Bloom or bust: Search for phytoplankton community drivers using long-term time-series observations and field measurements in a model Great Lakes estuary

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Dedication

I would like to dedicate this thesis, and all the work, time, and energy that went into the final product, to two people I lost during my time in graduate school. My great grandmother, Loretta M. Kallil (1924-2019), passed away at 94 years old in July of 2019. She will forever be remembered for her sweet demeanor, her never-ending love for her family, her stylish outfits and bright red lipstick, and her insistence that you must try whatever Lebanese food she was offering. In August of 2019, I unexpectedly lost a good friend - Jeffrey A. Langolf (1995-2019). Though gone far too soon, Jeff taught me a lifetime's worth. Jeff was kind to all, never failed to make us laugh, turned mundane tasks into fun memories, and would have made the most caring, compassionate, and dedicated medical doctor.

Memory Eternal.

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Abstract

As sentinels of climate change and other anthropogenic effects, freshwater lakes are experiencing ecosystem disruptions at every level of the food web, beginning with the phytoplankton. One of the major threats to waterbodies around the world are cyanobacterial harmful algal blooms (HABs) resulting from anthropogenic eutrophication and exacerbated by climate change. Muskegon Lake, a drowned river mouth Great Lakes estuary on the east coast of Lake Michigan, is no exception and was declared an Area of Concern by the EPA in 1987 with nuisance algal blooms cited as a beneficial use impairment. Using long-term data and additional 2019 sampling, we performed multivariate and univariate analyses on environmental and phytoplankton data in order to visualize variation over the study period. The objective of this thesis was two-fold: 1) we aimed to quantify changes in HAB prevalence and community composition in Muskegon Lake over 16 years (2003-2019) and explore the environmental factors potentially driving the change, and 2) recognizing 2019 as a year of anomalous weather patterns, we explored the effects of heavy precipitation and cool temperatures on the phytoplankton community and cyanobacterial HABs in particular.

For our first objective, we used two long-term data sets: the Muskegon Lake Observatory (MLO; 2011-2019) buoy data and data from the Muskegon Lake monitoring program (MLMP; 2003-2019) in addition to 2019 sampling. Principal component analysis (PCA) was used to visualize variation and patterns in environmental variables over time, non-metric multidimensional scaling (NMDS) was used to assess associations between HAB community composition and environmental variables, and a univariate comparison (paired T-test and Wilcoxon Rank Sum test) was made on environmental variables between a group of severe-bloom years and a group of mild-bloom years. Analyses revealed that, despite generally rising water temperatures, a reduction in nutrient concentrations likely led to decreases in HAB

abundance over time. Additionally, HAB community composition appears to be driven by nutrient form and concentration and temperature, with *Microcystis* often being dominant. These results indicate that, while increasing temperatures in the future may enhance HABs and alter their community composition, it may be possible to manage their severity through sustained nutrient reductions in the watershed.

For our second objective, we used biweekly sampling in 2019 at three locations on the lake to disentangle the connections between environmental conditions and phytoplankton community composition using multivariate analyses. Additionally, the long-term datasets from the MLO and MLMP allowed us to compare 2019 to previous years to capture how the aberrant weather of 2019 affected the phytoplankton community of Muskegon Lake. With the prevailing uncertainty regarding how future climate scenarios will impact HABs, knowledge of phytoplankton composition in years that experience anomalous weather patterns may be valuable. In 2019, the Muskegon Lake watershed experienced record-breaking amounts of precipitation and a relatively cool temperature regime. The cool spring and late onset of stratification delayed phytoplankton growth overall. Unexpectedly, diatoms were the dominant division throughout the entire 2019 study period, and the cyanobacteria community was diverse but negligible compared to previous years, likely as a result of frequent rain events that reduced residence time and cool temperatures that inhibited their growth. These results may provide insight into how phytoplankton communities, diatoms and HABs in particular, in temperate freshwater lakes may respond to a future climate change scenario in which precipitation is frequent and intense, water levels are highly variable, and some regions experience unexpected cooling.

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Abbreviations

ANOVA – analysis of variance

BOD – biological oxygen demand

DO – dissolved oxygen

GLERL - Great Lakes Environmental Research Laboratory

HAB – harmful algal bloom

L – liter

m – meter

mg – milligram

mL – milliliter

MLMP – Muskegon Lake monitoring program

MLO – Muskegon Lake Observatory

N – nitrogen

NMDS – non-metric multidimensional scaling

EPA – Environmental Protection Agency

NOAA– National Oceanic and Atmospheric Administration

ORP – oxidation reduction potential

P- phosphorus

PAR – photosynthetic active radiation

PCA – principal component analysis

PERMANOVA - permutation multivariate analysis of variance

SIMPER – similarity percentages

SRP – soluble reactive phosphorus

TDS – total dissolved solids

TKN – total Kjeldahl nitrogen

TP – total phosphorus

Chapter 1: Introduction

Introduction

As the main primary producers of aquatic ecosystems, phytoplankton form the foundational layer of the aquatic food web, using sunlight and mainly inorganic forms of nutrients to photosynthesize and produce biomass. Phytoplankton play an important role in aquatic food webs and carbon cycling. Like land plants, they contain the pigment chlorophyll and produce oxygen as a byproduct of photosynthesis, but unlike land plants, they are free-floating organisms that colonize the pelagic zone of aquatic ecosystems and are highly influenced by their surrounding environment (Sigee, 2005; Dodds and Whiles, 2019; Lyons et al., 2014). It is their quick growth cycles, small size, high abundance, and high reactivity that make them useful indicators of change in environmental conditions in a waterbody.

The phytoplankton community is broken down into divisions, a loose group of taxonomically similar individuals. The major divisions of freshwater phytoplankton include blue-greens, greens, euglenoids, yellow-greens, dinoflagellates, cryptomonads, chrysophytes, diatoms, red algae, and brown algae. Phytoplankton have a wide range of preferred habitat, mode of motility, morphology, and appearance and some are naturally more abundant than others (Sigee, 2005). In an unaltered, properly functioning temperate lake, the phytoplankton community shifts over seasons in a predictable pattern in response to changing environmental conditions (Dodds and Whiles, 2019; Sigee, 2005; Sommer, 1989). Maintaining spatial and temporal diversity is key to upholding balance and proper function (i.e. carbon cycling) within the phytoplankton community (Sigee, 2005). Though ubiquitous in nature, it has been discovered that phytoplankton diversity in the continental United States varies systematically across large-scale latitudinal, longitudinal, and altitudinal gradients (Stomp et al., 2011). This diversity is largely controlled by bottom-up mechanisms, such as space, nutrients, temperature, and light, which are affected by climate and land use change (Stomp et al., 2011; Zhang et al., 2016; Ptacnik et al., 2008). Furthermore, diversity positively correlates with resource use efficiency (i.e. carbon cycling), which is especially important in light of the realization that fresh waterbodies are hot spots for carbon cycling (Ptacnik et al., 2008; Ye et al., 2019; Williamson et al., 2009; Anderson et al., 2020a). As ecosystems are subject to intensifying anthropogenic stress, the phytoplankton community is responding, shifting towards decreased taxa diversity and, often, single-taxon dominance (Sigee, 2005; Urrutia-Cordero et al., 2017; Bergkemper at al., 2018); their ability to maintain homeostasis and resist changes to their internal environment is diminishing (Sigee, 2005). One of the greatest threats to the stability and biological integrity of phytoplankton communities in freshwater lakes is the combined effects of anthropogenic eutrophication and climate change.

Freshwater lakes around the world are increasingly suffering the consequences of anthropogenically induced eutrophication (Glibert, 2017; Sinha et al., 2017; Smith, 2003; Anderson, 2002; O'Neil et al., 2012). Eutrophication refers to the excess inputs of nutrients into a waterbody, which increases primary productivity above natural levels (Le Moal et al., 2019; Nixon, 2009; Heisler et al., 2008). Harmful algal blooms (HABs), the excessive growth of a phytoplankton division, are the most concerning symptom of eutrophication and have been increasing around the world for decades (Anderson, 2002; Brooks et al., 2016; Heisler et al., 2008; Paerl and Otten, 2013; Luring et al., 2017; Le Moal et al., 2019; Ho et al., 2019). HABs pose an on-going global threat to fresh waterbodies, and their associated direct and indirect effects on the ecosystems they afflict have both economic and ecological consequences.

As two of the major elements required for phytoplankton growth, nitrogen and phosphorus are the main nutrients stimulating eutrophication and causing HABs. Excessive nutrients entering aquatic ecosystems originate from agricultural fertilizers and wastes, urban runoff and wastewater treatment plant effluent, and industrial pollutants. They are mobilized through atmospheric deposition, groundwater discharge, surface water runoff, and erosion, which are exacerbated by deforestation, destruction of riparian zones, water diversion projects, and climate change (Glibert, 2017; Jenny et al., 2016; Wagner and Adrian, 2009; Lowrance et al., 2018; Paerl and Otten, 2013; Anderson, 2002; Weber et al., 2020). Nitrogen has a complex, biologically driven cycle that includes organic, inorganic, and gaseous forms. Nitrate (NO_3) is generally the most abundant form of nitrogen available to phytoplankton. Other biologically available forms include ammonium (NH_4) and nitrite (NO_2). Organic nitrogen is recycled within the food web, ultimately taking the form of detritus and assimilating into the sediments. Remineralization is the process that returns organic nitrogen to a usable, inorganic form, generally ammonia (NH_3), which can be oxidized to nitrate in the process of nitrification under aerobic conditions. Under anaerobic conditions, nitrogen oxides (NO_3 and NO_2) can be converted to dinitrogen gas (N_2), representing a loss of available nitrogen in the system. Lastly, the process of nitrogen fixation can be carried out by prokaryotes and involves converting $N_{2,}$ an unusable form, to NH_4 (Sigee, 2005). Therefore, nitrogen, in its various forms, can enter a system both internally and externally. One of the major anthropogenic influences on the nitrogen cycle was the invention of the artificial nitrogen fixation process to create fertilizer in the early 1900s. After the mid-1940s, nitrogen-based fertilizer was commercialized and the use of nitrogen expanded rapidly, leading to significant amounts of nitrogen leaching into waterways through groundwater or surface water flow (Glibert, 2017). Atmospheric deposition of nitrogen into lakes is also increasing due to anthropogenic activities, such as fossil fuel combustion and agricultural emissions (Howarth et al., 2002; Paerl, 2017).

Phosphorus is less available in aquatic systems than is nitrogen, only has one biologically usable form (orthophosphate), and cannot be fixed into a biologically available form (Sigee, 2005). Phosphorus can enter a waterbody externally, generally being transferred from land to water through erosion of cultivated land during flow events; small amounts of P added to a waterbody can contribute to eutrophication since ambient levels of phosphorus in freshwater ecosystems are generally low (Howarth et al., 2002). Internal loading of phosphorus from organic material in the sediments of a waterbody due to bacterial decomposition is also of concern. The release of phosphate into the water column is most notably a function of oxygen – under anaerobic conditions, phosphorus is easily diffused from sediments, but under aerobic conditions, phosphorus is adsorbed to sediments – but is also influenced by pH and redox potential (Colborne et al., 2019; North et al., 2014; Sigee, 2005). Phosphorus is also recycled within the waterbody when it is released by lake biota through lysis/death of phytoplankton cells or excretion from higher trophic levels (Sigee, 2005).

Phosphorus has long been considered the limiting nutrient in freshwater lakes; however, nitrogen has been found to also promote eutrophication, can especially benefit certain non-diazotrophic HAB species, and can enhance HAB toxin production, making it important to include both in nutrient management plans (Deng et al., 2014; Glibert, 2017; Chaffin et al., 2018; Le Moal et al., 2019; Filstrup et al., 2017; Jankowiak et al., 2019). Furthermore, HAB species are inclusive of both autotrophs and mixotrophs, as some can utilize both organic and inorganic forms of nutrients for their nitrogen, phosphorus, and carbon demands (Heisler et al., 2008; Paerl, 1988; Sigee, 2005). Overall, the anthropogenically perturbed influx of nutrients shifts the nitrogen:phosphorus ratio, alters the amounts of different forms of nutrients, and shifts the nature of nutrient limitation in the ecosystem, favoring opportunistic species, which outcompete others in the form of a HAB (Elser et al., 2009; Anderson, 2002).

The most common phytoplankton division creating HABs in freshwater lakes around the world is cyanobacteria (*Cyanophyta*, blue-green algae; Anderson, 2002). At 3.5 billion years old, they are the oldest known oxygen-producing organisms on earth and are largely responsible for the Great Oxygenation Event (Paerl and Huisman, 2009; Lyons et al., 2014). Cyanobacteria are prokaryotes, exist in unicellular and colonial forms, and thrive in nearly all freshwater, marine, and terrestrial habitats around the world (Sigee, 2005). Their widespread success is due to their myriad adaptations that allow them to outcompete other phytoplankton under favorable conditions. These adaptations include a higher temperature tolerance than other phytoplankton, the production of over-wintering akinetes, the ability to use buoyancy control to regulate their position in the water column, efficient nutrient uptake and use, the ability to access organic phosphorus and store luxury phosphorus, the ability to fix nitrogen in some species, the ability to photosynthesize at low carbon dioxide concentrations and high pH, efficient light harvesting due to accessory pigments, and resistance to damaging radiation (Carey et al., 2011; O’Neil et al., 2012; Paerl and Otten, 2013; Smith, 2003; Sigee, 2005). Unsurprisingly, the cyanobacteria are a diverse group. Species have differing preferred nutrient regimes, and the amount, form, and ratio of nutrients in a waterbody, along with temperature and other environmental parameters, will influence their community composition. Therefore, climate and land use change will alter and dictate the composition of the cyanobacteria community as well as that of the phytoplankton community as a whole (Glibert, 2017; Heisler et al., 2008; Rigosi et al., 2014). *Microcystis* Lemmermann is one of the most common cyanobacteria genera found in freshwaters worldwide, and its abundance is increasing in response to climate change and eutrophication. *Microcystis* can form nuisance blooms, produce toxins, regulate buoyancy, use organic phosphorus, and store luxury phosphorus (Šejnohová and Maršálek, 2012). A common species of *Microcystis*, *M. aeruginosa*, displays wide genetic diversity and a highly plastic genome that has allowed it to thrive under variable environmental conditions world-wide (Humbert et al., 2013; Xu et al., 2010). *Microcystis* growth has been shown to positively correlate primarily with high water temperatures (20-25 °C) and with several forms of both phosphorus and nitrogen (Donald et al., 2013; Davis et al., 2009; Xu et al., 2010).

The effects of climate change are exacerbating the severity of HABs because they create conditions in which cyanobacteria have an advantage over other phytoplankton (Sinha et al., 2017; Paerl and Otten, 2013; Paerl and Huisman, 2009; Moss et al., 2011). Importantly, inland waterbodies have rapidly warmed since 1985 (Schneider and Hook, 2010; O’Reilly et al., 2015). Warmer water temperatures benefit cyanobacteria in several ways, including catering to their higher temperature preference, extending the growing season, increasing their geographic range, and strengthening vertical stratification in the water column (Rigosi et al., 2014; Deng et al., 2014; Jöhnk et al., 2008; Ho and Michalak, 2019; Paerl and Huisman, 2008). Enhanced stratification has several benefits, including allowing buoyancy-regulating cyanobacteria easier navigation in a more stable water column, which leads to longer bloom duration, and intensifying hypoxic conditions (dissolved oxygen < 2 mg/L), which can lead to internal nutrient loading (Carey et al., 2011; Peeters et al., 2007; Paerl and Huisman, 2008; Wagner and Adrian, 2009; Watson et al., 2016; Diaz and Rosenberg, 2008). Besides increasing temperatures, the other major way climate change benefits cyanobacteria is through altered precipitation regimes. Several models predict increased precipitation and decreased return times on rain events (e.g. a 100-year storm will occur more frequently than every 100 years) (Sinha et al., 2017; Huntingford et al., 2003; Byun and Hamlet, 2018). Increased precipitation events may mitigate blooms due to a dilution and flushing effect (Ho and Michalak, 2019), but it is possible that heavy precipitation events interspersed by droughts will flush nutrients into waterbodies where they will subsequently concentrate and promote blooms (Paerl and Huisman, 2008), a phenomenon that has been observed in the San Francisco Estuary and several Florida lakes (Lehman et al., 2017; Havens et al., 2019). Droughts increase water residence time and decrease water depth, promoting persistent stratification and increasing water temperatures, which buoyancy-regulating, temperature-tolerant cyanobacteria can take advantage of (Carey et al., 2011; Paerl and Huisman, 2009). Therefore, it is projected that cyanobacterial HABs are likely to increase in duration, severity, and range under future climate scenarios, making management of HABs more difficult (Havens and Paerl, 2015; Carey et al., 2011).

HABs have long been noted in the coastal ocean, particularly in the Gulf of Mexico where a large "dead zone" forms due to hypoxia, but they are becoming a major threat to inland waterbodies and are expected to increase in severity (Rabalais et al., 2002; Paerl, 1988; Brooks et al., 2016; Chapra et al., 2017; Hallegraeff, 1993). The reason HABs are concerning is the myriad negative effects they have on ecosystems, wildlife, and humans. HABs cause a physical disturbance in the ecosystem by reaching high biomass to create unsightly blooms, mats, foams, or scums. This diminishes the photic zone, reducing the light environment for macrophytes and other phytoplankton, and can contribute to hypoxia upon bloom die-back and subsequent decomposition (Scavia et al., 2014; Sweerts et al., 1991; Biddanda et al., 2018; Diaz and Rosenberg, 2008). Hypoxia negatively impacts the food web through alteration of organism behavior to avoid hypoxic zones, which can impact organism interactions, and, in severe cases, can cause mass organism mortality (i.e. fish kills; Watson et al., 2016; North et al., 2014; Weinke and Biddanda, 2018). Some cyanobacteria produce toxins, which have caused illness and mortality in wild and farmed fish and shellfish, human illness and death, illness and death in domestic animals, and other wildlife deaths in addition to closing beaches and contaminating drinking water (Backer et al., 2015; Azevedo et al, 2002; Carmichael et al., 2016). HABs can also alter the food web through their resistance to grazing, ability to create microhabitats that are not suitable for growth of other phytoplankton, and tendency to comprise a large portion of the phytoplankton community (Wilson et al., 2006; Šejnohová and Maršálek, 2012; Arnold, 1971; Sigee, 2005). This effectively lowers the quality of the base of the food web, constrains energy transfer to higher trophic levels, and may force shifts in co-evolutionary dynamics between cyanobacteria and planktivorous grazers (Dickman et al., 2008; Ger et al., 2016). In addition to being ecologically harmful, HABs cause significant economic damage through decreasing water quality, inhibiting recreational opportunities (fishing, swimming, boating, etc.); endangering wildlife health, forcing recovery efforts; causing fish kills due to hypoxia, which affects both fisheries and tourism; contaminating drinking water, leading to costly clean up and filtration processes; and decreasing property values due to diminished aesthetics and safety in the waterbody (Dodds et al., 2008; Hoagland et al., 2002).

Muskegon Lake, Michigan, is a drowned river mouth Great Lakes estuary with annual occurrences of HABs, often dominated by *Microcystis*. Its main inflow is the Muskegon River, its outflow is through a navigation channel into Lake Michigan, and it drains the second largest watershed in the state (Figure 1). Intense abuse of the lake began in the mid-1800s, starting with immense lumber activity and transitioning into paper, coal, metal, foundry, and other industries, which lead to impairments such as hardening of the shoreline, filling/destruction of wetlands, filling of the littoral zone by sawmill debris and foundry slag, and contamination of the sediments with heavy metals (copper, lead, cadmium, and mercury), nutrients, and polycyclic aromatic hydrocarbon compounds (Gillet and Steinman, 2011; Rediske et al., 2002). Muskegon Lake was designated as an Area of Concern by the EPA in 1987. As such, a remedial action plan was developed, indicating nine beneficial use impairments targeted for improvement. Restoration efforts ensued, including several large projects that involved softening and restoration of the shoreline; sediment remediation and removal; restoration of wetlands, open water habitats, and streambanks; and re-establishment of fish passages and hydrological connectivity with surrounding waterbodies (Evans et al., 2018). The major ongoing monitoring efforts of Muskegon Lake include the long-term monitoring program, which began in in 2003 (Steinman et al., 2008). This monitoring is headed by the Steinman Lab at the Annis Water Resources Institute (AWRI) and samples six sites on Muskegon Lake three times per year. Additionally, a state-of-the-art observatory buoy, the Muskegon Lake Observatory (MLO, www.gvsu.edu/buoy), was installed in the geomorphic "middle" of the lake in 2011 (Figure 2). The MLO buoy is operated by the Biddanda Lab at AWRI and provides scientists and the public with high-frequency time-series meteorological data and water quality data from throughout the water column. Both the long-term monitoring program and the MLO have been the basis of many research projects at AWRI and play a significant role in this thesis. While the HAB impairment in the lake has largely been improved upon as a result of the intensive restoration efforts, annual occurrences of blooms still remain and toxin-producing species have been documented, warranting their continued study, especially under the impending threat of climate change.

Purpose

The purpose of this research was three-fold. First, we aimed to detail historical occurrences of cyanobacterial harmful algal blooms (HABs) in Muskegon Lake. This historical perspective provided an encouraging update on the progress being made by restoration efforts, allowed us to connect HAB dynamics with environmental variables, and provided a basis by which to compare our main study year, 2019. The second purpose of this thesis was to quantify and understand the phytoplankton community in Muskegon Lake, Michigan, in 2019 through spatial and temporal dynamics. Specifically, we aimed to describe changes in the phytoplankton community over time and space through correlated environmental variables. Lastly, an unexpected purpose that emerged throughout our study year was to understand how the anomalous weather patterns of 2019, which was characterized by high precipitation and low temperatures, affected the phytoplankton community. This provided insight into a much larger objective: an alternate understanding of how climate change may impact cyanobacterial HABs and the phytoplankton community as a whole if some regions differ from the global patterns or annual weather patterns become more variable. Fulfilling these objectives may provide insight into phytoplankton community patterns in other similar drowned river mouth estuaries in a temperate climate zone, particularly in the Great Lakes region.

Scope

Conclusions made from this research on Muskegon Lake, Michigan can be applied to other drowned river mouth Great Lakes estuaries along the east coast of Lake Michigan and, to a lesser extent, other freshwater estuaries within a temperate climate zone. Cautiously, conclusions drawn here may also be used to aid understanding of quantitative and qualitative cyanobacterial harmful algal bloom (HAB) patterns in temperate, dimictic, inland lakes that are morphometrically similar to Muskegon Lake. As most phytoplankton, and particularly the cyanobacteria, are ubiquitous, it is possible to apply the knowledge gained about the behavior of specific genera more broadly. It is also our goal to encourage the use and development of long-term data sets, such as those used in this thesis, to identify patterns in the phytoplankton communities of a wide range of aquatic habitats and to understand how climate change is affecting them.

Assumptions

1) We assume that, for all environmental, chemical, and phytoplankton samples in all data sets used, the values recorded are accurately representative of the general values of the location and time in which they were recorded; we assume that our values are not outliers.

2) We assume that measurements collected by the Muskegon Lake Observatory buoy in the geographic center of the lake are generally representative of the whole lake.

3) We assume no significant variation in technique between the two analysts (J. M. and I. S.) performing pigment analysis.

4) We assume that the values reported by the spectrophotometer and the wet chemistry analyst (B. S.) are correct.

5) We assume that our identifications of the phytoplankton genera present are correct and do not vary significantly between analysts (J. M, I. S., and M. L.).

6) We assume that there is no significant difference in phytoplankton community composition between 2019 biweekly sites and the three sites used from the Muskegon Lake monitoring program.

Hypothesis

Historical trends of HABs in Muskegon Lake

1) HABs have quantitatively decreased over time in terms of biovolume and percentage of the phytoplankton community in response to remediation efforts (reduction in nutrients).

2) Annual cyanobacteria community composition is driven by variation in temperature and nutrient form and amount since genera have varying preferences and requirements regarding temperature and nutrients.

2019 – spatiotemporal trends and effects of a cold, wet year – a posteriori

3) Community composition will be similar at the Muskegon Lake Observatory (MLO) and Channel sites and different at the River site due to the markedly different hydrologic and physical conditions regarding turbidity, flow rate, temperature, and stratification.

4) Community composition will be similar in the spring and summer but different in the fall because water temperature was low in the spring and summer relative to previous years and did not reach average values until the fall; temperature is often a main driver of phytoplankton community composition.

5) HABs will peak at a later date than usual due to the cool spring.

6) The influx of nutrients from the unusually heavy precipitation of the year 2019 will lead to severe HABs in the late summer and fall as temperatures rise.

7) The HAB community will be dominated by genera more tolerant of cooler water temperatures rather than *Microcystis*, which is usually dominant in Muskegon Lake.

Significance

This research will aid in understanding the long-term effects that remediation and management efforts have on cyanobacterial harmful algal bloom (HAB) abundance and composition in a Great Lakes drowned river mouth estuary. Specifically, this study highlights the marked improvements Muskegon Lake, Michigan, has experienced in terms of HABs, which may aid in its eventual removal as an Area of Concern. Additionally, the mixed effects that management efforts and impending climate change will have on cyanobacteria is a highly relevant topic in today’s world. Finally, the aberrant weather patterns of the year 2019 offered the opportunity to provide an alternate take on how climate change may affect HABs, especially in the Great Lakes region.

Definitions

Cyanobacteria – a division of microorganisms; prokaryotic and related to bacteria but are capable of photosynthesis and considered part of the phytoplankton community.

Diatoms – a division of microorganisms; eukaryotic and part of the phytoplankton community; characterized by silica cell walls.

Diazotrophic – capable of fixing nitrogen.

Drowned river mouth estuary – the interface between a river and the waterbody it is draining into in which a slow-moving lake forms.

Eutrophication – an introduction of excessive amounts of nutrients into an aquatic system, usually originating from urban, agricultural, and industrial sources, that causes excessive primary production and, often, hypoxia.

Harmful algal blooms (HABs) – the excessive growth of a division of algae such that harm is caused through alteration of the ecology of the system, presentation of health threats, and existence of a nuisance or threat to humans.

Hypoxia – low dissolved oxygen (DO) concentration; < 4 mg/L DO = mild, < 2 mg/L DO = severe.

Inorganic nitrogen – non-carbon containing nitrogenous compounds; many forms usable by plants and algae; includes nitrate, nitrite, ammonium, ammonia, and di-nitrogen gas.

Non-metric multidimensional scaling (NMDS) – a non-eigenvector-based method that operates on a dissimilarity matrix in which objects are represented along a predetermined number of axes, preserving the ordering relationships among them.

Organic nitrogen – nitrogen occurring in organic compounds, including urea, proteins, nucleic acids, amino acids, and nucleotides; generally non-usable by plants and algae.

Phytoplankton – free-floating photosynthetic microorganisms, including algae and cyanobacteria (Sigee, 2005).

Principal component analysis (PCA) – an eigenvector-based method that operates on an association matrix in which raw data is displayed in a multidimensional ordination such that dissimilarity is preserved among sites and variables; maximizes variation explained by variables.

Soluble reactive phosphorus – measure of orthophosphate; the chemically active, dissolved, inorganic form of phosphorus used by plants and algae.

Total Kjeldahl nitrogen – nitrogen occurring in organic compounds, ammonia, and ammonium.

Total phosphorus – inclusive of all organic (within plant and animal biomass) and inorganic. (orthophosphate) forms of phosphorus.

Figure Legends

Figure 1. A bathymetric map of Muskegon Lake. The shade of blue indicates relative depth, with darker shades indicating greater depth. The Muskegon River, which inflows into Muskegon Lake, is in the upper right corner. The navigation channel, which outflows into Lake Michigan, is in the lower left corner. The site of the Muskegon Lake Observatory buoy is denoted by a red circle.

Figure 2. A schematic diagram of the Muskegon Lake Observatory buoy. The meteorological sensors are situated atop the large buoy. The water quality sensors are located throughout the water column on a smaller buoy that is attached to the larger buoy (the power source and anchor) with a cable. Sensors acquire and report weather data every five minutes and water quality data every 15 minutes. The acoustic doppler current profiler (ADCP) is located on the lake bottom. However, ADCP data was not used in this thesis.

Figures

Figure 1.

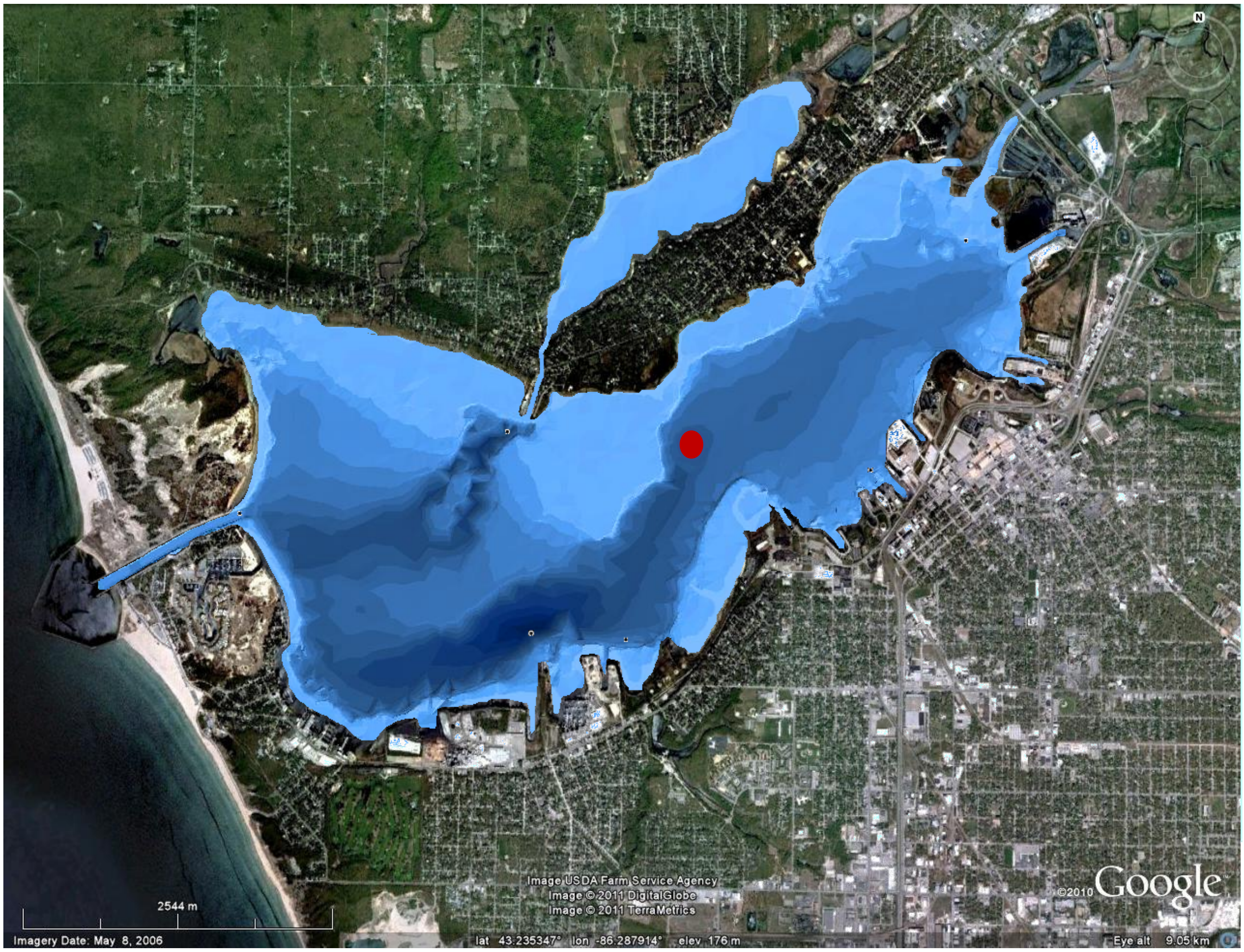
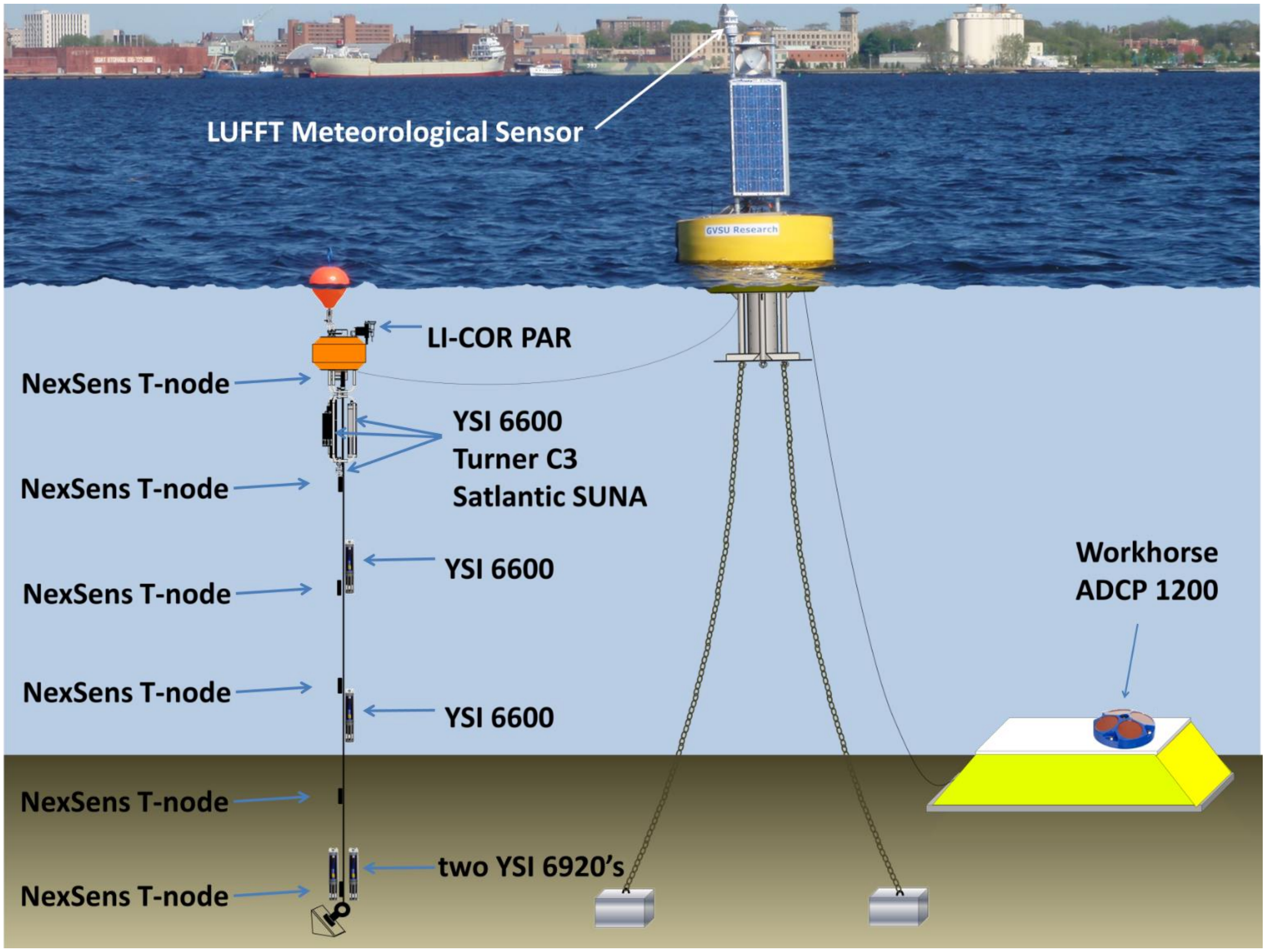


Figure 2.



