

Bloom and bust: Historical trends of harmful algal blooms in Muskegon Lake, Michigan, a Great Lakes estuary

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Abstract: Around the world, freshwater lakes are increasingly suffering from harmful algal blooms (HABs) as a result of anthropogenic eutrophication. Muskegon Lake (Muskegon, Michigan, USA), a drowned river mouth, Great Lakes estuary, was declared an Area of Concern by the Environmental Protection Agency in 1987 with nuisance algal blooms cited as a beneficial-use impairment. The objective of this study was to quantify changes in HAB prevalence and assemblage composition in Muskegon Lake over 14 y (2003–2016) and explore the environmental factors potentially driving the changes. We performed univariate and multivariate analyses on long-term monitoring data, including environmental and phytoplankton data, to understand their relationships and temporal variation. Using linear regressions, we assessed changes in environmental variables over time and relationships between cyanobacteria biovolume and environmental variables. We used non-metric multidimensional scaling to visualize variation in annual cyanobacteria assemblage composition and association with environmental variables. Analyses revealed that, despite generally rising water temperatures, which would be expected to increase HABs, a reduction in nutrient concentrations caused by restoration efforts has likely led to substantial decreases in HAB abundance over time. Additionally, HAB assemblage composition appears to be driven by temperature and nutrient form and amount, with *Microcystis* (Lemmermann, 1907) often dominating. HABs in freshwater bodies should be closely monitored into the future as we enter an era of uncertain climatic conditions.

Key words: cyanobacteria, harmful algal blooms, eutrophication, Muskegon Lake, Great Lakes estuary, drowned river mouth, Area of Concern

The world's freshwaters are increasingly afflicted by harmful algal blooms (HABs). These HABs are a result of anthropogenic eutrophication, which promotes excessive primary production mainly due to increased N and P loading from agricultural, urban, and industrial sources (Anderson et al. 2002, Smith 2003, Heisler et al. 2008, O'Neil et al. 2012, Paerl and Otten 2013, Glibert 2017). Nutrient influx shifts the nature of nutrient limitation in freshwater systems and benefits opportunistic or adaptive HAB-forming taxa (Anderson et al. 2002, Elser et al. 2009, Filstrup et al. 2016). Although eutrophication appears to be the main catalyst of HAB formation, blooms occur as a result of a complex process involving a number of interacting variables (Heisler et al.

2008, Le Moal et al. 2019). Ecologically, HABs impair systems by reaching high biomass, which reduces photic-zone depth and induces hypoxia (<2 mg/L dissolved oxygen; Sweerts et al. 1991, Scavia et al. 2014, Le Moal et al. 2019). Additionally, many taxa are resistant to grazing and constrain energy transfer through trophic levels (Dickman et al. 2008, Ger et al. 2016). Of major concern is the toxin production of many taxa, causing mortality and illness in wildlife and humans, unsafe conditions for swimming, and contaminated drinking water (Backer et al. 2015, Carmichael and Boyer 2016, Chaffin et al. 2019). Economically, HABs affect the recreational and aesthetic values of water bodies, waterfront real estate, wildlife, and drinking water sources (Dodds et al. 2008).

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Received 15 September 2020; Accepted 26 March 2021; Published online 17 August 2021. Associate Editor, Nathan J. Smucker

Freshwater Science, volume 40, number 3, September 2021. © 2021 The Society for Freshwater Science. All rights reserved. Published by The University of Chicago Press for the Society for Freshwater Science. <https://doi.org/10.1086/716236>

The most common HAB-forming phytoplankton division in freshwaters is cyanobacteria, the oldest photosynthesizing organisms on Earth at ~3.5 billion y of age (Anderson et al. 2002, Lyons et al. 2014). Cyanobacteria have developed a wide variety of adaptations that, under favorable conditions, allow them to outcompete other phytoplankton (Paerl and Huisman 2009, Carey et al. 2011, O'Neil et al. 2012, Paerl and Otten 2013, Paerl 2017). Cyanobacteria are a diverse phylum in which traits vary among taxa, and changes in climate and nutrient regimes alter the composition of the cyanobacteria assemblage, as well as that of the phytoplankton as a whole (Jankowiak et al. 2019).

Microcystis (Lemmermann, 1907) is one of the most common cyanobacteria genera found in freshwaters globally because its high genetic diversity within and between species allows it to thrive under myriad environmental conditions (Xu et al. 2010, Humbert et al. 2013, Harke et al. 2016). *Microcystis* is colonial and can form large blooms, produce potent toxins, regulate buoyancy, use organic P, and store luxury P (Šejnohová and Maršálek 2012). Its growth rate positively correlates primarily with high water temperatures (optimal growth rates reported to be ~20–25°C) and several forms of both N and P (Davis et al. 2009, Xu et al. 2010, Donald et al. 2013). *Microcystis* is a non-diazotrophic taxon, and studies indicate a preference for reduced forms of inorganic N (Šejnohová and Maršálek 2012, Gobler et al. 2016, Newell et al. 2019); however, it uses nitrate (NO₃⁻) when necessary, which it may prefer under saturated-P conditions (Donald et al. 2013). *Microcystis* prefers orthophosphates but can use organic P and requires <0.03 mg/L P for bloom formation (Šejnohová and Maršálek 2012, Donald et al. 2013).

Climate change is exacerbating the severity of HABs. Inland water bodies have experienced rapid warming since 1985 (Schneider and Hook 2010, O'Reilly et al. 2015), providing longer growth seasons and affording cyanobacteria an advantage over other taxa because of their higher temperature optima range (Paerl and Huisman 2008, Deng et al. 2014, Ho and Michalak 2019). Higher temperatures also increase the duration and strength of thermal stratification in lakes, allowing buoyant cyanobacteria and those with a low sinking velocity easier navigation and less risk of sedimentation (Carey et al. 2011). Enhanced stratification combined with increased decomposition caused by bloom die-backs can lead to intensified periods of hypolimnetic hypoxia (Diaz and Rosenberg 2008, Scavia et al. 2014, Biddanda et al. 2018). In turn, hypoxia may induce nutrient release from bottom sediments, further promoting blooms (Diaz and Rosenberg 2008, Watson et al. 2016, Steinman and Spears 2020). Global and regional changes in precipitation patterns may also enhance bloom formation. Heavy precipitation events interspersed within droughts flush nutrients into waterbodies, where they concentrate and fuel HABs (Paerl and Huisman 2008, Reichwaldt and Ghadouani 2012, Lehman et al. 2017, Havens et al. 2019). Globally, increased duration, severity,

and range of cyanobacterial HABs are likely to continue under future climate scenarios, making their management challenging (Carey et al. 2011, Havens and Paerl 2015, Huisman et al. 2018).

