ASSESSING THE OCCURRENCE OF CYANOTOXINS IN TWO URBAN
RESERVOIRS THROUGH A COMMUNITY SCIENCE MONITORING PROGRAM

A thesis
presented to
the Faculty of the Graduate School
At the University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
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JULY 2021

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ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Rebecca North, for making this work possible. She was always available when I needed assistance, gave me the space to ask questions, and provided many opportunities for professional growth. I would also like to thank Dr. Jennifer Graham, Dr. Binbin Wang, and Dr. Peter Motavalli for their service on my thesis committee and valuable input on this project.

Thank you also to members of the MU Limnology Lab for the camaraderie and helping me get through the hardest parts of graduate school. Specifically, I would like to thank Tony Thorpe for always being eager to answer my many questions about running a community science program and the hours spent training me on cyanotoxin analysis. Thank you to Carol Pollard for her eternal patience while training me on lab analyses. I would like to thank Clare Vanderwerken and Matt Sauer for the wonderful company on long road trips to conduct field work and assistance with training high school students.

Thanks to my family and friends for taking an interest in my work and always being available to talk. Biggest thanks to my husband, Justin Kinzinger, for his steady encouragement throughout the years. He didn't hesitate to support my decision to quit my full time job in pursuit of graduate school, and his confidence in my success has never wavered. Finally, thank you to my son, Otto, who provided inspiration to push through when things got challenging and shifted the way I see the world.

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Abstract

Cyanobacterial blooms are an ongoing threat to aquatic systems worldwide. These potentially toxic blooms have typically been observed in summer to early fall, but there are increasing reports of blooms in colder seasons. From the few studies available, we know that blooms can produce toxins in the winter. The objective of this research was to assess the year-round presence, concentration, and environmental drivers of the cyanotoxins microcystin and cylindrospermopsin in two reservoirs located in Columbia, Missouri, USA. To fulfill this objective, I coordinated and managed the Reservoir Observer Student Scientists (ROSS) program. This community science initiative combined educating high school students about limnology and cyanobacterial blooms with training students hands-on to collect weekly water quality samples. Data for this project was collected over two years in Bethel Lake and one year in Stephens Lake. We found detectable concentrations of the cyanotoxins year-round. There was no relationship between microcystin nor cylindrospermopsin and chlorophyll-*a* in either reservoir. Presence and concentration of microcystin and cylindrospermopsin were related to physical water quality parameters in Bethel Lake. This study provides evidence for the necessity of year-round water quality monitoring.

Chapter 1: Introduction, Objectives, and Literature Review

Introduction

Eutrophication, or over-enrichment of plant nutrients (Anderson et al., 2002), contributes to water quality issues on a global scale (Wiltsie et al., 2018). Anthropogenic inputs of nutrients from agriculture, industrialization, and urbanization to surface waters creates a growing problem that can lead to blooms of cyanobacteria (Downing et al., 2001; Paerl and Huisman, 2008); the most prevalent harmful phytoplankton in lakes and reservoirs (Anderson et al., 2002). Cyanobacterial blooms form mucilaginous scums on the surface of lakes and reservoirs that can shade submerged vegetation and deplete waters of oxygen, leading to fish kills. These foul-smelling blooms can also produce adverse taste and odor compounds and increase turbidity. Cyanobacterial blooms impact human and animal health through production of toxins, increase the cost of drinking water treatment, result in economic losses from a decrease in recreation, and decrease property values.

One of the main dangers associated with cyanobacterial blooms is their ability to produce and release toxins in the water (Wejnerowski et al., 2018). Approximately 75 % of cyanobacterial blooms are dominated by species that have the capability to produce toxins (Wejnerowski et al., 2018). Cyanotoxins can impair liver function, damage the nervous system, damage or irritate skin, and be fatal to animals. In rare cases, cyanotoxin consumption can even lead to human fatalities (Wiltsie et al., 2018).

While cyanobacterial blooms typically occur in the summer, they have also been observed in the shoulder seasons (spring and fall) and winter (Wejnerowski et al., 2018). Temperate, dimictic lakes - those that mix twice a year and are covered by ice in the

winter - generally exhibit minimum phytoplankton biomass in the winter (Wetzel, 2001). Warm monomictic lakes thermally stratify during the summer and mix from top to bottom the rest of the year (Wetzel 2001). Annual maximums of phytoplankton biomass typically occur in the spring, with a second peak during the late summer (Wetzel, 2001). The limiting factor for algal growth in the winter is often light, and some cyanobacteria have adapted to these low light conditions, allowing them to bloom under ice (Bertilsson et al., 2013). In addition, climate change is resulting in shorter winters and warmer water temperatures, with a reduction in ice cover (Sharma et al., 2019), potentially leading to an increase in cyanobacterial blooms.

Although cyanobacterial blooms have been observed in eutrophic lakes during the winter (Ma et al., 2016; Wejnerowski et al., 2018), cyanobacterial ecology during this season is understudied (Hampton et al., 2017). Cyanobacterial blooms in the winter months can produce toxins (Wejnerowski et al., 2018). The cyanotoxins microcystin and anatoxin were found year-round in a warm monomictic drinking water reservoir in North Carolina (Wiltsie et al., 2018). While concentrations of microcystin were below the US Environmental Protection Agency (EPA) 10-Day Drinking Water Health Advisory (HA) level for microcystin of $0.3 \mu\text{gL}^{-1}$ for pre-school age children, they did not vary significantly by season (Wiltsie et al., 2018).

Understanding the seasonal dynamics of cyanobacterial blooms and cyanotoxin occurrence is important for informing sound management of lakes and reservoirs and predicting future changes to these systems. Therefore, I assessed the year-round presence and concentration of cyanotoxins in two urban reservoirs located in central Missouri. Information from this study can help natural resource managers better understand the

presence of toxins in drinking and recreational waters so appropriate action can be taken to protect human and animal health.

Objective, Hypotheses, and Thesis Structure

Objectives

The overall purpose of this thesis is to assess the year-round presence, concentration, and environmental drivers of cyanotoxins in two urban reservoirs through the implementation of a community science program. Two years of water quality data were collected from Bethel Lake (Columbia, MO) and one year of data was collected from Stephens Lake (Columbia, MO) to draw connections among cyanotoxin presence, concentration, and environmental drivers.

Hypotheses

H₀: Cyanotoxins are only present in the summer.

H₁: Cyanotoxins are present year-round.

H₀: Algal biomass will not vary significantly through the seasons.

H₁: Algal biomass will differ significantly among the seasons.

H₀: Concentrations of cyanotoxins will not be associated with algal biomass (chlorophyll *a*)

H₁: Concentrations of cyanotoxins will be associated with algal biomass.

H₀: Neither light, phosphorus, nitrogen, nor water temperature will influence cyanotoxin presence or concentrations.

H₁: Cyanotoxin presence and concentration will be influenced by light, phosphorus, nitrogen, and/or water temperature.

Thesis Structure

This thesis is divided into four chapters. Chapter 1 provides background information to frame the research. Chapter 2 is a stand-alone research paper that examines the environmental drivers of cyanotoxin occurrence and concentration year-round in two reservoirs. In Chapter 3, I present information about the Reservoir Observer Student Scientist (ROSS) Program, a community science initiative that I managed and through which data collection was made possible. Chapter 4 is a concluding chapter in which I discuss main findings and reflections from my research.

Literature Review

Lake Mixing Patterns and Phytoplankton Succession

The phytoplankton consists of several taxonomic groups of algae and one major group of photosynthetic bacteria, cyanobacteria (Kalff, 2002; Wetzel, 2001).

Cyanobacteria are functionally similar to planktonic algae, so although they are not true algae, they are considered part of the phytoplankton (Kalff, 2002; Wetzel, 2001).

Hereafter in this document, the term algae will include cyanobacteria. Seasonal changes in physical, chemical, and biological factors, including light, temperature, nutrients, and grazing determine the dominant phytoplankton taxa in lakes and reservoirs (Wetzel, 2001).

