavily influenced by greenhouse agriculture. Previous studies showed that total phosphorus (TP) concentrations in this area were typical of similar agricultural streams prior to 1996. Following rapid growth of the greenhouse sector, TP concentrations have been substantially and consistently higher than nearby agricultural streams. In this study, TP loads and concentrations for the Leamington Tributaries were assessed from 2018 to 2022. Loading estimates were also compared to the other priority watersheds (Thames River and Sydenham River) to understand the Leamington Tributaries' TP contribution to Lake Erie. Updated TP loads will be presented for the Thames River, Sydenham River, and Leamington Tributaries based on calculations from Environment and Climate Change Canada's Erie Loading Tool . Unique challenges that face all

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three watersheds will be addressed, highlighting efforts made by local conservation groups and stakeholders to reduce phosphorus. Importantly, this work is a collaborative effort between local Conservation Authorities, and Federal and Provincial scientists.

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## Linking tributary nutrients, sediment, and cyanobacteria to Lake Superior nearshore algal blooms

Lake Superior, the most oligotrophic, or low nutrient, of the Laurentian Great Lakes, has experienced repeated nearshore cyanobacterial blooms along its southwestern shore over the past decade. Previous studies suggest that storm-driven fluxes of nutrients, sediment, and cyanobacteria may play an important role in driving these blooms. To investigate further, the U.S. Geological Survey participated in a large multi-institution partnership under the Collaborative Science and Monitoring Initiative. We focused on two key tributaries to south-shore Lake Superior, the Bois Brule and Siskiwit Rivers, and collected streamflow, discrete and continuous water quality, sediment-nutrient cycling, and microbial genetics data. Relationships between streamflow and concentrations of suspended sediment, total phosphorus, and total nitrogen were evaluated using regression models. Statistical analyses confirmed that these parameters increase with increasing streamflow, and also indicated that concentrations of total phosphorus increase with suspended sediment, suggesting that suspended sediment may serve as a vehicle for phosphorus transport from tributaries to the nearshore. Furthermore, sediments collected from the water column and streambed were analyzed for their potential to bind and release phosphorus. Results showed variability across years, upstream to downstream, and between tributaries, and suggested that sediment may serve to hold onto phosphorus from the water column within the stream environment but may have the potential to release this phosphorus downstream in the nearshore. Evaluation of microbial community dynamics also indicated changes in the communities present over time, which may be linked to nutrient availability.

[Anna Baker (abaker@usgs.gov)<sup>1</sup>, Rebecca Kreiling<sup>2</sup>, Carrie Givens<sup>1</sup>, Richard Kiesling<sup>1</sup>, Eric Dantoin<sup>1</sup>, Patrik Perner<sup>1</sup>, Shelby Sterner<sup>1</sup>, Krimson Anderson<sup>1</sup> and Gerald Storey<sup>1</sup>, <sup>1</sup>U.S. Geological Survey Upper Midwest Water Science Center, <sup>2</sup>U.S. Geological Survey Upper Midwest Environmental Sciences Center.]

## The genome characteristics and distribution of *Dolichospermum circinale* in the Great Lakes

Harmful cyanobacterial blooms have and continue to be a serious ecological and human health concern in the Great Lakes region. While the cyanobacterial communities of such blooms are often dominated by *Microcystis*, particularly in Lake Erie, there are notable exceptions. In November 2022 a bloom of *Dolichospermum* covered a substantial portion of Western Lake Erie. Here, we describe

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the genetic and evolutionary characteristics of this species, identified as *Dolichospermum circinale*, along with those of another related species, identified as *Dolichospermum heterosporum*, which was sampled from a cyanobacterial bloom in Lake Erie in 2011. Genetic analysis results characterize both species' abilities to produce biological compounds; *Dolichospermum heterosporum* was found to produce the metabolite anabaenopeptin, which is similar in structure and function to the toxin microcystin. Using the Great Lakes Atlas of Multi Omics Research (GLAMR) as a genetic database, we also characterize the abundance and distribution of both taxa across the Great Lakes. Collectively, these data provide valuable insight regarding the potential of bloom-forming members of this genus to dominate the cyanobacterial communities of the Great Lakes.

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## Genomic insights into bloom-forming Nostocales taxa in the Lake Erie watershed

Recent changes in cyanobacterial bloom composition in waterbodies within the Lake Erie watershed have seen filamentous cyanobacteria associated with nitrogen fixation becoming major bloom-forming taxa. We explore two case studies in northern Ohio: (1) early summer *Aphanizomenon* blooms have occurred since 2019, following the recent disappearance of the perennial *Planktothrix* bloom in Sandusky Bay (Lake Erie); (2) late summer mixed algal blooms with moderate microcystin and saxitoxin concentrations in a small lake. From bloom events, we have obtained cyanobacterial samples and undertaken genetic analysis to gain greater understandings into the ecology, biological processes, and toxin production potentials of the cyanobacterial taxa.

[Christopher S. Ward (chrward@bgsu.edu), Katelyn M. Brown, Ryan S. Wagner and George S. Bullerjahn, Bowling Green State.]

## The Other Algal Bloom Problem: Elucidating Effects of Cyanobacterial Induced "Lake Basification" on *Microcystis* spp.