Muskegon Lake (Muskegon, Michigan, USA), located on the west coast of Michigan's lower peninsula, provides a useful case study for understanding the dynamics of HABs in freshwater systems over time. The lake, which connects to Lake Michigan through a navigational channel, is economically important and has been subjected to significant anthropogenic stress. Muskegon Lake has historically suffered from annual occurrences of HABs, hypolimnetic hypoxia, winter fish kills, nuisance macrophyte growth, low water quality, and diminished wildlife diversity. These impairments stem from a long history of lumber, industry, and wastewater influx along the lakeshore that led to shoreline hardening, near-shore and open water filling by sawmill debris and foundry slag, ample wetland destruction, and widespread sediment contamination (Rediske et al. 2002, Gillett and Steinman 2011). After the lake's designation as an Area of Concern (AOC) by the Environmental Protection Agency per the US–Canada Great Lakes Water Quality Agreement in 1987, 9 beneficial use impairments (BUI) were identified: fish and wildlife consumption restrictions, loss of habitat, degradation of fish and wildlife populations, degradation of benthos, dredging restrictions, degradation of aesthetics, beach closings, eutrophication or undesirable algae, and drinking water consumption restrictions resulting from groundwater contamination (Steinman et al. 2008, USEPA 2020). A remedial action plan that included delisting targets for 5 of the BUIs was developed by the Michigan Department of Environmental Quality (now Michigan Department of Environment, Great Lakes, and Energy) and the Muskegon Lake Public Advisory Council (Steinman and Ogdahl 2004, Steinman et al. 2008). Eleven restoration actions were identified and scheduled to be completed in 2021, at which time delisting can be considered (Muskegon Lake Watershed Partnership, Muskegon, Michigan, personal communication). Management efforts occurred within the lake and along the shoreline and included removing contaminated sediment, historical lumber debris, and unnatural fill; major habitat restoration and conservation projects; re-establishing hydrological connectivity between surrounding waterways and Muskegon Lake; and improvement of water quality (Evans et al. 2018, USEPA 2020). A long-term monitoring program at Grand Valley State University's Annis Water Resources Institute began in 2003 to provide annual reports on the lake's progress and help inform management decisions (Steinman et al. 2008). The overall condition of the Muskegon Lake ecosystem has improved. As of 2018, 4 of the 9 BUIs (beach closings, fish and wildlife consumption restrictions, dredging restrictions, and drinking water consumption restrictions) were removed, and the remaining 5 are expected to be resolved upon completion of current projects (Hartig et al. 2018; Muskegon Lake Watershed Partnership, personal communication).

Through habitat restoration and reduction of nutrient inputs, several Canadian and US AOCs, including Bay of Quinte, Green Bay, and the Maumee River, have resolved their eutrophication BUIs (Hartig et al. 2018). Although the status of Muskegon Lake has improved substantially and the eutrophication BUI nears completion, isolated blooms are still an annual occurrence (Muskegon Lake Watershed Partnership, personal communication). The objectives of this study were to: 1) quantify the temporal changes in HAB abundance and cyanobacteria assemblage composition in the lake over 14 y (2003–2016) and 2) understand seasonal patterns of water physicochemical variables and their relationships with HABs. We hypothesized that the HAB impairment in Muskegon Lake has largely improved over the years as a result of remediation efforts and that their abundance and assemblage composition are associated with variation in temperature and nutrient form and amount.

METHODS

Our study used a comprehensive long-term dataset from a seasonal monitoring program on Muskegon Lake, including environmental variables and phytoplankton samples that are collected at various sites. We examined how HABs have changed over time in the lake, both in terms of abundance and assemblage composition. Using a multivariate approach, we visualized how changes in cyanobacteria assemblage com-

position associated with changes in physicochemical variables at different sites and in different seasons. We also used linear modeling to explore how water physicochemical variables changed over the study period and their relationships with HAB abundance.

Study site

Muskegon Lake is a mesotrophic, drowned river mouth, Great Lakes estuary that drains Michigan's 2nd-largest watershed into Lake Michigan (Fig. 1). Its main inflow is the Muskegon River, and it outflows through a human-made navigational channel. It has a surface area of 17 km², a water volume of 119 million m³, a mean depth of 7 m, a maximum depth of 21 m, and a seasonally variable hydraulic residence time of 23 d (Liu et al. 2018b). As a drowned river mouth, Muskegon Lake is an interface between lotic and lentic ecosystems and provides an array of habitats along gradients of physicochemical properties (Larson et al. 2013). Management, research, and restoration efforts aim to improve the quality of Muskegon Lake toward delisting as an AOC (Steinman et al. 2008).

Data collection: Environmental variables and phytoplankton

Data used in this study come from the Muskegon Lake Monitoring Program (MLMP), inclusive of years 2003 to 2016. MLMP data are collected onboard the W. G. Jackson

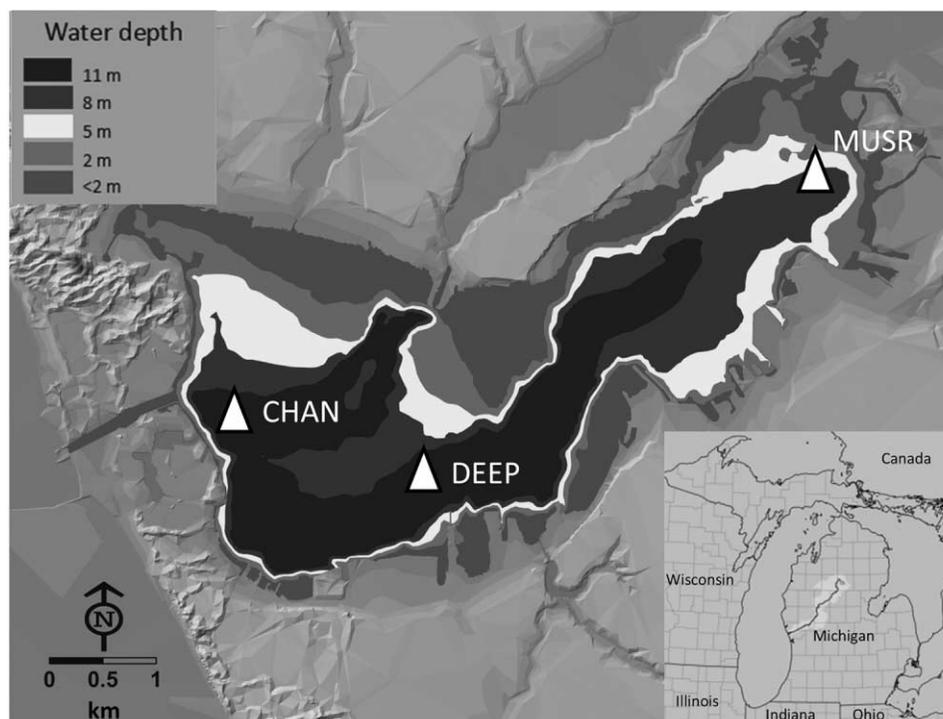


Figure 1. A bathymetric map of the study site, Muskegon Lake, Michigan, USA, including points of collection for the Muskegon Lake Monitoring Program sampling (white triangles: MUSR = Muskegon River, DEEP = deepest site in the lake, CHAN = Muskegon Lake channel). The inset in the lower-right corner is a map of Michigan with the Muskegon River shown and the extent of the watershed delineated. See the online edition for a color version of this image.

research vessel 3× annually during the spring (May), summer (July), and autumn (September/October). For the current study, we used data from 3 of the sampling sites—near the Muskegon River inflow (MUSR), near the Muskegon Lake navigational channel outflow (CHAN), and over the deepest point in the lake (DEEP; Fig. 1)—to account for the influence of the main inflow, the status of the main lake, and potential intrusion events from Lake Michigan.