The productivity of phytoplankton and structure of the phytoplankton community are influenced by lake stratification and mixing patterns. Thermal stratification occurs when surface water is warmed by the sun and the cold, dense water sinks to the bottom, forming the hypolimnion. The density difference between the hypolimnion and the upper layer, known as the epilimnion, creates a resistance to mixing that is greater than the power of wind-induced turbulence to mix the water. The transition zone between the epilimnion and hypolimnion is the metalimnion. The metalimnion restricts nutrient and gas circulation throughout the water column and impacts phytoplankton distribution. When a lake is thermally stratified, mixing is generally restricted to the epilimnion. In fall, as surface water cools, the density difference is reduced until resistance to mixing is less than the mixing power of wind. As a result, the entire water column mixes. Since currents move phytoplankton through the mixed layer, the depth of this layer is important for determining phytoplankton productivity and vertical positioning. If the euphotic zone,

the portion of the water column where photosynthesis is greater than respiration, is at the same depth or deeper than the mixing zone, phytoplankton are positioned to receive enough light for photosynthesis. If the mixing zone is deeper than the euphotic zone, phytoplankton spend a portion of their time without access to adequate light (Kalff, 2002).

Lakes can be classified by the frequency with which they experience thermal stratification and mixing. The reservoirs sampled during this study are classified as warm monomictic. Dimictic lakes are covered in ice in the winter, stably stratified during the summer, and mixed from top to bottom in spring and fall. Warm monomictic lakes are stably stratified during summer, but mix throughout the rest of the year and do not have extended periods of ice cover in the winter (Kalff, 2002).

Although phytoplankton dynamics change throughout the year, temperate regions exhibit somewhat predictable seasonal patterns in phytoplankton biomass. Wetzel (2001) describes phytoplankton seasonal succession in a temperate, dimictic lake through eight periods: 1) midwinter; 2) late winter; 3) spring circulation; 4) initial summer stratification; 5) summer clear water phase; 6) late summer stratification; 7) fall circulation; and 8) late autumn decline. Typical algal groups found in each season are identified in Figure 1.1. Midwinter is characterized by low light and water temperatures, a stable water column, low zooplankton grazing, and moderate nutrient concentrations. The phytoplankton are dominated by small, motile algae, but algal blooms can occur if the lake receives enough light penetration and nutrient concentrations are high enough. The late winter period exhibits high light intensity and day length, with an increase in small bacillariophytes and motile dinoflagellates. Spring circulation brings cold, but

increasing water temperatures, increasing light intensity, a mixing water column, high nutrient concentrations, and an annual maximum in bacillariophyte biomass. During the initial summer stratification water temperatures rise, light intensity increases, the water column stabilizes, and nutrient concentrations decline. The sedimentation of bacillariophytes results in a decrease in algal biomass. In the summer clear water phase, there is high light intensity, high water temperatures, a highly stable water column, and low nutrient availability. Zooplankton grazing increases and phytoplankton biomass declines. Late summer stratification brings high water temperatures, high water column stability, high, but decreasing light intensity, low nutrient availability, and a reduction in zooplankton. In more eutrophic lakes, there is often an abundance of cyanobacterial growth during this phase that persists until the water column is mixed. In the fall, water temperatures and light intensity decrease, and the water column mixes. This increases nutrient availability and there is an increase in algal biomass, primarily bacillariophytes. Cyanobacterial biomass declines as the mixing depth of the water column is greater than the euphotic depth. The late fall exhibits low water temperatures, light intensity, and nutrient concentrations and thus reduced algal biomass. There is a knowledge gap regarding fall and winter phytoplankton populations. More research is necessary to understand phytoplankton survival during fall and winter, in addition to how winter phytoplankton composition impacts spring succession (Sommer et al., 2012).

Eutrophication and Cyanobacteria

Eutrophication of surface waters is an ongoing water quality issue that impacts lakes and reservoirs on a global scale (Anderson, 2014; Schindler, 2006). Anthropogenic nutrient inputs to surface waters have accelerated eutrophication of freshwater resources

throughout the last several centuries (Paerl and Paul, 2012). Eutrophication can result in excessive algal production, leading to increased turbidity, depletion of oxygen in subsurface waters as the algae are decomposed, and fish kills (Schindler et al., 2008). Eutrophication can also promote a shift in the composition of phytoplankton communities to cyanobacteria (Smith, 2003).

Cyanobacteria are among the oldest organisms on Earth, dating back over 3 billion years (Carmichael, 1994), and are well adapted to exploit freshwater resources to achieve high growth rates (Watson et al., 2015). These photoautotrophic, oxygen-producing bacteria are naturally occurring in both planktonic and benthic communities of lakes, reservoirs, ponds, and streams. Cyanobacteria can be unicellular, colonial, or filamentous in form and are typically enclosed in mucilaginous sheaths (Wetzel, 2001). They can produce resting cells, called akinetes, to survive unfavorable growth conditions, while others are able to survive in a vegetative state in sediments (Kalff, 2002). Some cyanobacteria, called diazotrophs, can also fix atmospheric nitrogen (N), providing a competitive advantage in lakes with low available N concentrations. Further, some cyanobacteria can regulate buoyancy in calm waters, allowing them to move throughout the water column to optimal nutrient and light conditions (Kalff, 2002).

Freshwater algal blooms are typically dominated by cyanobacteria (Paerl et al., 2001). Cyanobacterial blooms can be characterized by dense, green, mucilaginous scums on surface waters. These blooms can be considered harmful as they may cause hypolimnetic hypoxia or anoxia, leading to fish kills, shade submerged vegetation, produce taste and odor compounds that are difficult to remove during drinking water treatment, create a visual nuisance leading to loss of revenue from recreation and

decreased property values, and produce toxins that are a serious threat to human and animal health (Anderson et al., 2002; Paerl et al., 2001). Cyanobacterial blooms are detrimental to lakes and reservoirs utilized for drinking water, recreation, or agriculture.

Factors affecting cyanobacteria growth

Light

Cyanobacteria growth is largely controlled by light, temperature, nutrients, and grazing. Solar radiation plays a critical role in the functioning of aquatic ecosystems (Wetzel, 2001). It drives photosynthesis (Kalff, 2002), influences thermal structure and stratification of lakes, and represents the largest input of energy to aquatic systems (Wetzel, 2001). Light is defined as the radiant energy of the electromagnetic spectrum (Wetzel, 2001). Photosynthetically available radiation (PAR) describes the wavelengths of light from 380 to 710 nanometers which allow for photosynthesis to take place (Kalff, 2002). While 50 percent of the sunlight that reaches surface waters is within the photosynthetic range (Kalff, 2002), active photosynthesis is restricted to the population of phytoplankton confined in the euphotic zone (Reynolds and Walsby, 1975). In this zone, phytoplankton photosynthesis is greater than respiration, and the lower boundary of the euphotic zone is defined as the depth where one percent of the surface irradiance penetrates (Kalff, 2002). When the mixing zone is deeper than the euphotic zone, phytoplankton spend more time in the dark, reducing photosynthesis. At high densities, cyanobacterial blooms can impair light penetration, resulting in a competitive advantage over other algal groups, reduce the depth of the euphotic zone and potentially self-shade (Reynolds and Walsby, 1975).

Water Temperature

Temperature plays a vital role in cyanobacterial ecology (Raven and Geider, 1988). In general, cyanobacteria growth has a positive relationship with increasing water temperature, but temperature requirements vary by species. Cyanobacterial growth is often highest during late summer, when water temperatures are warmer and growth of other phytoplankton has declined (Figure 1.1). Warmer water temperatures tend to favor cyanobacterial growth (Paerl and Huisman, 2008). While optimal temperature growth rates for cyanobacteria tend to occur between about 25.0 and 28.0 °C (Mehner et al., 2010), some can even grow in temperatures up to 35 °C (Butterwick et al., 2005). Temperature also indirectly influences cyanobacterial growth through promotion of stratification. High temperatures can result in stable stratification in eutrophic waters. Stratification provides buoyant cyanobacteria a competitive advantage for light and nutrients (Johnk et al., 2008) as they can benefit from exposure to light at the surface and migrate to deeper waters to obtain nutrients (Huisman et al., 2018).

Despite the evidence for cyanobacterial growth favoring warmer water temperatures, some species can tolerate a wide range of temperatures and sustain populations under ice (Wejnerowski et al., 2018). The presence of cyanobacteria under ice has been well documented, (Bertilsson et al., 2013; Kalinowska and Grabowska, 2016; Phillips and Fawley, 2002; Somogyi et al., 2014; Üveges et al., 2012; Wejnerowski et al., 2018). This indicates that low temperatures, while not considered optimal, can support cyanobacterial growth. Further, cyanobacteria can produce toxins in the winter (Ernst et al., 2001; Wejnerowski et al., 2018; Welch et al., 2000; Wiltsie et al., 2018).