LUFFT Meteorological Sensor
GVSU Research
LI-COR PAR
NexSens T-node
YSI 6600
Turner C3
Satlantic SUNA
NexSens T-node
YSI 6600
NexSens T-node
NexSens T-node
YSI 6600
Workhorse
ADCP 1200
NexSens T-node
two YSI 6920's
NexSens T-node

Chapter 2: Manuscripts

Chapter 2.1

Title

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

Abstract

Around the world, freshwater lakes are increasingly suffering from harmful algal blooms (HABs) as a result of anthropogenic eutrophication. Muskegon Lake, a drowned river mouth Great Lakes estuary, was declared an Area of Concern by the EPA in 1987 with nuisance algal blooms cited as a beneficial use impairment. Using long-term data and additional 2019 sampling, we performed multivariate and univariate analyses on environmental and phytoplankton data to visualize variation over time. The objective of this study was to quantify changes in HAB prevalence and community composition in Muskegon Lake over 16 years (2003-2019) and explore the environmental factors potentially driving the change. Principal component analysis (PCA) was used to visualize variation and patterns in environmental variables over time, non-metric multidimensional scaling (NMDS) was used to assess associations between annual community composition and environmental variables, and a univariate comparison (paired T-test or Wilcoxon Rank Sum test) was made on environmental variables between a group of severe-HAB years and a group of mild-HAB years. Analyses revealed that, despite rising water temperatures, a reduction in nutrient concentrations has led to decreases in HAB abundance over time. Additionally, HAB community composition appears to be driven by nutrient form and concentration and temperature, with *Microcystis* Lemmermann often being dominant. These results indicate that, while increasing temperatures in the future may enhance HABs and alter their community composition, it is possible to manage their severity through sustained nutrient reductions.

Introduction

Around the world, freshwater lakes are increasingly suffering from the presence of harmful algal blooms (HABs) as a result of anthropogenic activity (Smith, 2003; Anderson, 2002; O’Neil et al., 2012; Ho et al., 2019). HABs are a result of eutrophication, or the introduction of excess nutrients into a waterbody (Heisler et al., 2008). Eutrophication causes an imbalance in ecosystem function as waterbodies shift from a nutrient-limited environment to an environment characterized by nutrient saturation, increased primary production, and limited light penetration (Filstrup et al., 2017; Le Moal et al., 2019). Nutrients responsible for eutrophication originate from agricultural fertilizers and wastes, urban runoff and wastewater treatment plant effluent, and industrial pollution and are exacerbated by deforestation, the removal of riparian zones, water diversion projects, and climate change (Glibert, 2017; Lowrance et al., 2018; Paerl and Otten, 2013; Weber et al., 2020).

When these nutrients enter aquatic ecosystems through atmospheric deposition, groundwater discharge, surface water runoff, and soil erosion, they contain different nutrient concentrations, forms, and ratios than that of the waterbodies they are entering (Anderson, 2002). While it is agreed that nitrogen (N) and phosphorus (P) are the nutrients that stimulate primary production in waterbodies as two of the main elements required for phytoplankton growth, there is still some debate surrounding the question of which nutrient and which forms of each nutrient are of more concern, whether total amounts or ratios are more important, and whether management should focus on a duel- or single-nutrient plan (Heisler et al., 2008; Filstrup et al., 2017; Paerl et al., 2016; Søndergaard, et al., 2017; Chaffin et al., 2018). At any rate, the influx of nutrients shifts the N:P ratio, alters the amounts of nutrient forms, and shifts the nature of nutrient limitation in the ecosystem, potentially favoring opportunistic species, which outcompete and replace their counterparts in the form of a HAB (Elser et al., 2009; Anderson, 2002).

The most common division of phytoplankton creating HABs in freshwater lakes is cyanobacteria, also known as blue-green algae (Anderson, 2002). Cyanobacteria are the oldest known oxygen-producing organisms on Earth at 3.5 billion years old and are largely responsible for the Great Oxygenation Event (Lyons et al., 2014). Cyanobacteria have a wide variety of adaptations that allow them to outcompete other phytoplankton under favorable conditions; these include high temperature tolerance, ability to produce over-wintering akinetes, ability to regulate their position in the water column via buoyancy control, ability to access organic P and store luxury P, N-fixing ability for some species, and efficient light harvesting (Carey et al., 2011; O'Neil et al., 2012; Paerl and Otten, 2013). These physiological traits may afford cyanobacteria several advantages over other phytoplankton in a world altered by climate change and other anthropogenic effects. However, the cyanobacteria are an extremely diverse group, and their traits and adaptations vary widely between species, indicating that climate and land use change will alter the composition of the cyanobacteria community as well as that of the phytoplankton community as a whole. *Microcystis* Lemmermann is one of the most common cyanobacteria genera found in freshwaters worldwide. *Microcystis* can form nuisance blooms, produce toxins, regulate buoyancy, use organic P, and store luxury P (Šejnohová and Maršálek, 2012). It displays no ecotype differentiation; its wide genetic diversity within and between species has allowed it to thrive under myriad environmental conditions (Humbert et al., 2013; Xu et al., 2010). *Microcystis* growth has been shown to positively correlate primarily with high water temperatures, with optimal growth rates reported to be around 20–25 °C, and with several forms of both P and N (Donald et al., 2013; Davis et al., 2009; Xu et al., 2010). Studies have shown that *Microcystis* generally prefers reduced forms of N (Šejnohová and Maršálek, 2012; Newell et al., 2019; Gobler et al., 2016) but switches to NO_3 when necessary and may prefer it under saturated P conditions (Donald et al., 2013). They can use all forms of inorganic N except for N_2, as they are non-diazotrophic. Additionally, they are generally more limited by N than P. *Microcystis* prefers orthophosphates but can use organic P and usually requires less than 0.03 mg/L P for bloom-formation (Donald et al., 2013; Šejnohová and Maršálek, 2012).

Climate change is exacerbating the severity of HABs. Inland waterbodies around the world have experienced rapid warming since 1985 (Schneider and Hook, 2010; O'Reilly et al., 2015). With increasing temperatures, particularly over 20º C, cyanobacteria will have an advantage over other algae due to their high growth rates in a higher temperature range. Additionally, warming temperatures may lead to a longer growing season, which perpetuates earlier bloom onset and longer duration (Deng et al., 2014; Paerl and Huisman, 2008; Ho and Michalak, 2019). Higher temperatures also increase the intensity and duration of stratification within lakes, allowing buoyancy-regulating cyanobacteria, such as *Microcystis,* and those with a low sinking velocity easier navigation and less sedimentation within a more stable water column (Carey et al., 2011). Additionally, longer periods of stratification combined with increased decomposition within the hypolimnion due to bloom diebacks can lead to intensified periods of hypoxia. Hypoxia is the condition of low dissolved oxygen (DO) in a waterbody, generally referring to DO below 2 mg/L, as this is, generally, the threshold at which life can no longer be sustained (Scavia et al., 2014; Biddanda et al., 2018; Diaz and Rosenberg, 2008). In addition to reducing oxygen availability to organisms, hypoxia may induce nutrient release from the sediments, particularly P since the P-binding capacity of sediments is, in part, affected by DO concentration, which buoyant cyanobacteria can access and use (Watson et al., 2016; Diaz and

Rosenberg, 2008). Occurrences and severity of hypoxia are increasing due to eutrophication - globally, the number of hypoxic and anoxic zones has doubled each decade since the 1960s - with detrimental effects on fisheries, biodiversity, and food webs (Diaz, 2001; Diaz and Rosenberg, 2008). It is also theorized that warming water temperatures may increase diazotrophic cyanobacteria species' ability to fix nitrogen and that increased atmospheric carbon dioxide concentrations may benefit the growth of intercepting cyanobacteria near the water surface (Visser et al., 2016). In addition to temperature, global changes in precipitation events may enhance bloom formation. While some have found that increased precipitation events may mitigate blooms due to dilution and flushing effects (Ho and Michalak, 2019), others have theorized that heavy precipitation events interspersed by droughts will flush nutrients into waterbodies where they will subsequently concentrate and promote blooms (Paerl and Huisman, 2008), a phenomenon that has been observed in the San Francisco Estuary and several Florida lakes (Lehman et al., 2017; Havens et al., 2019). It is projected that cyanobacterial HABs are likely to increase in duration, severity, and range under future climate scenarios, making management of them more difficult (Havens and Paerl, 2015; Carey et al., 2011).

As their name suggests, HABs are ecologically and economically harmful. Non-toxic HAB species cause harm through their physical structure by reaching high biomass, causing unsightly blooms, mats, foams or scums; diminishing the photic zone, which reduces the amount of macrophytes and other algae; and inducing hypoxia by increasing phytoplankton biomass, which eventually dies and sinks to the hypolimnion where benthic heterotrophs deplete the oxygen supply during decomposition (Sweerts et al., 1991; Scavia et al., 2014; Le Moal et al., 2019). Some HAB species produce toxins and may cause illness and death in wildlife, domestic animals, and humans in addition to closing beaches and contaminating drinking water (Backer et al., 2015; Carmichael et al., 2016). The most prevalent toxins are hepatotoxins (affecting the liver) followed by neurotoxins (affecting the brain) (O'Neil et al., 2012). In addition, some cyanobacteria species produce toxins that affect the skin (dermatotoxins), kidneys (nephrotoxins), cells (cytotoxins), and can cause gastroenteritis. Some HAB toxins are potent and dangerous at low concentrations, and toxin production may increase under atypical nutrient regimes (Anderson, 2002; Hallegraeff, 1993; Newell et al, 2019). Some of the most common toxin-producing genera include *Dolichospermum* Wacklin, Hoffmann, & Komárek (formerly *Anabaena*), *Microcystis, Oscillatoria* Vaucher ex Gamont, *Planktothrix* Anagnostidis & Komárek, *Nostoc* Vaucher ex Bornet & Flahault, *Aphanizomenon* Morren ex Bornet & Flahault, *Raphidiopsis* Fritsch & Rich (formerly *Cylindrospermopsis*), and *Anabaenopsis* Miller species. Another way HABs cause harm is by altering the food web. Many HAB species are resistant to grazing due to their morphology causing mechanical interference in feeding appendages, the toxins they produce, and/or their comparatively low levels of essential nutrients (Wilson et al., 2006; Šejnohová and Maršálek, 2012). Their resistance to grazing, combined with their ability to outcompete other algae, create microhabitats that are not suitable for the growth of other algae, and comprise a large portion of the primary producer trophic level, lowers the quality of the base of the food web (Sigee, 2005). As a low-quality food source, HAB species constrain energy transfer up the food web and may force shifts in co-evolutionary dynamics between cyanobacteria and their grazers (Dickman et al., 2008; Ger et al., 2016). In addition to causing ecological harm, HABs cause economic damage through diminishing the recreational (boating, fishing, swimming, etc.) and aesthetic value of waterbodies, decreasing waterfront real estate, forcing recovery of threatened and endangered species, and contaminating drinking water sources, resulting in costly clean up processes. Combined economic losses resulting from eutrophication in freshwaters of the U.S. is estimated to be in the range of $2.2 billion to greater than $4 billion annually (Dodds et al., 2008; Kudela et al., 2015).

Muskegon Lake (Muskegon, Michigan, USA) is a mesotrophic drowned river mouth Great Lakes estuary along the eastern shore of Lake Michigan. A drowned river mouth estuary is the interface between a lentic and lotic system in which a river essentially widens into a lake before emptying into a larger lake (Larson et al., 2013). Drowned river mouth estuaries provide a unique ecosystem in which physical and chemical attributes create predictable spatial patterns. They are highly productive, their wide range of habitats enables them to host many species, they are often focal areas of economic importance, and they undergo significant anthropogenic stress (Larson et al., 2013). Muskegon Lake has historically suffered from annual occurrences of HABs due to a history of lumber and industry along the lakeshore that degraded the quality of the lake (Gillett and Steinman, 2011). After being designated as an Area of Concern by the Environmental Protection Agency in 1987, remedial action plans were developed, with nuisance algae being cited as one of the beneficial use impairments targeted for improvement. Between 1987 and 2002, nine beneficial use impairments were developed as part of a remedial action plan, and delisting targets were identified for five of them, inspiring management, research, and restoration efforts for the lake (Steinman et al., 2008). Among these efforts were the implementation of the long-term Muskegon Lake monitoring program (MLMP; 2003-present) and the installation of the Muskegon Lake Observatory buoy (MLO, 2011-present), the data from which allow scientists, managers, and the public to monitor the status of the lake. The objectives of this study were 1) to quantify the changes in HAB abundance and community composition in Muskegon Lake over the past 16 years (2003-2019) using long-term data sets and 2) to understand the environmental variables potentially driving the change. We hypothesize that the HAB impairment in Muskegon Lake has largely improved with remediation efforts and that their abundance and community composition are driven by variation in temperature and nutrient form and amount.

Methods

Study Site

Muskegon Lake (43.23° N, 86.29° W), located in Muskegon, Michigan, is a mesotrophic drowned river mouth Great Lakes estuary that drains Michigan's second largest watershed into Lake Michigan. Its main inflow is the Muskegon River, and it outflows through a man-made navigation channel (Figure 1). Muskegon Lake has a surface area of 17 km^2 and a water volume of 119 million m^3. It has a mean water depth of 7 m, a maximum water depth of 21 m, and a seasonally variable average hydraulic residence time of 23 days (Liu et al., 2018). Management, research, and restoration efforts aim to improve the quality of Muskegon Lake and eventually delist it as an AOC (Steinman et al., 2008).

Overview of Data Sets Used

Data used in this study spans 16 years (2003-2019) and comes from two separate data sets: the Muskegon Lake monitoring program ("MLMP") and data collected separately during the 2019 field season ("2019 data"). Additionally, high-frequency, time-series data from the MLO is used for supporting or explaining trends over the study period when applicable.

MLMP data has been collected three times per year (during the spring [May], summer [July], and fall [September/October]) since 2003. Sample collection occurs at six sites on the lake: near the Muskegon River inflow (MUSR), the Muskegon Lake outflow channel (CHAN), the inflow from Ruddiman Creek (RUDD), the inflow from Bear Lake (BEAR), the Annis Water Resources Institute (AWRI), and over the deepest point in Muskegon Lake (DEEP; Figure 1). 2019 data was collected biweekly from 30 April to 18 October at three sites on the lake: the Muskegon River inflow (River site), the Muskegon Lake Observatory buoy (MLO site), and the

Muskegon channel outflow (Channel site; Figure 1). The MLO (www.gvsu.edu/buoy) is located in the morphometric center of the lake (43.23º N, 86.28º W; Figure 1) and is managed by the Biddanda Lab at the Annis Water Resources Institute of Grand Valley State University with assistance from NOAA GLERL's Lake Michigan Field Station. Since 2011, the MLO has been delivering high-frequency, time-series meteorological and water quality data throughout the water column for about seven months each year (Biddanda et al., 2018). The MLO is equipped with YSI (Yellow Springs Instruments) 6600/6920 datasondes throughout the water column at depths of 2, 5, 8, and 11 m, a C3 Submersible Fluorometer (Turner Designs) at 2 m, a Submersible Ultraviolet Nitrate Analyzer (Satlantic) at 2 m, a photosynthetically active radiation sensor (LI-COR) at 2 m, and temperatures nodes (NEXENS) at 2, 4, 6, 8, 10, and 11 m. The MLO measures water quality and meteorological data every 15 minutes and 5 minutes, respectively; however, data used in this study are daily-averaged values. For an overview of the data used in this study, see Table 1.

Data Collection

Environmental Variables

All environmental data for the entire study period (2003-2019) is from the MLMP data set. Water quality parameters considered for this study were collected at 1 m at each site and include dissolved oxygen (DO), pH, temperature, specific conductivity, chlorophyll *a*, and nutrients (soluble reactive P [SRP], sulfate [SO_4], total P [TP], ammonia [NH_3], nitrate [NO_3], total Kjeldahl N [TKN], and chloride). Data was collected by and retrieved from the Steinman Lab at the Annis Water Resources Institute (see Steinman et al., 2008 for methodology).

Time-series data from the MLO was used in this study to add clarification to seasonal or yearly trends of variables in the lake that may have relevance to the severity of the HABs. In particular, trends in temperature, chlorophyll *a* concentration, and phycocyanin concentration at 2 m in the water column are displayed here. However, MLO data is only useful in describing trends in 2011 and subsequent years, as this is the year the MLO was installed. Periods of missing data are due to necessary maintenance and service, and no attempt was made to extrapolate data to fill the gaps as to preserve the accuracy of interpretation.

Phytoplankton

Phytoplankton data was provided by both the MLMP and 2019 data sets. For years 2003-2016, phytoplankton data was provided by the MLMP. Whole water phytoplankton samples were collected from near the surface and bottom at each site with a van Dorn sampler and subsequently analyzed by Dr. Mark Luttenton at Grand Valley State University (see Steinman et al., 2008 for methodology). MLMP phytoplankton data for subsequent years has not been analyzed. Phytoplankton data for the year 2019 is provided by the 2019 dataset. Samples were collected at each site using a 20 µm mesh plankton net to sample 10 m of the water column. Due to the varying depths at the three sites, two meters of the water column was sampled five times at the River and Channel sites, and 10 meters of the water column was sampled one time at the MLO site. Samples were concentrated to 100 mL and stored in a 125 mL brown Nalgene bottle in the field. All equipment was rinsed with site water prior to use. Bottles were placed in a cooler immediately after collection, and 3%-5% Lugol’s Iodine solution was added upon return to the lab for preservation. To enumerate the phytoplankton, we gently inverted the sample several times before placing 1 mL of sample in a Sedgewick rafter counting chamber using a pipette, let it settle for at least 10 minutes, and used the 20X and 40X objectives of the Nikon Ti-U and Nikon Eclipse Ni-U DIC/Epi-fluorescence Compound microscopes to identify individual phytoplankton to genus until reaching 350 natural algal units. We used Wehr et al. (2002), Prescott (1973), and Bellinger and Sigee (2010) as reference texts. We then scaled each sample up to represent the number of natural algal units per mL of water. Using biovolume data collected from the MLMP, we converted our abundance data (count/mL) to biovolume data (µg/mL) to make the two data sets comparable. As 2019 data was collected at three sites (River, MLO, and Channel) and MLMP data was collected at six sites, phytoplankton data for the three MLMP sites that most closely matched the 2019 sampling sites were used: MUSR, DEEP, and CHAN (Figure 1). The phytoplankton community composition for each year (2003-2016 and 2019) was analyzed to genus level; here, we consider only the division cyanobacteria, inclusive of genera comprising over 5% of the total biovolume.

Statistical Analysis

Changes in environmental variables over time

Environmental data from the MLMP from all six sites and 17 years was used in a principle component analysis (PCA) correlation biplot to visualize variation and patterns in the dataset over time. An ordination was created for each season (spring, summer, and fall) as to account for seasonal variability. Years are grouped using the *ordiellipse* function in the R package vegan, creating a polygon for each year based on the standard deviations of the points. Variables originally included in the ordinations were SRP, turbidity, TKN, NO_3, TKN:NO_3, chloride, temperature, SO_4, specific conductivity, total dissolved solids (TDS), ORP, DO, NH_3, TP, pH, and chlorophyll *a*. Variables were removed if they lacked explanatory power (i.e. had short eigenvector lengths). Years 2006 and 2015 were omitted from the spring PCA due to missing data. Variables kept in the spring ordination included SRP, turbidity, TKN:NO_3, chloride, temperature, SO_4, specific conductivity, TDS, TKN, ORP, and NO_3. Those omitted were DO, NH_3, TP, pH, and chlorophyll *a*. Variables kept in the summer ordination include SRP, chloride, SO_4, specific conductivity, TDS, pH, DO, temperature, chlorophyll *a*, TKN, NH_3, and TP. Variables omitted were NO_3, TKN:NO_3, turbidity, and ORP. Variables kept in the fall ordination include SO_4, NO_3, TKN:NO_3, TKN, SRP, chlorophyll *a*, temperature, pH, specific conductivity, and TDS. Variables omitted were turbidity, NH_3, ORP, TP, chloride, and DO. The R package vegan and was used in RStudio Version 1.1.453 to create the ordination and figures (Oksanen et al., 2018; R Core Team, 2018).

Chlorophyll *a* concentration, used as a proxy for phytoplankton abundance; phycocyanin concentration, used as a proxy for cyanobacteria abundance; and temperature data collected at 2 m by the MLO are displayed in time-series graphs to demonstrate the change in these variables over time and to help explain trends seen in the multivariate ordinations. These data go back to 2011, the first year of operation for the MLO buoy.

Changes in the cyanobacteria community over time

The change in the cyanobacteria community was assessed both quantitatively and qualitatively. Quantitatively, the percentage of the phytoplankton community that was comprised of cyanobacteria was calculated for each year based on biovolume. Additionally, the total annual cyanobacterial biovolume was calculated by adding the cyanobacterial biovolume of all sampling dates each year. Again, for the purpose of making the two datasets as comparable as possible, only data from the MUSR, DEEP, and CHAN sites were used from the MLMP data, as these sites most closely match the locations of the 2019 sampling sites. Total cyanobacterial biovolumes between years 2003-2016 are comparable, but some caution should be used when comparing cyanobacterial biovolume from 2019 to 2003-2016 since data was not collected in the same manner and the sites are not identical, as they came from two different datasets.

For a qualitative evaluation, the composition of the cyanobacteria community for each year was analyzed down to genus, including genera that comprised over 5% of the total biovolume. In order to try to illuminate the reason for the differences in the cyanobacteria communities between years, non-metric multidimensional scaling (NMDS) was used on the cyanobacteria community data described above. Environmental variables from the MLMP dataset were added to the ordination using the *envfit()* function in the vegan package in R and contained data from all seasons (spring, summer, and fall) from two sites (MUSR and DEEP). The justification for using only two of the three (MUSR, DEEP, and CHAN) sites from which phytoplankton data is used is that the DEEP and CHAN sites were likely similar in terms of environmental conditions and would be redundant. The MUSR site is representative of the influence of river conditions, and the DEEP site is representative of the influence of lake conditions on the cyanobacteria community. Environmental variables used were NH_3, TP, SRP, NO_3, TKN, and temperature. Variables kept in the ordination were weened down to those with the most influence, identified as variables with eigenvectors longer than 0.30. It should be noted that the year 2008 was omitted from the NMDS ordination; due to its comparatively exceptionally high cyanobacteria biovolume, it was alienated in the ordination and caused all other years to group together, making it impossible to distinguish patterns. The R packages vegan and ggplot2 were used in RStudio Version 1.1.453 to create the ordination and figures (Oksanen et al., 2018; R Core Team, 2018; Wickham et al., 2016).

Comparison between severe-HAB and mild-HAB years

To determine the potential drivers of HAB severity in Muskegon Lake, a univariate comparison was made between years with the most severe HABs (2004, 2005, 2008) and the least severe HABs (2010, 2016, 2019). Severity was determined by the percentage of the phytoplankton community that was comprised of cyanobacteria, such that the severe HAB category includes the three years for which there was sufficient data with the highest percentage of cyanobacteria (2004, 56.63%; 2005, 67.22%; 2008, 63.35%), and the mild HAB category includes the three years for which there was sufficient data with the lowest percentage of cyanobacteria (2010, 2.61%; 2016, 2.38%; 2019, 1.59%). Within each category (“severe-HAB” and “mild-HAB”), there was often statistically significant variation between the years as determined by a one-way analysis of variance (ANOVA) and post hoc Tukey HSD test, but this issue was deemed unavoidable, and statistical analysis proceeded with the pooled datasets. Prior to the univariate analysis, I created a scatter matrix for each season including the following variables: chloride, TKN, SRP, TP, temperature, specific conductivity, NH_3, DO, turbidity, and NO_3. If any two variables appeared strongly related, a linear regression was created for those two variables to further explore the strength of the relationship, and one of those variables was subsequently removed to reduce autocorrelation and improve the power of the tests. After removing redundant variables, comparisons were made between the two categories (severe-HAB and mild-HAB) using a paired T-test or the parametric equivalent, the Wilcoxon Rank Sum test. Comparisons were made seasonally, in the spring, summer, and fall, as to account for seasonal variability. Outliers, values falling outside of the range of three standard deviations above or below the mean, were identified and subsequently removed prior to analysis. If the assumption of normality, as determined by the Shapiro-Wilk test, was not met and could not be met through transformation by one or both groups, the Wilcoxon Rank Sum test was performed rather than the paired T-test. Levene’s Test was used to assess the equality of variances between groups for each variable. The R packages UsingR, car, and stats were used in RStudio Version 1.1.453 to perform analyses (R Core Team, 2018; Verzani, 2018; Fox and Weisberg, 2011). The alpha value used to assess significance was maintained at 0.05.

Results

Changes in environmental variables over time

The PCAs revealed seasonal trends in environmental variables over the study period in Muskegon Lake. The spring PCA showed close grouping within years and an interesting, multidirectional temporal trend. The first two axes explained 56.94% of the variation in the data (PC1=38.54%, PC2=18.40%; Figure 2). From 2003 to 2014, the data points seem to move in a general trend from the upper left quadrant to the lower right quadrant. This variation appears to be driven by a reduction in SRP, chloride, TDS, SO_4, and specific conductivity and an increase in temperature and, to a lesser extent, TKN. After 2014, data points are notably drawn upward, indicative of a shift away from NO_3 and towards TKN with increasing turbidity values. ORP is a significant vector and correlates with NO_3 and temperature but does not appear to follow a strong temporal trend.

The summer PCA revealed the strongest temporal trends in environmental variables. The first two axes explained 52.55% of the variation in the data (PC1=28.44%, PC2=24.11%; Figure 3). From 2003-2019, data points move in the general direction of the upper left quadrant to the lower right quadrant, demonstrating gradual and generally consistent change in environmental variables over time. This variation appears driven by a reduction in TP, SRP, NH_3, chloride, and SO_4 and an increase in DO, pH, chlorophyll *a*, pH, and temperature; these two groups of variables are negatively correlated. TKN was not strongly correlated with either of these two groups but does seem to correlate slightly more with the latter. Increasing TKN values of the past several years influenced this vector. Specific conductivity and TDS were expectedly correlated with one another but were parallel to the temporal trend; they were generally uncoordinated with most other variables and negatively correlated with TKN.

The fall PCA revealed very little in terms of trends in environmental variables over the study period. There was far more variation between sites within years than the spring and summer PCAs, and no strong temporal trend is evident. The first two axes explained 51.09% of the variation in the data (PC1=28.75%, PC2=22.34%; Figure 4). Similar to the other PCAs, specific conductivity and TDS are positively correlated. TKN, chlorophyll *a*, temperature, SO_4, pH, and SRP were positively correlated. NO_3 was negatively correlated with all other variables except for specific conductivity and TDS, with which it has no correlation. 2013 and 2014 were years with high NO_3 values, and, similar to other seasons, subsequent years saw increasing TKN and chlorophyll *a* concentrations.

MLO water temperature data displays no consistent trend over the 2011-2019 period (Figure 5). However, the trends of the time-series graph generally match those seen in the PCAs, especially when considering the exact dates of the MLMP samplings. As MLMP samplings only occur three times per year (spring, summer, and fall), the MLO time-series graphs can be useful in determining if the MLMP values are anomalous to the rest of the year or if they are in-line with the trend.

The time-series graph of MLO chlorophyll *a* data shows a decreasing trend from 2011 to 2018 with a slight rebound in 2019 (Figure 6a). There appear to be three peaks in concentration for each year - early to mid-June, late July, and late September - and a concentration crash around early July and early October. Every year, the chlorophyll *a* concentration reached or exceeded 10 µg/L, which is often the level at which a lake is considered possibly in a eutrophic state (Dodds and Whiles, 2019). The trends of the time-series graph match those seen in the

PCAs, especially when considering the exact dates of the MLMP samplings. The phycocyanin time-series graph (Figure 6b) shows that cyanobacteria populations do not peak until early August. Additionally, with the exception of years 2011 and 2017, concentrations have remained relatively low in recent years.

Changes in the cyanobacteria community over time

The cyanobacteria changed quantitatively over time. There appears to be a clear distinction in the percentage of the phytoplankton community that is comprised of cyanobacteria (hereafter, percent cyanobacteria) between the years 2003 to 2008 and 2009 to 2019 (data from years 2017 and 2018 unavailable). From 2003 to 2008, percent cyanobacteria ranges from 25.16% to 63.35% (Table 2). From 2009 to 2019, percent cyanobacteria ranges from 0.71% to 7.85%, with 2011 being a year of exception and having a percent cyanobacteria value of 39.29% (Table 2). 2015 had the lowest value, and 2019 had the second lowest value. Additionally, the biovolume of the cyanobacteria community was variable from year to year (Table 3). There was not a continuous decrease in cyanobacteria biovolume from 2003 through 2019, although the overall trend was negative.

The composition of the cyanobacteria community was also variable from year to year. Interestingly, three groups of years can be observed (2003-2008, 2009-2012, and 2013-2019) with three exceptions to those groups (2003, 2015, and 2019), remembering that data for 2017 and 2018 was not available (Figure 7). 2003 was the only year that the cyanobacteria community was dominated by *Aphanocapsa* Nägeli. From 2004 to 2008 (Group 1), *Microcystis* was the dominant genus. Other genera found during those years include *Dolichospermum, Raphidiopsis, Merismopedia* Meyen, *Coelosphaerium* Nägeli, *Aphanocapsa*, and *Gomphosphaeria* Kützing. Between 2009 and 2012 (Group 2), the cyanobacteria community was far more diverse, and no single genus was dominant. Genera present include *Lyngbya* Agardh ex Gomont, *Microcystis, Limnothrix* Meffert, *Aphanocapsa, Chroococcus* Nägeli, *Dolichospermum, Merismopedia, Gomphosphaeria,* and *Gloeoethece* Nägeli. Years 2013, 2014, and 2016 (Group 3) were once again dominated or co-dominated by *Microcystis.* 2014 was co-dominated by *Microcystis* and *Aphanizomenon,* and 2016 was co-dominated by *Microcystis* and *Lyngbya*. The cyanobacteria community in 2015 had low biovolume and is roughly equally comprised of *Aphanizomenon, Chroococcus, Limnothrix,* and *Microcystis.* The year 2019 was dominated by *Dolichospermum,* with *Microcystis, Planktothrix,* and *Aphanizomenon* also present.

The NMDS of the annual cyanobacteria community composition (described above) showed similar trends to the PCA and had a low stress value of 7.26, indicating little distortion (Figure 8). There is a pattern of Group 1 data points occurring in the upper right corner, Group 2 data points being centrally located, and Group 3 data points occurring in the lower left corner, with the outlier years described above (2003, 2015, 2019) not conforming to this pattern. NH_3 in the spring at both sites (MUSR and DEEP), TP in the summer at MUSR, and TKN in the fall at MUSR were positively correlated and associated with Group 1. TP in the summer at DEEP also appears to associate with Group 1 but is pulled to the right of the other variables, possibly by its association with 2019. Group 2's central location indicates intermediate values of each variable. NO_3 in the fall at both sites and in the summer at MUSR, temperature in the spring at both sites, and TP and SRP in the fall at DEEP were positively correlated and generally associated with Group 3. SRP in the spring at MUSR, SRP in the summer at DEEP, and temperature in the fall at DEEP were positively correlated and associated with 2003. TKN in the spring at MUSR, which associates with 2015, and TP in the summer at DEEP are negatively correlated with each other and generally not correlated to other variables.

Comparison between severe-HAB and mild-HAB years

The paired T-tests and Wilcoxon Rank Sum tests revealed significant differences in some variables between the severe-HAB and mild-HAB categories within seasons (Table 4).

Beginning with the spring, the scatter matrix and subsequent linear regressions revealed that temperature was significantly positively correlated with specific conductivity and significantly negatively correlated with DO, turbidity, and TKN. Therefore, temperature was removed from the spring analysis. Turbidity was significantly positively correlated with DO and TKN and was also removed. SRP values were either 0 or 0.01 mg/L, the limit of detection, throughout the entire dataset. While the number of values that were 0.01 mg/L was higher in the severe-HAB category, the lack of variation did not justify including SRP in the tests; it was removed. The remaining variables tested were chloride, TP, NH_3, NO_3, TKN, specific conductivity, and DO. Chloride was significantly higher in the severe-HAB category (t=7.25, p-value=2.81e-06). TP was significantly higher in the severe-HAB category (V=1, p-value=2.20e-3). NH_3 was significantly higher in the severe-HAB category (V=6, p-value=1.43e-3). Values of NO_3, TKN, specific conductivity, and DO were not significantly different between the severe-HAB and mild-HAB categories.

For the summer data, the scatter matrix and subsequent linear regressions revealed that specific conductivity was significantly negatively correlated with chloride and NH_3 and significantly positively correlated with temperature and DO. Specific conductivity was removed from the summer analysis. DO was significantly negatively correlate with NH_3, chloride, and NO_3 and significantly positively correlated with specific conductivity. DO was removed from the summer analysis. Chloride was significantly negatively correlated with specific conductivity, DO, and NO_3 and was removed from the summer analysis. SRP was removed from the summer analysis for the same reason it was removed from the spring analysis. The remaining variables tested were TP, temperature, NH_3, NO_3, TKN, and turbidity. TP was significantly higher in the severe-HAB category (V=0, p-value=2.48e-03). Temperature was significantly higher in the mild-HAB category (t=-2.82, p-value=0.012). NH_3 was significantly higher in the severe-HAB category (V=0, p-value=3.10e-04). NO_3 was significantly higher in the severe-HAB category (V=15, p-value=2.29e-03). TKN and turbidity were not significantly different between the severe-HAB and mild-HAB categories.

The scatter matrix of fall data revealed no autocorrelation between any two variables. However, to increase the power of the tests, we removed the variables we did not believe would substantially contribute to explaining variation in the severity of the HABs in the fall. SRP was removed for the same reasons as in the spring and summer tests, except the distribution of values displayed little difference between the categories. Other variables we decided to remove were chloride and specific conductivity. The remaining variables tested were NH_3, TKN, TP, temperature, DO, turbidity, and NO_3. NH_3 was significantly higher in the severe-HAB category (V=24, p-value=0.041). TKN was significantly higher in the mild-HAB category (t=-2.57, p-value=0.020). TP was significantly higher in the mild-HAB category (V=60.5, p-value=0.015). Temperature was significantly higher in the mild-HAB category (V=124.5, p-value=0.02). DO was significantly higher in the mild-HAB category (V=260, p-value=1.44e-03). Turbidity and NO_3 were not significantly different between categories.

Discussion

The HAB impairment in Muskegon Lake has decidedly improved in terms of both reduction in the percentage of the phytoplankton community that is comprised of cyanobacteria and the annual biovolume of cyanobacteria. Additionally, the cyanobacteria community composition was variable over the study period. In looking at compositional data, three phases are evident: 1) domination by *Microcystis* (Group 1, 2003-2008), 2) high diversity with no single dominant genus (Group 2, 2009-2012), and 3) domination by *Microcystis* (Group 3, 2013, 2014, and 2016), with exceptions being years 2003, 2015, and 2019 when *Aphanocapsa* dominated, there was very low biovolume and high diversity, and *Dolichospermum* dominated, respectively. This study aimed to link the quantitative and qualitative changes in the HAB community in Muskegon Lake to environmental variables that may have driven them.