See Steinman et al. (2008) for detailed sampling and laboratory methods, but here we provide a brief summary. Samples were collected and analyzed by the Steinman Lab at Annis Water Resources Institute. Water samples for physicochemical measurements were collected near the surface at each site. Water quality parameters considered for this study include temperature, chlorophyll *a* pigment concentration, and nutrients (soluble reactive P [SRP], total P [TP], ammonia [NH₃], nitrate [NO₃⁻], and total Kjeldahl N [TKN]). It should be noted that instruments used to measure nutrient concentrations were upgraded during the study period, leading to more precise measurements in later years. Phytoplankton samples were collected at each site from near the surface and bottom of the water column to account for both planktonic and deep-water forms of phytoplankton. A Van Dorn sampler was used to collect wholewater samples, and samples were subsequently analyzed for assemblage composition and biovolume. The phytoplankton assemblage composition for each year was identified to genus level; herein, we consider only the cyanobacteria, inclusive of genera comprising >5% of the annual cumulative cyanobacteria biovolume.

We used phytoplankton biovolume data to calculate seasonal and cumulative cyanobacteria biovolume for each year. We calculated seasonal (spring, summer, and autumn) cyanobacteria biovolume values by adding the cyanobacteria biovolume values of all 3 sites for each sampling event. We calculated cumulative cyanobacteria biovolume values by adding each year's seasonal cyanobacteria biovolume values from the 3 annual sampling events. As such, cumulative cyanobacteria biovolume values are only useful in comparison with each other and not to values found in other lakes. We then used this value to determine the percentage of the phytoplankton assemblage that was composed of cyanobacteria on an annual basis (% cyanobacteria = (cumulative cyanobacteria biovolume / cumulative phytoplankton biovolume) × 100).

HAB abundance and cyanobacteria assemblage composition over time

To address the 1st part of our 1st objective, to understand shifts in HAB abundance, we used linear regression modeling of cumulative cyanobacteria biovolume and % cyanobacteria biovolume over time. We used the *UsingR* package (version 2.0–6; Verzani 2018) in R (version 4.0.2; R Project for Statistical Computing, Vienna, Austria) to perform simple linear regression to model the relationships between

both % cyanobacteria and cumulative cyanobacteria biovolume values vs year. We also built linear regression models for cyanobacteria biovolume vs year for each season (spring, summer, and autumn) to examine seasonal trends over time. We checked for normality of residuals with the Shapiro–Wilk test (*stats* package version 4.0.4) and through visual assessment for normality of quantile–quantile plots. We checked for homoscedasticity of residuals with the Breusch–Pagan test (*lmtest* package version 0.9.38; Zeileis and Hothorn 2002). If the assumptions of normality and homoscedasticity were not met or could not be achieved through log-transformation, we ran a Kendall–Theil regression (*mblm* package version 0.12.1; Komsta 2019) in lieu of simple linear regression because the Kendall–Theil regression is robust to issues of non-normality of residuals and of outliers (Helsel and Hirsch 1992).

To address the 2nd part of our 1st objective, to understand shifts in cyanobacteria assemblage over time, we used non-metric multidimensional scaling (NMDS) in the *vegan* package (version 2.5-2; Oksanen et al. 2018) in R with a Bray–Curtis dissimilarity matrix of cyanobacteria species counts to visualize similarity of cyanobacteria assemblages among years (2003–2016). Bray–Curtis dissimilarity is recommended for use with ecological data because it provides non-Euclidean distance and accounts for both species composition and abundance (Borcard et al. 2018). To visualize the relationships of environmental factors with cyanobacterial assemblage over time, addressing our 2nd research objective, we added environmental data, including NH₃, TP, SRP, NO₃⁻, TKN, and water temperature, to the NMDS ordination with the *envfit* function. Data were from all seasons (spring, summer, and autumn) and sites (MUSR, DEEP, and CHAN). We removed variables with vector lengths <0.40. Because of its comparatively exceptionally high cyanobacteria biovolume, we excluded the year 2008. The data from 2008 were so distantly located from all other years along the *x*-axis of the ordination that it caused all other years to group together, making visualization and interpretation of results impossible.

Relationships of physicochemical variables with HAB abundance

To further address our 2nd research objective, we used linear regression modeling to assess temporal trends in environmental variables, pooled across all years and sites, as well as their relationships with changing HAB abundance over time. First, we modeled the relationships between the variables: NO₃⁻, TKN, NH₃, SRP, TP, water temperature, and chlorophyll *a* vs year for each season (spring, summer, and autumn). Then we individually modeled the relationships between cyanobacteria biovolume values and values of NO₃⁻, NH₃, TP, and water temperature from all years and sites to assess how each parameter individually contributed to variation in HAB abundance. We addressed normality and homoscedasticity in the same manner as the previous regressions. Additionally, to reduce risk of Type I error, we adjusted

Table 1. Annual percentage of the phytoplankton community that was composed of cyanobacteria (based on biovolume) and annual cumulative cyanobacterial biovolume from the spring, summer, and autumn monitoring events at Muskegon Lake, Michigan, USA (sites at Muskegon River, the deepest site in the lake, and Muskegon Lake channel; 2003–2016). Biovolume values are summed cyanobacteria biovolume of all sampling events within each year. Data were pooled within each year across sites and sampling days.

Year	% cyanobacteria	Cyanobacteria biovolume ($\mu\text{m}^3/\text{mL}$)
2003	36.32	9.91×10^6
2004	56.63	4.49×10^7
2005	67.22	5.97×10^7
2006	25.16	5.45×10^6
2007	32.71	5.29×10^6
2008	63.35	5.56×10^{10}
2009	5.74	1.16×10^6
2010	2.61	1.38×10^6
2011	39.29	1.00×10^7
2012	7.52	2.89×10^6
2013	5.91	5.11×10^5
2014	7.85	3.51×10^5
2015	0.71	4.83×10^4
2016	2.38	6.95×10^5

our p -values with a Bonferroni correction on the 2nd set of regressions. We did not include SRP and TKN in these analyses because of low variation in SRP values and a lack of change in TKN concentrations over time. We removed data from the year 2008 from all analyses that included seasonal cyanobacterial biovolume because it was an exceptional outlier (see Table 1, Fig. 2A, B).

We performed all data analyses and visualization in RStudio[®] (version 1.1.453; Boston, Massachusetts) and used the *ggplot2* package (Wickham 2016) to create figures.

RESULTS

Cyanobacteria assemblage shifts over time

We observed quantitative and qualitative temporal shifts in the cyanobacteria over the study period. Percent cyanobacteria decreased from 2003 to 2016 ($p = 0.003$; Fig. 2A). There was a distinction between the years 2003 through 2008 and 2009 through 2016: from 2003 through 2008, % cyanobacteria values ranged from 25.16 to 67.22%, and from 2009 through 2016, % cyanobacteria values ranged from 0.71 to 7.85%, with 2011 being an exception at 39.29% (Table 1). Similarly, cumulative cyanobacteria biovolume was annually variable, displaying a recurring undulating pattern. Overall, the trend was negative ($p = 0.04$; Fig. 2B), decreasing by several orders of magnitude over the study period (Table 1). The year 2008 had the highest cumulative cyanobacteria biovolume, with an exceptionally high value

of $5.56 \times 10^{10} \mu\text{m}^3/\text{mL}$. The lowest cumulative cyanobacteria biovolume occurred in 2015, with a value of $4.83 \times 10^4 \mu\text{m}^3/\text{mL}$. Additionally, cyanobacteria biovolume decreased substantially over time for all seasons (spring and autumn, $p < 0.001$; summer, $p = 0.003$; Table S1, Fig. 3B).