Cyanobacterial Harmful Algal Blooms (cHABs) are well-known for their prolific biomass accumulation and cyanotoxin production. Research has revealed cHAB ecological success is spurred by factors including anthropogenic nutrient loading and elevated temperatures. Yet, one factor that has remained absent from the discussion is their ability to manipulate water column pH (i.e., by rapidly consuming CO<sub>2</sub> (aq)). Elevated pH conditions have been widely recorded - yet seldomly investigated - in the literature. While we now know cHABs drive water column pH from 7.7-8.2 to 9.2-10 in various freshwater systems, we know little concerning how this affects the cyanobacteria and their competitors. Here, we investigated how alkaline pH levels alter transcription patterns and physiology of cyanobacteria (*Microcystis aeruginosa*) in continuous

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laboratory cultures. Chemostats were maintained at pH 7.75 to establish baseline physiology then shifted to pH 9.25. To this end, we were able to monitor both the transition to higher pH conditions as well as the new “steady state” physiology of the cells. In conjunction with our previous efforts, our work has demonstrated how *Microcystis* driven changes in pH provide a competitive advantage that enables cyanobacteria to exclude competitors (e.g., diatoms) while serving as a potential positive feedback loop for these cyanobacteria during late-stage bloom maintenance. Going forward, understanding the shifts in both biology and chemistry that occur during these contrasting conditions is necessary in the development of management strategies for cHABs.

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## **Distribution, Environmental Drivers and Risks of *Microseira wollei***

Noxious growths of the cyanobacterium, *Microseira wollei* have recently increased within the western portion of Lake St. Clair, MI. However, there are uncertainties related to historical spatial distribution of *M. wollei* within Lake St. Clair and throughout North America, environmental drivers stimulating blooms, and potential human and ecological health risks. Therefore, to address these uncertainties, a strategic literature review was performed, and information gained from this review will inform future management. Occurrences of *M. wollei* in North America have been reported from Canada to Florida, with anecdotal observations fitting the general description of *M. wollei* within Lake St. Clair dating back 30 years. Based on laboratory and field data, the environmental tolerances of *M. wollei* are relatively broad for pH, conductivity, light intensity, temperature, and nutrient concentration. Environmental drivers may be site-specific and specific to the *M. wollei* population. Human health risks from *M. wollei* may originate from toxins and disinfection byproducts. *M. wollei* has the potential to produce a range of toxins; however, the most prevalent toxins are saxitoxins, a group of neurotoxins. The potential human health risks associated with toxin production of *M. wollei* highlight the need for adaptive management.

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## **Mitigating cyanobacterial harmful algal blooms: water soluble chitosan derivative and clay-based coagulation and flocculation treatment**

Cyanobacterial harmful algal blooms (cHABs) can lead to concerns regarding water quality, treatment, and public health. Addressing cHABs requires targeted removal at their source to prevent complication in drinking water treatment. Particularly, in-lake treatment methods require

environmentally friendly chemicals/materials. Coagulation and flocculation are commonly employed due to their ability to remove whole cells while minimizing the release of cyanotoxins. Although chitosan-assisted coagulation methods hold promise, its solubility limitations at higher pH values pose challenges in cHAB-prone lakes with pH values above 7. Within this context, this project focuses on addressing cHABs through a treatment approach using a water-soluble chitosan derivative, hydroxyl trimethyl ammonium chitosan chloride (HTCC), in combination with bentonite clay. HTCC functions as a coagulant while clay acts as a flocculant, augmenting the size and strength of flocs, or larger particles. This innovative treatment yields buoyant floc that rises to the water surface after a short settling period. These flocs, laden with cyanobacteria, can then be skimmed off the water surface and removed. This study investigates the interactions between HTCC, clay, and cyanobacteria, proposing a treatment strategy aimed at removing cyanobacteria cells from cHABs-prone lakes and reservoirs.

[Katelin Weitzel (weitzeka@mail.uc.edu) and Soryong Chae, University of Cincinnati.]

## IAGLR 2024: Cyanotoxins and Off-Flavors in Freshwater: Synthesis Mechanism to Toxicology Assessment

Chairs: Xuexiu Chang, Arthur Zastepa, Lin Li

### Bacterial community and cyanotoxin gene distribution of the Winam Gulf, Lake Victoria, Kenya

The Winam Gulf of Lake Victoria, Kenya is frequently impaired by cyanobacterial harmful algal blooms (cHABs) due to inadequate wastewater treatment and excess agricultural nutrient input. Our aim of this project is to identify potential toxin-producing cyanobacteria using molecular approaches. The Gulf was sampled over two successive summer seasons, and rRNA gene sequencing was performed. Additionally, key genes involved in production of cyanotoxins were examined by quantitative PCR. On the eastern side of the Gulf, samples exhibited elevated *cyrA* gene abundances, indicating genetic capability of cylindrospermopsin toxin synthesis. Indeed, near the Nyando River mouth in 2022, high *cyrA* detections correlated to high *Cylindrospermopsis* spp. cell counts. In contrast, the southwestern region had elevated microcystin synthesis gene detections near Homa Bay where a small community of *Microcystis* spp. was observed. In this location, a strain of *Microcystis panniformis* was isolated and the whole genome was sequenced. This resulted in a gene analysis that correlates with frequent detections of microcystins in the Gulf. These findings show that within a relatively small embayment, composition and toxin synthesis potential of cHABs can vary dramatically. This underscores the need for multifaceted management approaches and frequent cyanotoxin monitoring to reduce human health impacts.