Quantitative changes in the HAB community and drivers of change

Quantitative changes in the HAB community are likely explained by the marked improvements in water quality, as evidenced by the nutrient and chloride reductions over the years. It is likely that the nutrient reduction (NO_3, SRP, TP, NH_3) in Muskegon Lake is the main driver of the decrease in cyanobacteria abundance, with support from the spring and summer PCAs and the univariate tests (Glibert, 2017; Anderson, 2002; Paerl and Otten, 2013). It appears that spring and summer nutrients are more influential than fall nutrients on the HAB community, as most nutrients in the fall univariate analysis were either not significantly different between the mild- and severe-HAB groups or were higher in the mild-HAB group. Additionally, the summer PCA is the only PCA that showed a strong trend of decreasing nutrients over the study period. It is possible that, since cyanobacteria do not experience substantial growth until the late summer and early fall, spring nutrients are less influential on their population. It is also important to note that the fall sampling occurred in September and October, which often occurs during the time period that the lake is mixing; therefore, these values are not truly from the fall season and experience high interannual variability, making it difficult to draw conclusions about the importance of fall nutrients to the cyanobacteria community in Muskegon Lake.

Both N and P appear influential on the HAB community in Muskegon Lake, as the decrease in cyanobacteria abundance coincided with decreases in both N and P concentrations in the water. While Muskegon Lake is naturally P-limited and P is often cited as the growth-limiting nutrient for cyanobacteria (Schindler et al., 2016), this does not exclude N as a productivity-boosting nutrient. It has been demonstrated that N plays an important role in phytoplankton growth and community composition at both high and low P levels (Glibert et al., 2016; Newell et al., 2019; Gobler et al., 2016). Donald et al. (2013) found that over 30% of the 140 phytoplankton taxa included in their mesocosm study significantly increased in abundance in response to N addition. In particular, cyanobacteria responded positively to reduced forms of N. Other studies have also documented a cyanobacterial dominance and increase in toxin production under conditions of high concentrations of reduced N (Lehman et al., 2017; McCarthy et al., 2009; Glibert et al. 2016; Newell et al., 2019). Therefore, reductions in N, especially the consistent reduction in NH_3, within Muskegon Lake likely contributed more to the reduction in cyanobacteria abundance than did P reductions, especially considering the low P levels and low variation in P levels over the study period. However, while most nutrient forms have decreased over the years, TKN, which is inclusive of organic N and NH_3, appears to have increased recently. In the spring, TKN has increased compared to NO_3 in recent years. While TKN:NO_3 had a relatively short vector length in the summer PCA and was removed, it correlated positively with TKN and chlorophyll *a*. Although no clean trends are apparent in the fall PCA, the shift towards higher TKN levels is still decipherable and is confirmed by the univariate analysis. Since NH_3 was either shown to decrease over time, as in the summer PCA and univariate comparisons, or was not a significant vector and was, thus, not included in the PCA, it can be reasonably inferred that the proportion of TKN that has increased over time is the organic N component. The recent shift towards organic N in the system may be a result of increased productivity (discussed blow), but since organic N can be recycled rapidly, it may also be indicative of a potential reduced N source that could benefit non-diazotrophic cyanobacteria, such as *Microcystis*, which can be very competitive for reduced N (Newell et al., 2019).

Despite the reduction in cyanobacteria, the PCAs show increasing chlorophyll *a* concentration, a proxy for phytoplankton growth, over time. As chlorophyll *a* is a pigment created by all phytoplankton and is not specific to cyanobacteria, this may indicate that phytoplankton biomass is increasing in response to rising temperatures in the lake, but a smaller proportion of it is comprised of cyanobacteria (Hampton et al., 2008). This may also qualify as a reasonable explanation for the increase in TKN, as more N would be bound up in phytoplankton biomass. Phytoplankton growth rates increase with temperature; therefore, this pattern unsurprising. However, many studies have demonstrated or predicted that higher temperatures resulting from global climate change will exacerbate the severity of HABs (Kosten et al., 2012; Paerl and Huisman, 2008; Visser et al., 2016). This has not been the case in Muskegon Lake, indicating that the conditions in the lake are no longer conducive to cyanobacterial dominance, despite rising temperatures. It can tentatively be concluded that nutrients are currently a more important variable in driving cyanobacterial abundance than temperature in Muskegon Lake. However, it is worth mentioning that the MLMP only samples three times per year (spring, summer, and fall). As such, caution must be exercised when interpreting chlorophyll *a* data from the MLMP since phytoplankton growth is spatially and temporally heterogenous, and such infrequent sampling could potentially be misleading. Additionally, chlorophyll *a* data from the MLO buoy (2011-2019) shows decreasing trends in the past several years.

Qualitative changes in the HAB community and drivers of change

While explaining quantitative changes in the cyanobacteria community is relatively straight-forward, understanding the drivers of community composition is more challenging due to the multitude of contributing variables, including nutrient form and concentration and temperature. Nutrient concentrations have generally decreased over time in Muskegon Lake, but nutrient forms have shifted, and water temperatures have steadily increased over the 2003-2019 time period, potentially explaining the shift in composition within the diminishing cyanobacteria community.

In the multivariate ordinations (PCAs and NMDS), except for the fall PCA, there is a general pattern of years moving consecutively in one direction. Therefore, just as it is in time, the group of years that had a highly diverse cyanobacteria community (Group 2) is positioned between the two groups of years in which *Microcystis* dominated (Group 1 and Group 3). Additionally, the years that were "outliers" in terms of cyanobacteria community composition (2003, 2015, 2019) deviate from the described pattern, not conforming to the three groups. This indicates that environmental factors drove the cyanobacteria community composition, but the factors contributing to *Microcystis'* advantage differed between the two *Microcystis*-dominated groups of years. The NMDS also demonstrated that influence from nutrients and temperature in the river and the lake are important factors that shape cyanobacteria community composition in Muskegon Lake. However, the description of the changes in the cyanobacteria community composition should be accompanied by the recognition that, after the year 2008, the cyanobacteria comprised a relatively small proportion of the phytoplankton community. Therefore, the caveat is that the composition in later years is comparatively less important because the cyanobacteria are having less of an influence on the phytoplankton community and the lake ecosystem as a whole.

While *Microcystis* can use organic P, such as that included in TP, it prefers orthophosphates (inorganic P; SRP); at any rate, it often requires less than 0.03 mg/L P to form a bloom (Šejnohová and Maršálek, 2012). Therefore, the combination of organic and inorganic P concentrations in Muskegon Lake proved sufficient for *Microcystis* in both Group 1 and Group 3. Gobler et al. (2016) found that at low P levels, such as those in Muskegon Lake, non-diazotrophic cyanobacteria (e.g. *Microcystis*), have an advantage due to their ability to thrive in low P environments. Additionally, Lehman et al. (2017) found no correlation between *Microcystis* biomass and SRP in the San Francisco Estuary, even in 2014 when SRP levels were abnormally high. As such, it can be concluded that *Microcystis* was never disadvantaged by the P supply in Muskegon Lake, and it may be argued that it had an advantage over other phytoplankton due to its ability to thrive in low P environments and use both organic and inorganic forms.

It is likely that *Microcystis'* abundance was more influenced by N forms and concentrations. In terms of N, *Microcystis* can use all inorganic forms, is highly competitive, and experiences rapid growth in response to inputs (Chaffin et al. 2018; Newell et al., 2019). Most studies suggest a preference for reduced forms, even when NO_3 is more abundant (Lehman et al., 2017; Šejnohová and Maršálek, 2012; Newell et al., 2019; Gobler et al., 2016). In earlier years in Muskegon Lake, reduced forms of N (NH_3 and TKN) were more abundant in the spring and fall, and in later years, NO_3 was more abundant in the summer and fall. This is potentially a result of a shift in the sources of N inputs or increasing DO in surface waters over time, which would create an oxidizing environment in which nitrification of reduced N occurs (Sterner et al., 2007;

Sigee, 2005). *Microcystis* often blooms in the late summer and fall (Chaffin et al., 2003; Dila and Biddanda, 2015; Lehman et al., 2017; Coffer et al., 2020). Therefore, *Microcystis* would have lost its advantage in terms of N form availability over this period, as reduced forms shifted to oxidized forms in their growth season. Worth mentioning is the fact that the river was a more significant source of organic nutrients (TKN and TP) in earlier years, which may have provided recyclable reduced nutrients that *Microcystis* could benefit from (Newell et al., 2019). We believe that the shift in N form and overall decrease in nutrient concentration is what drove the decrease in *Microcystis* abundance after Group 1 and led to a more diverse cyanobacteria community during the middle group of years, Group 2.

In Group 2, when the cyanobacteria community 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 PCA and NMDS. It does not appear that temperatures were ever necessarily insufficient for *Microcystis* growth during this time. Therefore, it is possible that moderate amounts of several nutrient forms allowed for diversity, not giving any one genus a competitive advantage, as it has been demonstrated that nutrient form preference and requirement varies by taxa, and nutrient forms and amounts are important drivers of phytoplankton community composition (Berg et al., 2003; Anderson et al., 2020a; Donald et al., 2013; Rothenberger et al., 2009). Other possible explanations may be meteorological in nature, such as ice cover duration, precipitation amount, or frequent storms or high winds that reduced stability in the water column (Weirich et al., 2019).

Temperature is often argued to be the most important factor influencing the success of *Microcystis*, especially toxin-producing species (Davis et al., 2009; Šejnohová and Maršálek, 2012). We propose that *Microcystis* regained its advantage in Group 3 due to increasing temperatures, as demonstrated by the PCA and NMDS ordinations. A secondary reason for *Microcystis'* regained advantage may be the increase in TKN concentrations in recent years. While this may reflect the increased primary production in the lake, it may also represent a recyclable pool of reduced nutrients, for which *Microcystis* is highly competitive. Due to their myriad adaptations and ecological flexibility, several environmental conditions are conducive to a cyanobacteria community dominated by *Microcystis*, which led to the two *Microcystis*-dominated time periods seen in this study. Over a relatively short period of time and small temperature increase, *Microcystis* has regained an advantage in the cyanobacteria community in Muskegon Lake; given more time and larger temperature increases due to climate change, we may find more difficulty in managing for HABs and a loss of progress in staving off *Microcystis* dominance in the future (Ho et al., 2019), which is particularly concerning due to their ability to produce toxins and form buoyant blooms.

Lastly, the cyanobacteria community composition of the years that qualified as exceptions to the pattern (2003, 2015, 2019) can also be explained by environmental variables. *Aphanocapsa* may have dominated in 2003 due to an unusually warm fall, which would have allowed this picoplankton to stay entrained in the water column; high chloride levels, which may have given *Aphanocapsa* an advantage since some species are tolerant of salinity; and high SRP concentrations (Wang et al., 2018). The presence of *Aphanocapsa* may explain the high sulfate levels, as some species have demonstrated the ability to produce sulfated extracellular polysaccharides (Matsunaga et al., 1996). 2015 was a year of very low phytoplankton growth in general, likely due to the comparatively cool spring and summer of that year, which would have specifically put cyanobacteria at a disadvantage and not allowed for *Microcystis* to dominate. It is likely that 2019 was an anomalous year for cyanobacteria due to aberrant weather patterns, characterized by record-breaking precipitation amounts and a cool temperature regime, especially in the spring; this created an unprecedented, diluted environment in which *Dolichospermum*, a toxin-producing, diazotrophic genus with a lower temperature tolerance range, benefited (Weirich et al., 2019).

Future work is needed to further understand what environmental factors – individually or interactively- drive the cyanobacteria community abundance and composition in Muskegon Lake and should include meteorological factors, such as precipitation and wind events, along with additional physical factors, such as Schmidt stability and photosynthetically active radiation (PAR). A closer look into the seasonal and spatial patterns of cyanobacteria community composition is also warranted and may aid in explaining how environmental factors impact the cyanobacteria community. Therefore, additional research should focus on monitoring and developing HAB management efforts, specifically as they pertain to future climate scenarios.

Conclusions

The HAB impairment in Muskegon Lake has decidedly improved from 2003 to 2019 both in terms of reductions in biovolume and proportion of the phytoplankton community, making the restoration of the lake in these terms a success story. Even though temperatures in the lake are increasing, the decreased nutrient concentrations in the lake are maintaining cyanobacteria at a low biovolume at present. Additionally, the cyanobacteria community composition appeared to be associated with shifts in nutrient forms and temperatures. *Microcystis* originally had an advantage at the beginning of the study period due to its ability to use organic P, thrive in low SRP environments, and benefit from the abundance of reduced N. It lost its advantage as nutrient concentrations decreased and nutrient forms diversified but regained an advantage with increasing temperature (Figure 9). However, some driving factors were likely missed in this study as we could not fully explain all compositional trends. HABs in

Muskegon Lake should be monitored into the future as we enter an era of uncertain climatic conditions.

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Figure Legends

Figure 1. A map of the study site, Muskegon Lake, including points of collection for the MLMP sampling (blue squares), the 2019 biweekly sampling (red triangles), and the site of the Muskegon Lake Observatory (MLO) buoy (light blue circle). The yellow stars indicate the three MLMP sampling sites from which phytoplankton data was used.

Figure 2. PCA of environmental data collected during the spring MLMP sampling, inclusive of all six sites and years for which sufficient data was available (2003-2019, excepting 2006 and 2015). PC1=38.54%, PC2=18.40%. Cumulative proportion of variation explained by the first two axes=56.93%. Years are grouped using the *ordiellipse* function in the R package vegan, creating a polygon for each year based on the standard deviations of the points. Variable abbreviations: SRP = soluble reactive phosphorus, Cl= chloride, SpC= specific conductivity, TDS= total dissolved solids, SO4= sulfate, NO3= nitrate, ORP= oxidation reduction potential, Temp= temperature, TKN= total Kjeldahl nitrogen, TKN:NO_3= ratio of total Kjeldahl nitrogen to nitrate, Turb= turbidity.

Figure 3. PCA of environmental data collected during the summer MLMP sampling, inclusive of all six sites and years for which sufficient data was available (2003-2019). PC1=28.75%, PC2=22.34%. Cumulative proportion of variation explained by the first two axes=51.09%. Years are grouped using the *ordiellipse* function in the R package vegan, creating a polygon for each year based on the standard deviations of the points. Variable abbreviations: DO= dissolved oxygen, Chl= Chlorophyll *a*, NH3= ammonia, TP=total phosphorus. See Figure 2 legend for remaining variable abbreviations.

Figure 4. PCA of environmental data collected during the fall MLMP sampling, inclusive of all six sites and years for which sufficient data was available (2003-2019). PC1=28.75%, PC2=22.34%. Cumulative proportion of variation explained by the first two axes=51.09%. Years

are grouped using the *ordiellipse* function in the R package vegan, creating a polygon for each year based on the standard deviations of the points. Variable abbreviations: See Figure 2 and Figure 3 legends.

Figure 5. A time-series graph displaying water temperature (2 m) data from the MLO for years 2011-2019.

Figure 6. (a.) A time-series graph displaying chlorophyll *a* concentrations (a proxy for phytoplankton abundance) at 2 m. (b.) A time-series graph displaying phycocyanin concentrations (a proxy for cyanobacteria abundance) at 2 m. Data is provided from the MLO and includes years 2011-2019, excepting 2016 and 2018 due to insufficient data.

Figure 7. Stacked bar graphs displaying the present cyanobacteria genera and total cyanobacterial biovolume per year from 2003-2016 and 2019. Data is provided by the MLMP and the 2019 datasets and includes genera comprising greater than 5% of the cyanobacteria biovolume per year. The years are categorized into three groups based on the patterns seen in community composition: Group 1 (2003-2008) was dominated by *Microcystis,* Group 2 (2009-2012) was highly diverse, and Group 3 (2013-2019) was dominated by *Microcystis.* There were three exceptions to these groups, and they are denoted by circles around the year. The varying x-axes should be noted, as cyanobacteria abundance decreased over the study period.

Figure 8. NMDS of cyanobacteria community composition data for each year, excepting 2008 due to it being an outlier that compromised the figure. Environmental data is provided by the MLMP and includes data from the spring, summer, and fall of all represented years from two sites: MUSR and DEEP. The first letter(s) of the variable indicates the season (Sp=spring, Su=summer, F=fall), the second letter indicates the site (R=river, D=deep), and the remainder of the description is the variable measured. The circles indicate the three groups of years from the

community composition bar graphs (Figure 7) and the squares indicate years that were exceptions to the groups.

Figure 9. This schematic presents a summary of the major findings of this study, demonstrating changing environmental conditions and cyanobacteria community composition over the study period (2003-2019; data unavailable for 2017 and 2018).

Tables

Table 1. Overview of data used in this study.

Data Set	Type of Data	Years of Available Data	Sites Used
Muskegon Lake Monitoring Program (MLMP)	Environmental (water)	2003-2019	MUSR, CHAN, DEEP, AWRI, RUDD, BEAR
	Phytoplankton	2003-2016	MUSR, DEEP, CHAN
2019 Data	Phytoplankton	2019	River, MLO, Channel
MLO Data	Environmental (water) & Meteorological	2011-2019	MLO

Table 2. This table contains the percentage of the phytoplankton community that was comprised of cyanobacteria based on biovolume from the spring, summer, and fall seasonal monitoring events (2003-2016) and data collected biweekly during 2019 at three sites: the Muskegon River mouth, near the MLO buoy, and in the Muskegon channel (note missing years, 2017 and 2018). (note missing years, 2017 and 2018). Data was pooled within each year across sites and sampling days. The line between years 2008 and 2009 represents the shift from relatively high percent cyanobacteria to relatively low percent cyanobacteria.

Year	***Percent Cyanobacteria***
2003	36.32
2004	56.63
2005	67.22
2006	25.16
2007	32.71
2008	63.35
2009	5.74
2010	2.61
2011	39.29
2012	7.52
2013	5.91
2014	7.85
2015	0.71
2016	2.38
2019	1.59

Table 3. This table displays the cyanobacterial biovolume for each year from 2003-2016 and 2019. Data from 2003-2016 was collected during the Muskegon Lake long-term monitoring events and is inclusive of data collected at sites MUSR, DEEP, and CHAN from the top and bottom of the water column. Data was for 2019 was collected biweekly from late April to mid-October at three sites: the Muskegon River mouth, near the MLO buoy, and in the Muskegon channel (note missing years, 2017 and 2018).

Year	***Cyanobacteria Biovolume***
2003	9.91E+06
2004	4.49E+07
2005	5.97E+07
2006	5.45E+06
2007	5.29E+06
2008	5.56E+10
2009	1.16E+06
2010	1.38E+06
2011	1.00E+07
2012	2.89E+06
2013	5.11E+05
2014	3.51E+05
2015	4.83E+04
2016	6.95E+05
2019	2.96E+04

Table 4. This table displays the results of the paired T-tests (T) and Wilcoxon Rank Sum tests (W) performed on two groups of data from the Muskegon Lake long-term monitoring program: a category containing three years that experienced severe HABs (2004, 2005, and 2008) and a category containing three years that experienced mild HABs (2010, 2016, and 2019). Data for each group was pooled within each season.

Season	*Variable*	*Severe-HAB category mean*	*Mild-HAB category mean*	*Test*	*Test Statistic*	*p-value*
Spring	Chloride (mg/L)	24.25	18.61	T	7.25	**2.81E-06**
	TKN (mg/L)	0.484	0.526	T	-1.26	0.226
	TP (mg/L)	0.020	0.014	W	1	**2.20E-03**
	Specific Conductivity (µS/cm)	0.314	0.335	W	104.5	0.190
	Dissolved oxygen (mg/L)	9.713	9.714	W	59.5	0.679
Summer	NH_3 (mg/L)	0.056	0.032	W	6	**1.44E-03**
	NO_3 (mg/L)	0.387	0.305	T	1.88	**0.044**
	TKN (mg/L)	0.605	0.601	T	0.094	0.927
	TP (mg/L)	0.038	0.026	W	0	**2.47E-03**
	Temperature (ºC)	23.47	25.30	T	-2.82	**0.012**
	Specific Conductivity (µS/cm)	0.391	0.358	W	114	**2.32E-03**
	Turbidity (NTU)	4.900	4.082	W	50.5	0.609
Fall	NH_3 (mg/L)	0.052	0.017	W	0	**3.10E-04**
	NO_3 (mg/L)	0.253	0.136	W	15	**2.29E-03**
	TKN (mg/L)	0.527	0.586	T	-2.572	**0.020**
	TP (mg/L)	0.025	0.034	W	60.5	**0.015**
	Temperature (ºC)	20.11	20.33	W	124.5	**0.025**
	Turbidity (NTU)	7.439	6.689	T	1.372	0.188
	Dissolved oxygen (mg/L)	8.348	9.278	W	260	**1.45E-03**
	NH_3 (mg/L)	0.028	0.018	W	24	**0.041**
	NO_3 (mg/L)	0.135	0.166	W	116.5	0.184

Figures

Figure 1.

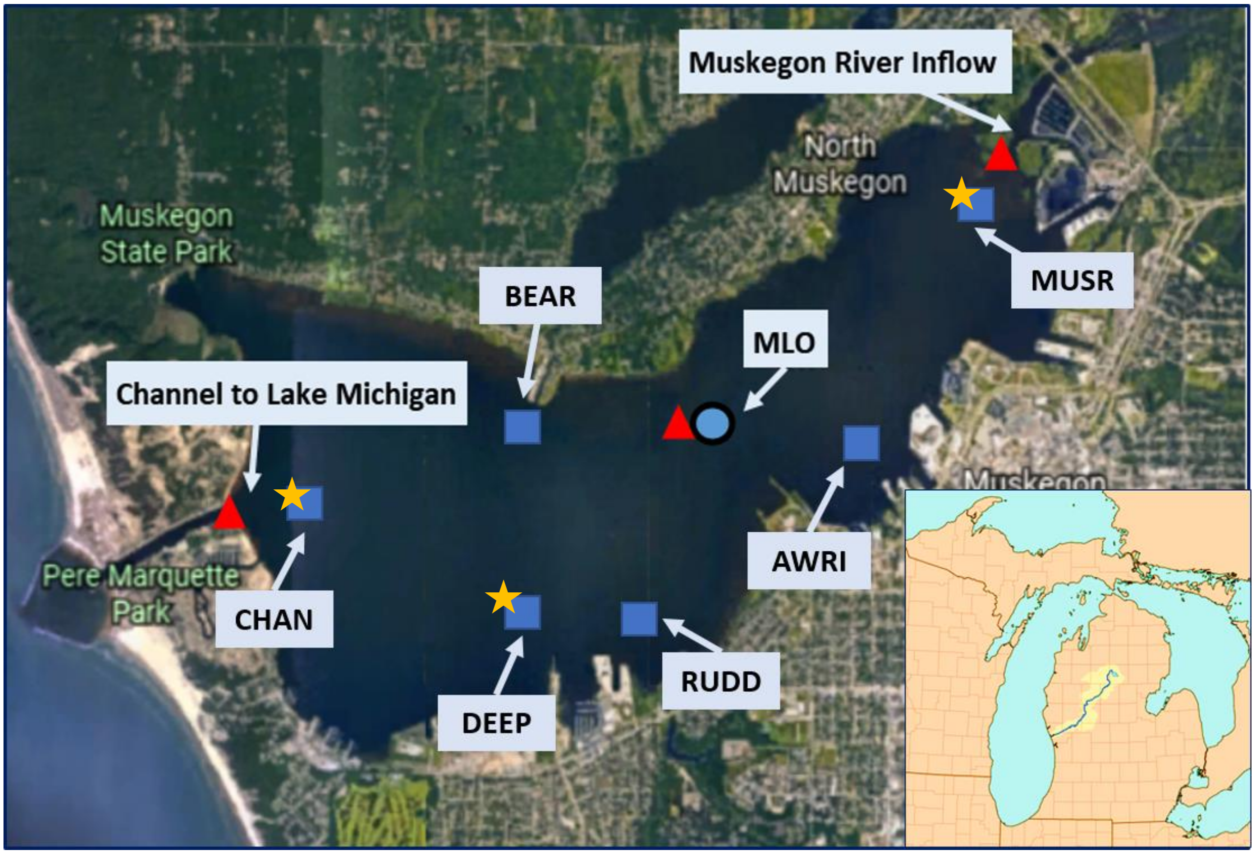
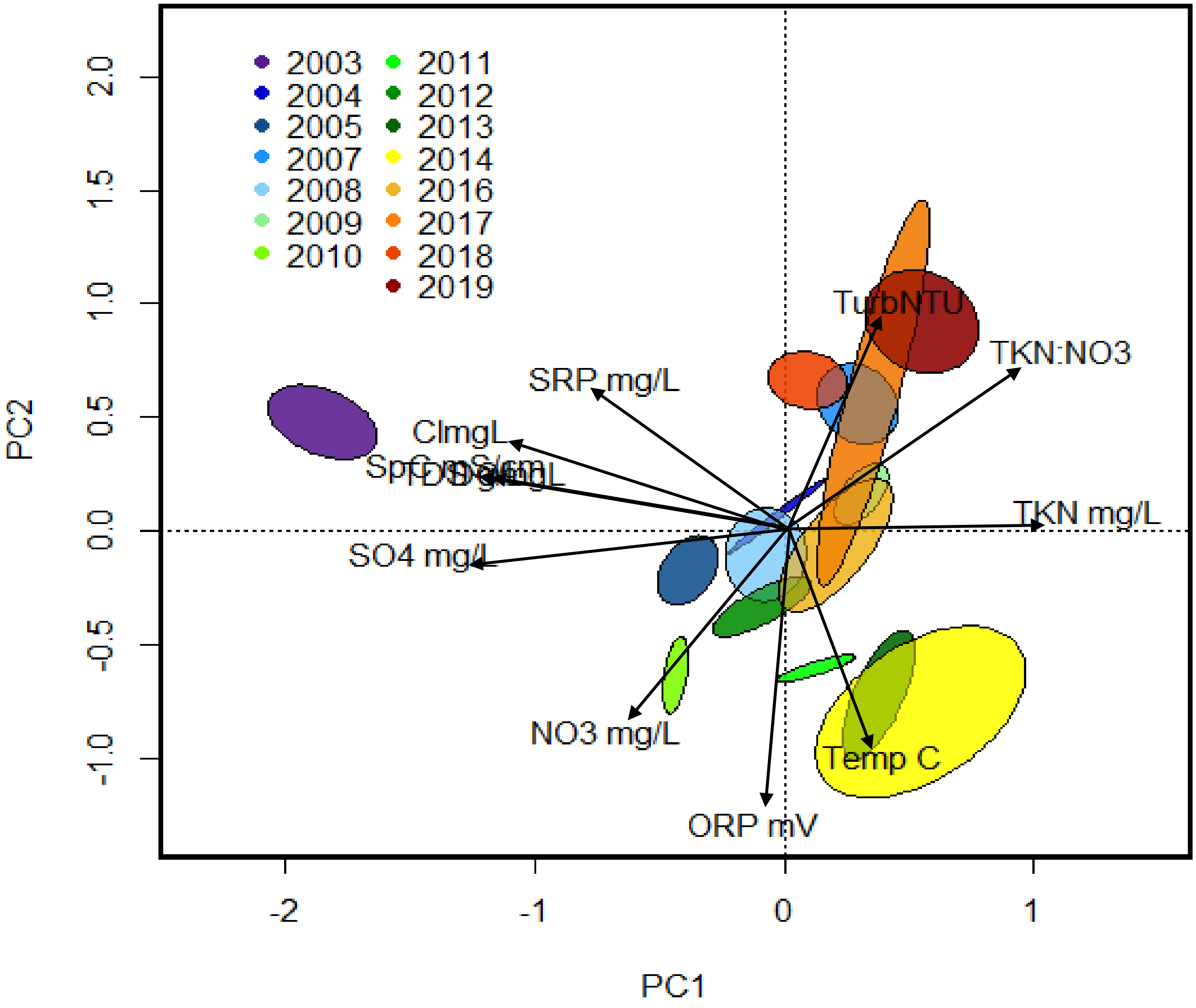
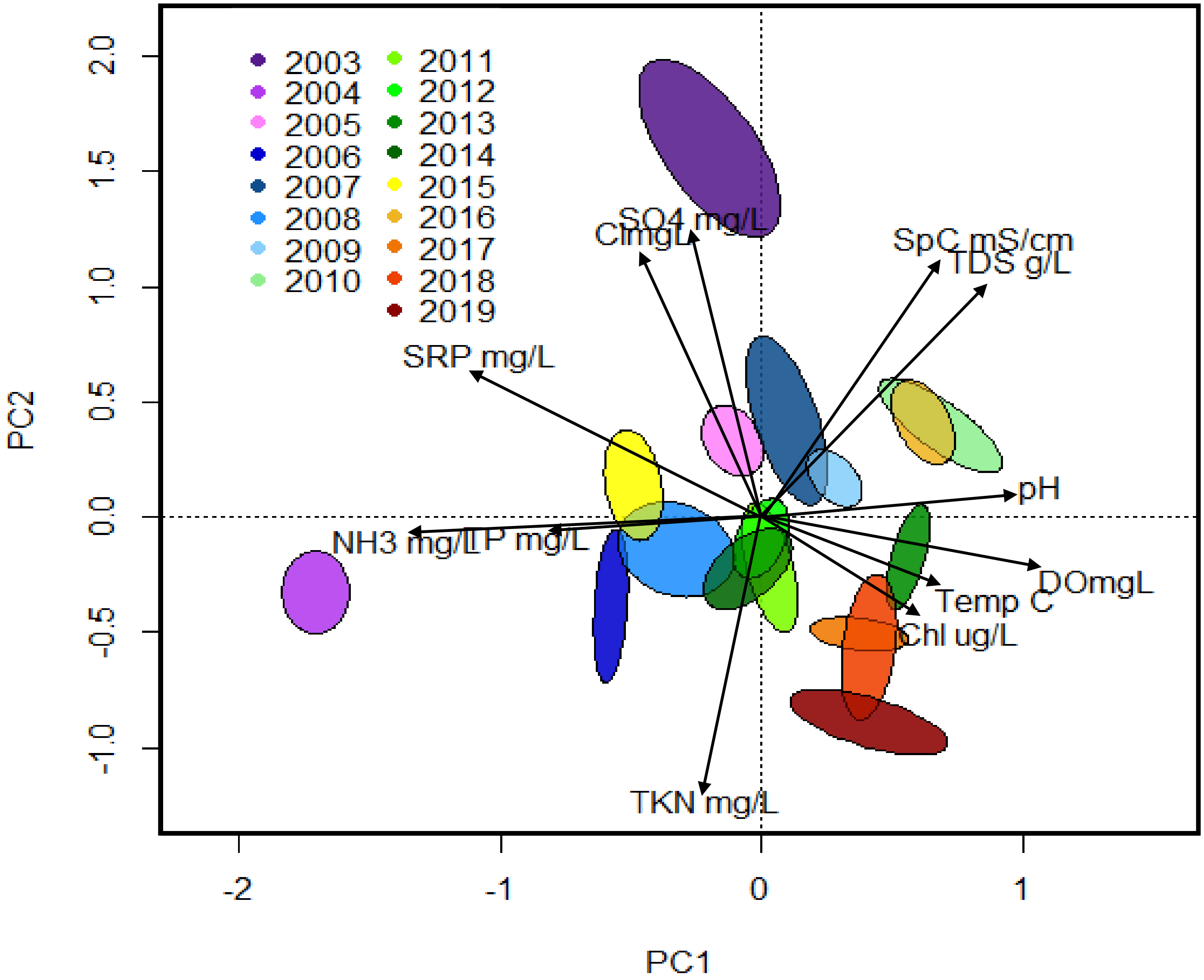


Figure 2.



2003
2004
2005
2007
2008
2009
2010
2011
2012
2013
2014
2016
2017
2018
2019
TurbNTU
TKN:NO3
SRP mg/L
ClmgL
SpC mS/cm
TDS g/L
TKN mg/L
SO4 mg/L
NO3 mg/L
Temp C
ORP mV
PC1
PC2
-2
-1
0
1
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

Figure 3.



2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
SO4 mg/L
ClmgL
SpC mS/cm
TDS g/L
SRP mg/L
pH
NH3 mg/L
TP mg/L
DOmgL
Temp C
Chl ug/L
TKN mg/L
PC2
PC1
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1

Figure 4.

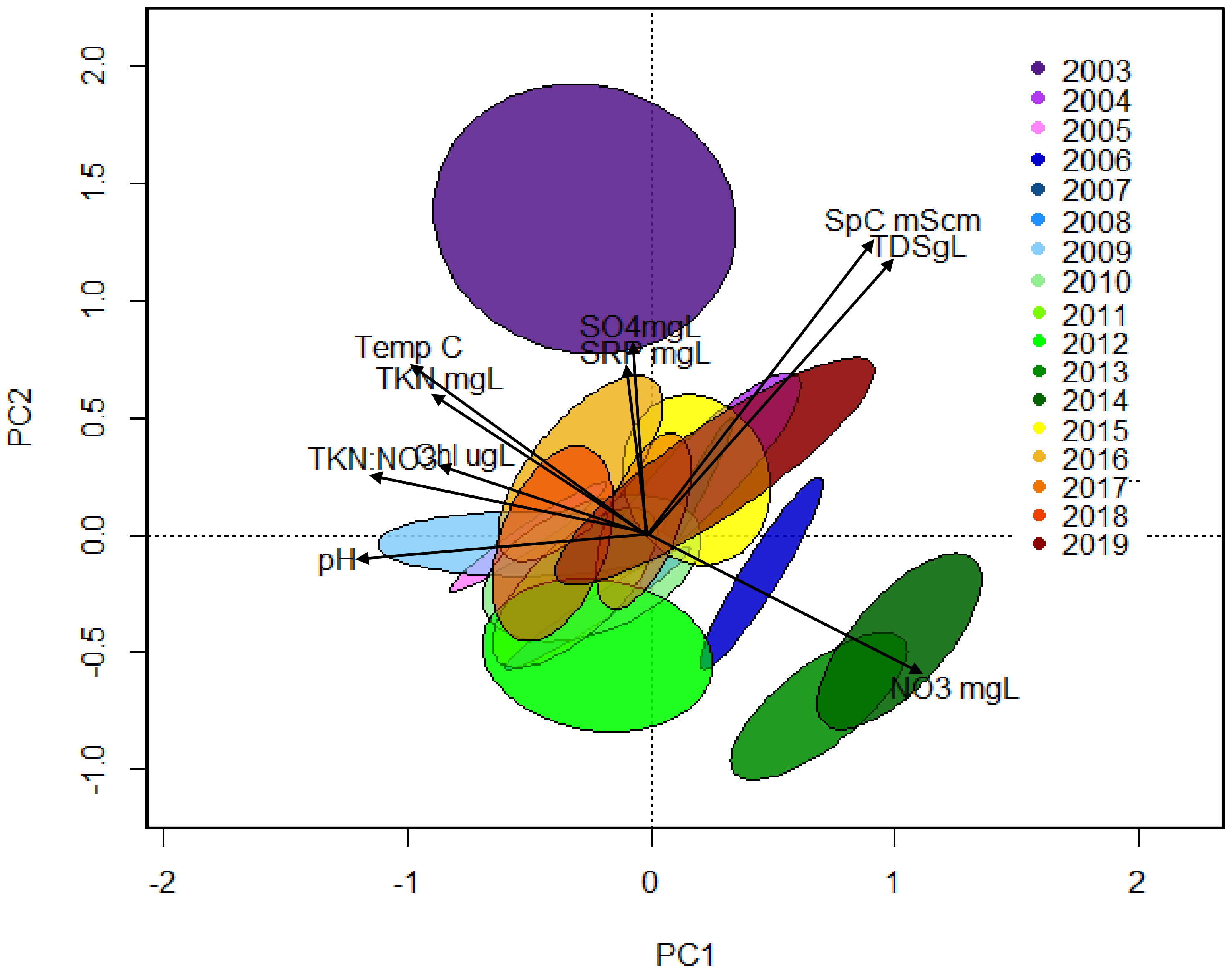

PC2
PC1
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1
2
SpC mScm
TDSgL
SO4mgL
SRP mgL
Temp C
TKN mgL
TKN:NO3
Chl ugL
pH
NO3 mgL
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019

Figure 5.