Thus, given the wide thermal tolerance of cyanobacteria, winter and shoulder season water quality monitoring is necessary and important.

Nutrients

In aquatic systems, N and phosphorus (P) are the nutrients that are frequently responsible for limiting phytoplankton growth. Increased inputs of these nutrients, largely from cultural eutrophication, to surface waters accordingly increases primary productivity in these systems. P has traditionally been identified as the limiting nutrient for freshwater cyanobacterial growth, and most CyanoHAB management strategies focus on reducing P inputs (Kalff, 2002). One reason for this is because some cyanobacteria can fix atmospheric N, which enables growth under low concentrations of N (Higgins et al., 2018). The main forms of dissolved N present in lakes and reservoirs are typically ammonium, nitrate, nitrite, and urea. Ammonium is the most biologically available form of N, but it is often present at relatively low concentrations because it is either oxidized to nitrate or taken up by phytoplankton. Many of the cyanobacteria species that form blooms are not N fixers and can be limited by a combination of P and N (O'Neil et al., 2012). The presence of diazotrophic cyanobacteria species is likely in systems with a molar N:P ratio less than 20. In highly eutrophic systems, non-diazotrophic cyanobacteria will likely dominate as nutrients are present in nonlimiting concentrations (Paerl et al., 2001). Cyanobacteria may use inorganic and organic compounds including ammonium, nitrate, and urea as sources of N.

Grazing

Phytoplankton populations can be significantly impacted through consumption by zooplankton grazers, including protozoa, rotifers, and crustaceans (Kalff, 2002; Wetzel, 2001). Through filtering and selectively feeding on phytoplankton, zooplankton can influence the size and composition of algal populations. For instance, cyanobacteria that have mucilaginous sheaths, form large colonies, or produce toxins can be resistant to zooplankton grazing (Wetzel, 2001). By selecting smaller, easier to digest phytoplankton, populations of larger or grazer resistant phytoplankton, like cyanobacteria, are less affected. Further, some phytoplankton ingested by zooplankton may not be digested, thus allowing them to pass through unharmed.

Cyanotoxins

Under certain conditions, some cyanobacteria may produce harmful secondary metabolites known as cyanotoxins (Rastogi et al., 2015; Westrick et al., 2010). Approximately 120 species of cyanobacteria have been identified that are capable of producing toxins (Chapman and Foss, 2019), but even if a potentially toxin-producing species is present, it may not be producing toxins. Further, both toxin producing and non-toxin producing strains can co-exist within some species of cyanobacteria (Sivonen and Jones, 1999). Cyanotoxins occur naturally in cyanobacteria and can be found intracellularly, within the cytoplasm or in cell walls, or extracellularly (Westrick et al., 2010). When the cell dies or ruptures, the toxins are released into the water and are considered extracellular. In this study, we are focusing on two cyanotoxins: microcystin and cylindrospermopsin. Microcystin is contained intracellularly about 95 percent of the time during growth and is released extracellularly upon senescence (United States

Environmental Protection Agency, 2014). Cylindrospermopsin, however, is excreted from cyanobacterial cells naturally and extracellular cylindrospermopsin can account for 50 percent or more of the total toxin (Griffiths and Saker, 2003).

There are generally three main groups of cyanotoxins: hepatotoxins (liver toxins), neurotoxins, and dermatoxins (Watson et al., 2015). Microcystin and cylindrospermopsin are both hepatotoxins and dermatoxins (Watson et al., 2015). Exposure to cyanotoxins can be harmful to both humans and animals. Humans can be exposed to cyanotoxins through ingestion of contaminated drinking water, recreation, or dietary exposure (Rastogi et al., 2014). There are world-wide cases of health ailments from contaminated drinking water, primarily gastrointestinal issues like stomach pain, nausea, vomiting, and diarrhea (Carmichael, 2008; Griffiths and Saker, 2003; Tisdale, 1931). Chronic exposure to low doses of cyanotoxins have been found to promote tumor formation and may be responsible for the development of cancer (United States Environmental Protection Agency, 2014; Zhou et al., 2002). One study identified that the risk of colorectal cancer was multiplied by 7.9 when water with more than $0.05 \mu\text{g L}^{-1}$ of microcystin was consumed (Zhou et al., 2002). Recreational activities like swimming or boating can lead to accidental ingestion, inhalation, or dermal contact with cyanotoxins. This can cause skin irritation, fever, headaches, asthma, eye irritation, muscle and joint pain, coughing, and gastrointestinal symptoms (Drobac et al., 2013; United States Environmental Protection Agency, 2014). It can be difficult to attribute illness to cyanotoxin exposure, but in 2011, two patients were confirmed to be hospitalized from direct recreational exposure to microcystin in a reservoir in Kansas, USA (Trevino-Garrison et al., 2015). Animal health risks are primarily associated with consumption of water contaminated

with cyanotoxins. Hundreds of documented cases of animal poisonings have been reported in literature (Wood, 2016). Deaths of wild and domestic animals like livestock, dogs, and migrating ducks and geese have been linked to consumption of water contaminated with cyanotoxins (Carmichael, 2008; Wood, 2016).

It is not well understood what causes cyanobacteria to produce toxins.

Microcystin is the most common cyanotoxin, and thus, the most studied (Niamien-Ebrott et al., 2015; Westrick et al., 2010). Although there is no consensus on what environmental parameters most directly impact the production of cyanotoxins, studies suggest that cyanobacteria have higher toxin production under conditions most favorable for cyanobacterial growth (Sivonen and Jones, 1999). The high volume of cyanobacterial cells in blooms may lead to higher concentrations of cyanotoxins than are found in waters surrounding the bloom (Paerl and Huisman, 2009). Cylindrospermopsin concentrations, however, are not highest under favorable growth conditions, but are related to growth limitations (Rzymiski and Poniedziałek, 2014). Possible drivers of cyanotoxin production include light intensity, water temperature, nutrients, and mixing events.

Light intensity can have a two- to three-fold impact on toxin concentration (Sivonen and Jones, 1999). Studies have identified positive relationships between microcystin production and PAR. Wiedner et al. (2003) identified an increase of microcystin production in response to PAR during the growth phase of *Microcystis*, but beyond the maximum growth rate, microcystin production was hindered at higher levels of PAR. Similarly, Ding et al. (2013) found that cyanobacterial blooms were most impacted by increased PAR during bloom formation. An experiment with both toxic and non-toxic strains of *Microcystis* found that the non-toxic strains were better light

competitors (Kardinaal et al., 2007), suggesting that non-toxic strains prevail under light limitation. Unlike microcystin, maximum cylindrospermopsin concentrations were observed at light intensities two times higher than those associated with the most optimal growth rate (Dyble et al., 2006a; Rzymiski and Poniedzialek, 2014).

Similar to light, there is a positive relationship between water temperature and microcystin. In general, studies have identified that temperatures between 18 and 25 °C resulted in the greatest production of microcystin (Sivonen and Jones, 1999; Walls et al., 2018). In a study that exposed both toxic and non-toxic *Microcystis* strains to experimentally manipulated temperatures, toxic strains grew more rapidly at higher temperatures than non-toxic strains (Davis et al., 2009). Concentrations of extracellular microcystin increase at higher temperatures, likely due to bloom senescence. The relationship between cylindrospermopsin concentrations and water temperatures is variable. In one study, cylindrospermopsin production decreased as temperature increased from 20 to 35 °C, until production stopped at 35 °C (Rzymiski and Poniedzialek, 2014; Saker and Griffiths, 2000). Toxin production restarted when temperatures were decreased to below 35 °C (Rzymiski and Poniedzialek, 2014; Saker and Griffiths, 2000). In a Chinese field study, no relationship was found between cylindrospermopsin concentration and temperature, but the highest concentrations were observed at the lowest mean water temperature of the study (17.6 °C; Lei et al., 2014; Rzymiski and Poniedzialek, 2014).