Additionally, the composition of the cyanobacteria community was annually variable (Fig. 4). Three groups of years can be distinguished (2003–2008, 2009–2012, and 2013–2016), with 2 exceptions (2003 and 2015). From 2004 to 2008 (Group 1), *Microcystis* was the dominant genus. From 2009 to 2012 (Group 2), there was more diversity, and no single genus dominated. Genera present include *Microseira* (Farlow ex Gomont) G.B. McGregor & Sendall ex Kenins, 2014 (formerly *Lyngbya*); *Microcystis*, *Limnothrix* Meffert, 1988; *Aphanocapsa* Nägeli, 1849; *Chroococcus* Nägeli, 1849; *Dolichospermum* Wacklin, Hoffmann, & Komárek, 2009

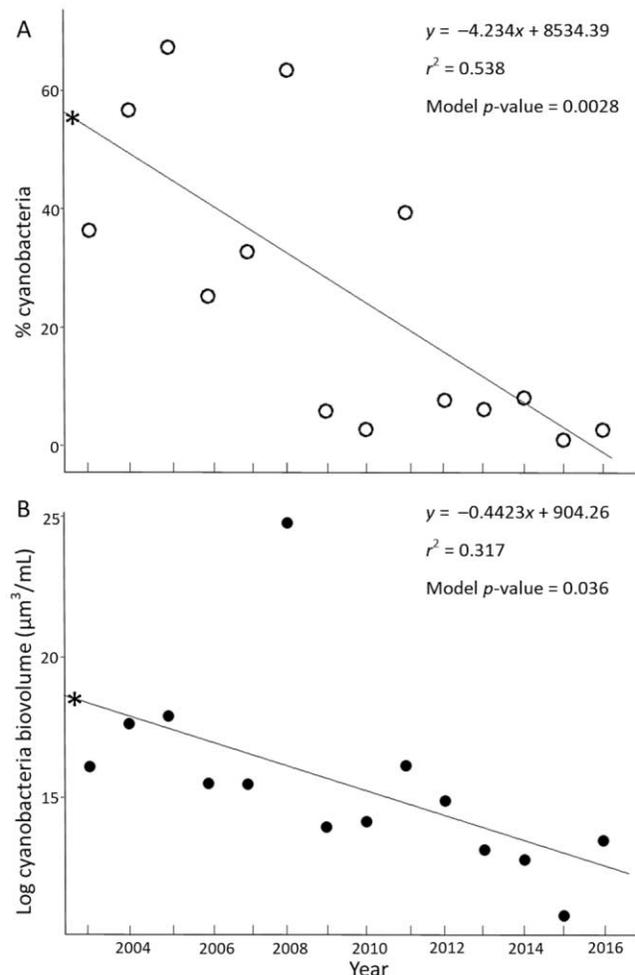


Figure 2. Simple linear regression models of the relationship between the annual percentage of the phytoplankton assemblage composed of cyanobacteria and year (A) and annual cumulative cyanobacteria biovolume values and year (B). Note that data were transformed to better meet assumptions of normality of residuals. See Table 1 for data.

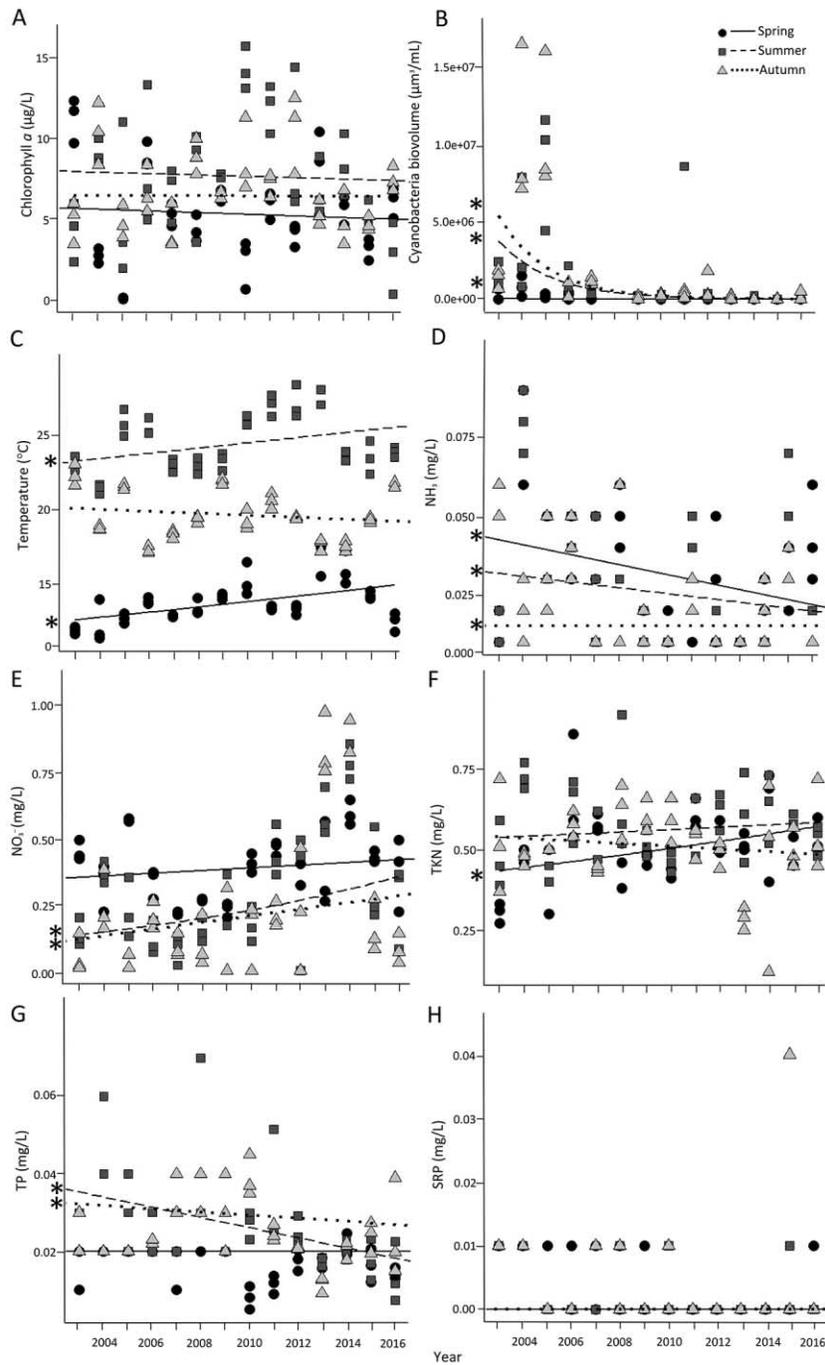


Figure 3. Simple linear regression and Kendall–Theil regression models of the relationship between seasonal values of chlorophyll *a* concentration (A), cyanobacteria biovolume (B), water temperature (C), ammonia (NH₃) concentration (D), nitrate (NO₃⁻) concentration (E), total Kjeldahl nitrogen (TKN) concentration (F), total phosphorus (TP) concentration (G), and soluble reactive phosphorus (SRP) concentration (H) with year. Asterisks on the left end of the trend line denote equations with $p < 0.05$. Note that some data were transformed to better meet assumptions of normality and homoscedasticity; as such, the equation built on the transformed data is displayed. See Table S1 for equations.