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## **A New Dominant *Microcystis* in Dianchi Lake (China) Disrupts Food Intake in Fish by Regulating Neurotransmitters and Hormones**

Cyanobacterial harmful algal blooms (cHABs) are pervasive sources of stress resulting in neurotoxicity for fish. *Microcystis wesenbergii* has become a new dominant bloom-forming species of cyanobacteria found in many freshwater lakes, including Dianchi Lake (China). However, unlike its more well-known counterpart *M. aeruginosa*, the effects of dense *M. wesenbergii* blooms are seldom studied. The disturbance of appetite and feeding behaviour can have downstream effects on the growth of teleost fish, posing a significant challenge to aquaculture and conservation efforts. This study examined the effects of *M. wesenbergii* blooms on the food intake of *Acrossocheilus yunnanensis*, a native fish cyprinid in southern China. This fish has disappeared in Dianchi Lake, and its reintroduction might be negatively affected by the presence of this new dominant *Microcystis* species. We co-cultured juvenile *A. yunnanensis* with *M. wesenbergii* at initial densities ranging from 5×10<sup>4</sup> to 1×10<sup>6</sup> cells/mL and monitored feeding behaviors, and protein levels of neurotransmitters and hormones. High- density *M. wesenbergii* cultures increased the feeding rate of co-cultured fish, elevating concentrations of appetite-stimulating signaling molecules (Agouti-related protein and γ-aminobutyric acid), while decreasing inhibitory ones (leptin). These changes coincided with histopathological alterations and reduced somatic indices in brain and intestinal tissues. Given this potential for detrimental effects and dysregulation of food intake, further studies are necessary to determine the impacts of chronic exposure of *M. wesenbergii* in fish.

[Wenyu Long<sup>1</sup>, Keira Harshaw<sup>2</sup>, Yunfeng Wang<sup>3</sup>, Qianqian Xiang<sup>1</sup>, Hugh MacIsaac<sup>2</sup>, Minmin Niu<sup>1</sup>, Qiwen Xi<sup>3</sup> and Xuexiu Chang (xchang@uwindsor.ca)<sup>2</sup>, <sup>1</sup>Yunnan Collaborative Innovation Center for Plateau Lake Ecology and Environmental Health, College of Agronomy and Life Sciences, Kunming University, <sup>2</sup>Great Lakes Institute for Environmental Research, University of Windsor, <sup>3</sup>Fishery Technology Extension Station of Yunnan]

## **Response of an endangered macrophytes to co-occurring cyanobacterial bloom and invasive fish**

Submerged microphytes play a crucial role in aquatic ecosystems, facing challenges from cyanobacterial harmful algal blooms (cHABs) and invasive fish. *Microcystis aeruginosa* is a common dominant species of cHABs, while *Pseudorasbora parva* is classified as an invasive alien fish. These two stressors coexist in Dianchi Lake (China), which was once dominated by the native macrophyte *Ottelia acuminata*. However, *O. acuminata* disappeared from the lake decades ago and

is now classified as an endangered plant. To understand how these dual stressors affect the reintroduction of *O. acuminata* to Dianchi Lake, we conducted mesocosm experiments, co-culturing *O. acuminata* with *M. aeruginosa* and *P. parva*. We found that: 1) both individual and combined exposures to *M. aeruginosa* and *P. parva* significantly reduced the biomass and chlorophyll content of *O. acuminata*, with the combined stress causing more pronounced inhibition and greater damage than individual stress; 2) *M. aeruginosa* and *P. parva*, individually or in combination, significantly increased the levels of soluble sugars, tannins, and total phenols in *O. acuminata*, with markedly higher content under combined stress; 3) *M. aeruginosa* treatment elevated the flavonoid content in *O. acuminata*, which was further intensified by the co-occurrence of *P. parva*, although no significant change was observed under *P. parva* alone. These findings contribute valuable knowledge for the protection of native plants in lakes under multiple stressors.

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## IAGLR 2024: The Role of Microbial Processes in the Assessment of the Great Lakes Ecosystem Health

Chairs: Sophie Crevecoeur, Thomas Reid

### Comparison of HAB metatranscriptomes in Sandusky Bay, Lake Erie from 2018 and 2019

Harmful algal blooms have plagued Sandusky Bay on Lake Erie since the early 2000's. *Planktothrix agardhii* is usually the dominant cHABs species. The bay averages a depth of 2m and has high rates of sedimentary denitrification that leads to nitrogen limitation much of the summer. Microcystin toxin concentrations have on occasion exceeded the recreational contact guidelines. Sampling was conducted in the bay every two weeks from June to September in 2018 and 2019. DNA was extracted from these sites and sequenced for genes to identify the taxonomy of the bacterial community. The analysis revealed a *Planktothrix* bloom in 2018, but there was a more diverse cyanobacterial community in 2019. RNA was extracted from separate filters, genetically sequenced, and then compared for nitrogen cycling and other pathways. *Planktothrix* was predominant in 2018. In early June of 2019 *Planktothrix* showed high expression rates, but *Anabaena* and *Microcystis* typically had a greater expression than *Planktothrix* for these pathways in July, August and September.