Nutrients can impact microcystin production in a variety of ways (Dolman et al., 2012). In general, high concentrations of P and N promote growth of toxic strains of *Microcystis* (Rapala et al., 1997; Vezie et al., 2002), but the production of toxins can

change with different types of nutrient limitations (Anderson et al., 2002). Microcystin concentrations have a positive relationship with increased P concentrations (Orihel et al., 2013; Rapala et al., 1997; Rastogi et al., 2014), but the relationship with N can vary among N forms and between diazotrophic and non-diazotrophic cyanobacteria. Diazotrophic cyanobacteria produce higher concentrations of toxins under N deplete conditions, while non-N fixers produce higher concentrations of toxins when in N rich conditions (Melanie Kaebernick and Neilan, 2001). In a study of three eutrophic lakes, Monchamp et al. (2014) found that different forms of N influenced microcystin concentrations. The systems with higher dissolved organic N and lower ammonium had higher microcystin concentrations (Monchamp et al., 2014). Conversely, a nutrient enrichment experiment by Chaffin et al. (2018) found that while N enrichments significantly increased microcystin concentrations, N form (ammonium, nitrate, and urea) did not significantly impact microcystin concentrations. The contradictory nature of the relationship between microcystin and N form in the literature could be related to presence of different microcystin producing species and strains of microcystin.

There is less information available regarding the relationship between cylindrospermopsin and nutrients. Two German field studies have identified positive relationships between cylindrospermopsin content and total N and P (Rücker et al., 2007; Rzymiski and Poniedzialek, 2014; Wiedner et al., 2008). In Polish field studies, cylindrospermopsin content was positively related to total P, but negatively related to total N (Kokociński et al., 2013; Rzymiski and Poniedzialek, 2014). Lab studies indicate that cylindrospermopsin production is triggered by N limitation in diazotrophic species of

cylindrospermopsin producers (Griffiths and Saker, 2003; Rzymiski and Poniedziałek, 2014).

Turbulent waters can be responsible for an increase in microcystin as well. A mesocosm experiment identified microcystin concentrations 3.4 times higher in turbulent water than calm water (Zhou et al., 2016). It is possible that the turbulence is responsible for damaging cells and allowing a release of intracellular toxins into the water (Zhou et al., 2016). An experiment by Orihel et al. (2013) identified a relationship between internal P loading and microcystin concentrations, so polymictic systems, which mix frequently, may be more susceptible to increased microcystin concentrations.

The reasons why cyanobacteria produce toxins is still unclear. Cyanotoxins may be produced in response to grazing to deter predation (Holland and Kinnear, 2013). The toxicity of microcystin to zooplankton, specifically *Daphnia*, is well established (Rohrlack et al., 1999). Microcystin production, however, likely pre-dates the evolution of common cyanobacterial predators like copepods and cladocerans (Rantala et al., 2004). Some studies have identified negative relationships between some forms of microcystin and other cyanobacteria or green algae, so allelopathy is another theory for why cyanobacteria may produce toxins (Holland and Kinnear, 2013). There is also evidence suggesting that cyanotoxins aid in nutrient uptake. Under P limitation, for example, cyanobacteria can secrete an alkaline phosphatase enzyme to obtain inorganic phosphate from organic molecules (Raven, 2010). A study by Bar-Yosef et al. (2010) found that cylindrospermopsin produced by *Aphanizomenon ovalisporum* caused other phytoplankton to secrete alkaline phosphatase, increasing the availability of inorganic

phosphate to *A. ovalisporum*. Further, Van de Waal et al. (2014) found that toxins may be produced as a response to nutrient limitation for assistance with nutrient acquisition.

Climate Change

Lake conditions are changing as a response to climate change (Livingstone, 2008). Over the last 60 years, surface water temperatures of lakes and rivers have increased between 0.2 and 2.0 °C, and they are projected to continue warming (Bates et al., 2008). Consequences of this warming include an increase in thermal stability and longer periods of more frequent thermal stratification (Livingstone, 2008; O’Neil et al., 2012). Implications of this include less nutrient availability in surface waters, plus a decrease in hypolimnetic oxygen. This would allow for increased P release from sediments, providing a competitive advantage to cyanobacteria that can fix atmospheric N and/or regulate buoyancy and obtain nutrients from deeper waters (Bates et al., 2008; O’Neil et al., 2012).

Climate change is also shifting patterns in lake ice cover (Sharma et al., 2019). Studies are showing a reduction in the ice cover phenology (Sharma et al., 2019). Ice is forming increasingly later in the year and melting earlier in the year (Livingstone, 2008; Magnuson et al., 2000); thus, resulting in decreased durations of ice cover (Magnuson et al., 2000). The greatest predictor of annual versus intermittent winter ice cover is mean annual air temperature (Sharma et al., 2019). Historical data was used to create projections identifying lakes in the Northern Hemisphere that could experience less frequent ice cover due to climate warming. Their results indicated that the trend towards intermittent ice cover is moving northward, and there is a potential for a rapid, exponential increase in lakes projected to experience less frequent ice cover. Their

projections indicated that the timing in loss of lake ice for lakes with similar mean depths was driven by a latitudinal gradient in air temperature. Winter processes set the stage for subsequent seasons (Hampton et al., 2017), so loss of duration and frequency of ice cover can impact year-round primary production in aquatic systems. Primary production in the winter is often limited by light (Hampton et al., 2017), so loss of lake ice could mean increased winter cyanobacterial biomass. As noted above, in some cases cyanobacteria can thrive under ice and primary production in the winter rivals that of summer (Wejnerowski et al., 2018).

References

- Anderson, D. M., Glibert, P. M., and Burkholder, J. M. (2002). Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences. *Estuaries* 25, 704–726.
- Anderson, D.M. (2014). HABs in a changing world: a perspective on harmful algal blooms, their impacts, and research and management in a dynamic era of climactic and environmental change. *Harmful Algae* 21, 309–317.
- Bar-Yosef, Y., Sukenik, A., Hadas, O., Viner-Mozzini, Y., and Kaplan, A. (2010). Enslavement in the water body by toxic aphanizomenon ovalisporum, inducing alkaline phosphatase in phytoplanktons. *Curr. Biol.* 20, 1557–1561. doi:10.1016/j.cub.2010.07.032.
- Bates, B., Kundzewicz, Z. W., Wu, S., and Palutikof, J. (2008). Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change.
- Bertilsson, S., Burgin, A., Carey, C. C., Fey, S. B., Grossart, H. P., Grubisic, L. M., et al. (2013). The under-ice microbiome of seasonally frozen lakes. *Limnol. Oceanogr.* 58, 1998–2012. doi:10.4319/lo.2013.58.6.1998.
- Butterwick, C., Heaney, S. I., and Talling, J. F. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. 291–300. doi:10.1111/j.1365-2427.2004.01317.x.
- Carmichael, W. W. (1994). The toxins of cyanobacteria. *Sci. Am.* 270, 78–86. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8284661>.
- Carmichael, W. W. (2008). A world overview-one hundred-twenty seven years of reserach on toxic cyanobacteira-where do we go from here? *Adv. Exp. Med. Biol.*, 105–125. doi:10.1007/978-0-387-75865-7.
- Chaffin, J. D., Davis, T. W., Smith, D. J., Baer, M. M., and Dick, Gregory, J. (2018). Interactions between nitrogen form, loading rate, and light intensity on Microcystis and Planktothrix growth and microcystin production. *Harmful Algae* 73, 84–97. doi:10.1016/j.hal.2018.02.001.
- Chapman, A. D., and Foss, A. J. (2019). GreenWater Laboratories Potentially Toxigenic (PTOX) Cyanobacteria List. 1–13. doi:10.13140/RG.2.2.32214.50243.
- Davis, T. W., Berry, D. L., Boyer, G. L., and Gobler, C. J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of Microcystis during cyanobacteria blooms. *Harmful Algae* 8, 715–725. doi:10.1016/j.hal.2009.02.004.
- Ding, Y., Song, L., and Sedmak, B. (2013). UVB Radiation as a Potential Selective Factor Favoring Microcystin Producing Bloom Forming Cyanobacteria. *PLoS One* 8. doi:10.1371/journal.pone.0073919.
- Dolman, A. M., Rücker, J., Pick, F. R., Fastner, J., Rohrlack, T., Mischke, U., et al. (2012). Cyanobacteria and cyanotoxins: The influence of nitrogen versus phosphorus. *PLoS One* 7. doi:10.1371/journal.pone.0038757.
- Downing, J. A., Watson, S. B., and McCauley, E. (2001). Predicting Cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* 58, 1905–1908. doi:10.1139/f01-143.