(formerly *Anabaena*); *Merismopedia* Meyen, 1839; *Gomphosphaeria* Kützing, 1836; and *Gloeothece* Nägeli, 1849. Years 2013, 2014, and 2016 (Group 3) were once again dominated or co-dominated by *Microcystis*. Groups 1 and 3 ap-

pear to have reduced taxa evenness and reduced taxa diversity among those represented compared with Group 2. The only year that was dominated by *Aphanocapsa* was 2003. The cyanobacteria community in 2015 was roughly equally

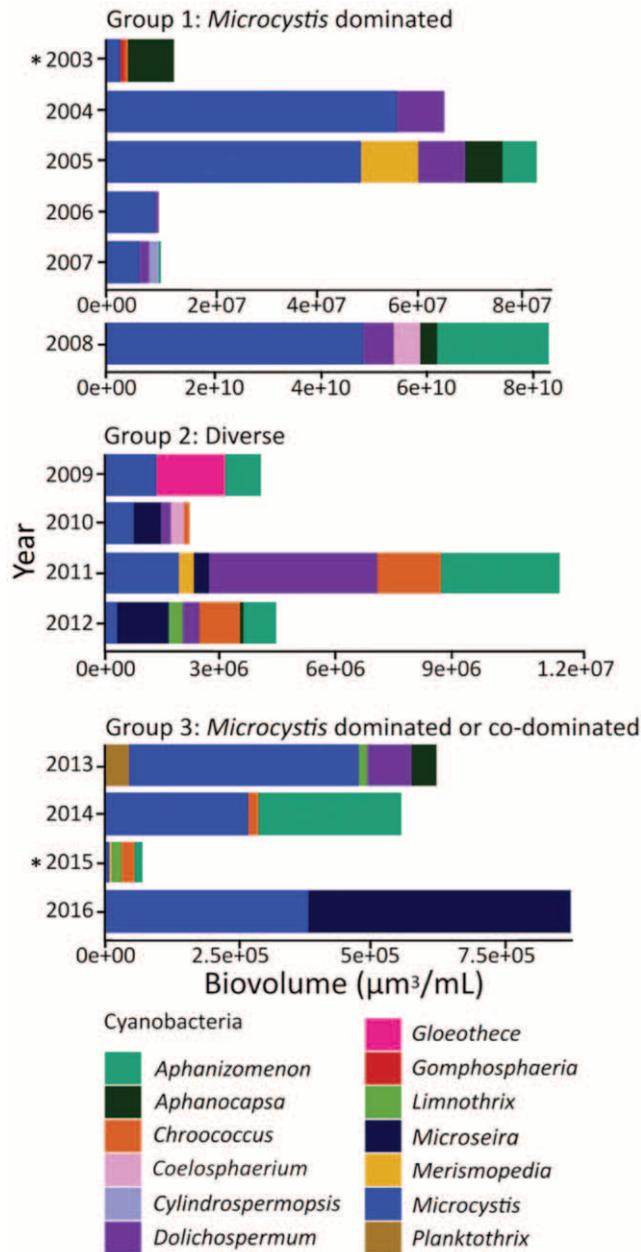


Figure 4. Stacked bar graphs displaying cyanobacteria assemblage composition at the genus level and cumulative cyanobacteria biovolume values from 2003 to 2016 (calculated as an addition of cyanobacteria biovolumes from the seasonal samplings). Data include genera comprising >5% of the cumulative cyanobacteria biovolume. Years are categorized into 3 groups based on the patterns observed in assemblage composition: Group 1 (2003–2008) was dominated by *Microcystis*, Group 2 (2009–2012) was highly diverse, and Group 3 (2013–2016) was dominated or co-dominated by *Microcystis*. The 2 exceptions to these groups are denoted by an asterisk (*) next to the year. Note that the x -axis scale is different for each group because cyanobacteria abundance was variable over the study period.

composed of *Aphanizomenon* Morren ex Bornet & Flahault, 1886, *Chroococcus*, and *Limnothrix*, with *Microcystis* present to a lesser extent, but overall biovolume was low.

Shifts in assemblage composition over time were also evident in NMDS visualizations. The NMDS had a low stress value of 5.26, indicating little distortion of the ordination (Fig. 5). There was a temporal pattern of years moving from the upper-right corner to the lower-left corner, indicating consistent change in the cyanobacteria assemblage composition over time and that earlier years and later years were least similar. Group 1 years' (2003–2008) composition appear to be influenced by summer values of variables and associated with higher levels of P. In particular, all Group 1 years' compositions were associated with high spring SRP values at all sites and high summer SRP values at DEEP and CHAN. Composition in years 2004 and 2005 were associated with high summer TP values at CHAN and MUSR. Additionally, composition in 2004 and 2005 were associated with high spring NH_3 values at CHAN and MUSR. Group 2 years (2009–2012) were more centrally located, associating with intermediate values of each variable. Group 3 years (2013–2016) appear to be more influenced by autumn values of variables. They associate with higher spring temperatures at DEEP and CHAN, high autumn TP values at DEEP, high autumn NO_3^- values at all sites, and, to a lesser degree, high spring TKN values at MUSR.

Environmental variable trends over time

Linear modeling demonstrated seasonal trends in environmental variables over time. Linear regression showed no notable trend of chlorophyll a concentration over time for any season (spring $p = 0.659$; summer $p = 0.776$; autumn $p = 0.983$; Tables 2, S1, Fig. 3A). Water temperature for all 3 seasons displayed an undulating pattern and increased strongly over time in the spring ($p = 0.001$) and summer ($p < 0.001$), whereas autumn water temperature did not change substantially over time but showed a slight decreasing trend ($p = 0.322$; Fig. 3C). NH_3 concentrations decreased substantially over time in all seasons (spring $p = 0.034$; summer $p = 0.001$; autumn $p = 0.005$; Fig. 3D). NO_3^- concentrations increased substantially over time in the summer ($p = 0.033$) and autumn ($p < 0.001$) but showed no trend in the spring ($p = 0.295$; Fig. 3E). TKN concentrations increased in the spring ($p = 0.013$) but did not change substantially over time in the summer ($p = 0.412$) or autumn ($p = 0.382$; Fig. 3F). TP concentrations decreased over time in the summer ($p < 0.001$) and autumn ($p = 0.009$) but not spring ($p = 0.132$; Fig. 3G). SRP concentrations appear to have decreased over time, but not substantially (spring $p = 0.213$; summer $p = 0.168$; autumn $p = 0.054$; Fig. 3H).