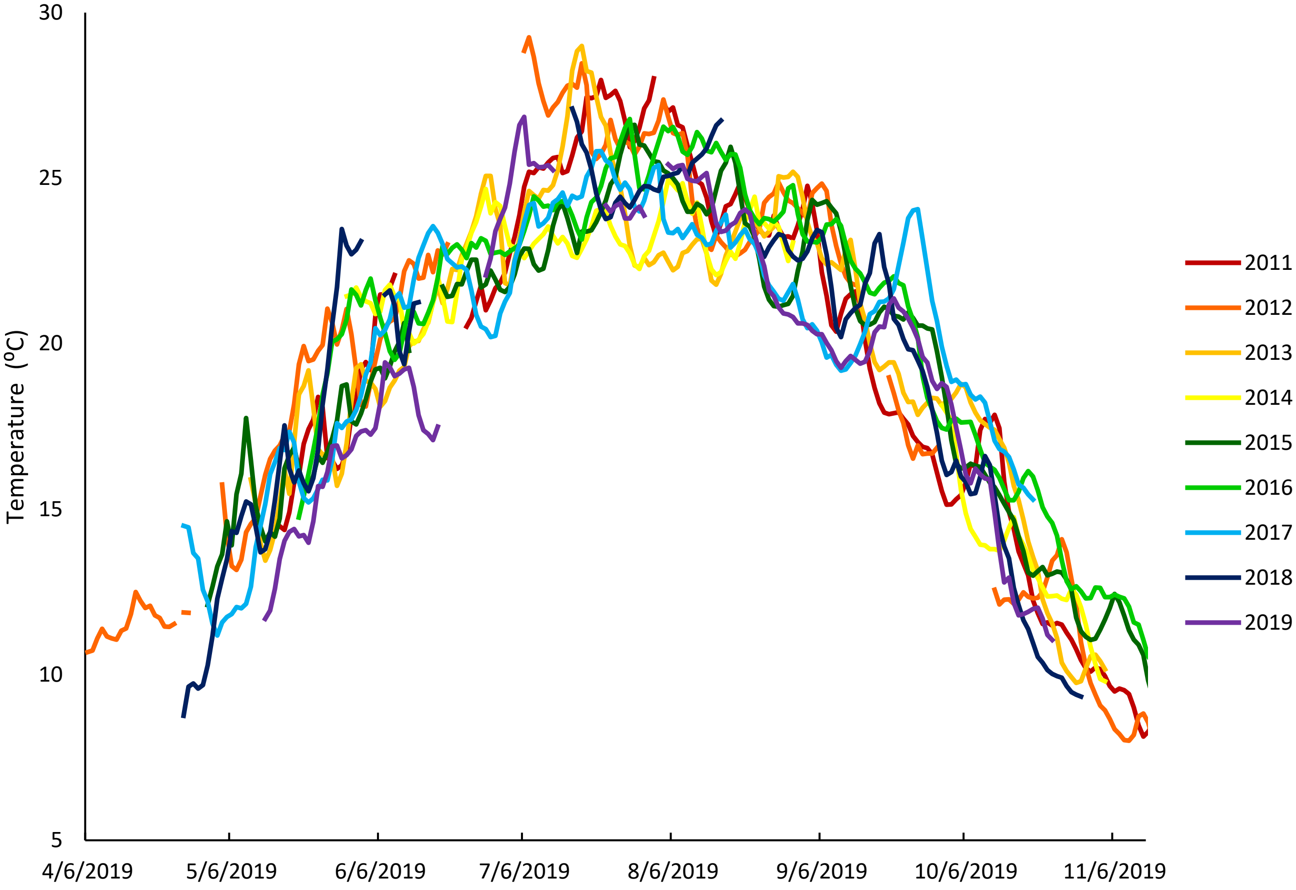

30
25
20
15
10
5
Temperature (°C)
4/6/2019
5/6/2019
6/6/2019
7/6/2019
8/6/2019
9/6/2019
10/6/2019
11/6/2019
2011
2012
2013
2014
2015
2016
2017
2018
2019

Figure 6.

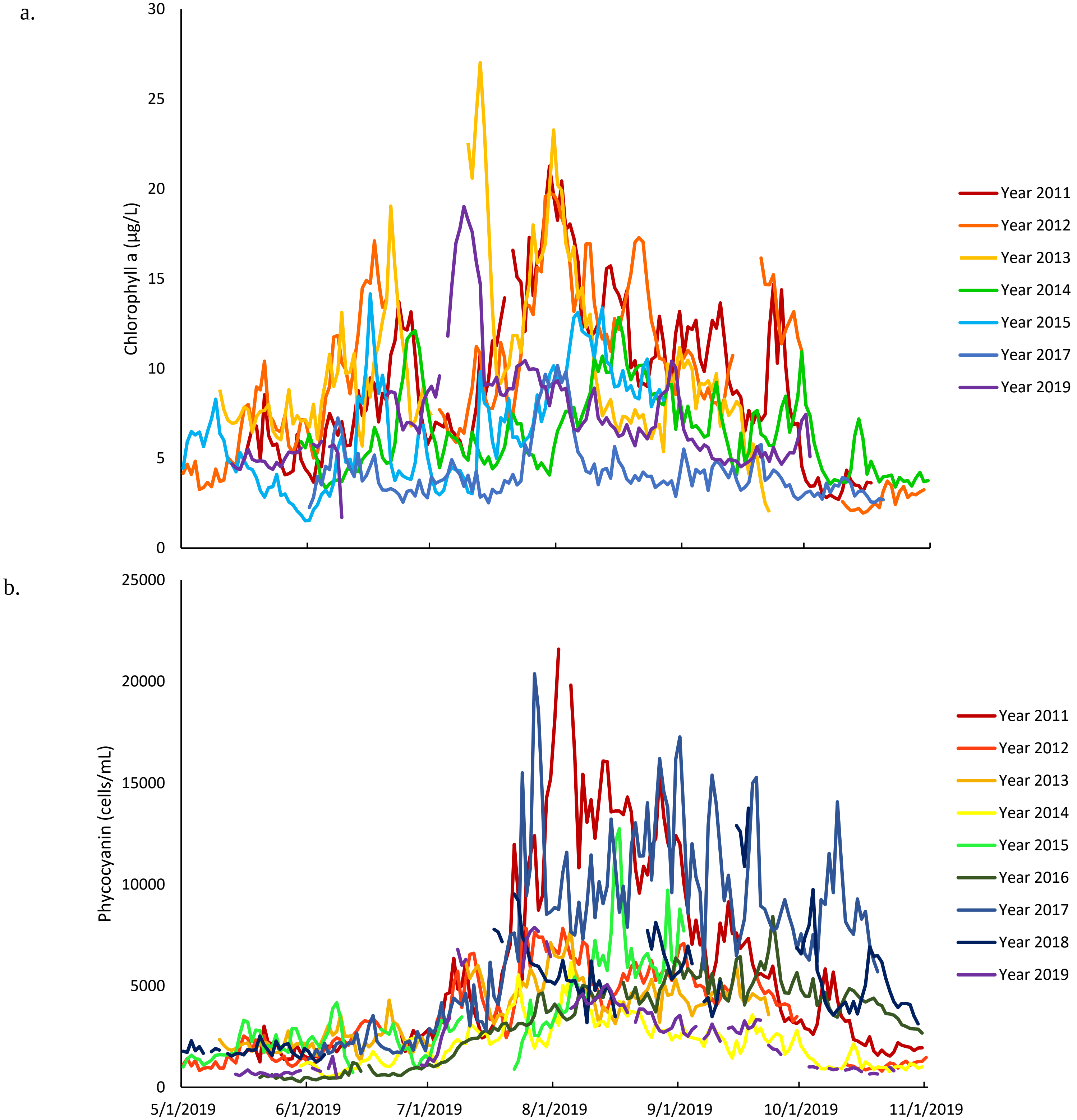

a.
Chlorophyll a (μg/L)
30
25
20
15
10
5
0
Year 2011
Year 2012
Year 2013
Year 2014
Year 2015
Year 2017
Year 2019
b.
Phycocyanin (cells/mL)
25000
20000
15000
10000
5000
0
5/1/2019
6/1/2019
7/1/2019
8/1/2019
9/1/2019
10/1/2019
11/1/2019
Year 2011
Year 2012
Year 2013
Year 2014
Year 2015
Year 2016
Year 2017
Year 2018
Year 2019

Figure 7.

Group 1: *Microcystis*-dominated

Group 2: Diverse

Group 3: *Microcystis*-dominated

Figure 8.

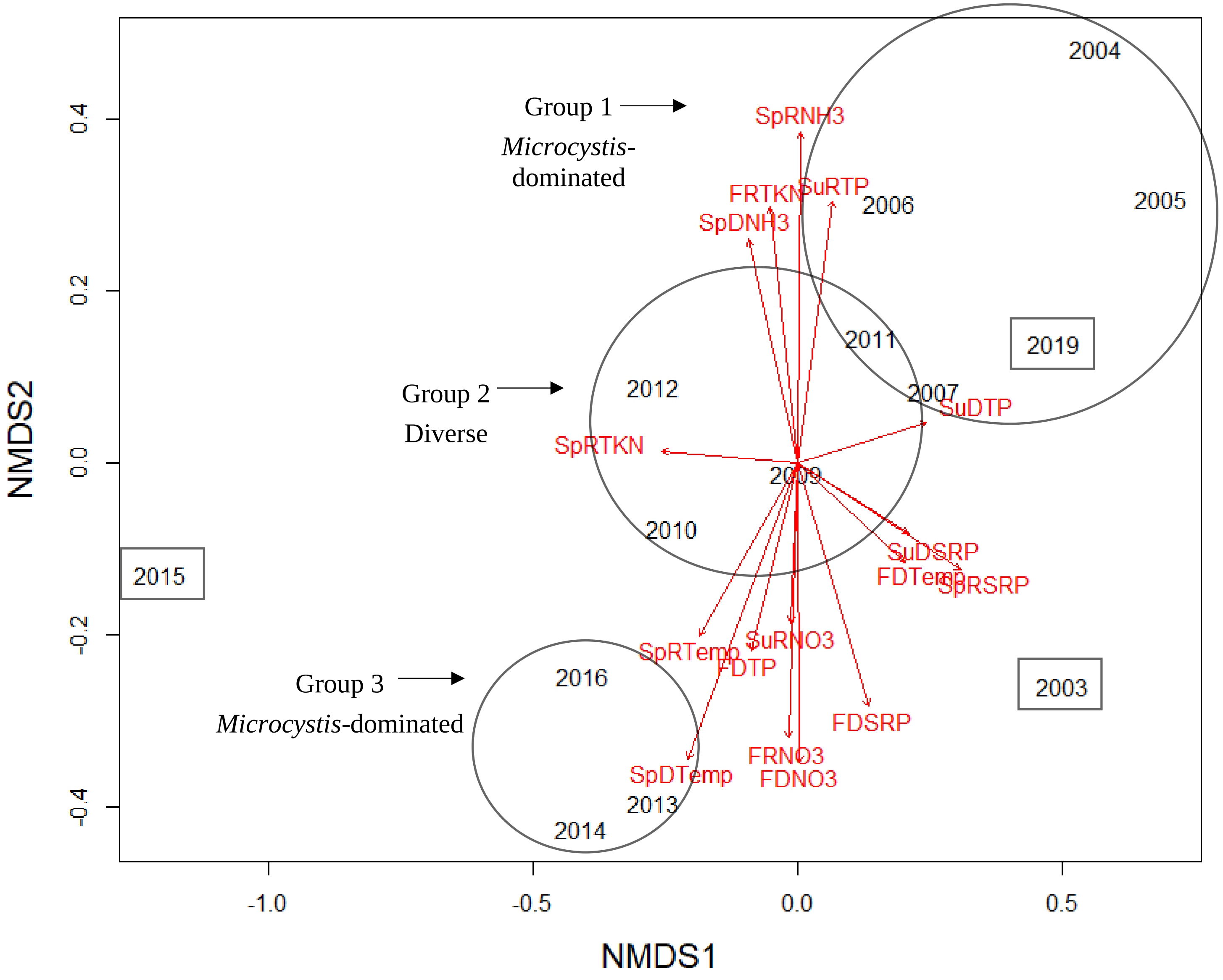
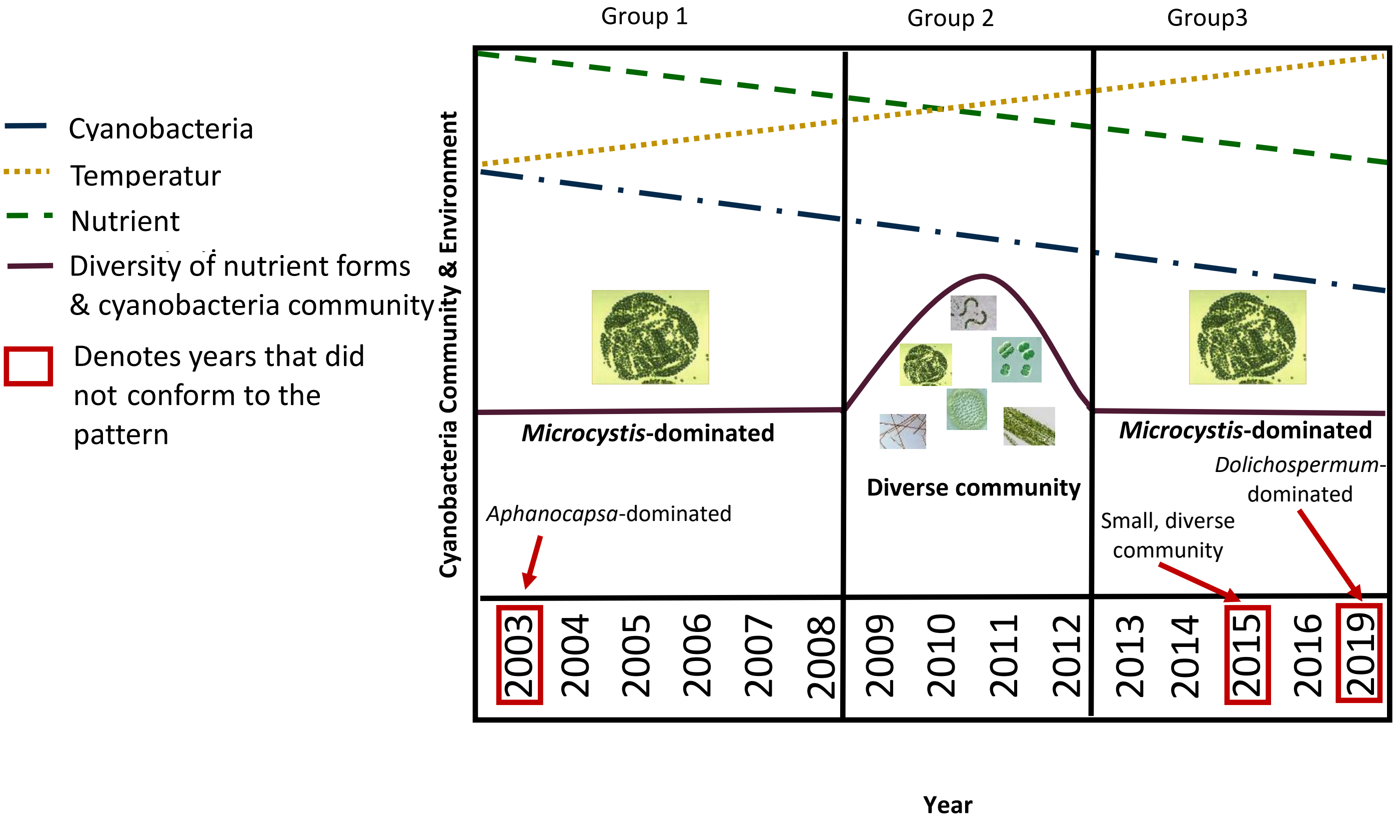

Group 1
Microcystis-dominated
Group 2
Diverse
Group 3
Microcystis-dominated
2004
2005
2006
2011
2019
2012
2007
SuDTP
SpRNH3
FRTKN
SuRTP
SpDNH3
SpRTKN
2009
2010
SuDSRP
FDTemp
SpRSRP
2015
SpRTemp
SuRNO3
FDTP
2016
FDSRP
2003
FRNO3
FDNO3
SpDTemp
2013
2014
0.4
0.2
0.0
-0.2
-0.4
-1.0
-0.5
0.0
0.5
NMDS1
NMDS2

Figure 9.


Group 1
Group 2
Group3
Cyanobacteria
Temperatur
Nutrient
Diversity of nutrient forms
& cyanobacteria community
Denotes years that did
not conform to the
pattern
Cyanobacteria Community & Environment
Microcystis-dominated
Diverse community
Microcystis-dominated
Aphanocapsa-dominated
Dolichospermum-
dominated
Small, diverse
community
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2019
Year

Chapter 2.2

Title

Cold and wet: Diatoms, not cyanobacteria, dominate the phytoplankton community in a year of anomalous weather in Muskegon Lake, a Great Lakes estuary

Abstract

As sentinels of the effects of climate change and other anthropogenic forces, freshwater lakes are experiencing ecosystem disruptions at every level of the food web, beginning with the phytoplankton. One of the main threats to freshwater lakes are cyanobacterial harmful algal blooms (HABs). Muskegon Lake, Michigan is a drowned river mouth Great Lakes estuary that connects to Lake Michigan. Designated as an Area of Concern by the EPA in 1987, Muskegon Lake's historical HAB impairment has largely been improved, but isolated, visible blooms are still an annual occurrence. With great uncertainty of how future climate scenarios will impact HABs, knowledge of phytoplankton composition in years that experience anomalous weather patterns may be valuable. In 2019, the Muskegon watershed experienced record-breaking amounts of precipitation and a relatively cool temperature regime. Through biweekly sampling at three locations on Muskegon Lake, we aimed to disentangle the connections between environmental conditions and phytoplankton community composition using multivariate analyses. Additionally, the long-term datasets from the Muskegon Lake Observatory buoy (2011-2019) and Muskegon Lake monitoring program (2003-2019) allowed us to compare 2019 to previous years to capture how the aberrant weather of 2019 affected the phytoplankton community of Muskegon Lake. The cool spring and late onset of stratification delayed phytoplankton growth overall. Unexpectedly, diatoms were the dominant division throughout the entire study period, and the cyanobacteria community was diverse but negligible compared to previous years, likely as a result of frequent rain events and insufficient temperatures. These

results may provide insight into how phytoplankton communities, diatoms and HABs in particular, in temperate freshwater lakes may respond to a future climate change scenario in which precipitation is frequent and some regions experience unexpected cooling.

Introduction

As primary producers, phytoplankton form the base of the major trophic sequence in the pelagic environment of freshwater aquatic systems. Similar to findings in other ecosystems, diversity that is maintained by species interactions, environmental conditions, and periodic disturbances is key to a healthy phytoplankton community. Interactions between microorganisms in the freshwater pelagic zone include competition for resources, such as light and nutrients; antagonistic behavior, such as cyanobacteria suppressing eukaryotic algae in blooms; trophic interactions, including ingestion and infection; and epiphytic associations between microorganisms (Sigee, 2005). According to Sommer (1989), there exists a predictable pattern of seasonal succession in the phytoplankton community of dimictic, freshwater lakes that responds to seasonal shifts in environmental conditions. Subsequent books have simplified this successional pattern: during the winter, nutrients build in the euphotic zone, but light is limiting and phytoplankton are largely metabolically inactive; in the early spring, a diatom bloom occurs, as diatoms thrive in turbulent, low-light environments, and variable nutrient concentrations; in late spring, a clear-water phase occurs due to increased zooplankton growth and limiting nutrients; small, fast-growing, inedible species occur in low populations near the end of the clearwater phase, usually inclusive of unicellular algae such as cryptomonads and green algae; in the summer, zooplankton decrease due to diminished food resources and increased fish growth; a mixed summer/fall bloom of inedible taxa, such as cyanobacteria and dinoflagellates, occurs followed by a resurgence of the diatoms; and in the fall, mixing can stimulate a bloom of a wide range of phytoplankton and zooplankton (Dodds and Whiles, 2019; Sigee, 2005).

Diversity is key to maintaining balance in the phytoplankton community and proper ecosystem functioning. Using a data set from the 1970s, Stomp et al. (2011) discovered that phytoplankton diversity varied across latitudinal, longitudinal, and altitudinal gradients in the continental United States. This study, along with others, indicates that changes in climate and land use that impact local environmental variables may impact phytoplankton diversity, as it is largely controlled by bottom-up mechanisms, such as nutrients, temperature, and light (Stomp et al., 2011; Zhang et al., 2016; Ptacnik et al., 2008). Furthermore, Ptacnik et al. (2008) and Ye et al. (2019) found that resource use efficiency by the phytoplankton community positively correlated with diversity in freshwater, marine, and brackish ecosystems and that fluctuations in community composition due to anthropogenic impairments may allow for single-taxon resource-domination and a reduction in carbon cycling efficiency. Thus, the effects of reduced phytoplankton diversity in freshwaters may prove to be detrimental in a time of increasing atmospheric carbon dioxide concentrations, especially in light of the recent realization that fresh waterbodies are hot spots of carbon cycling (Williamson et al., 2009)

Freshwater ecosystems are experiencing the deleterious effects of anthropogenic climate change and land use change. As systems are subject to intensifying environmental stress, the phytoplankton community is responding, shifting towards decreased taxa diversity and, often, single-taxon dominance (Sigee, 2005; Urrutia-Cordero et al., 2017; Bergkemper at al., 2018). Furthermore, their ability to maintain homeostasis and resist changes to their internal environment is diminishing (Sigee, 2005). One of the greatest threats to the stability and biological integrity of phytoplankton communities in freshwater lakes is the combined effects of anthropogenic eutrophication and climate change.

Eutrophication in waterbodies is a result of an excessive influx of nutrients, namely nitrogen (N) and phosphorus (P), from the surrounding watershed. The resulting increase in primary productivity introduces an imbalance in ecosystem function (Filstrup et al., 2017; Le Moal et al., 2019). The nutrients responsible for the eutrophication of waterbodies originate from agricultural, urban, and industrial sources in the form of fertilizers, pollutants, and waste (Glibert, 2017; Lowrance et al., 2018; Paerl and Otten, 2013). The influx of nutrients alters N:P ratios, the nutrient forms present, and shifts the nature of nutrient limitation in the ecosystem, favoring opportunistic species which outcompete and replace other algae in the form of a harmful algal bloom (HAB; Elser et al., 2009; Anderson, 2002). Around the world, freshwater lakes are increasingly suffering from the presence of HABs as a result of anthropogenic activity (Smith, 2003; Anderson, 2002; O'Neil et al., 2012; Ho et al., 2019). The most common division, or phylum, of phytoplankton creating HABs in freshwater lakes is cyanobacteria (Anderson, 2002). Cyanobacteria are prokaryotes and have a wide variety of adaptations that allow them to outcompete other phytoplankton under favorable conditions, including high temperature tolerance, production of over-wintering akinetes, buoyancy control, potential toxin production, ability to use organic P and store luxury P, N-fixing ability for some species, and efficient light harvesting (Carey et al., 2011; O'Neil et al., 2012; Paerl and Otten, 2013). However, the cyanobacteria are an extremely diverse group, and their traits and adaptations vary widely between taxa.

HABs are economically and ecologically harmful. Cyanobacteria can dominate the phytoplankton community and reach high biomass, lowering phytoplankton diversity, diminishing the photic zone, and inducing bottom-water hypoxia through increased aerobic decomposition (Sweerts et al., 1991; Scavia et al., 2014; Le Moal et al., 2019). Cyanobacteria are largely inedible, constraining energy transfer up the food web (Wilson et al., 2006; Šejnohová and Maršálek, 2012; Dickman et al., 2008; Ger et al., 2016). Additionally, many HAB species produce toxins that can cause illness and mortality in wildlife and humans in addition to contaminating drinking water and closing beaches (Backer et al., 2015; Carmichael et al., 2016). Combined economic losses resulting from eutrophication in freshwaters of the United States is estimated to be in the range of $2.2 billion to greater than $4 billion annually (Dodds et al., 2008; Kudela et al., 2015).

Climate change is altering phytoplankton communities globally, tending to favor cyanobacteria. Globally, inland waterbodies have experienced rapid warming since 1985 (Schneider and Hook, 2010; O'Reilly et al., 2015). With increasing temperatures, cyanobacteria have an advantage over other algae due to their higher temperature tolerance range. Additionally, warming temperatures may lead to a longer growing season, perpetuating earlier onset and longer duration of HABs (Deng et al., 2014; Paerl and Huisman, 2008; Ho and Michalak, 2019). Higher temperatures also increase the intensity and duration of stratification within lakes, allowing buoyancy-regulating cyanobacteria easier navigation towards light in the epilimnion and nutrients in the hypolimnion within a more stable water column (Carey et al., 2011). Longer periods of stratification combined with decomposition of HABs can further intensify hypoxia; this may lead to nutrient release from sediments, which benefits buoyant cyanobacteria (Scavia et al., 2014; Watson et al., 2016; Biddanda et al., 2018; Diaz and Rosenberg, 2008). Global changes in precipitation events as a result of climate change may also enhance bloom formation. While some have found that increased precipitation events may mitigate blooms due to a dilution and flushing effect (Ho and Michalak, 2019), others have theorized that heavy precipitation events interspersed by droughts will flush nutrients into waterbodies where they will subsequently concentrate and promote blooms (Paerl and Huisman, 2008; Havens et al., 2019).

It is projected that cyanobacterial HABs are likely to increase in duration, severity, and range under future climate scenarios, making their management more difficult (Havens and Paerl, 2015; Carey et al., 2011; Paerl and Huisman, 2008). However, uncertainty in predicting future patterns of temperature and precipitation, especially at the regional scale, warrant more investigations into how potential future climate scenarios will affect HABs and the phytoplankton community as a whole. It has been demonstrated that climate change is not progressing linearly, but rather manifests in extreme and record-breaking events, reduced predictability, and enhanced variability, especially at regional scales (Katz and Brown, 1992; Rahmstorf and Coumou, 2011; Wigley, 2009; Falvey and Garreaud, 2009). As such, studying years with anomalous weather patterns may offer insight into how this highly responsive, fast-growing group of organisms may respond under potential future climate scenarios. For example, Lehman et al. (2017) took advantage of the severe drought year of 2014 by studying the response of the cyanobacteria community in the San Francisco Estuary. Their results offered insights into how cyanobacterial HABs may be affected by a future climate scenario in which prolonged droughts are more common. Conversely, the year 2019 was an unusually wet year for most of the United States (Figure 1a). Our study site, Muskegon Lake, experienced record-breaking precipitation in much of its watershed during 2019. According to the National Oceanic and Atmospheric Administration (NOAA; 2020), Muskegon, Michigan received 121.95 cm of rain between 1 January and 31 December 2019, 36.88 cm higher than the long-term average, constituting 2019 as the wettest year for Muskegon in 115 years (Figure 1b, c). This heavy precipitation led to high lake levels and presumably shorter hydraulic residence times.

Additionally, 2019 was cooler than usual, especially in the spring. This unprecedented weather offered us an opportunity to examine the effects of a comparatively cold and wet year on the phytoplankton community, specifically the cyanobacteria.

Muskegon Lake (Muskegon, Michigan, USA) is a mesotrophic drowned river mouth Great Lakes estuary along the eastern shore of Lake Michigan. A drowned river mouth estuary is the interface between a lentic and lotic system, providing a unique ecosystem in which physical and chemical attributes create predictable spatial patterns. They are generally highly productive, and their wide range of habitats enables them to host a diversity of organisms and sustain high biomass (Dila and Biddanda, 2015; Defore et al., 2016). Furthermore, they are often focal areas of economic importance and undergo significant anthropogenic stress (Larson et al., 2013). Muskegon Lake has historically suffered from annual occurrences of HABs due to a history of lumber and industry along the lakeshore that degraded the quality of the lake (Gillett and Steinman, 2011). After being designated as a Great Lakes Area of Concern by the Environmental Protection Agency in 1987, remedial action plans were developed and revised from 1987-2002, with nuisance algae being cited as one of the beneficial use impairments targeted for improvement. The HAB impairment has been largely improved since 2003 as a result of the intense restoration projects on the lake (see Chapter 2.1). Monitoring efforts have been initiated to track the status of the lake, including the implementation of the long-term Muskegon Lake monitoring program (MLMP) in 2003 and the installation of the Muskegon Lake Observatory buoy (MLO) in 2011, the data of which is publicly available.

The uniqueness and significance of this study hinge on two ideas: 1) Muskegon Lake being a drowned river mouth estuary adds a spatiotemporal component that allows us to connect the phytoplankton community composition to the existing physiochemical gradient, and 2) 2019 was a year of aberrant weather, allowing a glimpse into the effects of an alternate climate change scenario on the phytoplankton community. The objectives of this study were to 1) quantify the spatial and temporal shifts in the phytoplankton community of Muskegon Lake and correlate them with shifts in environmental variables with an emphasis on cyanobacteria and 2) understand how the anomalous weather patterns of 2019 affected the phytoplankton community.

Methods

Study Site

Muskegon Lake (43.23º N, 86.29º W) is a mesotrophic drowned river mouth Great Lakes estuary in Muskegon, Michigan. Its main inflow is the Muskegon River, and it drains the second largest watershed in the state into Lake Michigan through a navigational channel (Figure 2). Muskegon Lake has a surface area of 17 km^2 and a water volume of 119 million m^3. It has a mean water depth of 7 m, a maximum water depth of 21 m, and a seasonally variable average hydraulic residence time of 23 days (Liu et al., 2018).

Muskegon Lake Observatory (MLO) buoy data

The Muskegon Lake Observatory (MLO; www.gvsu.edu/buoy) buoy is located in the geomorphic “middle” of the lake and is managed by the Biddanda Lab at the Annis Water Resources Institute of Grand Valley State University (Figure 2). Since its installation in 2011, the MLO has been delivering high-frequency, time-series meteorological and water quality data for about 7 months each year, excluding winter months (Biddanda et al., 2018). Water quality variables measured include temperature, dissolved oxygen (DO), nitrate (NO_3), photosynthetically active radiation (PAR), pH, specific conductivity, chlorophyll *a* concentration, phycocyanin concentration, and water current speed and direction. Meteorological variables measured include wind speed and direction, humidity, air temperature, and precipitation form and amount. The MLO is equipped with YSI (Yellow Springs Instruments)

6600/6920 datasondes at depths of 2, 5, 8, and 11 m, a C3 Submersible Fluorometer (Turner Designs) at 2 m, a Submersible Ultraviolet Nitrate Analyzer (Satlantic) at 2 m, a photosynthetically active radiation sensor (LI-COR) at 2 m, and temperatures nodes (NEXENS) at 2, 4, 6, 8, 10, and 11 m. The meteorological station is situated on top of the buoy. The MLO measures water quality and meteorological data every 15 minutes and 5 minutes, respectively; however, data used in this study are daily-averaged values. Periods of missing data are due to necessary maintenance, service, and biofouling; no attempt was made to extrapolate data to fill the gaps as to preserve the accuracy of interpretation.

2019 Biweekly Sampling

Sample collection

From 30 April to 18 October 2019, sampling occurred biweekly at three sites on the lake: the Muskegon Lake channel outflow (Channel site), the Muskegon River inflow (River site), and the site of the MLO buoy (MLO; Figure 2). Sites were sampled in the following order: Channel, River, and MLO sites. Water was collected from 1 m depth at the Channel and MLO sites and from the surface at the River site. Samples at the River site were collected at the surface rather than at 1 m depth because water here is expected to be well-mixed, and samples were collected from the M. Causeway bridge, where it was not feasible to use a Niskin sampler. Samples were stored in 2 L transparent Nalgene bottles, which were placed in a dark cooler until returning to the lab for nutrient and pigment analyses.

At each site, a YSI 6600 was used to collect physiochemical measurements. At the Channel and River sites, the YSI was maintained at a depth of 1 m for 2 minutes; at the MLO site, a profile of the water column was taken by slowly lowering a YSI over the side of the boat while taking an unattended sample at 2 second intervals until reaching the bottom of the lake, which is at about 12 m in this location. Secchi disk depth measurements were also taken at each site. All equipment was rinsed with site water prior to use.

At each site, we collected a phytoplankton sample using a 20 µm mesh plankton net to sample 10 m of the water column. At the Channel and River sites, we sampled 2 m of the water column five times consecutively, and at the MLO site, we sampled 10 m of the water column once due difference in depth. Each sample was concentrated to 100 mL and stored in a 125 mL brown Nalgene bottle. All equipment was rinsed with site water prior to use. Bottles were placed in a cooler after collection, and 3%-5% Lugol's iodine solution was added upon return to the lab for preservation. Samples were labeled and stored at room temperature until further analysis.

Phytoplankton Enumeration

To enumerate the phytoplankton, we gently inverted the sample several times before placing 1 mL of sample on a Sedgewick rafter counting chamber. After allowing the sample to settle for at least 10 minutes, we used the 20X and 40X objectives of the Nikon Ti-U and Nikon Eclipse Ni-U DIC/Epi-fluorescence Compound microscopes to identify individual phytoplankton to genus until reaching at least 350 natural algal units. We used Wehr et al. (2002), Prescott (1973), and Bellinger and Sigee (2010) as reference texts We then scaled each sample up to represent the number of natural algal units per L of unconcentrated lake water (individuals/L).

Nutrient and Pigment Analysis

Each water sample from all sites was analyzed for NO_3, NH_3, and SRP in the Wet Chemistry Lab at the Annis Water Resources Institute upon return to the lab. SRP and NH_3 were analyzed on a SEAL AQ2 discrete automated analyzer using USEPA Methods 365.1 and 350.1 Rev. 2.0 (1993), respectively (AQ2 Methods NO: EPA-118-A Rev. 4 and EPA-103-A Rev. 6, respectively). NO_3 was analyzed on a Dionex ICS 2100 Ion Chromatograph using USEPA Method 353.2 Rev. 2.0 (1993) (AQ2 Method NO: EPA-115-A Rev. 4).

Water samples from each site were then analyzed for pigments chlorophyll *a* and phycocyanin, which are used as a proxy for phytoplankton and cyanobacteria concentrations, respectively. Upon return to the lab, a measured amount of less than 500 mL of each sample was filtered through a 25 mm, .45µm microfiber GF/F glass fiber filter. Filters were individually stored in a -80 ºC freezer until analysis. Chlorophyll *a* analysis followed Standard Methods (APHA et al., 1992). Filters were prepared for chlorophyll *a* analysis by grinding the filter and 3 mL of buffered 90% acetone for one minute. The resulting slurry was left to extract at 5 ºC for 24 hours. Samples were centrifuged for 20 minutes at 4000 rpm. For each sample, 3 mL of supernatant were pipetted into a 10 mm quartz glass cuvette, and 3 mL of acetone buffer were pipetted into a separate cuvette to use as a blank. Absorbance wavelengths were measured at 750, 664, and 665 nm using a UV-VIS 2450 Shimadzu dual beam spectrophotometer. Chlorophyll *a* concentration (c) was calculated using the following equation:

c=26.7*(A664nm– A750nm)–(A665nm-A750nm)*Vol extracted (mL)/Vol filtered (L)

The procedure for phycocyanin analysis was developed based on findings in Horváth et al. (2013) and Lawrenz et al. (2011). Filters were prepared for phycocyanin analysis by completing two freeze thaw cycles to optimize pigment extraction (removed from -80 ºC, 5 ºC for 24 hours, -80 ºC for two hours, 5 ºC for 24 hours). At the end of the first cycle, 5 mL of phosphate buffer (0.1 M, pH=6.5) were added to each sample. Samples were sonicated for a total of 30 seconds. Phosphate buffer was used to rinse the sonicator into the centrifuge tube, bringing the final volume of the slurry to 10 mL. After sonication, samples were immediately placed in an ice bath for 15 seconds to cool. Samples were vortexed for a total of nine seconds and then allowed to extract at 5 ^{0}C for 96 hours. Samples were vortexed as before and centrifuged at 4000 rpm for 20 minutes. For each sample, 3 mL of supernatant were pipetted into a 10 mm quartz glass cuvette, and 3 mL of phosphate buffer were pipetted into a separate cuvette to use as a blank. Absorbance wavelengths were measured at 620 and 750 nm using a UV-VIS 2450 Shimadzu dual beam spectrophotometer. Phycocyanin concentration (p) was calculated using the following equation (corrected from Lawrenz et al., 2011):

$p=((A620-A750)/\varepsilon d)*(MW\ )*(Vol\ buffer\ (L)/Vol\ sample\ (L)*10^{6}$

where ε= molar extinction coefficient ($1.9*10^{6}$/mol cm), d= path length of the cuvette (1 cm), MW= Molar weight (264,000 g/mol). All pigment analysis was performed in the dark, and samples were wrapped in tin foil to prevent exposure to light.