- Drobac, D., Tokodi, N., Simeunovic, J., Baltic, V., Stanic, D., and Svircev, Z. (2013). Human exposure to cyanotoxins and their effects on health. *Arch. Ind. Hyg. Toxicol.* 64, 305–316. doi:10.2478/10004-1254-64-2013-2320.
- Dyble, J., Tester, P. A., and Litaker, R. W. (2006). Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. *African J. Mar. Sci.* 28, 309–312. doi:10.2989/18142320609504168.
- Ernst, B., Hitzfeld, B., and Dietrich, D. (2001). Presence of *Planktothrix* sp. and Cyanobacterial Toxins in Lake Ammersee, Germany and their Impact on Whitefish (*Coregonus lavaretus* L.). *Environ. Toxicol.* 16, 483–488. doi:10.1002/tox.10006.
- Griffiths, D. J., and Saker, M. L. (2003). The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicol.* 18, 78–93. doi:10.1002/tox.10103.
- Hampton, S. E., Galloway, A. W. E., Powers, S. M., Ozersky, T., Woo, K. H., Batt, R. D., et al. (2017). Ecology under lake ice. *Ecol. Lett.* 20, 98–111. doi:10.1111/ele.12699.
- Higgins, S. N., Paterson, M. J., Hecky, R. E., Schindler, D. W., Venkiteswaran, J. J., and Findlay, D. L. (2018). Biological Nitrogen Fixation Prevents the Response of a Eutrophic Lake to Reduced Loading of Nitrogen : Evidence from a 46-Year Whole-Lake Experiment. *Ecosystems* 21, 1088–1100. doi:10.1007/s10021-017-0204-2.
- Higgins, S. N., and Vander Zanden, M. J. (2010). What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecol. Monogr.* 80, 179–196. doi:10.1890/07-1861.1.
- Holland, A., and Kinnear, S. (2013). Interpreting the Possible Ecological Role(s) of Cyanotoxins: Compounds for Competitive Advantage and/or Physiological Aide? *Mar. Drugs* 11, 2239–2258. doi:10.3390/md11072239.
- Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., and Visser, P. M. (2018). Cyanobacterial blooms. *Nat. Rev. Microbiol.* 16, 471–483. doi:10.1038/s41579-018-0040-1.
- Johnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., and Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Glob. Chang. Biol.* 14, 495–512. doi:10.1111/j.1365-2486.2007.01510.x.
- Kaebernick, M., and Neilan, B. A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.* 35, 1–9. doi:10.1016/S0168-6496(00)00093-3.
- Kalff, J. (2002). *Limnology: inland water ecosystems*. Upper Saddle River (NJ): Prentice-Hall.
- Kalinowska, K., and Grabowska, M. (2016). Autotrophic and heterotrophic plankton under ice in a eutrophic temperate lake. *Hydrobiologia* 777, 111–118. doi:10.1007/s10750-016-2769-8.
- Kardinaal, W. E. A., Tonk, L., Janse, I., Hol, S., Slot, P., Huisman, J., et al. (2007). Competition for Light between Toxic and Nontoxic Strains of the Harmful Cyanobacterium *Microcystis*. *Appl. Environ. Microbiol.* 73, 2939–2946.

doi:10.1128/AEM.02892-06.

- Kokociński, M., Mankiewicz-Boczek, J., Jureczak, T., Spoof, L., Meriluoto, J., Rejmonczyk, E., et al. (2013). Aphanizomenon gracile (Nostocales), a cylindrospermopsin-producing cyanobacterium in Polish lakes. *Environ. Sci. Pollut. Res.* 20, 5243–5264. doi:10.1007/s11356-012-1426-7.
- Lei, L., Peng, L., Huang, X., and Han, B. P. (2014). Occurrence and dominance of Cylindrospermopsis raciborskii and dissolved cylindrospermopsin in urban reservoirs used for drinking water supply, South China. *Environ. Monit. Assess.* 186, 3079–3090. doi:10.1007/s10661-013-3602-8.
- Livingstone, D. M. (2008). Review Paper A Change of Climate Provokes a Change of Paradigm: Taking Leave of Two Tacit Assumptions about Physical Lake Forcing. *Int. Rev. Hydrobiol.*, 404–414. doi:10.1002/iroh.200811061.
- Ma, J., Qin, B., Paerl, H. W., Brookes, J. D., Hall, N. S., Shi, K., et al. (2016). The persistence of cyanobacterial (*Microcystis* spp.) blooms throughout winter in Lake Taihu, China. *Limnol. Oceanogr.* 61, 711–722. doi:10.1002/lno.10246.
- Magnuson, J. J., Robertson, D. M., Benson, B. J., Wynne, R. H., Livingstone, D. M., Arai, T., et al. (2000). Historical trends in lake and river ice cover in the Northern Hemisphere. *Science* (80-.). 289, 1743–1746. doi:10.1126/science.289.5485.1743.
- Mehnert, G., Leunert, F., Cires, S., Johnk, K. D., Rucker, J., Nixdorf, B., et al. (2010). Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. *J. Pla* 32, 1009–1021. doi:10.1093/plankt/fbq033.
- Monchamp, M., Pick, F. R., Beisner, B. E., and Maranger, R. (2014). Nitrogen Forms Influence Microcystin Concentration and Composition via Changes in Cyanobacterial Community Structure. *PLoS One* 9. doi:10.1371/journal.pone.0085573.
- Niamien-Ebrottie, J. E., Bhattacharyya, S., Deep, P. R., and Nayak, B. (2015). Cyanobacteria and cyanotoxins in the World : Review. *Int. J. Appl. Res.* 1, 563–569. doi:10.1038/nature25989.
- O’Neil, J. M., Davis, T. W., Burford, M. A., and Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14, 313–334. doi:10.1016/j.hal.2011.10.027.
- Orihel, D. M., Hadas, O., Pinkas, R., Viner-Mozzini, Y., and Sukenik, A. (2013). Internal nutrient loading may increase microcystin concentrations in freshwater lakes by promoting growth of *Microcystis* populations. *Ann. Limnol. - Int. J. Limnol.* 49, 225–235. doi:10.1051/limn/2013052.
- Ozersky, T., Barton, D. R., Hecky, R. E., and Guildford, S. J. (2013). Dreissenid mussels enhance nutrient efflux, periphyton quantity and production in the shallow littoral zone of a large lake. *Biol. Invasions* 15, 2799–2810. doi:10.1007/s10530-013-0494-z.
- Paerl, H. W., Fulton, R. S., Moisander, P. H., and Dyble, J. (2001). Harmful Freshwater Algal Blooms, With an Emphasis on Cyanobacteria. *Sci. World J.* 1, 76–113. doi:10.1100/tsw.2001.16.

- Paerl, H. W., and Huisman, J. (2008). Blooms Like It Hot. *Science* (80-.). 320, 57–58. doi:10.1126/science.1155398.
- Paerl, H. W., and Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* 1, 27–37. doi:10.1111/j.1758-2229.2008.00004.x.
- Paerl, H. W., and Paul, V. J. (2012). Climate change: Links to global expansion of harmful cyanobacteria. *Water Res.* 46, 1349–1363. doi:10.1016/j.watres.2011.08.002.
- Phillips, K. A., and Fawley, M. W. (2002). Winter phytoplankton community structure in three shallow temperate lakes during ice cover. *Hydrobiologia* 470, 97–113. doi:10.1023/A:1015667803372.
- Rantala, A., Fewer, D. P., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Borner, T., et al. (2004). Phylogenetic evidence for the early evolution of microcystin synthesis. *Proc. Natl. Acad. Sci.* 101, 568–573. doi:10.1073/pnas.0304489101.
- Rapala, J., Sivonen, K., Lyra, C., and Niemelä, S. I. (1997). Variation of microcystins in *Anabaena* spp as a function of growth stimuli. *Appl. Environ. Microbiol.* 63, 2206–2212.
- Rastogi, R. P., Madamwar, D., and Incharoensakdi, A. (2015). Bloom dynamics of cyanobacteria and their toxins: Environmental health impacts and mitigation strategies. *Front. Microbiol.* 6, 1–22. doi:10.3389/fmicb.2015.01254.
- Rastogi, R. P., Sinha, R. P., and Incharoensakdi, A. (2014). The cyanotoxin-microcystins: Current overview. *Rev. Environ. Sci. Biotechnol.* 13, 215–249. doi:10.1007/s11157-014-9334-6.
- Raven, J. A. (2010). Cyanotoxins: A poison that frees phosphate. *Curr. Biol.* 20, R850–R852. doi:10.1016/j.cub.2010.08.012.
- Raven, J. A., and Geider, R. J. (1988). Temperature and algal growth.
- Reynolds, C. S., and Walsby, A. E. (1975). Water blooms. *Biol. Rev. Camb. Philos. Soc.* 50, 437–481. doi:10.1111/j.1469-185X.1975.tb01060.x.
- Rohrback, T., Dittmann, E., Henning, M., Borner, T., and Kohl, J.-G. (1999). Role of Microcystins in Poisoning and Food Ingestion Inhibition of *Daphnia galeata* Caused by the Cyanobacterium *Microcystis aeruginosa*. 65, 737–739.
- Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I., and Wiedner, C. (2007). Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. *Toxicon* 50, 800–809. doi:10.1016/j.toxicon.2007.06.019.
- Rzymalski, P., and Poniedziałek, B. (2014). In search of environmental role of cylindrospermopsin: A review on global distribution and ecology of its producers. *Water Res.* 66, 320–337. doi:10.1016/j.watres.2014.08.029.
- Saker, M. L., and Griffiths, D. J. (2000). Seven isolates of *Cylindrospermopsis raciborskii* S-I (Beyan) [T (ml-I)], [W. 39, 349–354.
- Schindler, D. W. (2006). Recent advances in the understanding and management of eutrophication. *Limnol. Oceanogr.* 51, 356–363.