Relationships of HAB abundance with environmental variables

Kendall–Thiel regressions indicated strong relationships between cyanobacteria biovolume and some environmental

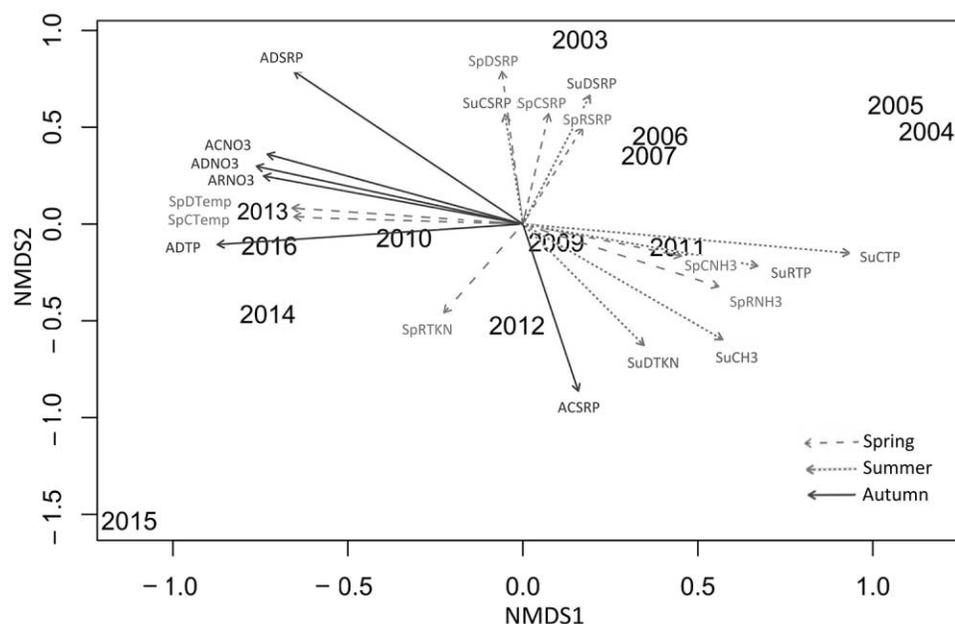


Figure 5. Non-metric multidimensional scaling of cyanobacteria assemblage composition data for each year, except 2008 because it was an outlier that compromised the clarity of results. Environmental data is from the Muskegon Lake Monitoring Program data set and includes data from all 3 sites and seasons. The 1st letter(s) of the variable indicates the season (Sp = spring, Su = summer, A = autumn), the 2nd letter indicates the site (R = MUSR, D = DEEP, C = CHAN), and the remainder of the description is the variable measured: TKN = total Kjeldahl nitrogen, NH₃ = ammonia, TP = total phosphorus, SRP = soluble reactive phosphorus, NO₃ = nitrate, Temp = temperature, MUSR = Muskegon River, DEEP = deepest site in the lake, CHAN = Muskegon Lake channel. See the online edition for a color version of this image.

variables. Cyanobacteria biovolume had a strong, negative relationship with NO₃⁻ concentration ($p < 0.001$; Table S2, Fig. 6A), a strong, positive relationship with NH₃ concentration ($p < 0.001$; Fig. 6B), a strong, positive relationship with TP ($p < 0.001$; Fig. 6C), and a strong, positive relationship with temperature ($p < 0.001$; Fig. 6D).

DISCUSSION

Over the years, the HAB impairment in Muskegon Lake has decidedly improved, both in reduction in the percentage of the phytoplankton community that is comprised of cyanobacteria and in overall cyanobacteria abundance. *Microcystis*, a toxin-producing taxon, was often present and appears to have regained dominance within the cyanobacteria assemblage, but the substantial reduction in cyanobacteria biovolume indicates this is likely of little current concern. Improvement in HAB impairment is a result of the considerable reduction in nutrient concentrations in Muskegon Lake, an outcome of careful and targeted restoration efforts. Despite rising spring and summer water temperatures, HABs appear to have been kept at bay in recent years.

Trends and factors associated with changes in cyanobacteria abundance

The quantitative changes of HABs over the study period are likely explained by the marked improvements in water

quality, which varied seasonally. We report a continuation of trends identified by Steinman et al. (2008), which assessed water quality changes in Muskegon Lake from 1972 to 2005. Steinman et al. (2008) reported decreased SRP, TP, and NH₃, increased NO₃⁻, reduced chlorophyll *a* concentration, and increased Secchi-disk depths in response to restoration activities and wastewater diversion to a tertiary treatment plant. For our study period, 2003 to 2016, the continued nutrient

Table 2. Summary of simple linear regression and Kendall–Theil regression results of seasonal trends of environmental variables and cyanobacteria biovolume over the study period (2003–2016). + indicates a notable positive trend, – indicates a notable negative trend, and n indicates no notable trend. NH₃ = ammonia, NO₃⁻ = nitrate, TKN = total Kjeldahl nitrogen, TP = total phosphorus, SRP = soluble reactive phosphorus.

Environmental variable	Spring	Summer	Autumn
Chlorophyll <i>a</i>	n	n	n
Cyanobacteria biovolume	–	–	–
Temperature	+	+	n
NH ₃	–	–	–
NO ₃ ⁻	n	+	+
TKN	+	n	n
TP	n	–	–
SRP	n	n	n

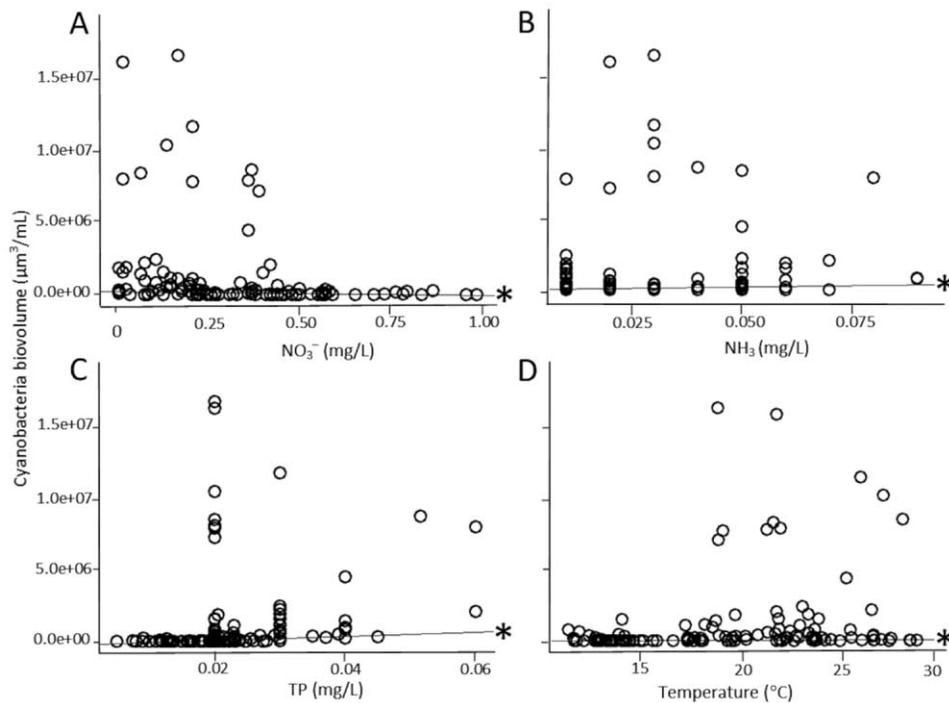


Figure 6. Kendall–Theil regression models of the relationship between the logarithm of cyanobacteria biovolume and NO_3^- concentration (A), NH_3 concentration (B), total phosphorus (TP) concentration (C), and water temperature (D). Asterisks (*) in the lower right corner of the plot denote equations with $p < 0.05$. See Table S2 for equations.

reduction in Muskegon Lake was likely the main driver of the observed decreases in cyanobacteria abundance (Anderson et al. 2002, Paerl and Otten 2013, Glibert 2017). A seasonal component of nutrient reductions was evident, with substantial P reductions over time occurring mainly in the summer and increases in NO_3^- over time occurring in the autumn. Spring SRP concentrations, which have been reliable indicators of cyanobacteria blooms in Lake Erie (Stumpf et al. 2012, 2016), also decreased over time in Muskegon Lake, though not substantially. Autumn concentrations of TP and SRP appear to have increased at DEEP, which may be a result of hypolimnetic nutrients being entrained in the water column during autumn mixing. Hypolimnetic P concentrations have been reported to increase in the autumn at DEEP and the geographic center of the lake (Steinman et al. 2008, Weinke and Biddanda 2018). However, internal P loading is considered to contribute a minor portion of the lake's overall P budget (Weinke and Biddanda 2018; A. D. Steinman, Grand Valley State University, Allendale, Michigan, personal communication).