Statistical Analysis

MLO buoy data – PCA and time-series graphs

We used a principal component analysis (PCA) correlation biplot to analyze MLO buoy data from the year 2019 to visualize variation in environmental variables in the lake over seasons. Data used in this PCA ranges from 14 May to 24 October and includes daily-averaged values. Variables kept in the ordination include dissolved oxygen (DO) at 2 and 11 m, maximum daily PAR, pH at 2 and 11 m, chlorophyll *a* at 2 m, phycocyanin at 2 m, temperature at 2 and 11 m, specific conductivity at 2 and 11 m, and NO_3 at 2 m. Variables removed from the PCA due to lack of explanatory power include pH at 5 and 8 m, DO at 5 and 8 m, specific conductivity at 5 and 8 m, and temperature at 4, 6, and 8 m. Color and shape were used to denote astronomical seasons. The R package vegan was used to create the ordination in RStudio Version 1.1.453 (Oksanen et al., 2018; R Core Team, 2018).

In order to monitor the status of stratification and hypoxia in the water column, daily-averaged values of water temperature at 2, 4, 6, 8, 10, and 11 m and dissolved oxygen at 2, 5, 8, and 11 m are displayed on time-series graphs. Additionally, a bar graph of daily precipitation accumulation was created to elucidate the frequency and amount of precipitation during the study season. Daily average air temperature is also included in this graph. These data are intended to help explain trends in HAB community dynamics.

2019 biweekly sampling – PCA, time-series graphs, NMDS, and stacked bar graphs

A PCA correlation biplot was created using environmental data collected at each site during biweekly sampling. The purpose of this analysis was to explore temporal and spatial variation in environmental variables in Muskegon Lake. As a PCA cannot be run on a matrix including missing observations, samples with missing data due to malfunctioning equipment were removed. Variables kept in the PCA include temperature, specific conductivity, pH, chlorophyll *a*, DO, NO_3, NH_3, and SRP. Phycocyanin was removed from the ordination due to lack of explanatory power, as evidenced by the short eigenvector length. The R package vegan was used to create the ordination in RStudio Version 1.1.453 (Oksanen et al., 2018; R Core Team, 2018).

In order to track chlorophyll *a* and phycocyanin concentrations at each site on the lake over time, time-series graphs were created for each of these variables. Water temperature, as an average of the water temperatures recorded at each site on the lake for each sampling day, was added to the phycocyanin and chlorophyll *a* time-series graphs.

Non-metric multidimensional scaling (NMDS) was used to visualize dissimilarity in the phytoplankton community in different seasons and at different sites in 2019. A Bray-Curtis dissimilarity matrix was used. A post-hoc adonis test was performed to determine if significant differences existed between seasons and sites. If significance was detected, a pairwise permutation multivariate analysis of variance (PERMANOVA) was performed to determine which dependent variables were significantly different. Subsequently, a similarity percentage (SIMPER) analysis was performed to determine the percent contribution of each phytoplankton genus to the dissimilarity matrix. Ellipses that enclose all points of each season were added to show differentiation between the seasons, and different shapes were used to denote site. The R packages vegan and RVAideMemoire were used to create the ordination in RStudio Version 1.1.453 (Hervé, 2020; Oksanen et al., 2018; R Core Team, 2018).

Stacked bar graphs were created using phytoplankton abundance data to visualize community composition at the division level for each season and site combination (i.e. spring, River). Abundance values for samples within each category were added together such that the value does not represent any one point in time but rather the cumulative abundance for the entire season at that site. Seasons were based on astronomical dates (spring: 30 April to 7 June; summer: 26 June to 5 September; and fall: 18 September to 18 October). Additionally, stacked bar graphs were created for the division cyanobacteria for each site and season combination to determine the spatial and temporal patterns in cyanobacteria community composition at the genus level. Abundance data was managed as described previously. The R package ggplot2 was used to create the figures in RStudio Version 1.1.453 (R Core Team, 2018; Wickham, 2016).

Comparison to previous years

To understand how the year 2019 differs from other years in terms of environmental variables, two long-term data sets were used: data from the MLO buoy and data from the Muskegon Lake monitoring program (MLMP; see Chapter 2.1 and Steinman et al., 2008).

Data from the MLO used here includes 2 m water temperature, chlorophyll *a*, and phycocyanin data, which was used to create overlapping time-series graphs that highlight the year 2019. Additionally, a table was created that summarizes 2 m water temperature data for each year of operation (2011-2019). The table consists of the maximum water temperature recorded by the buoy during its time of operation, the spring average, the summer average, the fall average, the number of days that had an average value over 25 ºC, and the number of days that had an average value over 20 ºC. The dates included within each season are based on astronomical seasons and are confined by the operational dates of the MLO (spring: 20 May to 19 June; summer: 20 June to 22 September; fall: 23 September to 20 October). Average temperature values reflect an average of the daily averaged temperatures within the season.

Using the MLMP data, the maximum, minimum, average, and 2019 values for nine variables (chloride, NO_3, NH_3, TKN, TP, temperature, DO, turbidity, and chlorophyll *a* concentration) for three seasons (spring, summer, and fall) collected at 1 m depth were calculated and distilled into a table. The subset of data used to create this table includes three of the six sites, MUSR, CHAN, and DEEP, because these sites are most comparable to the sites used in the 2019 season for biweekly sampling and avoid influence from inflowing tributaries (Figure 2). The purpose of this table is to compare 2019 environmental variable values to those of the prior 15 years in Muskegon Lake. A table of the percentage of the phytoplankton community that was comprised of cyanobacteria annually was calculated from MLMP phytoplankton data from 2003-2016 and biweekly sampling data from 2019 to compare 2019 to previous years. See Steinman et al. (2008) for sample MLMP collection methods.

Results

2019 MLO buoy data – PCA and time-series graphs

The first two axes of the PCA of 2019 MLO buoy data explained a cumulative 66.41% of the variation in the data (PC1=47.18%, PC2=19.23%; Figure 3). The PCA demonstrated distinct variation in values of environmental variables between seasons, shown by data points of each season grouping together. Summer data points correlated with high values of chlorophyll *a*, phycocyanin, and temperature at 2 m. To a lesser degree and with more variation, summer data points had high values of DO, maximum daily PAR, and specific conductivity at 2 m. Summer data points had low values of DO and pH at 11 m and nitrate at 2 m and mid to low values of temperature and specific conductivity at 11 m. Spring data points had high values of maximum daily PAR at 2 m and DO at 2 and 11 m and moderately high values of nitrate at 2 m and pH at 11 m. Spring data points had low values of temperature and specific conductivity at 2 and 11 m and moderately low values of all other variables (pH, chlorophyll *a*, and phycocyanin at 2 m). Fall data points had high values of pH and DO at 11 m and nitrate at 2 m. To a lesser degree and with more variation, fall data points had high temperature at 11 m and specific conductivity values at 2 m and 11 m. Fall data points had low values of DO, temperature, pH, chlorophyll *a*, and phycocyanin at 2 m and maximum daily PAR.

The time-series graph of water temperature at various depths in 2019 demonstrates the thermal regime of the lake (Figure 4a). Stratification began around late June, though it is difficult to pinpoint a narrower time frame due to missing data. Water temperature at all depths steadily rose until reaching a peak in early July after which they generally decreased until mid-August. After this, surface temperatures declined while deeper water temperatures increased. The lake almost completely mixed in late August, and the thermocline moved to a greater depth after re-stratification. Temperatures at 2 through 8 m were then nearly isothermal for the rest of the year.

After completely mixing and re-stratifying three more times, the lake became permanently isothermal in late September. The DO regime generally followed the same patterns as the temperature regime in terms of stratification causing differing concentrations at the different depths (Figure 4b). Depths of 8 and 11 m reached mild hypoxia (< 4 mg/L) in early July. 11 m reached severe hypoxia (<2 mg/L) in early July, and 8 m reached severe hypoxia for the first time in early August. Mild hypoxia was sustained at 8 and 11 m for the majority of July and August and lasted at 11 m through much of September. At least two Lake Michigan intrusions occurred, indicated by DO concentrations at 11 m increasing above that of 8 m. Mixing events coincide with those depicted on the temperature time-series graph. Precipitation data from the MLO demonstrates that rainfall was frequent, more so in the spring and fall than summer, and that air temperature often decreased after a rain event (Figure 1). The fall had higher daily accumulation than the spring. Out of the 165 days of MLO operation, 79 days experienced precipitation. The total precipitation accumulation during the MLO's operation was 65.05 cm, all as rain, which is 56.90 cm less than the yearly rain accumulation for 2019, 121.95 cm (NOAA, 2020), indicating the rest of the rainfall occurred in the early spring and late fall.

2019 biweekly sampling – PCA, time-series graphs, NMDS, and stacked bar graphs

The first two axes of the PCA of 2019 biweekly sampling data explained a cumulative 62.86% of the variation in the data (PC1=39.66%, PC2=23.20%; Figure 5). Variation between sites and between seasons was discernable. Spring data points were separate from the summer and fall sites, which largely overlap. The spring sites correlate with high values of NH_3 and DO and low values of temperature, specific conductivity, and chlorophyll *a*, though some data points have moderate values of chlorophyll *a.* Spring data points have moderate values of NO_3, SRP, and pH. While fall and summer data points overlap, summer data points tend towards higher values of chlorophyll *a*, pH, and temperature, while fall data points tend towards higher values of NO_3 and SRP. Specific conductivity is high in both the fall and the summer. Fall data points have low values of DO, and summer data points have low values of NH_3. Regarding sites, there is overlap, but the Channel and MLO tend to group together with little overlap with the River site. The variation between sites is driven by higher levels of DO at the Channel and MLO and higher levels of NO_3, SRP, and specific conductivity in the River.

Time-series graphs of chlorophyll *a* and phycocyanin concentrations showed similar patterns over time. Chlorophyll *a* concentrations were low at the River for the duration of the study season, and the peaks at the MLO and Channel coincided (Figure 6a). The first peak in chlorophyll *a* concentration occurred in late July, which coincided with the water temperature peak as well. The second peak occurred in mid-September but had lower chlorophyll *a* concentrations than the first peak. The phycocyanin concentrations from late April to early July were higher than chlorophyll *a* concentrations, which is highly improbable considering all phytoplankton, including cyanobacteria, produce chlorophyll *a* but only cyanobacteria create phycocyanin pigment (Figure 6b). Therefore, this data may be inaccurate. After early July, phycocyanin concentrations remained lower than chlorophyll *a* concentrations and peaked at the same times. Phycocyanin concentration in the River spiked in the summer, but then remained lower than MLO and Channel concentrations, which were similar and followed the same pattern.

The NMDS of the phytoplankton data collected during the biweekly sampling of Muskegon Lake had a stress value of 11.94, which indicates reasonably low distortion (Figure 7). The spring data points group together, and the fall data points group together with very little overlap, but the summer data points are widespread in the ordination and overlap significantly with spring and fall. There is no discernible pattern among the different sites overall, but within each season, the River sites group together separately from the Channel and MLO sites, which overlap. Confirming this visual analysis, the post-hoc adonis test revealed a significant difference in the phytoplankton community composition between seasons (F=3.882, R2=0.177, p=0.001), a marginally significant difference between sites (F=1.740, R2=0.088, p=0.051), and no significant interaction factor between season and site. However, the R^2 value of 0.1774 between seasons indicates that only 17.74% of the variation in the data is explained by the "season" effect and that there is significant overlap between seasons. The pairwise PERMANOVA revealed that there is a significant difference between spring and fall (p=0.003) and spring and summer (p=0.003) but not between fall and summer phytoplankton communities. While the adonis test revealed only marginal significance between sites, we continued with a pairwise PERMANOVA and found that there was a significant difference between the Channel and River sites (p=0.015). However, the R^2 value of the adonis test was low. The SIMPER analysis we ran considers the percentage each genus contributes to the Bray-Curtis dissimilarity matrix between the seasons and lists them in order of decreasing contribution. The genera driving 85.88% of the dissimilarity between the spring and summer were *Aulacoseira* Thwaites, *Fragilaria* Lyngbye, *Melosira* Agardh, *Dinobryon* Ehrenberg, *Nitzschia* Hassall, *Aphanizomenon* Morren ex Bornet & Flauhault, *Mallomonas* Perty, *Navicula* Bory, *Dolichospermum* Wacklin, Hoffman, & Komárek, *Planktothrix* Anagnostidis & Komárek, and *Microcystis* Lemmermann, all of which were more abundant in the summer. The genera driving 85.32% of the dissimilarity between the spring and fall were *Aulacoseira, Fragilaria,* centric diatoms (mainly *Cyclotella* Brébisson and *Thalassiosira* Cleve), *Melosira,* and *Planktothrix,* all of which were more abundant in the fall except for *Fragilaria*, which was more abundant in the spring. The genera driving 85.11% of the dissimilarity between the Channel and the River were *Aulacoseira, Fragilaria, Melosira,* *Dinobryon,* centric diatoms (mainly *Cyclotella* and *Thalassiosira*), *Planktothrix, Nitzschia, Tabularia* Williams & Round, and *Aphanizomenon,* all of which were more abundant in the Channel.

The stacked bar graphs depicting phytoplankton community composition over time and location demonstrated that diatoms were the dominant division throughout spring, summer, and fall at all sites (Figure 8). Hereafter, all other phytoplankton divisions found in the samples will be described in order of decreasing abundance. Due to the variable number of samples pooled within each seasonal time frame, comparisons can only be made between sites within each season, but not between seasons. In the spring, the phytoplankton abundance at the River and Channel were comparable to one another and higher than that at the MLO. While the large majority of the phytoplankton community for all sites and seasons was comprised of diatoms, some other divisions were present in lower abundance at each site. Other divisions that were present at the River were: chlorophytes, cyanobacteria, chrysophytes, dinoflagellates, and euglenoids; at the MLO, cyanobacteria, cryptophytes, chrysophytes, chlorophytes, and dinoflagellates; and at the Channel, chrysophytes, chlorophytes, cryptophytes, and dinoflagellates. In the summer, the River had significantly lower phytoplankton abundance than either the MLO or Channel sites and the Channel had much higher phytoplankton abundance than the MLO. The River was still dominated by diatoms but diversified slightly with slightly greater abundance of cyanobacteria, chlorophytes, chrysophytes, dinoflagellates, cryptophytes, and euglenoids. At the MLO in the summer, diatoms still dominated by a large degree, but some other divisions increased, including chrysophytes, cyanobacteria, chlorophytes, dinoflagellates, and cryptophytes. The phytoplankton community at the Channel was similar to that of the MLO. In the fall, the River had far lower phytoplankton abundance than either the MLO or Channel, and the Channel had slightly higher abundance than the MLO. The community at each site remained dominated by diatoms. Other phytoplankton divisions present at the River include cyanobacteria, chrysophytes, dinoflagellates, chlorophytes, and euglenoids; at the MLO, chrysophytes, cyanobacteria, chlorophytes, and dinoflagellates; and at the Channel, cyanobacteria, chlorophytes, chrysophytes, and dinoflagellates.

The stacked bar graphs depicting the cyanobacteria community composition by genus shows that no single genus truly achieved dominance throughout the study period of 2019 (Figure 9). Cyanobacteria abundance was low in the spring for the River and MLO sites and nonexistent in the Channel. In the River, only *Chroococcus* Nägeli was found, and at the MLO, *Dolichospermum* dominated the cyanobacteria community with very small amounts of *Chroococcus* and *Microcystis*. In the summer, cyanobacteria abundance was much lower at the River than the Channel or MLO and was slightly higher at the MLO than at the Channel. At the River, *Microcystis* and *Planktothrix* were the most abundant genera and had roughly equal counts. *Chroococcus* was the next most abundant. *Aphanizomenon* and *Dolichospermum* were much less abundant but also present in roughly equal counts, and very small amounts of *Coelosphaerium* Nägeli, and *Lyngbya* Agardh ex Gomont were present. The most abundant genus at the MLO and Channel was *Dolichospermum*, the second most abundant genus was *Aphanizomenon*, followed by roughly equal numbers of *Microcystis* and *Planktothrix.* The Channel, additionally, had small amounts of *Chroococcus, Gomphosphaeria* Kützing, and *Lyngbya.* In the fall, the cyanobacteria abundance was highest at the Channel, and the MLO had slightly higher abundance than the River. The cyanobacteria community of all three sites was over 50% composed of *Planktothrix*. The second most abundant genus at the River was *Microcystis,* followed by small amounts of *Chroococcus, Dolichospermum,* and *Oscillatoria.*

The second most abundant genus at the MLO was *Dolichospermum*, followed by *Microcystis, Gomphosphaeria,* and *Chroococcus.* Lastly, the second most abundant genus at the Channel was *Microcystis,* followed by *Dolichospermum.*

Comparison to previous years – MLO data and MLMP data

Regarding water temperature at 2 m, Table 1 and Figure 10 demonstrate that 2019 had a relatively cool temperature regime compared to previous years. The average spring temperature was the coolest on record in 2019 at 17.97 ºC, 1.21 ºC cooler than the next coolest year and 3.13 ºC cooler than the warmest recorded year. The summer average temperature for 2019 was lower than all but one year at 22.84 ºC. The fall average temperature was average, being warmer than four of the years on record and cooler than four of the years on record. The number of days above 25 ºC for 2019 was 15, which is higher than only two other years on record. The average number of days above 20 ºC for 2019 was 69, which is the lowest on record, with the next lowest value being 78. Regarding chlorophyll *a* concentrations in the lake, Figure 11a demonstrates that 2019 showed relatively low chlorophyll *a* concentrations throughout the growing season compared to previous years. Most notable is how late in the year the first peak occurred and how comparably low the chlorophyll *a* concentrations remained after the first peak. The phycocyanin concentration time-series graph shows that 2019 had exceptionally low phycocyanin concentrations compared to other years (Figure 11b). In the spring, 2019 concentrations were lower than any other year. The peak was around mid- to late-July, which is roughly the same time-period of the peak for other years but was much lower in comparison. After the peak, concentrations steadily decreased, saw a small increase around mid-September, and then decreased to below concentrations of other years.

From the MLMP data shown in Table 2, we can make comparisons between the year 2019 and the previous 15 years in terms of environmental variables in Muskegon Lake. Chloride levels in 2019 were consistently lower than average and always closer to the minimum value than the maximum value in all seasons and sites. NO_3 values for 2019 were below or equal to average for all sites and seasons and far closer to the minimum than the maximum value. NH_3 values for 2019 were around average in most cases except for at MUSR and CHAN in the spring, when they approached or equaled the maximum value, respectively. TKN values for 2019 were generally above average, in three cases equaling the maximum recorded value but never exceeding it. TP in 2019 was generally slightly above average during the summer and fall seasons and at MUSR and DEEP but remained around average during all seasons at CHAN. Water temperature for 2019 was below average at every site in the spring, around average for each site in the summer, and around average for each site in the fall except at DEEP, where it was significantly warmer than average and equaled the maximum recorded temperature. DO values for 2019 were similar to average in all sites and seasons, except for MUSR in the spring, where it was slightly above average. Turbidity values for 2019 were above average in the spring at MUSR, in all seasons at DEEP, and spring and summer at CHAN and was slightly below average in all other cases. Lastly, chlorophyll *a* concentrations were well above average during the summer for all sites, equaling the maximum recorded value. In all other cases, the 2019 values were about average. It is noted that there was an algal bloom present on the lake during the summer sampling date for 2019, which likely explains the unexpectedly high values.

There appears to be a clear distinction in the percentage of the phytoplankton community that is comprised of cyanobacteria (hereafter, percent cyanobacteria) between the years 2003 to 2008 and 2009 to 2019 (data from years 2017 and 2018 unavailable). From 2003 to 2008, percent cyanobacteria ranges from 25.16% to 63.35%. From 2009 to 2019, percent cyanobacteria ranges from 0.71% to 39.29%, with 2019 having the second lowest value at 1.59% (Table 3).

Discussion

Our objectives for this study were to 1) quantify the spatial and temporal shifts in the phytoplankton community on Muskegon Lake and correlate them with shifts in environmental variables with an emphasis on cyanobacteria and 2) understand how the anomalous weather patterns of 2019 shaped the phytoplankton community. Environmental data showed distinctions between some seasons and sites, which provided differing conditions to which the phytoplankton community responded both quantitatively and qualitatively. Additionally, the phytoplankton community as a whole responded to the aberrant weather of 2019 in unexpected ways.

Spatial patterns in the phytoplankton community

Our environmental data demonstrate that the MLO and Channel sites were similar to each other and distinct from the River site. Similarly, the phytoplankton communities of the River and Channel were significantly different. The River had substantially lower phytoplankton amounts compared to other sites, which is likely the main driver of the significant difference in the phytoplankton community between them. While the River site associated with high nutrient concentrations, it is likely that the phytoplankton community was unable to establish a strong population in the river due to high flow rates, short residence times, and high turbidity that diminished the photic zone. Though these attributes are inherent characteristics of river mouths, the frequent precipitation of 2019 would have provided a particularly unfavorable environment for phytoplankton in the river. Few genera were more abundant in the River than the other two sites; however, those that were more abundant were mostly single-celled forms, such as *Cocconeis* Ehrenberg, *Amphora* Ehrenberg ex Kützing, *Navicula*, *Rhoicosphenia* Grunow, and *Diatoma* Bory. It is possible that their high surface area to volume ratio and small size provided them with advantages, such as high nutrient uptake rates and entrainment in the water column, in the turbulent, nutrient-rich river (Abonyi et al., 2014). Additionally, several of the listed genera are, or can be, epiphytic, likely having been detached from a macrophyte host in the high-flow conditions, which would be more common in the river than in the pelagic zone of the lake.

The slower water velocity, longer residence times, influx of nutrients from the Muskegon River, and lower turbidity of water at the MLO and Channel sites allowed for higher phytoplankton growth rates and different genera to establish than in the River, namely filamentous and bloom-forming genera that benefit from stratification and slow water velocity (Mantzouki et al., 2015). The MLO consistently had lower phytoplankton abundance than the Channel, possibly due to a concentration effect of the water in the lake being moved into the shallower, narrow channel. Additionally, the Channel appeared to have higher NH_3 concentrations, which may represent a reduced nitrogen pool that increased phytoplankton growth rates. However, the difference in phytoplankton abundance could also be a result of the discrepancy in our phytoplankton sampling methods at these two sites due to the depth difference.

Temporal patterns in the phytoplankton community: Dominance of filamentous diatoms

It is clear that, in Muskegon Lake, environmental variables are distinct between seasons. Environmental data demonstrated that the summer and fall were more similar to each other than they were to the spring. Consequently, the phytoplankton communities of the summer and fall were also distinct from the spring. Over seasons, the time-series chlorophyll *a* concentration graphs show that phytoplankton growth was highest in the summer and lowest in the spring with high values in the fall as well. The seasonal pattern in both environmental data and phytoplankton community composition was likely driven primarily by variations in water temperature, which was exceptionally cool in the spring of 2019.

The phytoplankton community of Muskegon Lake in 2019 was dominated by diatoms for the duration of the study period (late-April through mid-October). This is clearly an exception to observed patterns in temperate dimictic lakes and in Muskegon Lake in particular, which usually has a fairly diverse phytoplankton community throughout the year (Sommer, 1989; Dodds and Whiles, 2019; Sigee, 2005; Gillett and Steinman, 2011). Though phyla diversity was low due to this single-division domination, there were temporal differences in the phytoplankton community at the genus level that reflect changes in environmental conditions.

The spring phytoplankton community was over 80% comprised of diatoms and was largely dominated by *Fragilaria* and, secondarily, *Aulacoseira* and *Melosira*, which are all filamentous diatoms. These results are unsurprising, as we sampled the pelagic zone, where filamentous genera are expected to be, and diatoms often dominate in the spring (Sommer, 1989; Sigee, 2005; Dodds and Whiles, 2019). *Fragilaria* is often a dominant genus in Lake Michigan, to which Muskegon Lake is connected, and has been shown to benefit from high N:P ratios (Stoermer et al., 1978; Saros et al., 2005); this may have been the driving factor of their success here, as spring data points correlated with high NH_3 and low SRP.

The difference between the spring and summer communities was driven by an increase in five diatom, two chrysophyte, and four cyanobacteria genera. Whereas diatoms should be minimally present in the summer, this indicates that the diatom bloom not only persisted, but grew in the summer. Again, the diatom bloom was driven by the three filamentous genera previously mentioned (*Fragilaria, Aulacoseira,* and *Melosira*), but within that group, *Aulacoseira* had increased in proportion substantially, followed by *Fragilaria* and *Melosira.* The summer only experienced mild increases in chrysophytes (*Dinobryon* and *Mallomonas*) and cyanobacteria (*Dolichospermum*, *Planktothrix*, and *Microcystis*), which deviates far from the expected pattern. The shift in dominance from *Fragilaria* to *Aulacoseira* may be explained by documented attributes of these two genera. Horn et al. (2011) found that *Fragilaria* was often dominant in the transition between spring and summer but was a poor competitor at low-light conditions. In contrast, it was found that *Aulacoseira* often dominated in years that experienced long, cold springs, a late onset of summer stratification, and low-light conditions. Muskegon Lake experienced an unusually cool spring in 2019, and water temperature warmed far later than in previous years, delaying stratification. This, combined with heavy precipitation that flushed organic matter into the water, increasing turbidity and decreasing PAR, gave *Aulacoseira* an advantage in the summer and fall of 2019. Additionally, *Melosira* unexpectedly increased in proportion in the summer as well. *Melosira* sinks quickly, is usually most abundant in the spring and fall, and is usually scarce in the euphotic zone during stratified conditions (Lund, 1954). However, it has been found that *Melosira* can make an appearance in the summer if mixing is sufficient (Lund, 1971). Therefore, it can be concluded that *Aulacoseira* and *Melosira* increased over *Fragilaria* due to conditions sustained by frequent precipitation, such as water column mixing that prevented sedimentation and high turbidity that provided a low light environment in which they have an advantage.

The difference between spring and fall communities was, likewise, driven by an increase in three diatoms; *Aulacoseira* continued to increase, centric diatoms (*Cyclotella* and *Thalassioseira*) became more prevalent, and *Melosira* increased from spring to fall as well. *Fragilaria,* however, was significantly more abundant in the spring than fall, indicating its continued decline. One cyanobacteria genus (*Planktothrix*) was also significantly more abundant in the fall than spring. While a resurgence of diatoms is a familiar scenario in the fall, they are usually accompanied by a significant proportion of cyanobacteria, which was not the case here. The lack of differentiation in phytoplankton community composition between the summer and fall further indicates an unwavering presence of diatoms throughout the entire growth season.

The most relevant question at this point is, “why did the diatoms dominate throughout the entire growing season?” Diatoms are characterized by a silica cell wall and the presence of a water-filled vacuole, which has both advantages and consequences (Sicko-Goad et al., 1984; Hansen and Visser, 2019). The silica cell wall protects against predation and is necessary to house the vacuole, but promotes sinking, and the vacuole increases nutrient affinity, size, possible buoyancy, and the potential for light capture, but is expensive to maintain (summarized in Hansen and Visser, 2019). Hansen and Visser (2019) hypothesize that these traits likely correlate with their success and growth. In broader terms, it is hypothesized that phytoplankton bloom development, coexistence among species, and single-taxon domination are largely driven by competition for light, which is directly influenced by mixing of the water column (Huisman et al., 1999; Mur and Schreurs, 1995). In effect, a bloom can develop in a deep lake if the turbulent mixing does not exceed the critical turbulence of the phytoplankton species present, and which taxa dominates is dependent on critical light intensity and the mixing regime. Most of the models in Huisman et al. (1999) found that competitive exclusion is far more common than coexistence, likely due to the restricted nature of the light environment in aquatic ecosystems. Therefore, we believe that the light environment and mixing conditions of 2019 provided diatoms with the advantage necessary to competitively exclude other divisions.

Diatoms have high population growth rates and are competitive at a variety of nutrient concentrations, lower temperatures, and low light intensity (Hansen and Visser, 2019; Huisman et al., 1999; Rothenberger et al., 2009). It is well-documented that diatoms are often favored by frequent mixing of the water column, as it reduces the risk for sedimentation, moderates water temperatures in the photic zone, lowers light intensity, and entrains nutrients (Huisman et al., 1999; Visser et al., 1996; Huisman et al., 2004; Lund, 1971). Additionally, it has been shown that, in estuaries, diatoms benefit from high rainfall, high river discharge rates, and short residence times, a phenomena that is well-documented in the Neuse River Estuary, North Carolina, USA (Rothenberger et al., 2009; Paerl et al., 2009; Paerl et al., 2010). The cool water temperatures experienced in Muskegon Lake in 2019, the long spring, and the late onset of stratification provided the temperature regime necessary for diatoms to dominate through June. After this, the frequent storm and wind events created sufficient mixing of the water column to keep filamentous diatoms entrained throughout the summer. Additionally, the heavy precipitation flushed organic matter into the water column, which increased turbidity and maintained a low-light environment in which diatoms are highly competitive. Diluted nutrient conditions caused by heavy precipitation, insufficient temperatures, and water column mixing concurrently prevented other genera from forming blooms (Huisman et al., 1999). In essence, the filamentous diatoms dominated throughout the entire growth season of 2019 because low temperatures gave them a strong advantage for several months in the spring and early summer that allowed them to build biomass, frequent precipitation provided an environment in which they stayed entrained in the water column, and no other division had enough of an advantage to outcompete them. It is also possible that top-down mechanisms were influencing the phytoplankton community, such as if fish and zooplankton communities were also affected by the aberrant weather of 2019. If decreases in the return periods of extreme rainfall events is a persistent pattern in the future, as is suggested by some climate models (Huntingford et al., 2003), specifically one calibrated for the Great Lakes region (Byun and Hamlet, 2018), diatom domination may become a more frequent occurrence. However, if mean temperatures increase in the Great Lakes region, this may not be the case.

Cyanobacteria community suppressed under cold, wet, turbid conditions

The cyanobacteria are of particular interest due to the unresolved beneficial use impairment of “undesirable algae” in Muskegon Lake, a Great Lakes Area of Concern. However, it should be noted that the cyanobacteria comprised a very small percentage of the phytoplankton community in Muskegon Lake in 2019, such that they likely had very little influence on the ecosystem. Additionally, the impact of removing the rest of the phytoplankton community for statistical analyses for the purpose of focusing on trends in the cyanobacteria community should be considered.

In terms of the relatively insignificant cyanobacteria community, it was fairly diverse, and no single genus dominated. Additionally, it followed a similar pattern to the expected seasonal succession, with peaks occurring in the summer and fall. Historically, Muskegon Lake suffered severe HABs until remediation efforts were instituted after being listed as an AOC by the EPA; from 2003-2016, blooms were often dominated by *Microcystis* except for a group of years (2009-2012) that had diverse cyanobacteria communities (see Chapter 2.1). 2019 had the second lowest percent cyanobacteria on record. This is not out of line with the trend in previous years, but was likely exacerbated by the low temperatures, frequent mixing, and dilution of nutrients in the water column that specifically disadvantaged the cyanobacteria community.

The River consistently had lower cyanobacteria abundance than the MLO and Channel sites, which had relatively similar counts. This is likely due to the fast water velocity and lack of thermal stratification that do not allow blooms to form, especially with the high precipitation experienced in 2019. In the summer, *Dolichospermum* comprised the largest proportion of the cyanobacteria community at the MLO and Channel sites, which may be concerning due to their ability to produce several toxins, including microcystins, anatoxin-a, anatoxin-a(S), saxitoxin, cylindrospermopsin, and lipopolysaccharide (Li et al., 2016). *Dolichospermum* is a bloom-forming diazotrophic genus with a lower and wider temperature tolerance range than other cyanobacteria (Weirich et al., 2019; Li et al., 2016). Because of its temperature tolerance range, *Dolichospermum* is often one of the earliest cyanobacteria present in temperate lakes (Chaffin et al., 2018). It also often correlates with high turbidity, due to its ability to regulate buoyancy, and low N concentrations, due to its diazotrophic capabilities. These attributes may have given it an advantage over other cyanobacteria in this case (Huisman et al., 2004; Smith, 1983; Li et al., 2016). Despite *Dolichospermum* being the most abundant cyanobacteria, several other genera were present in appreciable amounts, such as *Microcystis, Planktothrix,* and *Aphanizomenon*. *Aphanizomenon* is similar to *Dolichospermum* in that it is also a diazotrophic, filamentous cyanobacteria, which likely gave it a similar advantage as *Dolichospermum* (Rigosi et al., 2014). *Microcystis* and *Planktothrix* are both non-diazotrophic and both have adaptations to low-light conditions (Nixdorf et al., 2003; Mur and Schreurs, 1995; Mantzouki et al., 2015). *Microcystis* never dominated due to the few, non-sustained periods of time in which temperature was suitable for them and the continuous mixing of the water column due to precipitation events (Robarts and Zohary, 1987; Mantzouki et al., 2015). *Planktothrix* has a lower temperature tolerance than *Microcystis* and has been shown to be dominant under turbid, wind-mixed conditions. Additionally, N concentrations increased in the fall, taking the advantage away from diazotrophic genera, such as *Dolichospermum* and *Aphanizomenon*. This explains the dominance of *Planktothrx* in the fall of 2019 in Muskegon Lake (Donald et al., 2013; Nixdorf et al., 2003).

Several studies have documented the complex effects of weather patterns on cyanobacterial HABs. The general consensus is that warm, drought years tend to exacerbate HABs, and cool, wet years tend to ameliorate them (Havens et al., 2019; Lehman et al., 2017; Reichwaldt and Ghadouani, 2011). Therefore, the effects of future climate change scenarios on HABs are difficult to predict and are largely dependent on patterns of precipitation and temperature. In the present study, we not only elucidated the effects of an exceptionally wet and relatively cool year on the HAB community, but we found that these anomalous conditions fostered the domination of another phytoplankton group as well: the diatoms. The effects of a diatom-dominated ecosystem may radiate throughout the food web, as many of them are not prone to consumption due to their size and silica cell wall, though their high concentrations of fatty acids make them a valuable food source (Hansen and Visser, 2019).