- doi:10.4319/lo.2006.51.1_part_2.0356.
- Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., et al. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci.* 105, 11254 LP – 11258. doi:10.1073/pnas.0805108105.
- Sharma, S., Blagrove, K., Magnuson, J. J., Reilly, C. M. O., Oliver, S., Batt, R. D., et al. (2019). Widespread loss of lake ice around the Northern Hemisphere in a warming world. *Nat. Clim. Chang.* 9, 227–231. doi:10.1038/s41558-018-0393-5.
- Sivonen, K., and Jones, G. (1999). “Cyanobacterial toxins,” in *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*, 41–111. doi:10.1046/j.1365-2427.2003.01107.x.
- Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems. *Environ. Sci. Pollut. Res.* 10, 1–14. Available at: [http://www.limnoreferences.smsu.edu/Reprints/Smith Vall 2003 eutrophication.pdf](http://www.limnoreferences.smsu.edu/Reprints/Smith%20Vall%202003%20eutrophication.pdf).
- Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., et al. (2012). Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession. *Annu. Rev. Ecol. Evol. Syst.* 43, 429–448. doi:10.1146/annurev-ecolsys-110411-160251.
- Somogyi, B., Dokulil, M. T., Herzig, A., May, L., Vörös, L., Nöges, T., et al. (2014). Winter conditions in six European shallow lakes: a comparative synopsis. *Est. J. Ecol.* 63, 111. doi:10.3176/eco.2014.3.01.
- Tisdale, E. (1931). Epidemic of intestinal disorders in Charlston, W. VA., occurring simulataneously with unprecedented water supply conditions. *Am. J. Public Health Nations. Health* 21, 198–200.
- Trevino-Garrison, I., Dement, J., Ahmed, F. S., Haines-Lieber, P., Langer, T., Ménager, H., et al. (2015). Human illnesses and animal deaths associated with freshwater harmful algal blooms—Kansas. *Toxins (Basel)*. 7, 353–366. doi:10.3390/toxins7020353.
- United States Environmental Protection Agency (2014). Cyanobacteria and Cyanotoxins : Information for Drinking Water Systems. *United States Environ. Prot. Agency*, 1–9. doi:10.1007/s00535-004-1497-4.
- Üveges, V., Tapolczai, K., Krienitz, L., and Padisák, J. (2012). Photosynthetic characteristics and physiological plasticity of an *Aphanizomenon flos-aquae* (Cyanobacteria, Nostocaceae) winter bloom in a deep oligo-mesotrophic lake (Lake Stechlin, Germany). *Hydrobiologia* 698, 263–272. doi:10.1007/s10750-012-1103-3.
- Van de Waal, D. B., Smith, V. H., Declerck, S. A. J., Stam, E. C. M., and Elser, J. J. (2014). Stoichiometric regulation of phytoplankton toxins. *Ecol. Lett.* 17, 736–742. doi:10.1111/ele.12280.
- Vanderploeg, H. A., Liebig, J. R., Carmichael, W. W., Agy, M. A., Johengen, T. H., Fahnenstiel, G. L., et al. (2001). Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Can. J. Fish. Aquat. Sci.* 58, 1208–1221. doi:10.1139/cjfas-58-6-1208.
- Vezie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., and Sivonen, K. (2002). Effect of

- Nitrogen and Phosphorus on Growth of Toxic and Nontoxic *Microcystis* Strains and on Intracellular Microcystin Concentrations. *Microb. Ecol.* 43, 443–454. doi:10.1007/s00248-001-0041-9.
- Walls, J. T., Wyatt, K. H., Doll, J. C., Rubenstein, E. M., and Rober, A. R. (2018). Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Sci. Total Environ.* 610–611, 786–795. doi:10.1016/j.scitotenv.2017.08.149.
- Watson, S. B., Whitton, B. A., Higgins, S. N., Paerl, H. W., Brooks, B. W., and Wehre, J. D. (2015). “Harmful Algal Blooms,” in *Freshwater Algae of North America*, eds. J. D. Wehr, R. G. Sheath, and J. P. Kociolek (Academic Press), 1050.
- Wejnerowski, Ł., Rzymiski, P., Kokociński, M., and Meriluoto, J. (2018). The structure and toxicity of winter cyanobacterial bloom in a eutrophic lake of the temperate zone. *Ecotoxicology* 27, 752–760. doi:10.1007/s10646-018-1957-x.
- Welch, E. B., Crayton, M., Hardy, F. J., Jacoby, J. M., and Collier, D. C. (2000). Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. *Can. J. Fish. Aquat. Sci.* 57, 231–240. doi:10.1139/f99-234.
- Westrick, J. A., Szlag, D. C., Southwell, B. J., and Sinclair, J. (2010). A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Anal. Bioanal. Chem.* 397, 1705–1714. doi:10.1007/s00216-010-3709-5.
- Wetzel, R. G. (2001). *Limnology: Lake and River Ecosystems*. Third. Academic Press.
- Wiedner, C., Rücker, J., Fastner, J., Chorus, I., and Nixdorf, B. (2008). Seasonal dynamics of cylindrospermopsin and cyanobacteria in two German lakes. *Toxicon* 52, 677–686. doi:10.1016/j.toxicon.2008.07.017.
- Wiedner, C., Visser, P. M., Fastner, J., Metcalf, J. S., Mur, L. R., Metcalf, J. S., et al. (2003). Effects of Light on the Microcystin Content of *Microcystis* Strain PCC 7806. *Appl. Environ. Microbiol.* 69, 1475–1481. doi:10.1128/aem.69.3.1475-1481.2003.
- Wiltsie, D., Schnetzer, A., Green, J., Borgh, M. Vander, and Fensin, E. (2018). Algal blooms and cyanotoxins in Jordan Lake, North Carolina. *Toxins (Basel)*. 10. doi:10.3390/toxins10020092.
- Wood, R. (2016). Acute animal and human poisonings from cyanotoxin exposure - A review of the literature. *Environ. Int.* 91, 276–282. doi:10.1016/j.envint.2016.02.026.
- Zhou, J., Qin, B., Han, X., and Zhu, L. (2016). Turbulence increases the risk of microcystin exposure in a eutrophic lake (Lake Taihu) during cyanobacterial bloom periods. *Harmful Algae* 55, 213–220. doi:10.1016/j.hal.2016.03.016.
- Zhou, L., Hai, Y., and Kun, C. (2002). Relationship Between Microcystin in Drinking Water and Colorectal Cancer. *Biomed. Environ. Sci.* 15, 166–171.

Figure

Figure 1.1. Typical phytoplankton biomass and seasonal succession in a temperate lake (from Wetzel 2001).