[Michelle Neudeck (mneudec@bgsu.edu)<sup>1,2</sup> and George Bullerjahn<sup>1,2</sup>, <sup>1</sup>Bowling Green State University, <sup>2</sup>Great Lakes Center for Fresh Waters and Human Health]

# ASLO 2024: cHABS as a Response to Ecosystem Disturbance

Chairs: Mary Anne Evans, Elena Lichtman, Rebecca Gorney

## Evaluating the Interactive Effects of Dissolved Organic Matter and Nutrients on Cyanobacteria and their Toxins

Terrestrially derived dissolved organic matter (tDOM) enters lakes along with sediments and inorganic nutrients through runoff and tributaries draining wetlands and agricultural fields, contributing to eutrophication and potentially leading to Harmful Algal Blooms of cyanobacteria (cHABs). Although tDOM is a key regulator of nutrient and light regimes, its role in cyanoHAB development and toxin production is unclear. In this study, we augmented natural water with manure-derived tDOM and inorganic nutrients in large outdoor tanks for eight weeks.

Cyanobacteria and heterotrophic bacteria communities were profiled via genetic analysis. Cyanobacteria toxin concentration was quantified via laboratory analysis. The addition of manure-derived tDOM altered both microbial community structure and toxin production. Cyanobacteria communities were largely dominated by *Planktothrix spp.* in tDOM treated tanks regardless of nutrients, while those not amended with tDOM were more diverse and shifted toward non-toxic algal species. The ratio of toxin concentration to algal mass was also higher in tDOM-amended tanks, regardless of nutrient additions. These results indicate that DOM can contribute to cHABs and toxin production. Our results demonstrate the need to incorporate tDOM more fully into cyanoHAB management.

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## Observations of Benthic Cyanobacteria and Toxicity from New York State to New South Wales

Benthic (or bottom forming) cyanobacteria are an understudied yet important component to cyanobacterial harmful algal blooms (cHABs) research; they can form toxic mats that cover bottom substrate and are often found in waterbodies thought to be unideal for HAB development (e.g., river systems). Because cHAB research has been centered around planktonic cyanobacteria, we lack confidence and data on the conditions that promote benthic blooms. We surveyed nearshore zones in the Lake Ontario watershed (2022 and 2023) and a stream in New South Wales (NSW), Australia (2023) for benthic cyanobacteria and cyanotoxins. Populations of benthic cyanobacteria were found in the three Finger Lakes studied and in Lake Ontario embayments, but they were not found along either Lake Ontario shoreline that we surveyed. Cyanotoxins were detected in 46% of samples analyzed, with no detections from Lake Ontario. Our findings in NSW suggest that toxic

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benthic cyanobacteria are more widespread than previously thought. Here, we present our observations in New York State lakes and NSW in relation to their environmental conditions including water temperature, nutrient availability, and light intensity, and suggest future work involving the same suspected novel toxin producer found in these two locations under markedly dissimilar conditions. Upcoming steps include a genetic analysis approach to identify toxin gene clusters. This work contributes knowledge to an understudied but increasing field of research and will highlight a case of a suspected novel toxin producer in two geographically distinct populations.

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## **Temporal and Spatial Dynamics of Harmful Algal Bloom-Associated Microbial Communities in Eutrophic Clear Lake, California**

Clear Lake is a large, natural eutrophic (high nutrient) lake located in northern California, USA that serves as an important source of water for drinking, irrigation, recreation, cultural practices of indigenous people and aquatic wildlife diversity. Persistent, toxic cyanobacterial blooms have been reported in the lake since 2011, particularly during the summer and fall seasons. Despite numerous traditional biological surveys of Clear Lake, a thorough molecular investigation of the microbial community is still lacking. The specific taxa that are the cause of toxins (predominantly microcystins but also anatoxin-a) observed in the lake are not presently known, nor is the effect that these species and/or cyanotoxins have on the species richness and community composition of the co-occurring microbial assemblages. Similarly, the relative importance of biotic and abiotic factors in controlling microbial community structure and cyanoHAB occurrence patterns is not fully understood. We employed multi domain (16S and 18S) rRNA amplicon sequencing to 1) provide extensive characterization of the species richness and community composition of the planktonic bacterial and microbial eukaryotic community of the lake in the presence and absence of toxic cyanobacterial blooms; 2) investigate the spatiotemporal dynamics of the community; and 3) evaluate the influence of environmental factors and cyano-HAB proliferation on microbial community structure.