Conclusion

One year of anomalous weather patterns, 2019, resulted in an anomalous phytoplankton community. The phytoplankton community of Muskegon Lake became dominated by filamentous diatoms throughout the growing season, an unprecedented phenomenon that can be explained by the aberrant weather of that year. A cool, long spring that delayed summer stratification allowed diatoms to grow with little competition through June. The record-breaking rainfall diluted nutrients in the waterbody, increased turbidity, and, importantly, allowed for sufficient mixing. As such, the diatoms stayed entrained in the water column, and other divisions, such as cyanobacteria, were disadvantaged and outcompeted. This study provides insight into how the effects of eutrophication and climate change interact and affect HABs, demonstrating that short-term climatic oscillations can override the effects of nutrient inputs on the phytoplankton community if significant (Paerl et al., 2010; Figure 12). In a world experiencing aberrant weather patterns and an uncertain future in the face of climate change, studying years that experienced extreme weather events may provide clues to how future climate change scenarios will impact the phytoplankton community of temperate freshwater lakes with consequences for the aquatic food web and the global cycling of elements (Downing, 2010; Biddanda, 2017).

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Figure Legends

Figure 1. Precipitation data for 2019, including: a.) A map showing precipitation data collected from 1 January to 31 December in 2019; map provided by NOAA b.) Annual rain accumulation (1 Jan – 31 Dec) for years 2003-2019; data provided by NOAA c.) Daily rain accumulation and daily average air temperature (ºC); data provided by the Muskegon Lake Observatory (MLO) buoy.

Figure 2. A map of the study site, Muskegon Lake, including points of collection for the 2019 biweekly sampling (red triangles): the Muskegon River inflow, the Muskegon channel outflow, and near the Muskegon Lake Observatory (MLO) buoy (blue circle). Also included in the figure are the sampling sites for the MLMP from which data was used: MUSR, DEEP, and CHAN. The inset in the lower right corner denotes the boundaries of the watershed.

Figure 3. A PCA created from 2019 MLO data. The seasons (spring, summer, and fall) are denoted by differing shape and color combinations. The cumulative proportion of variation explained by the first two axes is 66.41% (PC1=47.18%, PC2=19.23%). Variable codes: Chl=chlorophyll *a*, PC=phycocyanin, Temp=temperature, SpCond=specific conductivity, DO=dissolved oxygen, MaxPAR=maximum photosynthetically active radiation, 2m=2 meter depth, 11m=11 meter depth.

Figure 4. a) Water temperature data at various depths provided by the MLO buoy during 2019 b) Dissolved oxygen data at various depths provided by the MLO buoy during 2019

Figure 5. PCA of environmental data collected biweekly during 2019 at the three sampling sites on the lake. Site is denoted by color, and season is denoted by shape. The cumulative proportion of variation explained by the first two axes is 62.86% (PC1=39.66%, PC2=23.20%). Variable

codes: NO3=nitrate, NH3=ammonia, SRP=soluble reactive phosphorus, DO Conc=dissolved oxygen concentration, SpCond=specific conductivity, Temp=temperature.

Figure 6. Time-series graphs of a) chlorophyll *a* and b) phycocyanin concentrations from 30 April to 18 October 2019 at three sampling locations, which are denoted by color. The temperature line is an average of the temperatures at those three locations on each sampling date.

Figure 7. NMDS of phytoplankton data collected during the 2019 biweekly sampling of Muskegon Lake. The ordination has a stress value of 11.938. Seasons are denoted by color, and sites are denoted by shape. The vectors represent environmental data collected during the 2019 sampling.

Figure 8. Stacked bar graphs of phytoplankton community composition from data collected during the 2019 biweekly sampling on Muskegon Lake. Each bar represents the phytoplankton community present at each site during each season at the division level. Spring includes sampling dates from 30 April to 7 June, summer includes sampling dates from 26 June to 5 September, and fall includes sampling dates from 18 September to 18 October. As such, quantitative comparisons can only be made within each season and not between seasons (denoted by dark boxes around each season).

Figure 9. Stacked bar graphs of the cyanobacteria community composition from data collected during the 2019 biweekly sampling on Muskegon Lake. Each bar represents the cyanobacteria community present at each site during each season at the genus level, inclusive of genera that comprised over 5% of the sample by count. Spring includes sampling dates from 30 April to 7 June, summer includes sampling dates from 26 June to 5 September, and fall includes sampling dates from 18 September to 18 October. As such, quantitative comparisons can only be made within each season and not between seasons (denoted by dark boxes around each season).

Figure 10. Time-series graph of 2 m water temperature data from the MLO. Years 2011-2019 are included, but only 2019 is highlighted for the purpose of comparing that year's temperature regime to previous years.

Figure 11. a) Time-series graph of 2 m chlorophyll *a* concentration (µg/L) data from the MLO. Years 2011-2019 are included, excepting 2016 and 2018 due to insufficient data, but only 2019 is highlighted for the purpose of comparing that year's temperature regime to previous years b) Time-series graph of 2 m phycocyanin concentration (cells/mL) data from the MLO. Years 2011-2019 are included, but only 2019 is highlighted for the purpose of comparing that year's temperature regime to previous years.

Figure 12. This schematic presents a summary of the major findings of this study, visually describing the differences in weather patterns and the cyanobacteria community between 2019 and an average year. It should be noted that the graphs do not depict actual values but rather general observed patterns.

Tables

Table 1. This table contains water temperature data from the MLO at 2 m depth from 2011-2019 with the purpose of comparing 2019 (bolded) to other years. Included is maximum recorded temperature (20 May - 20 October), spring average (20 May – 19 June), summer average (20 June – 22 September), fall average (23 September – 20 October), and number of days above 20 ºC and 25 ºC (20 May-20 October).

Year	*Max (ºC)*	*Spring average (ºC)*	*Summer average (ºC)*	*Fall average (ºC)*	*Days above 25 (ºC)*	*Days above 20 (ºC)*
2011	28.01	19.85	23.87	15.95	35	89
2012	29.25	20.95	24.90	14.45	36	91
2013	28.99	19.42	23.49	17.37	21	97
2014	24.95	21.10	23.29	14.84	0	94
2015	26.61	19.18	23.22	17.07	18	106
2016	26.79	20.99	24.07	17.45	28	121
2017	25.82	20.86	22.62	19.05	9	111
2018	27.10	21.47	23.73	15.90	19	78
2019	**26.85**	**17.97**	**22.84**	**16.38**	**15**	**69**

Table 2. MLMP data from near the surface at three of the six sampling locations: MUSR (river), DEEP (deep), and CHAN (channel). Included in the table are the maximum, minimum, average, and 2019 (bolded) values for each of nine variables at each site within each season. The purpose of this table is to compare 2019 to past years.

	River	*Cl mg/L*	*NO$_3$ mg/L*	*NH$_3$ mg/L*	*TKN mg/L*	*TP mg/L*	*Temp (C^0)*	*DO mg/L*	*Turb NTU*	*Chl ug/L*
Spring	Maximum	107.00	0.59	0.09	0.73	0.025	17.29	13.71	7.00	12.80
	Minimum	15.00	0.21	0.01	0.30	0.011	11.41	8.04	0.00	0.20
	Average	28.43	0.36	0.03	0.52	0.018	13.91	9.61	4.49	5.91
	2019	**17.00**	**0.23**	**0.05**	**0.56**	**0.015**	**11.41**	**11.46**	**6.40**	**5.60**
Summer	Maximum	60.00	0.86	0.07	0.92	0.070	28.49	10.04	36.00	14.40
	Minimum	7.00	0.03	0.01	0.40	0.018	21.16	6.87	2.10	2.40
	Average	26.79	0.37	0.03	0.58	0.032	25.08	8.38	8.12	9.41
	2019	**19.00**	**0.09**	**0.02**	**0.84**	**0.042**	**24.87**	**7.98**	**7.00**	**13.60**
Fall	Maximum	52.00	0.98	0.06	0.91	0.043	23.16	10.32	11.00	12.50
	Minimum	22.00	0.07	0.01	0.12	0.013	17.29	7.25	2.50	3.50
	Average	28.86	0.35	0.02	0.50	0.027	20.16	8.75	6.71	7.01
	2019	**22.00**	**0.25**	**0.03**	**0.61**	**0.043**	**20.10**	**7.92**	**5.10**	**5.40**
	Deep	*Cl mg/L*	*NO3 mg/L*	*NH3 mg/L*	*TKN mg/L*	*TP mg/L*	*Temp C*	*DO mg/L*	*Turb NTU*	*Chl ug/L*
Spring	Maximum	69.00	0.65	0.06	0.79	0.020	17.88	15.31	6.90	14.20
	Minimum	15.00	0.23	0.01	0.31	0.005	10.55	8.96	0.00	0.10
	Average	24.79	0.37	0.03	0.51	0.016	13.40	10.17	3.21	6.12
	2019	**17.00**	**0.24**	**0.03**	**0.79**	**0.014**	**11.71**	**9.93**	**5.90**	**6.40**
Summer	Maximum	42.00	0.78	0.09	0.84	0.040	28.16	10.53	24.90	15.00
	Minimum	18.00	0.01	0.01	0.39	0.007	21.75	7.21	1.40	2.00
	Average	24.86	0.23	0.02	0.59	0.025	24.89	9.13	6.13	8.04
	2019	**18.00**	**0.06**	**0.01**	**0.37**	**0.031**	**25.77**	**10.53**	**8.30**	**15.00**
Fall	Maximum	45.00	0.83	0.05	0.70	0.045	25.77	10.84	8.40	15.00
	Minimum	16.00	0.01	0.01	0.25	0.013	17.60	7.73	2.40	3.60
	Average	25.79	0.21	0.02	0.52	0.026	20.28	9.22	5.96	7.57
	2019	**21.00**	**0.21**	**0.03**	**0.58**	**0.034**	**25.77**	**10.53**	**8.30**	**15.00**
	Channel	*Cl mg/L*	*NO3 mg/L*	*NH3 mg/L*	*TKN mg/L*	*TP mg/L*	*Temp C*	*DO mg/L*	*Turb NTU*	*Chl ug/L*
Spring	Maximum	83.00	0.57	0.07	0.70	0.020	17.53	12.16	6.20	17.70
	Minimum	15.00	0.22	0.01	0.27	0.008	11.44	9.29	0.00	0.20
	Average	26.14	0.40	0.03	0.51	0.015	13.51	10.01	2.94	5.88
	2019	**19.00**	**0.22**	**0.07**	**0.70**	**0.016**	**11.89**	**10.40**	**6.00**	**5.80**
Summer	Maximum	36.00	0.73	0.08	0.75	0.060	27.13	10.99	34.70	24.00
	Minimum	18.00	0.08	0.01	0.40	0.012	21.63	7.12	0.80	0.40

	Average	24.86	0.27	0.02	0.58	0.027	24.32	9.18	6.95	8.68
	2019	**18.00**	**0.08**	**0.01**	**0.75**	**0.028**	**25.86**	**10.99**	**8.10**	**16.00**
Fall	Maximum	60.00	0.83	0.06	0.96	0.040	22.29	10.73	9.90	12.20
	Minimum	20.00	0.01	0.01	0.32	0.009	17.27	7.97	1.00	3.50
	Average	29.71	0.20	0.02	0.57	0.025	19.68	9.06	6.46	7.31
	2019	**20.00**	**0.16**	**0.02**	**0.60**	**0.027**	**19.66**	**8.04**	**4.40**	**7.60**

Table 3. This table contains the percentage of the phytoplankton community that was comprised of cyanobacteria based on biovolume from the spring, summer, and fall seasonal monitoring events (2003-2016) and data collected biweekly during 2019 at three sites: the Muskegon River mouth, near the MLO buoy, and in the Muskegon channel (note missing years, 2017 and 2018). (note missing years, 2017 and 2018). Data was pooled within each year across sites and sampling days.

Year	***Percent Cyanobacteria***
2003	36.32
2004	56.63
2005	67.22
2006	25.16
2007	32.71
2008	63.35
2009	5.74
2010	2.61
2011	39.29
2012	7.52
2013	5.91
2014	7.85
2015	0.71
2016	2.38
2019	1.59

Figures

Figure 1.

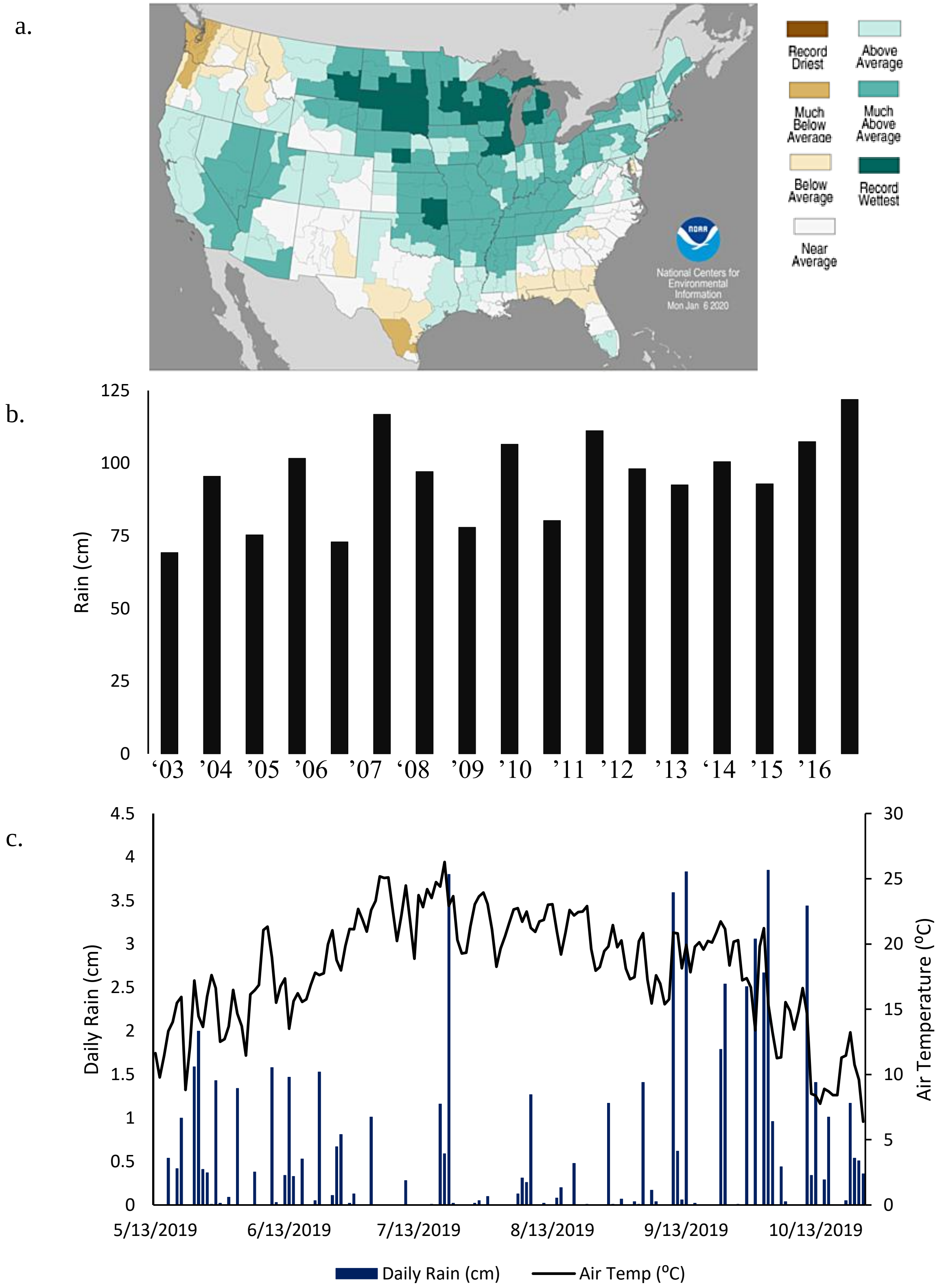


Figure 2.

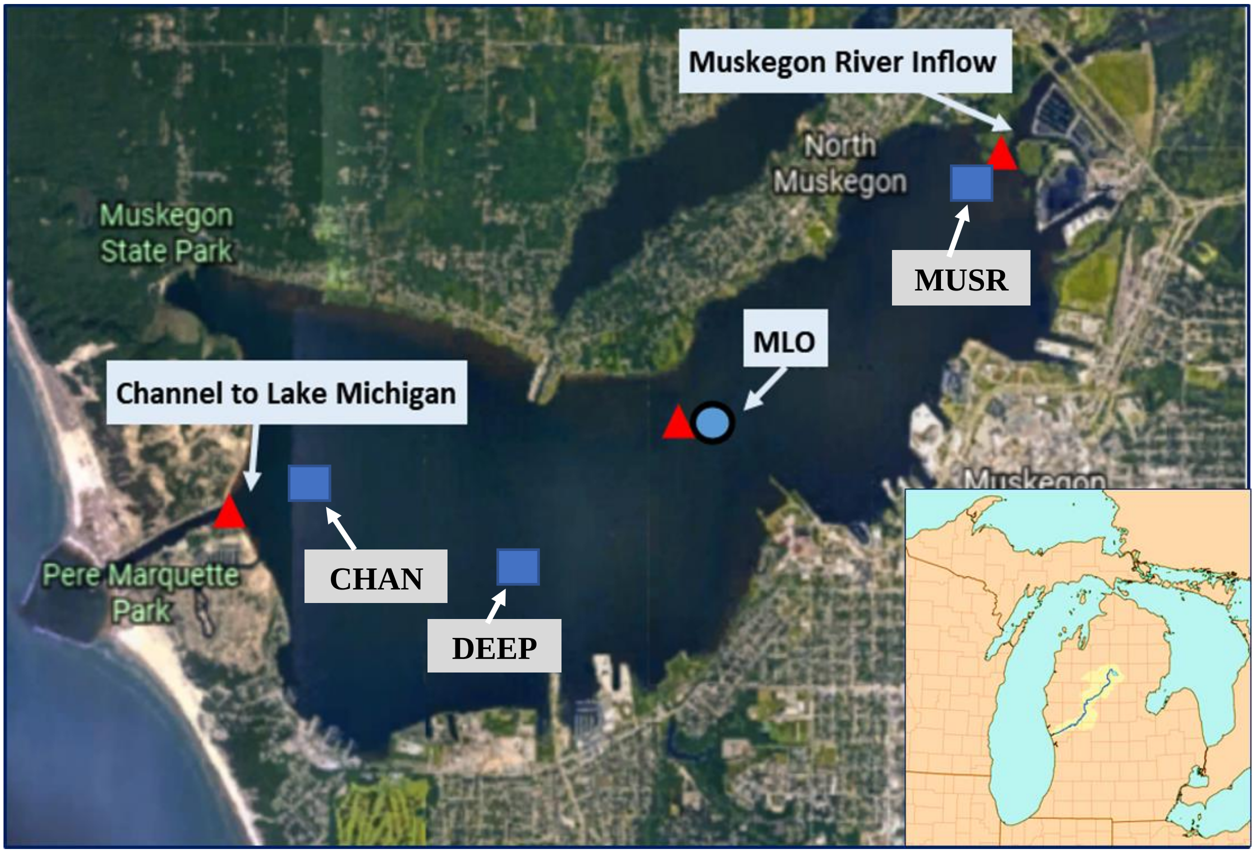

Muskegon River Inflow
North Muskegon
Muskegon State Park
MUSR
MLO
Channel to Lake Michigan
Muskegon
CHAN
Pere Marquette Park
DEEP

Figure 3.

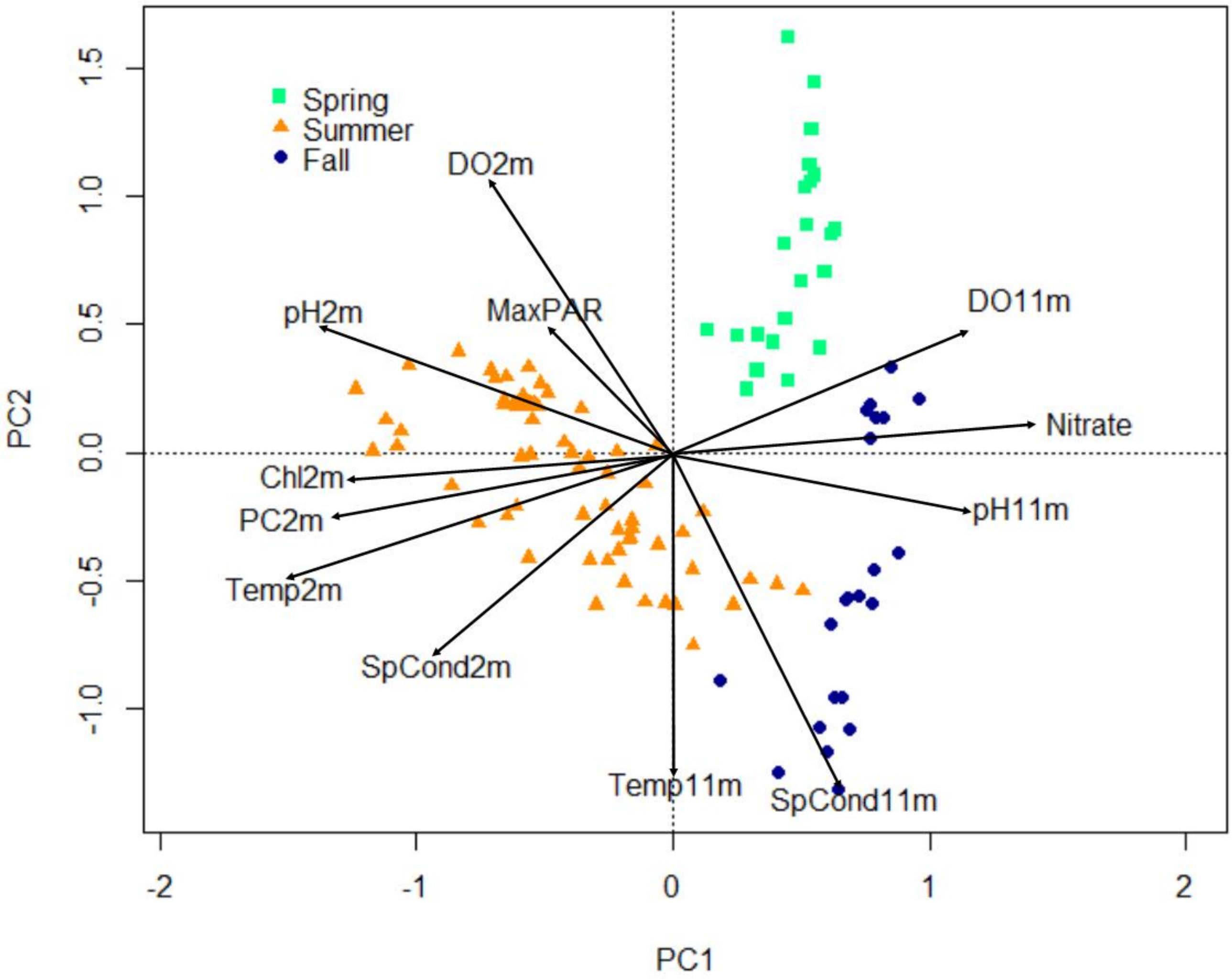
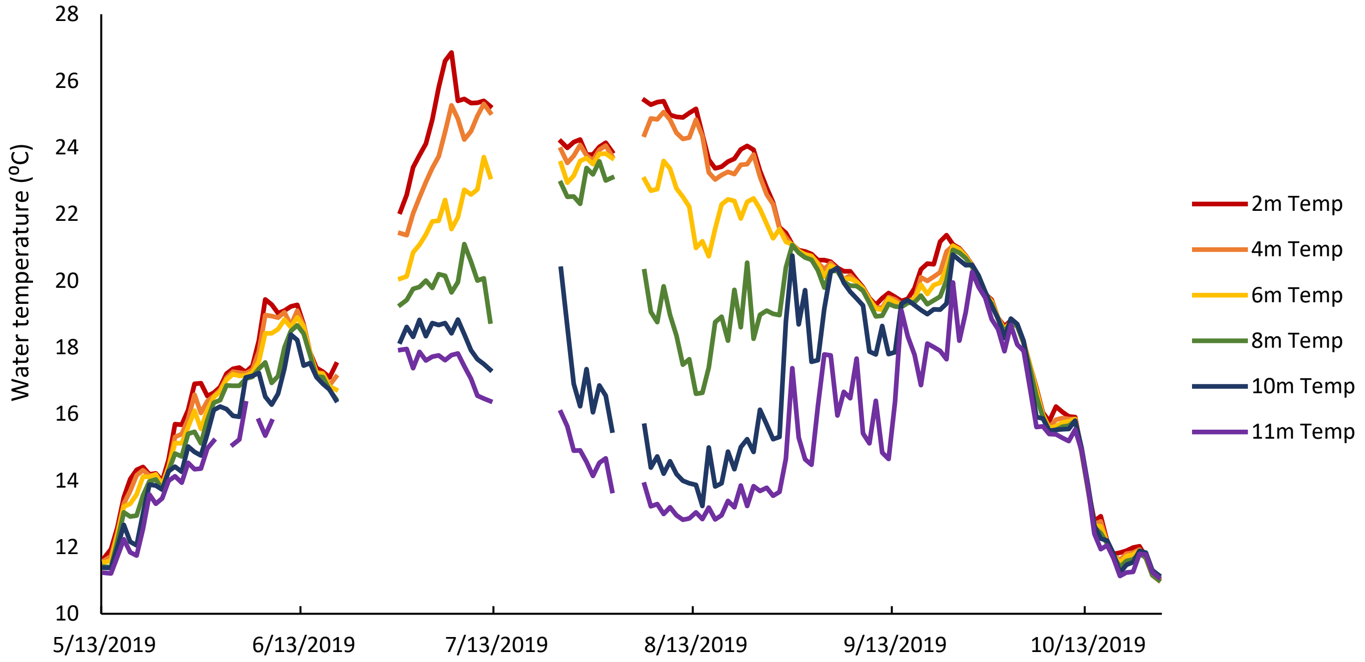

■ Spring
▲ Summer
● Fall
DO2m
MaxPAR
pH2m
DO11m
Nitrate
pH11m
Chl2m
PC2m
Temp2m
SpCond2m
Temp11m
SpCond11m
PC2
1.5
1.0
0.5
0.0
-0.5
-1.0
PC1
-2
-1
0
1
2

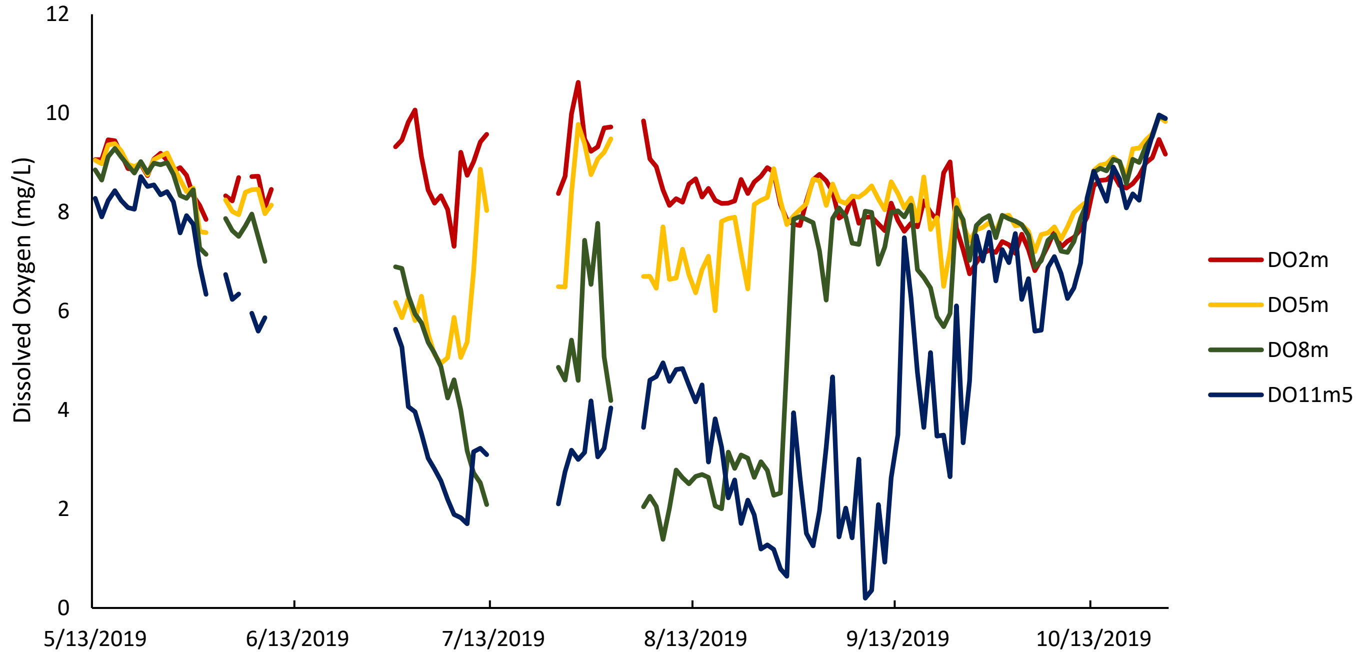
Figure 4.

a.



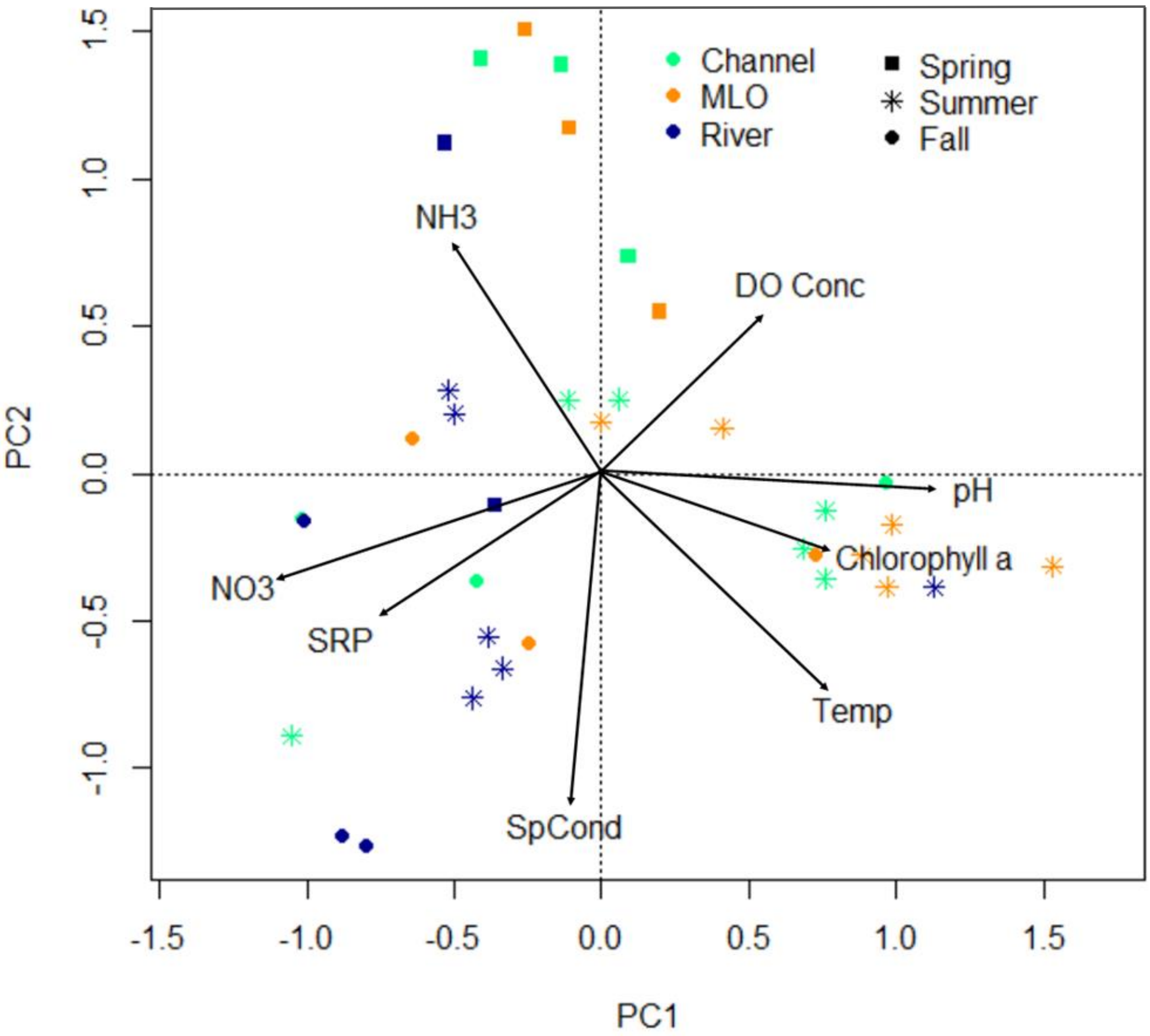
Water temperature (ºC)
28
26
24
22
20
18
16
14
12
10
5/13/2019
6/13/2019
7/13/2019
8/13/2019
9/13/2019
10/13/2019
2m Temp
4m Temp
6m Temp
8m Temp
10m Temp
11m Temp

b.



Dissolved Oxygen (mg/L)
12
10
8
6
4
2
0
5/13/2019
6/13/2019
7/13/2019
8/13/2019
9/13/2019
10/13/2019
DO2m
DO5m
DO8m
DO11m5

Figure 5.



Channel
MLO
River
Spring
Summer
Fall
NH3
DO Conc
pH
Chlorophyll a
Temp
SpCond
SRP
NO3
PC1
PC2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Figure 6.

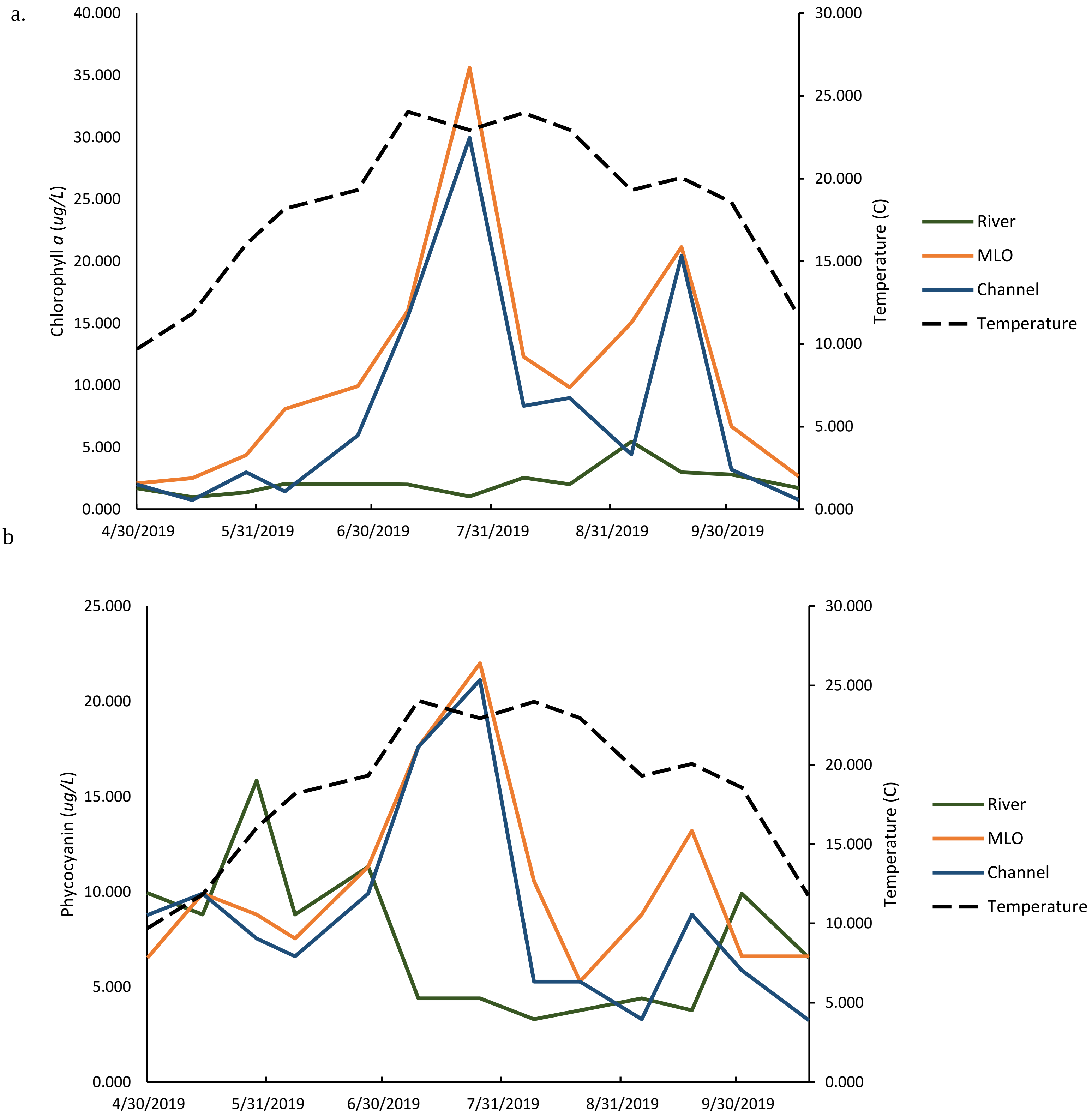

a.
Chlorophyll *a* (*ug/L*)
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Temperature (C)
30.000
25.000
20.000
15.000
10.000
5.000
0.000
4/30/2019
5/31/2019
6/30/2019
7/31/2019
8/31/2019
9/30/2019
River
MLO
Channel
Temperature
b
Phycocyanin (*ug/L*)
25.000
20.000
15.000
10.000
5.000
0.000
Temperature (C)
30.000
25.000
20.000
15.000
10.000
5.000
0.000
4/30/2019
5/31/2019
6/30/2019
7/31/2019
8/31/2019
9/30/2019
River
MLO
Channel
Temperature

Figure 7.