Chapter 2: Year-round Occurrence of Cyanotoxins in Urban Reservoirs

Abstract

Potentially toxic cyanobacterial blooms are an increasing water quality issue in freshwater systems across the globe. Though blooms are typically documented in the summer months, there are reports of blooms in the shoulder and winter seasons. Climatically induced seasonal changes to lakes and reservoirs may be facilitating cyanobacterial blooms in the off season, and from the little data available, blooms can produce toxins in the winter. We explored the year-round presence and drivers of the cyanotoxins microcystin and cylindrospermopsin in two eutrophic, urban reservoirs. Weekly water quality sampling was conducted over two years in Bethel Lake and one year in Stephens Lake in Columbia, Missouri, USA. We found that peak microcystin and cylindrospermopsin concentrations occurred when surface water temperatures were 0–15 °C, and there was no relationship between the cyanotoxins and chl-*a* in either reservoir. Physical water quality parameters, including surface water temperature, stratification, particulate inorganic matter (PIM), and mean daily mixed layer irradiance ($\bar{E}_{24}$) were important drivers of cyanotoxin presence and concentration in Bethel Lake. Microcystin and cylindrospermopsin were detectable year-round. Our study provides insight into seasonal cyanotoxin dynamics and demonstrates the necessity of year-round water quality monitoring.

Introduction

Eutrophication and climate change are contributing to an increase of cyanobacterial blooms in lakes and reservoirs worldwide (Heisler et al., 2008; Visser et al., 2016). Cyanobacteria can produce toxic secondary metabolites that are harmful to

humans and animals that use surface waters for drinking water or recreation (Sivonen and Jones, 1999). Exposure to cyanotoxins can impair liver function, damage the nervous system, and damage or irritate skin (Drobac et al., 2013). Chronic exposure to low doses of cyanotoxins can promote tumor formation and may be carcinogenic to humans (Zhou et al., 2002). Animal fatalities associated with cyanotoxins are well documented (Trevino-Garrison et al., 2015; Wood, 2016), and in extreme cases, cyanotoxins have resulted in human casualties (Carmichael et al., 2001). Recent evidence suggests inhalation of spray aerosols containing cyanotoxins is a public health threat (Plaas and Paerl, 2021), indicating that exposure can occur without drinking or being in the water.

Cyanobacterial blooms typically occur in the summer, but blooms have been documented in the shoulder seasons (spring and fall) and occasionally in winter (Ma et al., 2016; Üveges et al., 2012; Wejnerowski et al., 2018). While blooms like it hot (Davis et al., 2009; Paerl and Huisman, 2008), they also require sufficient light and nutrients. Nutrient deficiency is uncommon during the winter and shoulder seasons (Davies et al., 2004) and light is most often the limiting factor (Pernica et al., 2017). Some cyanobacteria (e.g., *Aphanizomenon flos-aquae*) have adapted to these low light conditions, allowing them to bloom under ice (Bertilsson et al., 2013). In addition, climate change is resulting in shorter winters (Magnuson et al., 2000) and warmer water temperatures (O'Reilly et al., 2015), with a reduction in ice cover (Sharma et al., 2019). Thus, leading to changes in year-round underwater light availability. Warmer water temperatures (Hayes et al., 2020) and increased light (Kaebernick and Neilan, 2001; Wiedner et al., 2003) have been attributed to elevated microcystin concentrations, the most pervasive cyanotoxin. Northern expansion of invasive cyanobacteria such as

Raphidiopsis raciborskii (formerly *Cylindrospermopsis raciborskii*) has resulted in newfound awareness of additional cyanotoxins, such as cylindrospermopsin (Loftin et al., 2016). A warming climate has influenced the spread of *Raphidiopsis raciborskii* (Sinha et al., 2012) from subtropical regions to temperate lakes and rivers in recent decades (Wood and Stirling, 2003); less is known about cylindrospermopsin in these systems.

Raphidiopsis is a dominant genera in Missouri reservoirs (Graham et al., 2010), accounting for up to 79 % of phytoplankton biovolume (Jones et al., 2008).

There is an identified lack in knowledge regarding aquatic processes during winter (Hampton et al., 2015) and shoulder seasons. Cyanobacterial blooms in the winter months can produce toxins (Wejnerowski et al., 2018). One of the few year-round studies in a warm monomictic reservoir detected multiple cyanotoxins with no significant differences between seasons for microcystin and anatoxin (Wiltsie et al., 2018). Public health guidance centers on advising people to stay out of green lakes to avoid potential cyanotoxin exposure (CDC, n.d., US EPA, n.d.). The assumption that chlorophyll *a* (chl-*a*; proxy for phytoplankton biomass) concentrations can be predictive of concentrations of cyanotoxins is largely based on summer sampling (Hollister and Kreakie, 2016), but does this relationship hold year-round? Understanding the seasonal dynamics of cyanotoxins is important for informing sound management of lakes and reservoirs, predicting future changes to these systems, and protecting public health.

We conducted a year-round assessment of the cyanotoxins microcystin and cylindrospermopsin in two urban reservoirs, Bethel and Stephens Lakes in Columbia, Missouri, USA. Our objectives were to: (1) Test for the year-round presence of cyanotoxins, (2) Determine if full-year chl-*a* concentrations are correlated with

cyanotoxin concentrations, and (3) Identify environmental drivers associated with cyanotoxin presence and concentration. To achieve these goals, we evaluated year-round concentrations of microcystin and cylindrospermopsin along with water quality parameters from two years of sampling at Bethel Lake and one year at Stephens Lake. This work highlights the importance of year-round water quality monitoring and increases our understanding of cyanotoxin dynamics in eutrophic reservoirs.

Materials and Methods

Study site description and field sampling

Our study focused on year-round sampling of two man-made reservoirs in Columbia, Missouri, United States. Bethel Lake has a surface area of 2.4 ha and is used primarily for angling (Table 2.1). Stephens Lake has a surface area of 4.9 ha and is used for angling and swimming with a public access beach. Both reservoirs are surrounded by walking trails, and Stephens Lake is an important local recreation destination, hosting large public gatherings throughout the year. Stephens Lake is a highly managed reservoir with three different aeration mechanisms: waterfalls, fountains, and a conventional aerator with five weighted bubblers, all of which run approximately April to November (M. Snyder, Columbia, Missouri Park Planning and Development Superintendent, personal communication, October 28, 2020).

One sampling site was established at each reservoir. Weekly shoreline samples were collected by students in the Reservoir Observer Student Scientists (ROSS) program, a novel community science initiative that engages high school and college undergraduate students in water quality sampling. Whole water, grab surface water samples were collected in polyethylene terephthalate glycol (PETG) plastic bottles and samples were

processed and frozen within 12 hours of collection. Bethel Lake was sampled from November 2017 – November 2019 by the Rock Bridge High School Senior Environmental Science students and Stephens Lake was sampled from November 2018 – November 2019 by a University of Missouri Soil and Water Conservation Club student volunteer. We sampled side-by-side with ROSS volunteers approximately quarterly (Table A.1).

Physical parameters

Each reservoir was profiled ~quarterly with a Yellow Springs Instruments EXO3 multi-parameter sonde to collect depth profiles (0.001 m resolution) of water temperature (accuracy of ± 0.01 °C with a 0.001 °C resolution), dissolved oxygen (accuracy of ± 0.1 mg L⁻¹ with a 0.01 mg L⁻¹ resolution), chl *a* fluorescence (range of 0–400 µg L⁻¹ with a 0.01 µg L⁻¹ resolution), and phycocyanin fluorescence (range of 0–400 µg L⁻¹ with a 0.01 µg L⁻¹ resolution). Onset HOBO UA-002-08 temperature data loggers were deployed in 0.5-m increments from the bottom of the reservoir to within 1 m of the surface in Bethel and Stephens Lakes to record year-round water temperatures. The Hobo data loggers have an accuracy of ± 0.53 °C from 0 to 50 °C and resolution of 0.14 °C at 25 °C. We recorded the surface water temperature (Table 2.2) for each sampling date from the Hobo data logger closest to the surface of the reservoir. Maximum depth (Z_{max} ; Table 1) at Bethel and Stephens Lakes was based on bathymetry conducted by the University of Missouri Limnology Lab in 2017 and 2018, respectively. A Lowrance Elite 7 TI sonar was used to take continuous, geolocated readings which were compiled using ReefMaster software with a depth resolution of 0.01 m.