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## **Cyanobacterial Blooms in an Oligotrophic Lake: Manifestation of Not-So-Visible Multi-Decadal Ecosystem Changes**

Freshwater cyanobacterial blooms are often associated with eutrophy (high nutrients) and high water temperature, but lakes that are considered oligotrophic (low nutrient) by all three major indices (chlorophyll a, total phosphorus, and Secchi depth) have been affected by recurrent cyanobacterial blooms, including when the water temperature is < 15°C (59°F). Otsego Lake, NY, USA experienced recurrent blooms of toxigenic *Microcystis aeruginosa* for the first time in the summer of 2022, which took the local community by surprise while the lake had continuously been



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invaded by a variety of non-indigenous aquatic species. The effort to control further introductions had not been fully supported financially or in terms of lake user compliance. The most serious example is the serial invasions by zebra (2007) and quagga (2018) mussels, both of which quickly colonized the entire 16 km<sup>2</sup> (4046 acres) lake. The altered food web structure and biogeochemical cycling as well as the unbalanced in-lake nitrogen to phosphorus (N:P) ratios after successful P load reduction appear to be the main drivers of cHABs that may be amplifying the effects of climate warming, reduced ice cover, and altered precipitation patterns. The Otsego Lake community is working towards a New York State Department of Environmental Conservation's Nine Element Plan, a comprehensive lake and watershed management plan that includes both watershed and in-lake modeling, which will enable data-driven decisions for watershed management.

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## **Revisiting the Drivers of Eutrophication and Cyanobacteria Blooms in Lake Champlain's Northeast Arm**

Lake Champlain has been the focus of extensive research and investment in recent decades, as the states and provinces within the catchment have attempted to protect and improve water quality in the lake. In much of the lake, phosphorus concentrations remain stable. A notable exception is the large "Inland Sea" basin in the lake's Northeast Arm, where phosphorus concentrations are significantly increasing and there are indications that there may be a regime shift occurring in the magnitude of seasonal internal loading. Using more than 30 years of consistent monitoring data coupled with several watershed and lake modeling approaches, we investigate lake water quality in the context of changing internal loads, spatially variable influence of external nutrient loads from several tributaries, and physical changes to the lake thermal structure resulting from climate patterns. Finally, we address the extent to which internal loading in the Inland Sea may contribute to chronic, severe cyanobacteria blooms in an adjacent shallow, eutrophic (high nutrient) bay.

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## **Cyanobacteria Harmful Algal Blooms in Low-Nutrient New York Lakes: Combined Approaches to Address an Ephemeral Phenomenon**

In response to increases in cyanobacterial harmful algal blooms (cyanoHABs) in lakes with relatively low nutrient concentrations, the US Geological Survey (USGS) has developed research projects in two areas of New York State (NY). In the Finger Lakes region, USGS conducted a pilot study in collaboration with the state Department of Environmental Conservation to assess traditional and innovative monitoring approaches. The goals were to inform future monitoring strategies and increase the understanding of factors related to the formation and proliferation of cyanoHABs. Monitoring methods included data collection platforms in open water, nearshore mapping surveys, passive samplers, multi-channel fluorescence sensors, imaging flow cytometry, and hyperspectral

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imagery analysis. USGS research projects being implemented within the Adirondack Park region are using water- and sediment-based methods appropriate to the challenges of capturing episodic cyanoHABs. These include measurement of cyanotoxin synthetase genes and paleolimnological approaches to investigate historical patterns. Results indicate that cyanoHAB detection in low nutrient systems is challenging. However, several lines of evidence converged to help inform observed patterns at the lake surface and within the water column within both regions. For example, two Finger Lake projects indicated the role of the thermocline in cyanotoxin dispersion through the water column. Findings across our NY-focused studies provide tools for managers and lake users to understand drivers and patterns of cyanoHABs in low nutrient systems.

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## **Vertical Distribution and Migration of Cyanobacteria and Cyanotoxins Across Diel to Seasonal Timescales in Lake Mendota**

Cyanobacteria, commonly known as “blue-green algae,” are a phylum of bacteria that produce toxic or otherwise bioactive peptides that can affect the brain, liver, and skin of humans and animals. Rapid growth of Cyanobacteria in water bodies—known as “blooms”—affect the health and recreation of many nutrient-rich lakes, including Lake Mendota (Madison, WI). Freshwater Cyanobacteria are known to grow and migrate throughout the water column to access varying light and nutrient conditions, but perceptions of blooms are often limited to lake surface observations. Many previous studies lack investigation of potential vertical heterogeneity of both Cyanobacteria and the cyanotoxins they produce, posing a challenge to water quality managers and health officials assessing the extent of toxic threat from cyanotoxins in freshwater lakes. In this study, we assess the vertical distribution and migration of Cyanobacteria and cyanotoxins in Lake Mendota on both a diel and seasonal time scale throughout the cyanobacterial growth season (May–Nov) 2021. To assess diel vertical migrations of the cyanobacterial community, we designed and constructed an in situ automated phycocyanin fluorescence water column profiler. We observed consistent yet taxonomically distinct diel migrations of Cyanobacteria throughout the epilimnion, as well as unique patterns in vertical distribution of cyanobacterial taxa across the growth season, observed via 16S rRNA gene amplicon sequencing. We also analyzed the concentration of 19 cyanotoxins using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) from weekly depth-discrete samples and across two 24-hour diel studies. We observed high seasonality of epilimnion cyanotoxins and high variability in concentration between layers of the stratified lake. This study provides insights into potential coupled strategies for light avoidance, nutrient access, and toxin production throughout the water column. The combination of vertical measurements across time scales of hours to seasons provides a detailed characterization of the distribution and migration of cyanobacterial taxa and cyanotoxins throughout the water column.