Both N and P appear to be influential on HAB dynamics in Muskegon Lake, as decreases in cyanobacteria abundance coincided with decreases in NH_3 , SRP, and TP concentrations. Although Muskegon Lake is naturally P limited, and P is often cited as the growth-limiting nutrient for cyanobacteria (Schindler et al. 2016), N also plays an important role in phytoplankton growth and assemblage composition at both high and low P levels (Glibert et al. 2016, Gobler et al. 2016, Newell

et al. 2019). In particular, cyanobacteria respond positively to reduced forms of N (Von Rückert and Giani 2004, Donald et al. 2013). Other studies have documented that cyanobacterial dominance and toxin production increase under high concentrations of reduced N (McCarthy et al. 2009, Glibert et al. 2016, Lehman et al. 2017, Newell et al. 2019), and the strong presence of non-diazotrophic genera, such as *Microcystis*, indicates N abundance (Paerl 2017). Therefore, the consistent reduction in NH_3 in all seasons in Muskegon Lake, combined with the TP and SRP reductions, appear to have resulted in reduced cyanobacteria abundance over the years.

NH_3 decreased over time, but NO_3^- increased over time in all seasons, substantially so in the summer and autumn—a trend also occurring in some of the Great Lakes and likely attributable to increasing atmospheric dry deposition caused by fossil-fuel combustion (Sternner et al. 2007, Steinman et al. 2008, Foley and Betterton 2019). Similar to the conclusion of Steinman et al. (2008), increased NO_3^- did not stimulate phytoplankton growth in Muskegon Lake, likely because of their preference for reduced forms of N (Dortch 1990). TKN concentrations, which are inclusive of organic N and NH_3 , remained steady, except for an increase in the spring. Because NH_3 concentrations decreased in all seasons over time, it can be inferred that the organic N component of TKN increased. This shift towards organic N may be a result of sustained primary productivity (discussed below), but because organic N can be recycled rapidly, it may also provide a potential reduced N source that could benefit non-diazotrophic

cyanobacteria, such as *Microcystis*, which can be very competitive for reduced N resources (Salk et al. 2018, Newell et al. 2019).

Despite the overall reduction in cyanobacteria abundance, chlorophyll *a* concentration, a proxy for phytoplankton growth, did not show a trend over time. Chlorophyll *a* is a pigment present in all phytoplankton and is not specific to cyanobacteria, so this suggests that phytoplankton biomass is being maintained, and a smaller proportion comprises cyanobacteria. The mirrored interannual patterns of chlorophyll *a* concentration and temperature in the regressions indicate a likely causal relationship. Phytoplankton growth rates increase with temperature; therefore, this pattern is not entirely surprising (Hampton et al. 2008).

Many studies have demonstrated or predicted that higher temperatures resulting from global climate change will exacerbate the severity of HABs (Paerl and Huisman 2008, Wagner and Adrian 2009, Kosten 2012, Visser et al. 2016); however, this effect has not been observed in Muskegon Lake, demonstrating that the conditions in the lake are not currently conducive to cyanobacterial dominance, despite rising temperatures. This phenomenon may be because, in part, temperature increases were seen in the spring and summer only, whereas autumn temperatures appear to have decreased slightly over the study period (though it should be noted that autumn sampling occurred during the mixing period of the lake, potentially providing misleading measurements). Cyanobacteria abundance peaks in the late summer or early autumn in Muskegon Lake (Muskegon Lake Observatory buoy time-series data, available at www.gvsu.edu/buoy/; Dila and Biddanda 2015); therefore, increases in spring and summer temperature may have allowed non-cyanobacteria phytoplankton growth to begin earlier in the year and maintain biomass (Peeters et al. 2007) despite nutrient reductions, which may also explain the increase in the organic N component of TKN in the spring. Although temperature is important in driving cyanobacteria abundance, the nutrient reduction in Muskegon Lake is presently maintaining low cyanobacteria abundance. However, the MLMP only samples 3×/y. As such, caution must be used when interpreting phytoplankton data because phytoplankton growth is spatially and temporally heterogeneous, and such infrequent sampling could be misleading (Rantajärvi et al. 1998).

Factors associated with changes in cyanobacteria assemblage structure

Understanding the drivers of assemblage composition is more challenging than those of quantitative reductions because of the multitude of contributing variables, including nutrient form and concentration and water temperature (McCarthy et al. 2009, Weirich et al. 2019). Nutrient concentrations have generally decreased over time in Muskegon Lake, but nutrient forms have shifted, and spring and summer water temperatures have steadily increased over the study

period, potentially explaining the shift in composition within the diminishing cyanobacteria assemblage.

Cyanobacteria compositional shifts reflected a general temporal trend (Fig. 5). In ordination space, the group of years that had a highly diverse cyanobacteria assemblage (Group 2) was positioned between the 2 groups of years in which *Microcystis* dominated the cyanobacteria assemblage and reduced its diversity and evenness (Groups 1 and 3). Additionally, the years that were outliers in terms of composition (2003 and 2015) deviated slightly from this pattern, with 2003 positioned further to the left than would be expected if it were in line with the temporal trend and 2015 positioned further away from 2014 and 2016 than any other 2 consecutive years. This pattern indicates that environmental factors drove the cyanobacteria assemblage composition, but the factors contributing to *Microcystis*' advantage differed between Group 1 and Group 3, and that the remainder of the cyanobacteria assemblage differed as well. It is important to note that after the year 2008, cyanobacteria composed a relatively small proportion of the phytoplankton assemblage. Therefore, the composition in later years is comparatively less important than that of earlier years because the cyanobacteria exerted less influence on the phytoplankton assemblage and the lake ecosystem as a whole.

We expected spatial factors to influence the HAB assemblage composition because drowned river mouths contain a gradient of physicochemical conditions (Larson et al. 2013, Dila and Biddanda 2015), but no strong spatial trends were evident. The NMDS biplot demonstrated that nutrients and temperature in the river, channel, and midlake were influential factors that shaped cyanobacteria assemblage composition, but no site appeared disproportionately influential. Although we expect that the Muskegon River, which accounts for 95% of inputs (Liu et al. 2018a), is the main source of nutrients to Muskegon Lake, nutrient loading from this site did not appear substantial. This finding coincides with other studies in the Muskegon River watershed, which found that wastewater influx as a source of P far surpassed agriculture, which has decreased in the watershed, and that the wastewater diversion in 1973, combined with the effect of dams, has reduced P levels to those before European settlement (Tang et al. 2005, Liu et al. 2018a).