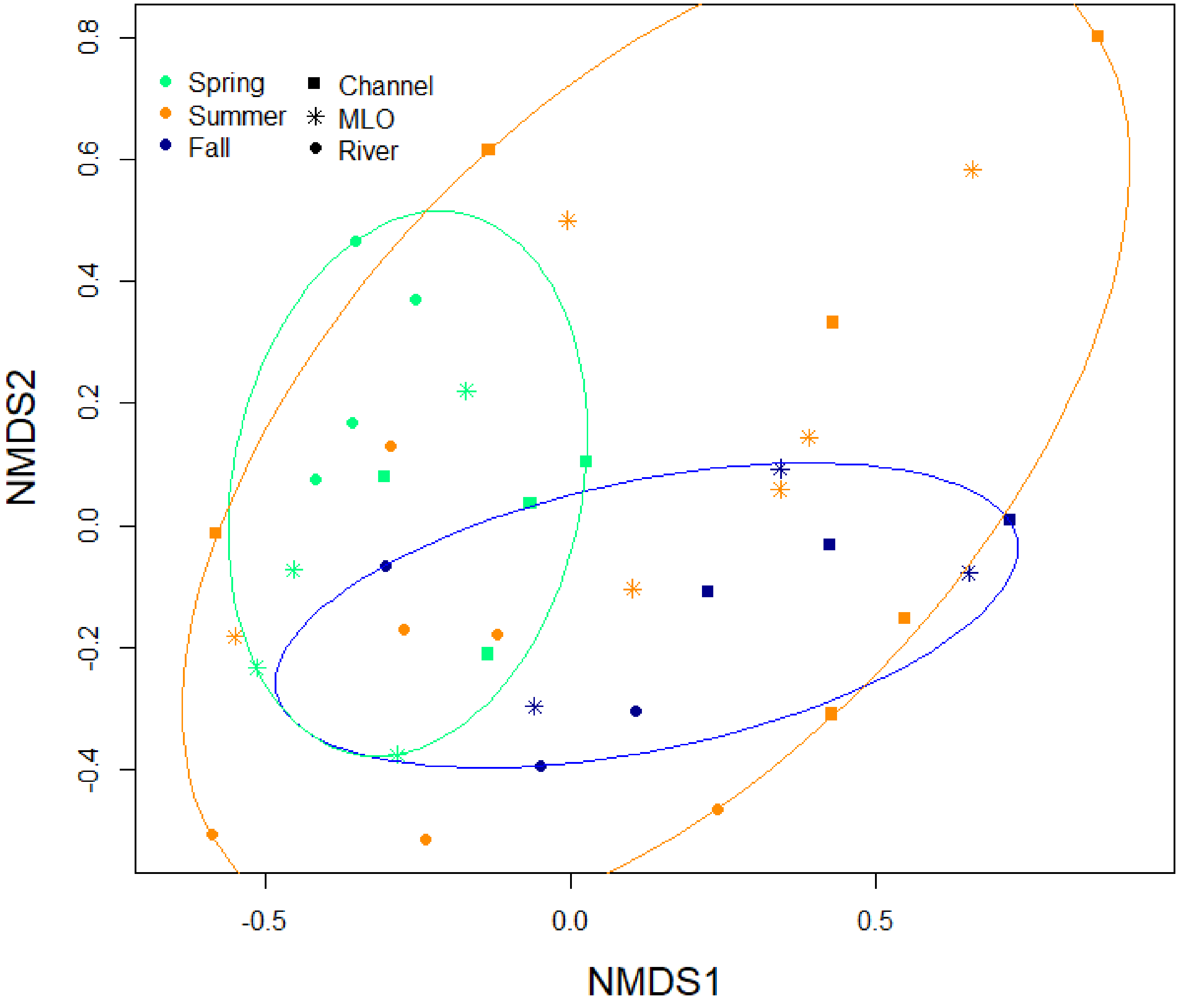

Spring
Summer
Fall
Channel
MLO
River
NMDS2
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.5
0.0
0.5
NMDS1

Figure 8.

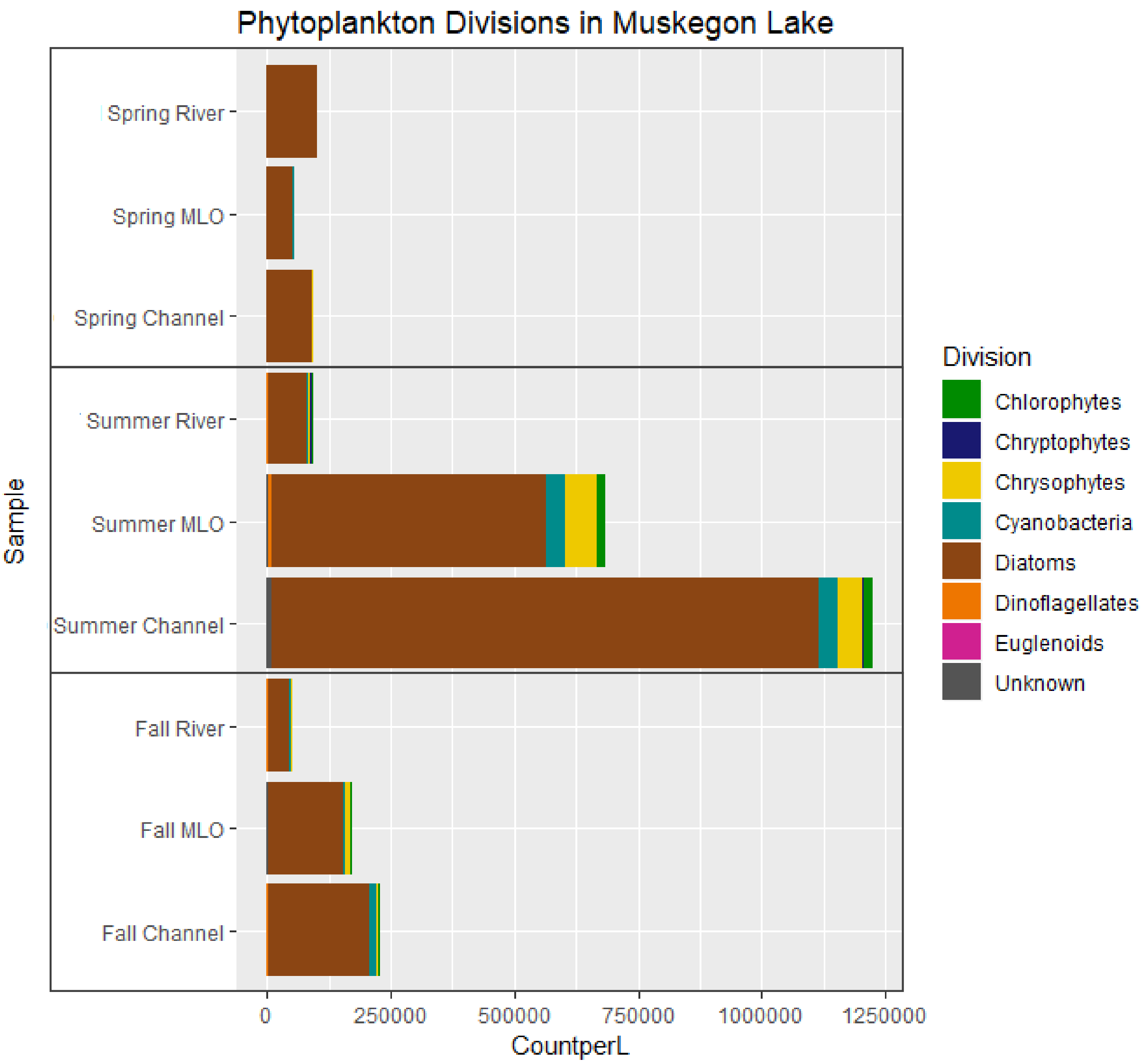

Phytoplankton Divisions in Muskegon Lake
Sample
Spring River
Spring MLO
Spring Channel
Summer River
Summer MLO
Summer Channel
Fall River
Fall MLO
Fall Channel
0
250000
500000
750000
1000000
1250000
CountperL
Division
Chlorophytes
Chryptophytes
Chrysophytes
Cyanobacteria
Diatoms
Dinoflagellates
Euglenoids
Unknown

Figure 9.

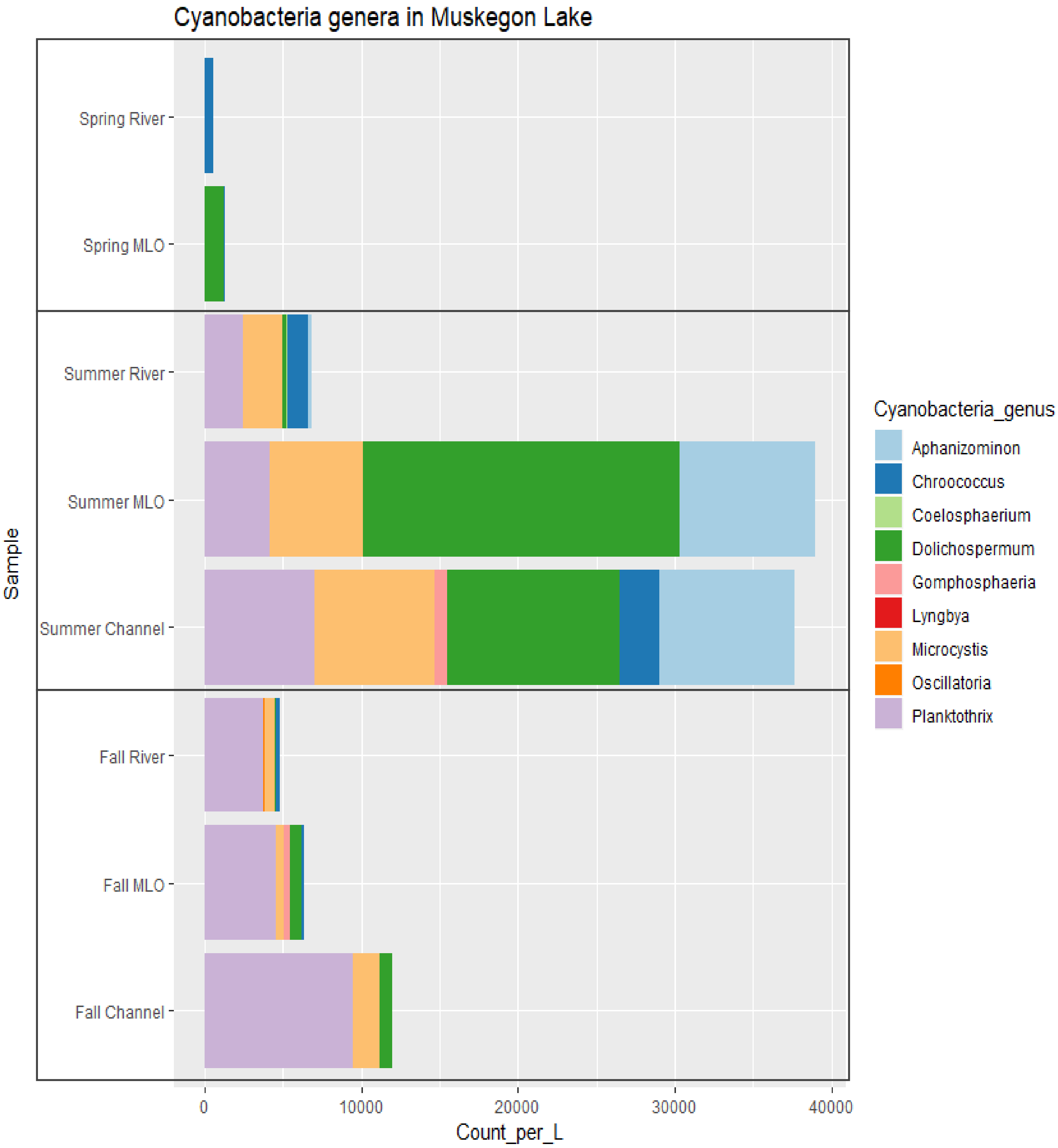

Cyanobacteria genera in Muskegon Lake
Sample
Spring River
Spring MLO
Summer River
Summer MLO
Summer Channel
Fall River
Fall MLO
Fall Channel
0
10000
20000
30000
40000
Count_per_L
Cyanobacteria_genus
Aphanizominon
Chroococcus
Coelosphaerium
Dolichospermum
Gomphosphaeria
Lyngbya
Microcystis
Oscillatoria
Planktothrix

Figure 10.

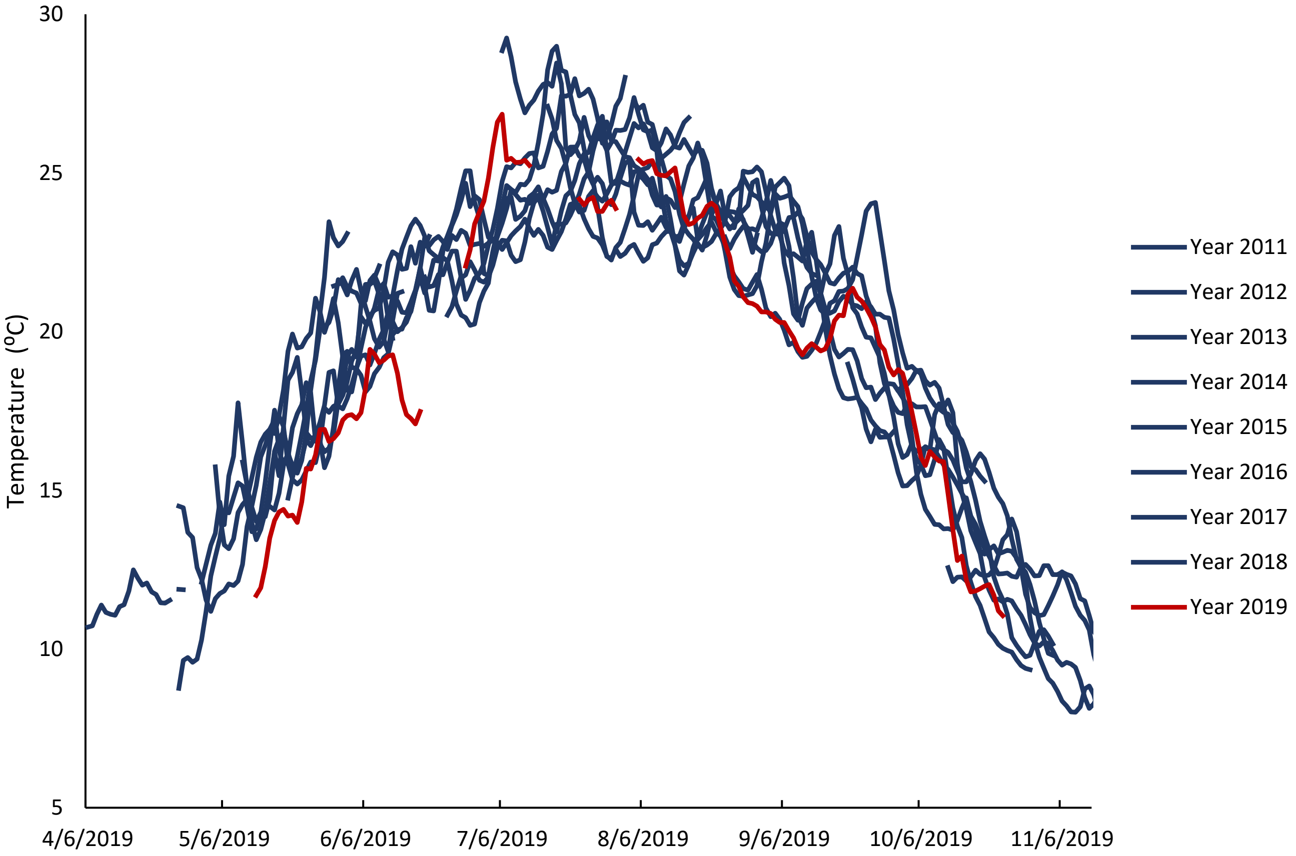
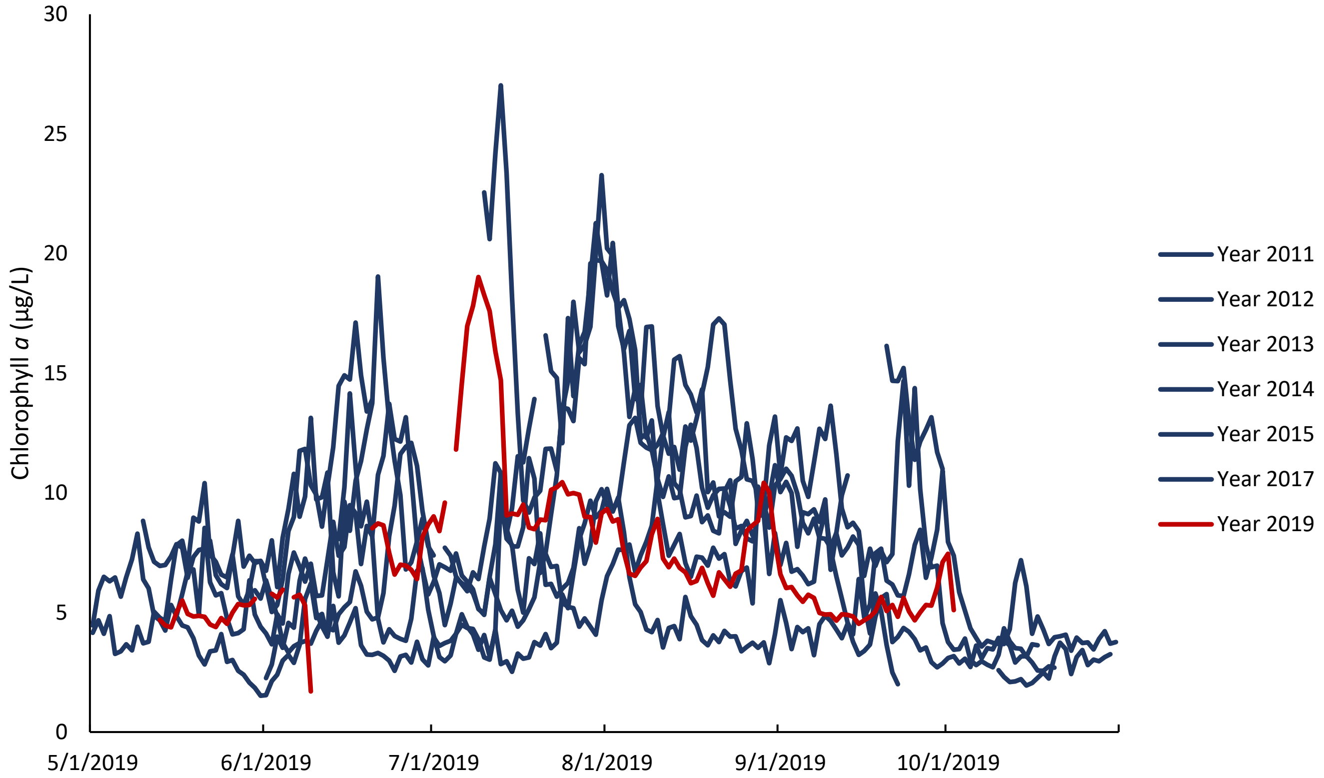

30
25
20
15
10
5
Temperature (°C)
4/6/2019
5/6/2019
6/6/2019
7/6/2019
8/6/2019
9/6/2019
10/6/2019
11/6/2019
Year 2011
Year 2012
Year 2013
Year 2014
Year 2015
Year 2016
Year 2017
Year 2018
Year 2019

Figure 11.

a.


30
25
20
15
10
5
0
Chlorophyll *a* (µg/L)
5/1/2019
6/1/2019
7/1/2019
8/1/2019
9/1/2019
10/1/2019
Year 2011
Year 2012
Year 2013
Year 2014
Year 2015
Year 2017
Year 2019

b.

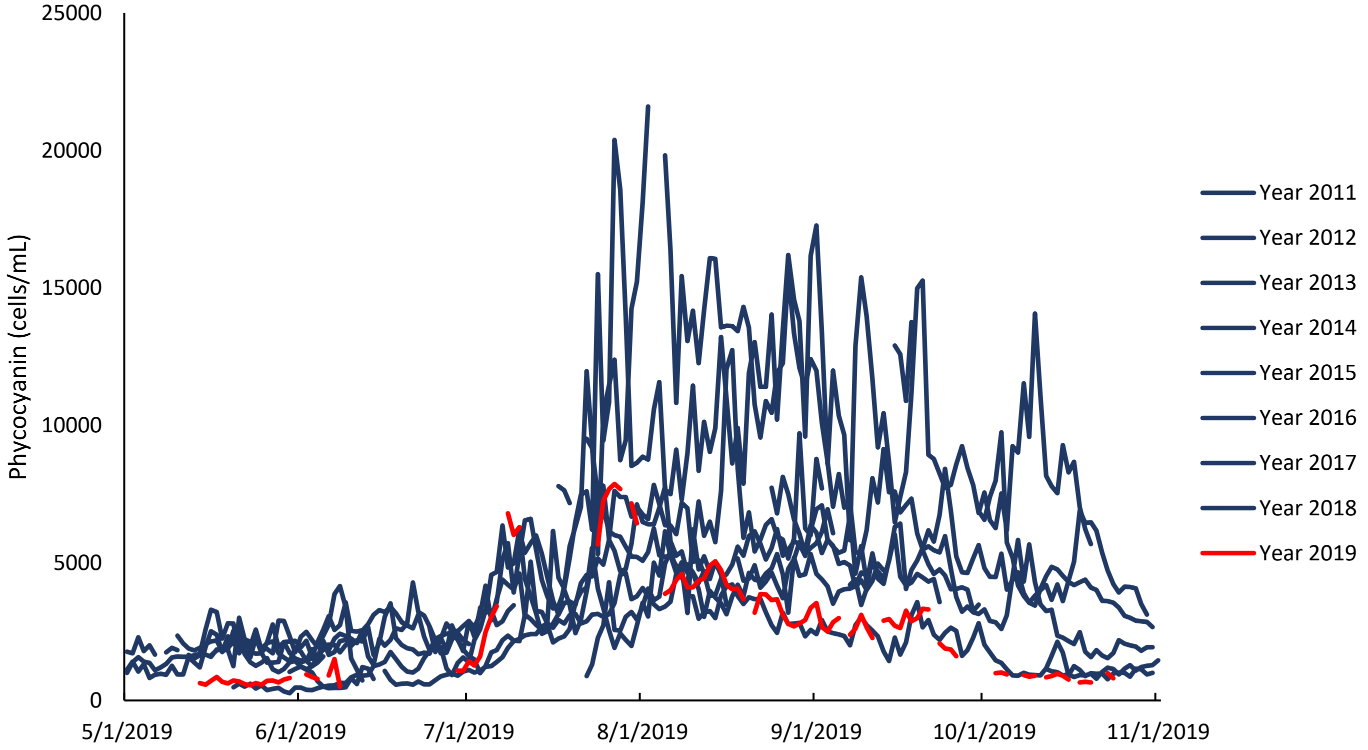
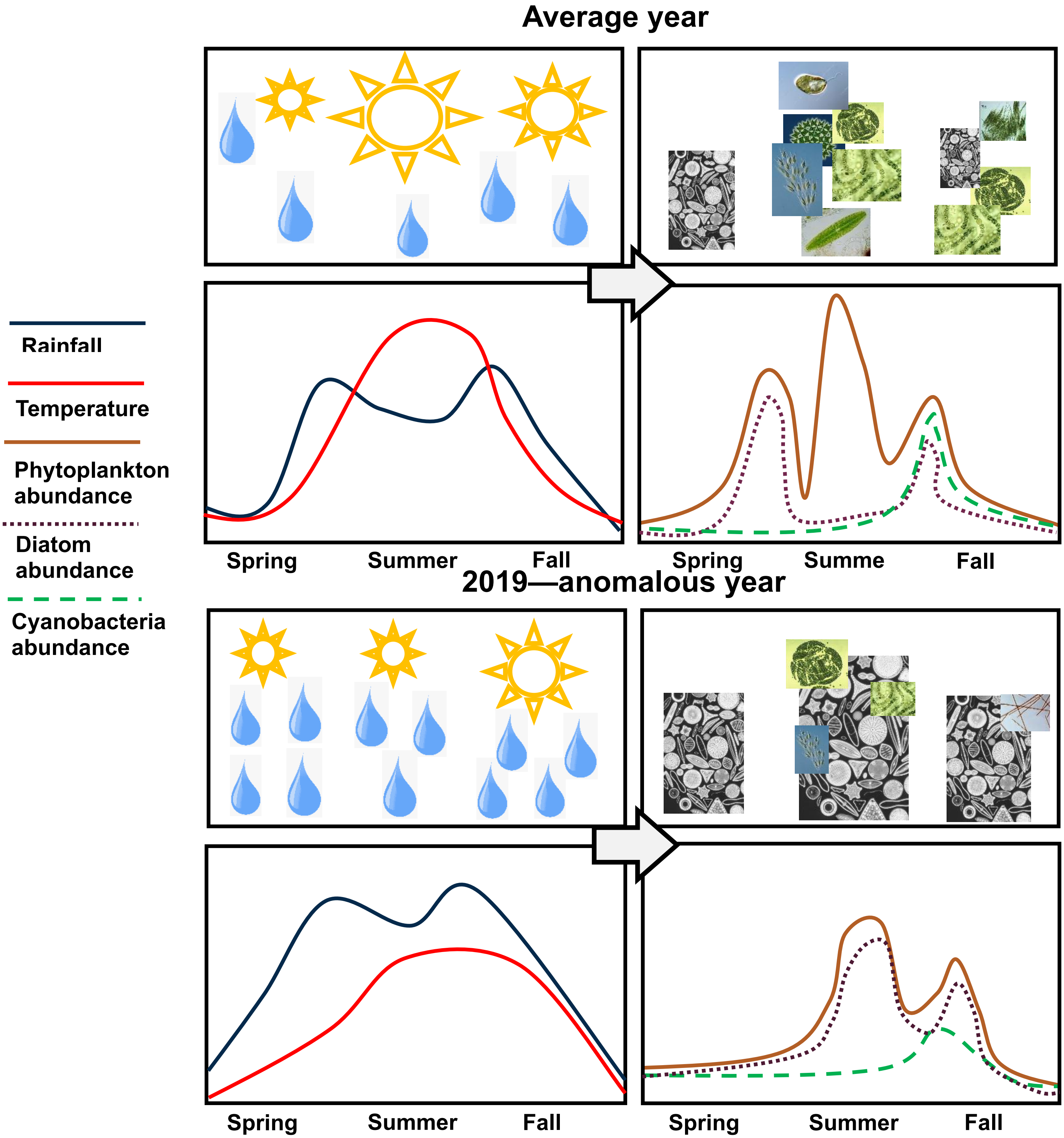

25000
20000
15000
10000
5000
0
Phycocyanin (cells/mL)
5/1/2019
6/1/2019
7/1/2019
8/1/2019
9/1/2019
10/1/2019
11/1/2019
Year 2011
Year 2012
Year 2013
Year 2014
Year 2015
Year 2016
Year 2017
Year 2018
Year 2019

Figure 12.


Average year
Rainfall
Temperature
Phytoplankton abundance
Diatom abundance
Cyanobacteria abundance
Spring
Summer
Fall
Spring
Summe
Fall
2019—anomalous year
Spring
Summer
Fall
Spring
Summer
Fall

Chapter 3: Extended Review of Literature and Extended Methodology

Extended Review of Literature

Introduction to phytoplankton

In aquatic systems, phytoplankton are the main primary producers and form the base of the major trophic sequence in the pelagic environment. Similar to terrestrial plants, they contain chlorophyll and photosynthesize, consuming carbon dioxide and producing oxygen, though both autotrophic and mixotrophic phytoplankton exist. As such, phytoplankton require light, nutrients (mainly inorganic forms of nitrogen and phosphorus), and carbon dioxide for growth. The major divisions of freshwater algae include blue-greens (*Cyanophyta*), greens (*Chlorophyta*), euglenoids (*Euglenophyta*), yellow-greens (*Raphidiophyta*, *Eustigmatophyta*, *Tribophyta*), dinoflagellates (*Dinophyta*), cryptomonads (*Cryptophyta*), chrysophytes (*Chrysophyta*), diatoms (*Bacillariophyta*), red algae (*Rhodophyta*), and brown algae (*Phaeophyta*). Phytoplankton taxa have a wide range of preferred habitats, modes of motility, morphologies, and appearance and some are naturally more abundant than others (Sigee, 2005). Overall, phytoplankton play an important role in global carbon cycling, oxygen production, and in aquatic food webs as primary producers (Sigee, 2005; Lyons et al., 2014).

According to Sommer (1989), there exists a predictable pattern of seasonal succession in the phytoplankton community of dimictic, freshwater lakes that responds to seasonal shifts in environmental conditions. Subsequent books have simplified this successional pattern: during the winter, nutrients build in the euphotic zone, but light is limiting and phytoplankton are largely metabolically inactive; in the early spring, a diatom bloom occurs, as diatoms thrive in turbulent, high-nutrient, low-light environments; in late spring, a clear-water phase occurs due to increased zooplankton growth, resulting in predation, and a decline in nutrients; small, fast-growing, inedible species occur in low populations near the end of the clearwater phase, usually inclusive of unicellular algae such as cryptomonads and green algae; in the summer, zooplankton decrease due to diminished food resources and increased fish growth, resulting in increased predation; a mixed summer/fall bloom of inedible taxa, such as cyanobacteria and dinoflagellates, occurs followed by a resurgence of the diatoms; and in the fall, mixing can stimulate a bloom of a wide variety of phytoplankton and zooplankton (Dodds and Whiles, 2019; Sigee, 2005).

Similar to findings in other ecosystems, spatial and temporal diversity that is maintained by species interactions, environmental conditions, and periodic disturbances is key to upholding balance and function in the phytoplankton community (Tilman and Downing, 1994; Sigee, 2005). Interactions between phytoplankton in the freshwater pelagic zone include competition for resources, such as light and nutrients; antagonistic behavior, such as cyanobacteria suppressing eukaryotic algae in blooms; trophic interactions, including ingestion and infection; and epiphytic associations between microorganisms (Sigee, 2005). Using a data set from the 1970s, Stomp et al. (2011) discovered that phytoplankton diversity varied across latitudinal, longitudinal, and altitudinal gradients in the continental United States. The driving factors underlying this geographic variation were temperature, lake surface area, and productivity – factors that also correlate with species richness of macroorganisms. Whereas it was once thought that the fast growth, long-range dispersal capabilities, small size, and high abundance of phytoplankton may override diversity gradients, this study indicates that changes in climate and land use that impact local environmental variables may impact phytoplankton diversity. Other studies have found similar results, citing diversity as being largely controlled by bottom-up mechanisms, such as nutrients, temperature, and light, which may be affected by climate and land use change (Zhang et al., 2016; Ptacnik et al., 2008). Furthermore, Ptacnik et al. (2008) and Ye et al. (2019) found that resource use efficiency (i.e. carbon cycling) by the phytoplankton community positively correlated with diversity in freshwater, marine, and brackish ecosystems. Thus, fluctuations in community composition due to anthropogenic impairments may allow for single-taxon resource-domination and result in a reduction in carbon cycling efficiency. The effects of reduced phytoplankton diversity in freshwaters will, thus, prove to be detrimental in a time of increasing atmospheric carbon dioxide concentrations, especially in light of the realization that fresh waterbodies are hot spots of carbon cycling (Williamson et al., 2009).

Freshwater ecosystems are experiencing the deleterious effects of anthropogenic climate change and land use change. As systems are subject to intensifying environmental stress, the phytoplankton community is responding, shifting towards decreased taxa diversity and, often, single-taxon dominance (Sigee, 2005; Urrutia-Cordero et al., 2017; Bergkemper at al., 2018). Furthermore, their ability to maintain homeostasis and resist changes to their internal environment is diminishing (Sigee, 2005). One of the greatest threats to the stability and biological integrity of phytoplankton communities in freshwater lakes are the combined effects of anthropogenic eutrophication and climate change.

Eutrophication and harmful algal blooms (HABs)

Freshwater lakes around the world are increasingly suffering the consequences of anthropogenically induced eutrophication (Glibert, 2017; Sinha et al., 2017; Smith, 2003; Anderson, 2002; O’Neil et al., 2012; Weber et al., 2020). The term “eutrophication” formerly referred to the natural aging process of aquatic ecosystems in which a large, deep, nutrient-poor lake eventually fills in with sediment and becomes increasingly productive, nutrient-rich, and shallow over hundreds or thousands of years. This process is being severely amplified by humans due to increased nutrient inputs, which increases the production of organic matter within aquatic ecosystems by fueling primary productivity, or algal growth (Le Moal et al., 2019; Nixon, 2009; Heisler et al., 2008). Eutrophication introduces a perturbation to the dynamic balance of an aquatic ecosystem, causing an imbalance in ecosystem function as waterbodies shift from nutrient limitation to nutrient saturation. Eutrophication and its subsequent symptoms deteriorate the integrity and quality of the waterbody. Harmful algal blooms (HABs) are the most concerning symptom of this sped-up process (Anderson, 2002; Brooks et al., 2016; Heisler et al., 2008; Paerl and Otten, 2013; Luring et al., 2017; Le Moal et al., 2019). HABs pose an on-going threat to fresh waterbodies all over the world, and their associated direct and indirect effects on the ecosystems they afflict have both economic and ecological consequences (Weinke and Biddanda, 2018; Hoagland et al., 2002; Glibert, 2017). Therefore, there is an urgent need for their continued study.