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## Making the Watershed Connection: The Influence of Cyanobacteria, Sediment, and Nutrient Loading and Hydrology on Cyanobacterial Bloom Initiation in the Nearshore Environment

Nearshore algal blooms appearing along the southern coastline of the western arm of Lake Superior ranging from Duluth Harbor to the Apostle Islands appear to coincide with large storm events that flush sediment and nutrients from the watershed into the nearshore. The concept of “fluvial seeding” (rivers flushing cyanobacteria into lakes) has been hypothesized, but the limited available data have not always agreed on the influence of riverine cyanobacteria on open lake algal bloom initiation. Using cultivation and genetic analysis, our work assessed fluvial flow of cyanobacteria with nutrient and sediment loading from the Siskiwit and Bois Brule tributaries to the Lake Superior nearshore. Water and sediment samples were collected and added to selective cyanobacteria media. Additionally, DNA from environmental samples and cultivations were sent for genetic analysis to characterize and compare the cyanobacteria community along the tributary to nearshore range. Additional cyanobacteria genetic analysis was analyzed to determine cyanobacteria relative abundance in relation to sediment and nutrient loading. Viable cyanobacteria cells were cultivated from tributary samples and genetically similar signatures of *Cyanobium*, *Pseudanabaena*, and *Nostocaceae* species were detected from tributary environmental samples and cultivations. Additional data are needed to understand whether cyanobacteria seeding originates in the tributaries themselves, whether fluvial flow is simply transporting these cells to the nearshore from upstream, or if there are multiple sources seeding the nearshore.

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## Multi-Omics Approach to Identify a Microbial Biomarker for *Planktothrix*-Dominated Cyanobacterial Harmful Algal Blooms in Freshwater Lakes

The global frequency and duration of cyanobacterial blooms are increasing, posing threats to drinking water supplies, disrupting recreational activities, and disturbing ecosystems. *Planktothrix*, a filamentous cyanobacterium, is responsible for blooms worldwide and produces toxins. Water parameters such as temperature, pH, turbidity, and nitrogen and phosphorus concentrations have been reported to influence *Planktothrix* blooms. However, there is still a lack of in-depth understanding regarding key microbial players of bloom dynamics and their symbiotic and competitive interactions within *Planktothrix*-dominated bloom. Therefore, we conducted biweekly surveillance over a three-year period in Grand Lake St. Marys in Ohio, USA, a freshwater lake with historical dominance of *Planktothrix*. We analyzed physicochemical parameters, toxins, and the fungal and bacterial microbiome using genetic analysis. The integrated analysis of the genetics data revealed a potential interaction among nitrogen-related bacteria, methane-producing archaea, and algicidal fungi/bacteria, with carbon- and nitrogen-associated metabolism serving as

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connecting factors. In addition, *Rhodobacter*, a species of bacteria, emerged as a candidate indicator to predict *Planktothrix*-dominated blooms, showing a consistent negative association with *Planktothrix* as well as with a toxin-producing gene abundance. Further studies to validate functional interactions across microbial players are warranted.

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## **Phytoplankton Responses to Nutrients and Increased Temperature in Three Great Lakes Regions Susceptible to Cyanobacterial Blooms**

There is increased recognition that cyanobacterial blooms occur under diverse environmental circumstances. In project CYBORG (CYano BLOoms dRivers and Genes) labs from three universities looked at factors driving blooms in Lake Superior, Green Bay, and Lake Erie. We performed short (2-4 day) nutrient addition assays in comparable ways in the three environments, with a total of 26 such experiments performed across the three lakes over two years. Treatments included adding P, several forms of N (nitrate, ammonium, urea), and incubating under both ambient and at plus 3-degree temperatures. Measuring chlorophyll for coarse community differentiation, we found that nutrient additions sometimes disfavored green algae compared to cyanobacteria, diatoms, and cryptophytes and that warmer temperatures sometimes favored cyanobacteria at the expense of green algae. Extracted chlorophyll measurements indicated that elevated temperatures increased growth in all three lakes, but with interactions with nutrients, especially in Green Bay. Lake Superior was consistently responsive to P and possibly slightly to N whereas both lower lakes responded more variably to P and to different forms of N. Our work helps to define the diverse limiting factors operating in steering algal communities in bloom-susceptible areas in the Great Lakes. It also shows how networked experimental work can provide insights into environmental variability at a large spatial scale.

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## **Responses of Great Lakes HAB Forming Algae to Shifts in Nutrients and Temperature**

The impact of increased temperature and nutrients on harmful algal blooms (HABs) has been extensively studied in recent decades. However, less is known about how changes in temperature, nitrogen and phosphorus impact the relative abundance of specific bloom forming species in the Laurentian Great Lakes. To better understand these shifts we conducted 4 identical 28-day nutrient addition bioassays in Mawikwe Bay Lake Superior and Sandusky Bay Lake Erie during 2022 and 2023. This timeframe was selected to allow for competition to take place. Chlorophyll measurements were used to track broad changes in communities every 3 days and microscopy was used to obtain detailed community information on days 14 and 28. Microscopy analyses allowed us to track



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changes in relative abundance of dominant bloom forming species, shifts in major phytoplankton groups, and changes in dominant functional traits in each treatment. In Lake Superior the dominant bloom forming genera *Dolichospermum* was significantly more abundant in treatments with lower N:P ratios. In Lake Erie, *Microcystis* was significantly more abundant in treatments with higher N:P ratios and *Dolichospermum* more abundant in treatments with lower N:P ratios.