Although *Microcystis* can use organic P, such as that included in TP, it prefers orthophosphates (inorganic P, SRP), and it often requires <0.03 mg/L P to form a bloom (Šejnová and Maršálek 2012). SRP concentrations in Muskegon Lake were low throughout the study period and had little variation; however, some studies, including Steinman et al. (2008), have demonstrated that *Microcystis* abundance positively corresponds with high TP concentrations (Wagner and Adrian 2009, Xu et al. 2010). Therefore, the combination of organic and inorganic P concentrations in Muskegon Lake were sufficient for *Microcystis* in earlier years of the study period. Gobler et al. (2016) found that at low P levels,

such as those in Muskegon Lake in the later years of the study period, non-diazotrophic cyanobacteria (e.g., *Microcystis*) have an advantage because of their ability to thrive in low P environments. The higher P concentrations in earlier years likely gave *Microcystis* an advantage, and it may be argued that it had an advantage over other cyanobacteria in later years as well because of its ability to thrive in low P environments and to use both organic and inorganic forms of P.

Microcystis' abundance was also likely influenced by N forms and concentrations. *Microcystis* can use all inorganic forms of N, is highly competitive for N resources, and experiences rapid growth in response to N inputs (Chaffin et al. 2018, Newell et al. 2019). Most studies suggest a preference for reduced forms of N, even when NO_3^- is more abundant (Von Ruckert and Giani 2004, Šejnohová and Maršálek 2012, Gobler et al. 2016, Lehman et al. 2017, Newell et al. 2019). In earlier years in Muskegon Lake, reduced forms of N (e.g., NH_3) were abundant in all seasons, and in later years, NO_3^- concentrations increased notably in summer and autumn. *Microcystis* often blooms in the late summer and autumn (Chaffin et al. 2013, Dila and Biddanda 2015, Lehman et al. 2017, Coffey et al. 2020); therefore, *Microcystis* would have lost its advantage in terms of N form availability over this period because reduced forms shifted to oxidized forms during their growth season. Our data indicate the shift in N form and overall decrease in nutrient concentration may have driven the decrease in *Microcystis* abundance after Group 1 years and led to a more diverse cyanobacteria assemblage in the years following.

Nutrient form preference and requirement vary by taxa, and nutrient forms and amounts are important drivers of phytoplankton assemblage composition (Berg et al. 2003, Rothenberger et al. 2009, Donald et al. 2013, Anderson et al. 2020, Erratt et al. 2020). During Group 2 years, when the cyanobacteria assemblage was diverse and no single genus dominated, there was also a diversity of nutrient forms, indicated by the central location of the data points for those years on the NMDS biplot. Moderate amounts of several nutrient forms may have allowed for diversity by not giving any single genus a competitive advantage (Hutchinson 1961). Also, temperature was sufficient for *Microcystis* growth during this time, so we infer that temperature was not the factor preventing their dominance during Group 2 years.

We propose that *Microcystis* regained its advantage over other cyanobacteria taxa during Group 3 years because of increasing water temperatures in the spring and summer, as demonstrated by the linear regressions and NMDS. Temperature is often argued to be the most important factor influencing the success of *Microcystis*. Species in this genus, and especially toxin-producing species, respond strongly to increased temperatures (Davis et al. 2009, Xu et al. 2010, Šejnohová and Maršálek 2012). Deng et al. (2014) found that increasing spring temperatures benefitted *Microcystis* because they have overwintering akinetes, which are stimulated when water temperatures become suitable for growth. Because of *Microcystis*'

myriad adaptations and ecological flexibility, multiple environmental conditions can be conducive to a cyanobacteria assemblage dominated by *Microcystis*. This adaptability to a range of conditions helps to explain the 2 *Microcystis*-dominated time periods, which differed in other environmental respects, seen in this 14-y study. Over a relatively short period of time and a small temperature increase, *Microcystis* regained an advantage in the cyanobacteria assemblage in Muskegon Lake. Because *Microcystis* abundance appears to reduce evenness and, possibly, diversity within the cyanobacteria assemblage, this phenomenon may have ecosystem-level consequences. Given more time and larger temperature increases associated with climate change, we may find more difficulty in managing for HABs and a loss of progress in staving off *Microcystis* dominance in the future, especially if autumn temperatures increase (Ho and Michalak 2019).

Last, the cyanobacteria assemblage composition of the years that qualified as exceptions to the pattern (2003 and 2015) can also be explained by environmental variables. For 2003, *Aphanocapsa* may have dominated because of high SRP concentrations and an unusually warm autumn, delaying autumn turnover and allowing this picoplankton to stay entrained in the water column (Wang et al. 2018). The year 2015 had very low phytoplankton growth overall, likely because of the comparatively cool, late summer that resulted from several storm events (Weinke and Biddanda 2018), which would have specifically put cyanobacteria at a growth disadvantage and not allowed for *Microcystis* to dominate.

Future research directions

Future work is needed to further understand which environmental factors individually or interactively drive the cyanobacteria assemblage abundance and composition in Muskegon Lake and should include meteorological factors (e.g., precipitation and wind events), along with additional physical factors (e.g., historical dredging and Schmidt stability; Weirich et al. 2019). A closer look into the seasonal and spatial patterns of cyanobacteria assemblage composition is also warranted and may provide insights into the ecosystem effects of reduced cyanobacteria assemblage diversity and evenness when *Microcystis* is the dominant taxon. Therefore, additional research should focus on continued monitoring and on developing HAB management efforts, specifically as they pertain to future climate scenarios. Regional long-term water-quality data will be invaluable for reliably validating remote-sensing approaches, such as AquaSat, and identifying patterns of multiannual and seasonal variability to reconstruct historical water quality changes and to forecast future changes, such as regime shifts, in the world's freshwater systems (Ross et al. 2019, Kelvey 2020, Ortiz et al. 2020, Wilkinson et al. 2020). Such advances will pave the way for detection, mitigation, and prevention of HAB events, providing socioeconomic benefits in terms of reducing health risks and saving costs associated with HAB exposure and improving

our understanding of freshwater ecosystem dynamics under increasing stress from anthropogenic and climate-change forcing (Stroming et al. 2020, Biddanda et al. 2021).

ACKNOWLEDGEMENTS

Author contributions: Development of study design: JLM, BAB, ADW, SEH, EBS, and MMWS. Execution of field work: JLM, BAB, ADW, and IPS. Execution of lab work and sample analyses: JLM, IPS, BAB, ADW, and SEH. Designing and executing data analyses: JLM, ADW, BAB, and MMWS. Writing, editing, and idea contribution to manuscript: all co-authors.

This research was funded by the Michigan Space Grant Consortium [NNX15AJ20H], the Community Foundation for Muskegon County, the Grand Valley State University Presidential Research Grant, and the Michigan Chapter of the North American Lake Management Society and Michigan Lakes and Streams Association Lake Research Student Grant. We thank Kurt Thompson for creating the Muskegon Lake bathymetric map. We thank Brian Scull at the Annis Water Resources Institute for analyzing nutrient samples. We are grateful to Drs. Alan Steinman and Mark Luttenton of the Annis Water Resources Institute for generously sharing historical Muskegon Lake Monitoring Program data and the Community Foundation for Muskegon County's Muskegon Lake Research Fund for funding the program.

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