Nutrients causing eutrophication

The nutrients affecting aquatic ecosystems and promoting eutrophication and subsequent HABs are mainly nitrogen and phosphorus. Their anthropogenic origins are from agricultural fertilizers and wastes, urban runoff and wastewater treatment plant effluent, and industrial sources, and their effects are exacerbated by deforestation, the removal of riparian zones, water diversion projects, and climate change (Glibert, 2017; Jenny et al., 2016; Wagner and Adrian, 2009; Lowrance et al., 2018; Paerl and Otten, 2013). Nutrients enter aquatic ecosystems via atmospheric deposition, groundwater discharge, surface water runoff, and soil erosion, all of which contain different nutrient concentrations, forms, and ratios than the waterbodies they enter (Anderson, 2002). Therefore, the influx of nutrients shifts the nitrogen:phosphorus ratio, alters the amounts of different forms of nitrogen and phosphorus in the waterbody, and shifts the nature of nutrient limitation in the ecosystem, favoring opportunistic phytoplankton, which may outcompete and replace their counterparts in the form of a HAB (Elser et al., 2009; Anderson, 2002).

As two of the major elements required for phytoplankton growth, nitrogen and phosphorus are the main nutrients stimulating eutrophication, but they have differing cycles and enter water bodies in different ways. Nitrogen has a complex, biologically driven cycle with five major routes of transformation. Nitrate (NO_3) is generally the most abundant form of nitrogen available to phytoplankton, and it enters aquatic systems through soil and rain deposition. Other biologically available forms include ammonium (NH_4) and nitrite (NO_2). Once nitrogen is transformed into a complex organic form bound up in phytoplankton biomass, it is recycled within the food web, ultimately taking the form of detritus and assimilating into the sediments. Remineralization is the process that returns organic nitrogen to a usable, inorganic form, generally ammonia (NH_3). NH_3 can then be oxidized to nitrate in the process of nitrification under aerobic conditions. Under anaerobic conditions, nitrogen oxides (NO_3 and NO_2) can be converted to dinitrogen gas, representing a loss of available nitrogen in the system. Lastly, the process of nitrogen fixation can be carried out by prokaryotes and involves converting N_2, an unusable form, to NH_4 (Sigee, 2005). Therefore, nitrogen, in its various forms, can enter a system both internally and externally. A major culprit of the increase of nutrients into waterbodies is the rapid intensification of agriculture, which began in the 1700s. From 1700 to 1980 alone, the total area of cultivated land around the world increased 466% (Matson et al., 1997). Nitrogen and phosphorus are applied to croplands in the form of fertilizers and manure, and surface and ground water carry excess nutrients into receiving waterbodies. After the mid-1940s, when nitrogen-based fertilizer was commercialized, the use of nitrogen expanded rapidly from less than 10 MT nitrogen per year in 1950 to over 170 MT nitrogen per year in 2013 to an expected over 220 MT nitrogen per year by 2020 (Glibert, 2017). Depending on soil type, between 10% and 80% of nitrogen-based fertilizer leaches into groundwater and surface waters; overall, this is the case for an estimated 20% of agriculturally applied fertilizer in North America. Atmospheric deposition of nitrogen into lakes is also increasing due to anthropogenic activities, such as fossil fuel combustion and agricultural emissions; for example, it has been estimated that 36% of the nitrogen excreted by farm animals is volatized to the atmosphere in the form of NH_3, and 14% of all nitrogen applied as fertilizer is volatized (Howarth et al., 2002; Paerl, 2017).

Phosphorus is less available in aquatic systems than is nitrogen, only has one biologically usable form (orthophosphate), and cannot be fixed into a biologically available form, making it the limiting nutrient in most systems (Sigee, 2005). Phosphorus is generally transferred from land to water through erosion of cultivated land during flow events – these fluxes comprise 60% to 90% of the phosphorus exported from cultivated land. Loss of phosphorus from applied fertilizer or manure is generally less than 5% because conditions for phosphorus loss are relatively specific compared to nitrogen; however, small amounts of phosphorus added to a waterbody can contribute to eutrophication, as ambient levels of phosphorus in freshwater ecosystems are generally low, and influxes from industrial and human effluent can alter the trophic state of a waterbody (Howarth et al., 2002; Sigee, 2005). Internal loading of phosphorus from organic material in the sediments of a waterbody due to decomposition is also of concern. The release of phosphate into the water column is most notably a function of oxygen – under anaerobic conditions, phosphorus is easily diffused from sediments, but under aerobic conditions, phosphorus is adsorbed to sediments – but is also influenced by pH and redox potential (Colborne et al., 2019; North et al., 2014; Sigee, 2005). Phosphorus is also recycled within the waterbody in cases when it is release by lake biota through lysis/death of phytoplankton cells or excretion from higher trophic levels (Sigee, 2005).

Excessive nutrients in a waterbody increase primary productivity by providing phytoplankton with nutrients that are usually limiting in lakes. Phosphorus has long been deemed the main limiting nutrient in freshwater lakes; however, nitrogen has been found to also promote eutrophication and can especially benefit certain non-nitrogen fixing HAB species (Schindler et al., 2016; Deng et al., 2014; Glibert, 2017). There is still some debate surrounding the question of which nutrient, nitrogen or phosphorus, and which forms are of more concern, whether total amounts or ratios are more important, and whether management should focus on a duel- or single-nutrient plan (Heisler et al., 2008; Filstrup et al., 2017; Paerl et al., 2016; Søndergaard, et al., 2017; Chaffin et al., 2018). Furthermore, HAB species can be both autotrophic and mixotrophic, and some species can utilize both organic and inorganic forms of nutrients for their nitrogen, phosphorus, and carbon demands (Heisler et al., 2008; Paerl, 1988). Therefore, it is likely that both nitrogen and phosphorus in their various forms have major implications for eutrophication in freshwater ecosystems, and management plans should include both (Glibert, 2017; O'Neil et al., 2012; Smith, 2003; Le Moal et al., 2019).

Cyanobacteria – the main culprits of HAB formation

Around the world, cyanobacteria (*Cyanophyta*, blue-green algae) are the most common division creating HABs in freshwater lakes (Anderson, 2002). Likely having evolved in the Precambrian era, they are the oldest known oxygen-producing organisms on Earth at 3.5 billion years old and are largely responsible for the Great Oxygenation Event (Paerl and Huisman, 2009; Lyons et al., 2014). Unlike other algae, cyanobacteria are prokaryotes rather than eukaryotes, and they have found great success in nearly all freshwater, marine, and terrestrial habitats around the world, including extreme environments (Sigee, 2005). Cyanobacteria can be unicellular or colonial, and they have a wide variety of adaptations that allow them to outcompete other phytoplankton under favorable conditions. These include a higher temperature tolerance than other phytoplankton, ability to produce over-wintering akinetes, ability to regulate their position in the water column via buoyancy control, efficient nutrient uptake, ability to access organic phosphorus and store luxury phosphorus, nitrogen-fixing ability for some species, ability to photosynthesize at low carbon dioxide concentrations and high pH, efficient light harvesting due to accessory pigments, and resistance to damaging radiation (Carey et al., 2011; O'Neil et al., 2012; Paerl and Otten, 2013; Smith, 2003; Sigee, 2005). Understanding these physiological traits has led scientists to believe that cyanobacteria will have several advantages in a world altered by climate change and other anthropogenic effects and will likely be able to outcompete other phytoplankton in future scenarios. Cyanobacteria species have differing preferred nutrient regimes due to their physiological adaptations, and specific species proliferate due to a complex process based on the amount, form, and ratio of nutrients entering the waterbody (Glibert, 2017; Heisler et al., 2008). The cyanobacteria are an extremely diverse group, and their traits and adaptations vary widely between species, indicating that climate and land use change will alter the composition of the cyanobacteria community as well as that of the phytoplankton community as a whole.

Microcystis Lemmermann is one of the most common cyanobacteria genera found in freshwaters worldwide. *Microcystis* can form nuisance blooms, produce toxins, regulate buoyancy, use organic phosphorus, and store luxury phosphorus (Šejnohová and Maršálek, 2012). It displays no ecotype differentiation; its wide genetic diversity within and between species has allowed it to thrive under myriad environmental conditions (Humbert et al., 2013; Xu et al., 2010). *Microcystis* growth has been shown to positively correlate primarily with high water temperatures, with optimal growth rates generally reported to be around 20–25 ^{o}C, and with several forms of both P and N (Donald et al., 2013; Davis et al., 2009; Xu et al., 2010).

Studies have shown that *Microcystis* generally prefers reduced forms of nitrogen (Šejnohová and Maršálek, 2012; Newell et al., 2019; Gobler et al., 2016) but switches to nitrate when necessary and may prefer it under phosphorus-saturated conditions (Donald et al., 2013). *Microcystis* can use all forms of inorganic nitrogen except for dinitrogen, as they are non-diazotrophic. Additionally, they are generally more limited by nitrogen than phosphorus. *Microcystis* prefers orthophosphates but can use organic phosphorus and usually requires less than 0.03 mg/L phosphorus for bloom formation (Donald et al., 2013; Šejnohová and Maršálek, 2012).

The negative effects of HABs

As their name suggest, HABs are ecologically and economically harmful. Non-toxic HABs cause harm through their physical structure by reaching high biomass, creating unsightly blooms, foams, mats, or scums; diminishing the photic zone, which reduces the amount of emergent vegetation and subsequent resources for other species; and inducing hypolimnetic hypoxia. Hypoxia commonly accompanies HABs and is the condition of low dissolved oxygen (DO) in a waterbody, generally referring to oxygen below 2 mg DO/L, as this is, for the most part, the threshold at which aquatic life can no longer be sustained (Scavia et al., 2014; Biddanda et al., 2018; Diaz and Rosenberg, 2008). Hypoxia requires two conditions to develop: thermal stratification of the waterbody and decomposition of organic matter in the hypolimnion, the lowest layer of water in a waterbody (Diaz, 2001; Diaz and Rosenberg, 2008). Thermal stratification of the waterbody creates a density gradient in which warm, low-density water in the epilimnion, the upper-most layer of water, floats on top of the cool, high-density water in the hypolimnion, with the boundary between them termed the thermocline. These two water masses do not mix due to their density difference, preventing the oxygen supply in the hypolimnion from being replenished by the epilimnion. In the epilimnion, high DO is maintained through photosynthesis, turbulence or advection, and oxygen exchange with the atmosphere. The hypolimnion, however, continues to lose oxygen to respiration by heterotrophs, degradation of organic matter, re-oxidation of reduced constituents, and chemoautotrophy (Zhang et al., 2010). Eutrophication and subsequent HABs induce hypoxia by increasing phytoplankton biomass, which eventually dies and sinks to the hypolimnion, where benthic heterotrophs deplete the oxygen supply during respiration (Sweerts et al., 1991; Scavia et al., 2014; Le Moal et al., 2019). Another concern regarding hypoxic bottom waters is the potential for legacy nutrients (namely phosphorus), metals, and gases to be released from anoxic sediments and refuel HABs or contaminate the waterbody when wind events mix the nutrients back into the epilimnion or when buoyancy-regulating cyanobacteria move to the hypolimnion (Colborne et al., 2019; Watson et al., 2016; Biddanda et al., 2018; Carmichael et al., 2016). Lakes around the world are experiencing hypoxia at higher rates due to a combination of increased HAB events and fundamental changes in their thermal regimes as a result of climate change; warming surface waters cause increased thermal stability and, consequently, the partial suppression of mixing in the waterbody, providing buoyant, warmth-loving HAB species with an advantage (Biddanda et al., 2018; Jenny et al, 2016; North et al., 2014; Schneider and Hook, 2010). Hypoxia serves as an indicator of ecosystem health, as its extent, duration, and frequency have increasingly negative effects on an ecosystem (North et al., 2014). Globally, the number of hypoxic and anoxic zones has doubled each decade since the 1960s with the combined effects of HABs and climate change to blame (Diaz and Rosenberg, 2008).

Some cyanobacteria species produce toxins. Toxic HABs are poisonous and may cause mass mortalities of wild and farmed fish and shellfish, human illness and death, illness and death in domestic animals, and other wildlife deaths in addition to closing beaches and contaminating drinking water (Backer et al., 2015; Carmichael et al., 2016). Many toxin groups exist, including microcystins (most common), nodularins, anatoxins, antillatoxins, aplysiatoxins, homoanatoxins, jamaicamides, kalkitoxins, cylindrospermopsins, cyanopeptolins, lyngbyatoxins, saxitoxins, BMAAs, and lipopolysaccharides (Rastogi et al., 2015; Babica et al, 2006; Codd et al., 2004). These toxins can be neurotoxic, cytotoxic, nephrotoxic, hepatotoxic, dermatotoxic, and gastrointestinal toxins. The most prevalent kinds of toxins produced by HABs are hepatotoxins (affecting the liver), which include microcystins, nodularins, and cylindrospermopsins, followed by neurotoxins (affecting the brain), which include anatoxin-a, anatoxin-a(S), and saxitoxins (O'Neil et al., 2012). The most common toxin produced by HABs are microcystins, a group of hepatotoxic cyclic peptides, and can be produced by *Dolichospermum* Wacklin, Hoffman, & Komárek (formerly *Anabaena*), *Microcystis, Oscillatoria* Vaucher ex Gomont, *Planktothrix* Anagnostidis & Komárek, *Nostoc* Vaucher ex Bornet & Flahault, and *Anabaenopsis* Miller species. Microcystins are particularly toxic to animals and humans, can be aerosolized, and pose a serious threat upon dermal contact, inhalation, and ingestion (Codd et al., 2004; Pearson et al., 2010; Olson et al., 2020; Gorham et al., 2020). Other toxin-producing cyanobacteria genera include *Aphanizomenon, Nodularia,* and *Raphidiopsis* Fritsch and Rich (formerly *Cylindrospermopsis*). Some algal toxins are potent and dangerous at low concentrations, and algal species that are not typically toxic may become toxic or increase in toxicity under atypical nutrient regimes (Anderson, 2002; Hallegraeff, 1993).

Another way in which HABs cause ecological harm is through altering the food web. Many HAB species are resistant to grazing due to their morphology causing mechanical interference in feeding appendages, the toxins they produce, and their comparatively low levels of essential nutrients (Wilson et al., 2006; Šejnohová and Maršálek, 2012; Arnold, 1971). Their resistance to grazing - combined with their ability to outcompete other algae, create microhabitats that are not suitable for the growth of other algae, and comprise a large portion of the primary producer trophic level - lowers the quality of the base of the food web (Sigee, 2005). As a low-quality food source, HABs species constrain energy transfer up the food web and may force shifts in co-evolutionary dynamics between cyanobacteria and their grazers (Dickman et al., 2008; Ger et al., 2016).

Lastly, HABs can be economically harmful. HABs can cause significant economic damage through: decreasing water quality, inhibiting recreational (fishing, swimming, boating, etc.) opportunities; endangering wildlife health, forcing recovery efforts; causing fish kills due to hypoxia, which affects both fisheries and tourism; contaminating drinking water, leading to costly clean up and filtration processes; and decreasing property values due to diminished aesthetics and safety in the waterbody (Dodds et al., 2008; Hagland et al., 2002). Combined economic losses resulting from eutrophication in freshwaters of the United States is estimated to be in the range of $2.2 billion to over $4 billion annually (Dodds et al., 2008; Kudela et al., 2015).

Climate change and HABs

While excessive nutrient inputs into waterbodies are the main cause of HABs, climate change is tending to exacerbate their severity. An emerging concern is how HABs will respond to and interact with the effects of climate change, such as increasing temperatures and altered precipitation patterns (Paerl and Otten, 2013; Sinha et al., 2017; Weber et al., 2020). Inland waterbodies have experienced rapid warming since 1985 (Schneider and Hook, 2010; O'Reilly et al., 2015). With increasing temperatures, particularly over 20 ºC, all phytoplankton will benefit from higher growth rates, but cyanobacteria will have an advantage over other algae due to their higher temperature range. Additionally, warming temperatures may lead to a longer growing season, which perpetuates earlier bloom times and longer bloom durations with a shorter winter season (Rigosi et al., 2014; Deng et al., 2014; Jöhnk et al., 2008; Ho and Michalak, 2019; Paerl and Huisman, 2008). Increased temperatures have also been shown to lead to higher toxin release from toxic cyanobacterial species and increased ability of diazotrophic cyanobacteria species to fix nitrogen (Walls et al., 2018; Wood et al., 2016; Lürling et al, 2016).

Another concern about increasing water temperatures is the strengthening, earlier onset, and increased duration of vertical stratification. This allows buoyancy-regulating cyanobacteria, such as *Microcystis,* and those with a low sinking velocity easier navigation and less sedimentation within a more stable water column; therefore, blooms have the potential to last longer in a waterbody (Carey et al., 2011; Peeters et al., 2007; Paerl and Huisman, 2008; Wagner and Adrian, 2009). Additionally, enhanced stratification combined with increased decomposition within the hypolimnion due to bloom diebacks can lead to intensified periods of hypoxia (Diaz and Rosenberg, 2008). Hypoxia allows for nutrient release from the sediments on the bottom of the lake, particularly phosphorus, from which buoyant cyanobacteria can access and benefit (Watson et al., 2016; Diaz and Rosenberg, 2008). Furthermore, episodic strong wind events that are projected to increase with climate change may mix the water column, entraining nutrient-rich bottom water at the surface and promoting growth of surface blooms (Weinke and Biddanda, 2019). Occurrences and severity of hypoxia are increasing worldwide due to eutrophication - globally, the number of hypoxic and anoxic zones has doubled each decade since the 1960s - with detrimental effects on fisheries, biodiversity, and food webs (Diaz, 2001; Diaz and Rosenberg, 2008).

Climate change also alters hydrologic regimes, influencing patterns of precipitation and drought. It has been demonstrated that climate change is not progressing linearly, but rather manifests in extreme and record-breaking events, reduced predictability, and enhanced variability, especially at regional scales (Katz and Brown, 1992; Rahmstorf and Coumou, 2011; Wigley, 2009; Falvey and Garreaud, 2009). Several models predict increased precipitation and decreased return times on rain events (e.g. a 100-year storm will occur more frequently than every 100 years) as a result of climate change (Sinha et al., 2017; Huntingford et al., 2003; Byun and Hamlet, 2018). It is likely that this will increase nutrient loading into waterbodies through surface runoff and groundwater discharge (Sinha et al., 2017), which may stimulate algal blooms (Paerl and Huisman, 2009). While some have found that increased precipitation events may mitigate blooms due to a dilution and flushing effect (Ho and Michalak, 2019), others have theorized that heavy precipitation events interspersed by droughts will flush nutrients into waterbodies where they will subsequently concentrate and promote blooms (Paerl and Huisman, 2008), a phenomenon that has been observed in the San Francisco Estuary (Lehman et al., 2017). Droughts increase water residence time in waterbodies, promoting persistent stratification and increasing water temperatures, of which buoyancy-regulating, temperature-tolerant cyanobacteria can take advantage (Carey et al., 2011; Paerl and Huisman, 2009; Lehman et al., 2017). Therefore, it is projected that cyanobacterial HABs are likely to increase in duration, severity, and range under future climate scenarios, making management of HABs more difficult (Havens and Paerl, 2015; Carey et al., 2011).

Study site – Muskegon Lake, Muskegon, Michigan

Muskegon Lake (W 86° 17′ 25.42″, N 43° 13′59.45″) is a drowned river mouth Great Lakes estuary located in Muskegon, Michigan that drains the second largest watershed in the state into Lake Michigan through a man-made navigation channel. Muskegon Lake has a surface area of 17 km^2, a water volume of 119 million m^3, a mean water depth of 7 m, a maximum water depth of 21 m, and a seasonally variable average hydraulic residence time of 23 days (Liu et al., 2018). The Muskegon Lake watershed was formed by the same glacial activity that formed the

Great Lakes 11,000 years ago, and Muskegon Lake subsequently formed as a drowned river mouth estuary as Lake Michigan water levels rose and back flowed up the Muskegon River (Rediske et al., 2002). A drowned river-mouth estuary is the interface between a lentic and lotic system in which a river essentially widens into a lake before emptying into a larger lake. Drowned river mouth estuaries provide a unique ecosystem in which physical and chemical attributes create predictable spatial patterns. They are often focal areas of economic importance, acting as shipping ports for various products and services, and undergo significant anthropogenic stress – Muskegon Lake is no exception (Larson et al., 2013).

The natural resources of the Muskegon Lake watershed were originally utilized by Native American tribes until the 1800s, when intense lumber activity began to drastically change the system, deforesting riparian zones and causing severe erosion problems. Additionally, the sawmills constructed along the coast filled the water with debris and waste. Unsustainable harvesting practices saw the downfall of the lumber era and the rise of industrial expansion, including foundries, metal finishing facilities, petrochemical production, shipping, a pulp and paper mill, plating companies, a coal gasification facility, an iron foundry, a coal storage operation, rail yards, and sand mining. Additionally, the wastewater discharge for the City of Muskegon, a municipal waste landfill, and the NPDES discharge for an engine-production company were located on the South Branch of the Muskegon River. However, in 1973, wastewater diversion to the Muskegon Wastewater Treatment Plant began. Historically, Muskegon Lake has suffered nuisance algal blooms, hypoxia in the hypolimnion, fish kills in the winter, nuisance macrophyte growth, and fish consumption restrictions (Rediske et al., 2002).

Per the U.S.-Canada Great Lakes Water Quality Agreement, Muskegon Lake was listed as a Great Lakes Area of Concern (AOC) in 1987 due to the intense lumber and industrial activity that lead to hardening of the shoreline, filling/destruction of wetlands, filling of the littoral zone by sawmill debris and foundry slag, and contamination of the sediments with heavy metals (copper, lead, cadmium, and mercury) and polycyclic aromatic hydrocarbon compounds (Gillet and Steinman, 2011; Rediske et al., 2002). Between 1987 and 2002, nine beneficial use impairments (BUIs) were developed as part of a remedial action plan, and delisting targets were identified for five of them, inspiring management, research, and restoration efforts for the lake (Steinman and Ogdahl, 2004). Among these efforts was to remediate contaminated sediment and debris, including 68,710 m^3 in Ruddiman Creek in 2006, 33,230 m^3 at the Division Street Outfall in 2012, and 33,640 m^3 near the Zephyr Oil Refinery in 2019. Several major habitat restoration and conservation projects have been undertaken, which include restoring wetland, open water, riparian, and shoreline/streambank habitats; reestablishing hydrological connectivity between surrounding waterways and with Muskegon Lake; removal of historical lumber debris, unnatural fill, and nutrient-rich sediment; and improvement of water quality (Evans, et al., 2018). A long-term monitoring program, headed by the Steinman Lab at the Annis Water Resources Institute (AWRI) of Grand Valley State University, began in 2003 to provide annual reports on the progress of the lake and help inform management decisions. Additionally, the Muskegon Lake Observatory (MLO) buoy, which is located at 43.23ºN, 86.28 ºW and is managed by the Biddanda Lab at AWRI (www.gvsu.edu/buoy), was installed. Since 2011, the MLO has been delivering high-frequency, time-series meteorological and water quality data throughout the water column each year from April/May to November/December, the data from which can be used by researchers and the public (Biddanda et al., 2018).

Though the overall condition of the lake ecosystem has improved and 4 of the 9 listed BUIs have been resolved as of 2018, eutrophication, HABs, and hypoxia are still prevalent processes in Muskegon Lake and are preventing it from reaching its delisting targets (Steinman et al., 2008; Gillett and Steinman, 2011). Stratification and subsequent hypoxia occur every summer in Muskegon Lake, and the monitoring data show that phytoplankton blooms are also prominent in the summer and fall (Biddanda et al., 2018). Several toxic and non-toxic nuisance cyanobacteria strains have been detected in Muskegon Lake, and fish populations have been shown to be affected by seasonal hypoxia, justifying a need to understand the processes of cultural eutrophication, its drivers and patterns, and its subsequent symptoms (Gillett and Steinman, 2011; Steinman et al., 2008; Hong, 2006; Weinke and Biddanda, 2018). The impending and persistent effects of climate change make the study of HABs even more pressing, especially as Muskegon Lake aims to be delisted as an AOC in the near future.

The importance of this study lies in the power of high-frequency time-series data, long-term data sets, and the need to understand the ecological processes within a unique ecosystem, a Great Lakes drowned river mouth estuary, to advance management and restoration practices on similar ecosystems world-wide. Globally, freshwater eutrophication and its symptoms are expected to increase due to increased nutrient deposition and the effects of climate change (Morrison et al., 2017; Ho and Michalak, 2015). Though occurrences of eutrophication have been recorded as early as biblical times (Exodus 7:17), its characteristics, drivers, and impacts have morphed with time, inspiring Le Moal et al. (2019) to deem “this new eutrophication process…a ‘new wine in an old bottle’”, highlighting the importance of continued research into the topic. More than ever before, it is imperative that our freshwater sources are protected and the processes that threaten them are understood.

Extended Methodology

Muskegon Lake Observatory (MLO) buoy

The Muskegon Lake Observatory (MLO) buoy is located at 43.23º N, 86.28º W and is managed by the Biddanda Lab at the Annis Water Resources Institute of Grand Valley State University with assistance from the NOAA GLERL's Lake Michigan Field Station in annual deployment and recovery. Since 2011, the MLO has been delivering high-frequency, time-series meteorological and water quality data throughout the water column for roughly seven months each year, excluding the winter months. Data from the buoy is publicly available on the Grand Valley State University website (www.gvsu.edu/buoy) and is part of the regional node of the Global Ocean Observing System (GOOS), the Great Lakes Observing System (GLOS). The MLO has sensors throughout the water column at depths of 2, 5, 8, and 11 m and measures temperature, oxygen, nitrate, light, pH, conductivity, chlorophyll *a*, phycocyanin, and water current speed and direction. The meteorological sensors, which are situated on top of the buoy, measure wind speed and direction, humidity, temperature, and precipitation type and amount. Water quality data is measured every 15 minutes and meteorological data is measured every five minutes. Periods of missing data are due to necessary maintenance, service, or biofouling, and no attempt was made to extrapolate data to fill in these periods so as to preserve the accuracy of interpretation. The MLO buoy is cleaned *in situ* approximately once per month to maintain the biofouling, which is mainly due to dreissenid mussels, at a low level.

MLO time-series data

Data from the MLO was first rid of any erroneous or suspect data. Then, data was distilled into a pivot table where daily averages were calculated. Cells for days of missing data were left empty. These daily averages were then made into a time-series graph.

Biweekly sampling

To maintain consistency, we began sampling days at 9:00 AM. We sampled the Muskegon channel site first, collecting our sample just west of the USS Silversides submarine museum, located on the south side of the channel. We then drove to the river site, parking at Veteran's Memorial Park and sampling off of the middle of the M. Causeway Bridge. After sampling these two sites, we returned to the lab and stored the water samples in the fridge and the phytoplankton samples at room temperature before taking a jon boat out to the third site. Here, we moored the boat to the MLO buoy and finished sampling.

Muskegon Lake monitoring program (MLMP) PCAs

The principal component analysis (PCAs) correlation biplots created from the spring, summer, and fall MLMP warrant brief further explanation. Data used in the PCAs was raw data collected from the MLMP and was not transformed or otherwise manipulated in any way. The only extrapolated data was the variable, $TKN:NO_3$, and this was simply a division between the two. In very few cases, a data point was an extreme outlier that confounded the figure significantly and was removed. Two types of chlorophyll *a* data were available: YSI measurements and the acetone extraction method. We used the data provided by the acetone extraction method in this case. Decisions to remove or keep variables was made simply by visual analysis of vector length.

Plankton enumeration

To enumerate the phytoplankton, we gently inverted the sample several times before extracting 1 mL of sample with a pipette and placing it in a Sedgewick rafter counting chamber, being careful to exclude any air bubbles and remove excess water using a Kimwipe. After allowing the sample to settle for at least 10 minutes, we used the 20X and 40X objectives of the Nikon Ti-U and Nikon Eclipse Ni-U DIC/Epi-fluorescence Compound microscopes. We mainly used three textbooks for identification: *Freshwater Algae of North America* 1st edition (Wehr et al., 2002), *Algae of the Western Great Lakes Area* (Prescott, 1973), and *Freshwater Algae: Identification and Use as Bioindicators* 1st edition (Bellinger and Sigee, 2010). We always used the camera function while identifying algae such that both analyzers (I. S. and J. M.) could confirm each identification. We followed the same sampling pattern each time, starting in the upper left-hand corner and moving five squares at a time until reaching the far-right side and then moving down two squares and continuing this same pattern moving to the left. We repeated this zig-zag pattern until we counted 350 natural algal units. Natural algal units were defined as filaments for filamentous phytoplankton, colonies for colonial phytoplankton, and cells for single-celled phytoplankton. One exception was *Fragilaria*, which is a filamentous phytoplankton that we counted as single cells due to the frequency of cells separating. Should we reach the bottom of the counting chamber before counting 350 natural algal units, we started the pattern over again starting with the square directly to the right of the first square we examined. Algal units completely within the squares and those lying on the top and left-hand borders, but not the bottom and right-hand borders, were counted to prevent counting the same phytoplankton twice. Specimens were identified to genus.

Converting phytoplankton raw data to count (abundance) data

We used a Sedgwick rafter counting chamber, which has holds 1 mL of water and contains a 100-square grid (50 X 20), to enumerate phytoplankton data. Since we counted up to a certain amount of natural algal units, we counted a different number of squares for each sample. Therefore, this must be included in the equation to convert raw counts of each genus to counts per L:

Count/L = ((g*100)/s)*1000)/176.7

g=the number of genus counted in the sample

s=the number of squares counted during enumeration of the sample

100=the number of squares on the Sedgwick rafter counting chamber

1000=scaling mL to L

176.7=the volume of water sampled (L)

Converting phytoplankton count (abundance) data to biovolume data

In instances of comparing count (abundance) data from the 2019 biweekly sampling to biovolume data from the long-term Muskegon Lake monitoring program (MLMP), it was necessary to convert it to biovolume (Chapter 1). To do this, I used biovolume data from the MLMP and applied it to the abundance data by simply multiplying the biovolume for each genus by the abundance of each genus to arrive at mg/L, which then could easily be converted to mg/mL where necessary. However, the MLMP phytoplankton data is species-level data, whereas ours is genus-level data. Therefore, first we looked up each species to make sure there were none we confidently had not seen before, and then we averaged the biovolumes of the species within each genus before multiplying it by our abundance data.

Annual cyanobacterial biovolume and percentage

The biovolume data explained previously was used to calculate annual cyanobacterial biovolume for the year 2019, which was compared to years 2003-2016. Cyanobacterial biovolume for 2019 was calculated by adding the cyanobacterial biovolume from each sampling day, inclusive of all three sites. Similarly, cyanobacterial biovolume for years 2003-2017 was calculated by adding the cyanobacterial biovolume at three sites, MUSR, CHAN, and DEEP, over the three sampling days (spring, summer, and fall). In this way, the cyanobacterial biovolumes calculated in this paper are rather arbitrary values only useful for making comparisons between years 2003-2016. The 2019 value cannot necessarily be compared to the other years.

To obtain the percentage of the phytoplankton community that was comprised of cyanobacteria, the total cyanobacterial biovolume was divided by the total phytoplankton biovolume found in sites MUSR, CHAN, and DEEP for all three seasons for 2003-2016. Data from both the top and bottom of the water column were used. For 2019, the total cyanobacterial biovolume was divided by the total phytoplankton biovolume found in the three sites for the entirety of the biweekly sampling.

Pigment Analysis

Water samples from sites Channel, River, and MLO surface were prepared for pigment analyses the day of collection. Upon return to the lab, a measured amount (no more than 500 mL) of each water sample was filtered through a 25 mm, 0.45 µm microfiber GF/F glass fiber filter using a vacuum and side-arm flask, such that two filters (one for phycocyanin and one for chlorophyll *a*) were produced for each water sample. Filters were individually stored in 15 mL polypropylene conical tubes and stored in a -80-degree ⁰C freezer until further analysis, no later than six months after collection. All pigment analysis was performed in the dark, and samples were wrapped in tinfoil. Filters were prepared for chlorophyll *a* analysis by adding the filter and 3 mL of a buffered acetone solution to a grinding tube and grinding the filter for one minute. If the tube were to get too warm, we would place it in an ice bath to cool and then resumed grinding. The slurry was poured back into the polypropylene tube and left to extract in the buffered acetone solution at 5 ⁰C for 24 hours. After 24 hours, samples were centrifuged at 4000 rpm for 20 minutes. For each sample, 3 mL of supernatant were pipetted into a 10 mm quartz glass cuvette, and 3 mL of acetone buffer were pipetted into a separate 10 mm quartz glass cuvette to use as a blank. Absorbance wavelengths were measured at 750 nm, 664 nm, and 665 nm using a UV-VIS 2450 Shimadzu dual beam spectrophotometer. Chlorophyll *a* concentration (*c*) was calculated using the following equation (APHA, 1992):

c=26.7*(A664nm–A750nm)–(A665nm-A750nm)*V extracted (mL)/V filtered (L)

A=absorbance value

V=volume

Filters were prepared for phycocyanin analysis by completing two freeze-thaw cycles, such that filters were removed from the -80-degree °C freezer (no later than six months after collection), thawed at 5 °C for 24 hours, placed back in the -80-degree °C freezer for two hours, and thawed at 5 °C for 24 hours. At the end of the first cycle (after thawing for 24 hours at 5 °C), 5 mL of phosphate buffer (0.1 M, pH=6.5) were added to each sample. After the freeze-thaw cycles were complete, samples were sonicated for 30 seconds total (six five-second intervals with 3-second breaks). Phosphate buffer was used to rinse the sonicator into the centrifuge tube, bringing the final volume of the slurry to 10 mL. After sonication, samples were immediately placed in an ice bath for 15 seconds to cool. Samples were vortexed for a total of nine seconds (three rounds of three seconds with three second breaks) and then allowed to extract at 5 °C for 96 hours. Samples were then vortexed as before and centrifuged at 4000 rpm for 20 minutes. For each sample, 3 mL of supernatant were pipetted into a 10 mm quartz glass cuvette, and 3 mL of phosphate buffer were pipetted into a separate 10 mm quartz glass cuvette to use as a blank. Absorbance wavelengths were measured at 620 and 750 using a UV-VIS 2450 Shimadzu dual beam spectrophotometer. Phycocyanin concentration (c) was calculated using the following equation (corrected from Lawrenz et al., 2011):

p=((A620-A750)/Ed)*(MW)*(Vol buffer (L)/Vol sample (L)*06

A=A620-A750

E=Molar extinction coefficient ($1.9*10^6$/mol cm; Lawrenz et al., 2011)

d=Path length of the cuvette (1 cm)

MW=Molar weight (264,000 g/mol; Lawrenz et al., 2001)

V buffer=Volume of buffer added

V sample=Volume of sample filtered

Nutrient analysis preparation

We prepared water samples for analysis in the wet chemistry lab at the Annis Water Resources Institute the day of collection. Before preparation, all water samples were inverted several times to homogenize the sample as much as possible. To prepare for SRP analysis, 25 mL of sample was filtered through a 25 mm, 0.45 µm nitrocellulose filter. The filter was first rinsed with 5 mL of 25% HCl (v/v) and then 25 mL of deionized water, followed by pushing air through the filter with a syringe to remove excess liquid. After filtering, samples were placed in a fridge at 4 °C until analysis. NO_3 samples were prepared the same way but were stored in a chest freezer until analysis. NH_3 samples were prepared by acidifying 250 mL of sample with 250 µL of concentrated H_2SO_4 and then inverting to mix. Samples were stored in a fridge at 4 °C until analysis.

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