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## **Nutrient Disturbances and Harmful Algal Blooms in Green Bay: Implications for the North American Great Lakes**

This research investigates the role of nutrients (nitrate, ammonia, and phosphorus) in promoting harmful algal blooms (HABs) and the production of cyanotoxins in Green Bay, an estuary of Lake Michigan. We conducted bioassays in which water samples from Green Bay were spiked with nitrogen and phosphorus. The samples were analyzed for toxins using Liquid Chromatography-Tandem Mass Spectrometry. Microcystins, cyanopeptolins, and anabaenopeptins were the dominant toxins detected. The samples spiked with N-rich nutrients and at elevated temperatures showed higher concentrations of cyanotoxins than samples spiked with P-nutrients or at ambient temperature. The quantified range of cyanotoxins was between 0.3 - 1.5 µg/L for microcystins, 0.5 - 27 µg/L for anabaenopeptins, and toxin concentration showed seasonal variations. The study showed that nutrients, primarily nitrogen, and increasing temperature impacted algal growth and cyanotoxin production. The results provide some insights into the impacts of nutrient management strategies and climate change on algal blooms and toxin production.

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## **Reconstructing Historical Phytoplankton Communities Within Lake Huron Through Pigment and Genomic Biomarkers**

In recent decades, the Laurentian Great Lakes have seen an increase in harmful and nuisance algal blooms. Despite increased offshore water quality, Lake Huron has seen a sustained prevalence of cyanobacterial blooms in the Georgian and Saginaw Bays. This raises questions about the potential factors leading to these blooms and whether the bloom-forming species have changed with time. Using dated sediment cores, identification of phytoplankton communities using rDNA sequencing, photosynthetic pigments (from cyanobacteria, green algae, and diatoms), and bulk elemental analysis, we seek to reconstruct historic communities to answer these questions. Three sediment cores were collected from Huron in the North Channel, Severn Sound, and Saginaw Bay. Preliminary results show that all cores contain a mix of freshwater algae and terrestrial organic matter inputs. In more recent sediments, there is a shift towards relatively more cyanobacteria-produced pigments and a minor shift away from diatom-specific pigments, indicating that at all

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core locations, cyanobacteria have become more dominant members of the phytoplankton community. These results show that in recent years the phytoplankton community within areas of concern in Lake Huron has veered toward potentially harmful cyanobacteria populations.

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## **Pigment Biomarkers of Historic Cyanobacterial Presence in the Southwestern Lake Superior Region**

Since the first documentation in 2012, cyanobacteria blooms in Lake Superior have raised concerns regarding the health of the lake. To address whether these blooms are a new phenomenon, a series of sediment cores were collected from Siskiwit Bay on Lake Superior's Wisconsin shoreline. Elemental isotopic and molecular (algal pigments) signatures were used to reconstruct historical profiles of productivity and bloom periods. Carotenoid pigments were extracted from water samples collected during a nearby bloom to develop a pigment profile for cyanobacterial blooms in Lake Superior and tracked downcore in the Siskiwit Bay core samples. Overall algal productivity, measured via the sum of chlorophyll and carotenoid pigments, has significantly increased towards the present day. Diatom-associated pigments have been in higher concentrations than cyanobacteria pigments since 170 years before present, aligning with previous studies indicating that diatoms are currently the dominating phytoplankton species in Lake Superior. Despite diatom community dominance, peaks in cyanobacteria-associated pigments correlate with the 2012 bloom and likely an earlier bloom around 470 years before present. As the first study of sedimentary pigments within the southwestern region of Lake Superior, this work provides historical context that informs our understanding of modern-day cyanobacteria presence.

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## **Spatio-Temporal Dynamics of Cyanobacterium *Dolichospermum lemmermannii* Populations in a Bloom-Prone Region of Lake Superior**

Cyanobacterial Harmful Algal Blooms (cHABs) are increasingly common in marine and freshwater environments, including the Laurentian Great Lakes. Lake Superior has seen two large-scale cHABs (2012 and 2018) along the Wisconsin shoreline west of the Apostle Islands, caused by the cyanobacterium *Dolichospermum lemmermannii*. The drivers of bloom formation in Lake Superior are unclear, with many factors known to trigger blooms elsewhere in the Great Lakes absent in Lake Superior. Furthermore, little is known about *D. lemmermannii*'s spatial structure or phenology (seasonal patterns). Thus, we tracked the seasonal population dynamics of *D. lemmermannii* to shed light on its growth, physiology, and abundance. In 2021, we used genetic analysis to characterize patterns of *D. lemmermannii* abundance and diversity along the bloom-prone Wisconsin shore of Lake Superior through geography and time. In 2022, we performed net tows and

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direct colony counts in another localized area. No large-scale bloom event was observed during either year, though several smaller blooms were observed. *D. lemmermannii* abundances were low at nearly all sites and sampling times. Spikes in abundance occurred in July and September, particularly near Siskiwit Bay, a hotspot of bloom formation. Most striking was the seasonal turnover of *D. lemmermannii* strains, suggesting strain adaptation to specific environmental conditions. These results offer valuable and actionable insights for managers and provide a foundation for additional work to clarify drivers of bloom formation in Lake Superior.

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## The Impact of Legacy Nutrient Loading from Lake Sediments on Cyanobacteria Bloom Severity

Harmful algal blooms of cyanobacteria (cHABs) affect more fresh waterbodies now than historically experienced. The greatest driver of cHAB formation is nutrient pollution. Nutrient pollution can come from sources external to the waterbody (e.g., overland runoff, stream loading, atmospheric deposition) and internally from legacy nutrients in sediments, yet nutrient reduction plans focus only on reducing external loading. To our knowledge, there is no standard method for impact analysis of the internal load (from lake sediments). The purpose of this study is to provide a framework for testing the impact of the internal load relative to the external load on cHAB severity and identifying upstream legacy stores to target nutrient reduction. We applied this methodology in a freshwater lake and found evidence that including the internal load improves predictability in cHAB severity. The variation in cHAB severity explained by the internal load varied among years, from 0.0% to 31.8% using chlorophyll-a to indicate cHAB severity, and from 0.0% to 42.4% using cyanobacteria density to indicate cHAB severity. In addition, we identified upstream sub-watersheds with consistently higher P export per contributing area, which may be driving the accumulation of legacy nutrients in lake sediments. This framework can be applied to other waterbodies to improve nutrient pollution reduction plans and enhance waterbody recovery from impairment.

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## Individual-Level Trait Response of Cyanobacteria to Environmental Variations

Trait-based approaches can provide a mechanistic understanding of species' responses to global change, such as climate change and shifts in resource availabilities. Here, we applied a functional trait approach at the cellular level to better understand the mechanisms underlying cyanobacteria populations and communities' dynamics along environmental gradients. First, we assessed trait

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plasticity of controlled laboratory cultures of the cyanobacteria *Microcystis* spp. placed under light, nitrogen, and phosphorus limitation and elevated CO<sub>2</sub> levels. Then, we scaled up to trait variation across populations and communities in natural samples. Univariate statistical analysis showed that the cellular levels of nitrogen-rich traits decrease under nitrogen limitation and increase under light and phosphorus limitation compared to control conditions. The multivariate response revealed a low overlap in the functional space between the control and both light and nitrogen limitation. This suggests that light and nitrogen limitation act as resource reallocation filters, promoting the occupation of a new functional space while reducing the size of this new space. Using the direction of trait-responses in this functional space as fingerprint, we explore how resource availability impact cyanobacteria across cells and populations within natural communities. Overall, this study reveals a general trait pattern that can be used to assess the ecological status of lakes.

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## Physiological Profiling of Novel *Microcystis* Species Reveals Species-Specific Nutrient and Light Growth Responses

Cyanobacterial harmful algal blooms (cyanoHABs) are expected to increase in frequency and magnitude with continued high nutrient loading and global warming. *Microcystis*, an often-toxigenic cyanobacterium, is one of the most prevalent cyanoHAB types globally, inhabiting aquatic systems across broad geographic and ecological ranges which suggests an equally wide range of physiological capabilities. To date, little connection has been found between morphologically based species identity and niche. A recently reported genome-based taxonomy (genetic species identification) reveals at least 16 distinct *Microcystis* genospecies (genetic species) and resolves substantial cryptic diversity within and across the numerous *Microcystis* morphospecies (structural species). We characterized growth of different genospecies grown in monocultures under varying nutrient and light conditions to assess the ecological validity of the new genome-based taxonomy. Results revealed biologically meaningful genospecies-specific responses to varying nutrient and light conditions across the genospecies tested. These results provide corroborating support for the new genome-based taxonomy and provide both a genetic and physiological basis linking genes to environment in this important HABs type. Coupled with recently observed variation in *Microcystis* genome size and the potential dependence of *Microcystis* on its associated microbiome, this new genome-based taxonomy may be useful in better understanding the success of *Microcystis* across broad environmental conditions, as well as its seemingly unexpected success in oligotrophic (low nutrient) systems.

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## Benthic Algae Response to Surface Harmful Algal Blooms

Despite occupying different habitats, benthic (bottom growing) and pelagic (open water growing) algae depend on the same resources for growth, including light, nutrients, and optimal temperatures. Their different growth habits (planktonic/floating versus attached) allow them to access different micro-habitats, limiting the amount of competition for resources. Thus, benthic and pelagic algae may exhibit similar seasonal patterns of succession and biomass. However, pelagic algae growth may limit light availability for benthic algae. Shading from surface harmful algal blooms (HABs) may cause a decline in attached algae when blooms are dense enough to limit light to the nearshore benthic zone, reducing seasonal cooccurrence. We collected benthic and surface algae using algal tiles and surface water samples to track pelagic and attached algae species composition, mass, dry mass, and chlorophyll concentration. Our study site was Chautauqua Lake, New York, a two-basin lake with a mesotrophic (moderate nutrient) North Basin and eutrophic (high nutrient) South Basin. Physical and chemical variables, such as temperature, light, and nutrients also provided insight into successional patterns. Phytoplankton counts allowed us to evaluate cooccurrence in species composition of benthic and pelagic phytoplankton communities. Chlorophyll results indicated that in areas with minimal surface HABs, benthic algae increased throughout the season. In contrast, light transmission decreased and benthic algae remained at stable levels through the season in areas with severe HABs.

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