Mixing depth (Z_{mix}) was calculated from the HOBO data loggers using the ‘rLakeAnalyzer’ R-package (Winslow et al., 2017) and is described as the depth from the water surface to the top of the metalimnion. The reservoirs were considered thermally stratified when the metalimnion was present and isothermal when no metalimnetic depth was returned. When the reservoirs were stratified for at least 80 percent of the days out of a given month, all samples collected that month were considered to come from a stratified reservoir. Missouri reservoirs typically exhibit hypolimnetic anoxia during summer stratification (Jones et al., 2011), so YSI profiles were examined to confirm a lack of DO in the hypolimnion and validate the stratified classification. The vertical attenuation coefficient (K_d) was calculated using the equation 1.44/Secchi (Koenings and Edmundson, 1991), where Secchi disk depths were estimated using the relationship between Secchi depth to TSS for Missouri reservoirs (Jones et al., 2008). Daily incident irradiance scaled to PAR ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was modeled using the ‘phytotools’ R-package (Silsbe and Malkin, 2015) and used to calculate 24-hour average incident irradiance ($\bar{E}_0$) on each sampling date. Mean daily mixed layer irradiance ($\bar{E}_{24}$; Table 2), the amount of light encountered by phytoplankton in the mixed layer, was calculated according to Guildford et al. (Guildford et al., 2000):

$$\bar{E}_{24} = \bar{E}_0 \times (1 - \exp(-1 \times K_d \times Z_{mix})) \times (K_d \times Z_{mix})^{-1} \quad (1)$$

Laboratory Analysis

TP and TN sub-samples from surface whole water samples were stored in acid-washed (5% hydrochloric acid) high-density polyethylene (HDPE) containers and immediately frozen until analysis. Defrosted samples were then pipetted into glass test

tubes and analyzed in triplicate. TP samples were digested by the ammonium peroxydisulfate method (APHA, 2017) (4500-P B) and analyzed spectrophotometrically with an ascorbic acid colorimetric method (APHA, 2017) (4500-P E) with a detection limit of 0.03 $\mu\text{mol L}^{-1}$. TN samples were analyzed spectrophotometrically following the second derivative method (Crumpton et al., 1992) with a detection limit of 2.50 $\mu\text{mol L}^{-1}$.

Total suspended solids (TSS; Table 2.2) were determined using a standard tare-weight method (APHA, 2017) (2540-D E) with a detection limit of 0.1 mg L^{-1} . Sample water was filtered through Whatman 934-AH filters (1.5 μm pore) in triplicate and frozen until analysis. Filters were then dried at 105 °C for 20–30 minutes and weighed for TSS. Filters were then placed in a muffle furnace at 550 °C for 20 minutes and then weighed for particulate inorganic matter (PIM; Table 2.2). Particulate organic matter (POM; Table 2.2) was determined by taking the difference between TSS and PIM.

Sample water was passed through 0.7 μm glass-fiber filters (GFF) in triplicate and frozen until analysis of chl-*a*. Ethanol was used for chl-*a* extraction, samples were acidified to correct for pheophytin, and concentrations were determined with a Turner Design Fluorometer (Bethel Lake: November 8, 2017 to September 13, 2019; Stephens Lake: November 16, 2018 to August 19, 2019) or a Cary Eclipse Fluorescence Spectrophotometer (Bethel Lake: September 24, 2019 to November 25, 2019; Stephens Lake: September 12, 2019 to November 19, 2019) at a detection limit of 0.3 or 0.7 $\mu\text{g L}^{-1}$, respectively (Knowlton, 1984; Sartory and Grobbelaar, 1984).

We measured microcystin and cylindrospermopsin concentrations from sample water that was stored in amber glass vials and immediately frozen until analysis. Samples were lysed by three freeze-thaw cycles, and then filtered through 0.45 μm GFF. Total

microcystin concentrations were measured using Abraxis Microcystins-ADDA enzyme-linked immunosorbent assays (ELISA) kits (detection limit: $0.10 \mu\text{g L}^{-1}$; congener-independent). Total cylindrospermopsin concentrations were analyzed by Abraxis Cylindrospermopsin ELISA kits (detection limit: $0.04 \mu\text{g L}^{-1}$).

Statistical Analysis

During preliminary data exploration, we identified a pattern of detectable cyanotoxin concentrations at different surface water temperatures. Based on this, we organized the presentation of our data around three surface water temperature groups: 0–15, 15–25, and 25–35 °C. Our data did not conform to a normal distribution, so we conducted a two-factor nonparametric Brunner-Dette-Munk (BDM) test using the ‘asbio’ package in R (Aho, 2020) as outlined in Helsel (Helsel et al., 2020) to explore significant differences in cyanotoxin concentrations and our environmental variables across reservoirs and temperature groups (Table 2.3). When significant ($p < 0.05$), we computed all possible pairwise Wilcoxon rank-sum tests of the factor of interest after subtracting the medians of the secondary factor. This adjusts the data for differences that can be attributed to the secondary factor (Helsel et al., 2020). We used Spearman Rank Correlation coefficients (r_s) to explore relationships between continuous environmental variables.

We have two years of data for Bethel Lake compared to one for Stephens Lake, and Stephens Lake is highly managed with aerators, so we decided to take a deeper look into which explanatory variables are driving cyanotoxin concentrations in Bethel Lake only. Concentrations of cyanotoxins in Bethel Lake were strongly-right skewed due to a high frequency of samples with microcystin and cylindrospermopsin below the detection

limits (approximately 36 and 78 %, respectively). To account for this, we conducted a two-part analysis for each cyanotoxin (Taranu et al., 2017). We first modeled the presence (samples above the detection limit) and absence (samples below the detection limit) of each toxin against our explanatory variables using a binary logistic model to determine which variables best explained the presence of each cyanotoxin. Then, we removed all observations of samples below the detection limit and used log-link Gamma models to model detectable cyanotoxins as a function of our explanatory variables to determine what predictors account for the variation in toxin presence once observed. All models were fit using generalized additive models (GAMs) from the ‘mgcv’ package in R (Wood, 2011). GAMs provide an approach to model non-linear relationships between explanatory and response variables.

A Spearman correlation matrix was used to guide which explanatory variables to include to avoid multicollinearity (Table A.2). For each part of the analysis, we compared nested models with different combinations of explanatory variables. To choose the best fit model, each model was ranked according to Akaike Information Criterion using the AIC function in the R base stats package.

To reduce skewness, all response variables were transformed (log, square-root, or reciprocal). Each response variable was then standardized by subtracting the overall mean from individual observations and dividing by the standard deviation using the *scale* base function in R. Microcystin and cylindrospermopsin concentrations above the detection limit were log-transformed. As gamma models require positive, real numbers, the log₁₀ detection limit for each cyanotoxin was subtracted from the log₁₀ cyanotoxin concentrations to make the value of the detection limit zero.

Results

Year-round reservoir water quality parameters

Both reservoirs were eutrophic based on mean chl-*a*, total nitrogen (TN), and total phosphorus (TP) concentrations during the study period (criteria from (Jones et al., 2008); Table 2.1). All samples from Stephens Lake were collected when the reservoir was isothermal and 54 % of the samples from Bethel Lake were collected when the reservoir was stratified. When Bethel Lake was stratified the hypolimnion was anoxic. We identified a pattern of detectable cyanotoxin concentrations at different temperature ranges during data exploration, so samples were categorized by surface water temperature at the time of collection (grouped by 0–15, 15–25, and 25–35 °C).

Microcystin concentrations ranged from below detection to 2.49 µg L⁻¹ in the two reservoirs during the study period and was detectable in all temperature groups (Table 2.2). Detectable microcystin concentrations at surface water temperatures of 25–35 °C were 1.1–2.2 times higher than when water temperatures were 0–15 or 15–25 °C (Table 2.2, Figure 2.1). The highest microcystin concentration, however, was in the 0–15 °C temperature group in Stephens Lake. Cylindrospermopsin concentrations ranged from below detection to 0.11 µg L⁻¹, and no cylindrospermopsin was detected in the 15–25 °C temperature group for either reservoir (Table 2.2, Figure 2.1). Mean detectable cylindrospermopsin concentrations did not vary significantly by reservoir nor temperature group. The highest cylindrospermopsin concentration was 0.11 µg L⁻¹ in Bethel Lake in the 0–15 °C temperature group (Table 2.2). Concentrations of microcystin and cylindrospermopsin were detectable in Bethel and Stephens Lakes year-round (Figure 2.2).

Chl-*a* is a proxy for phytoplankton biomass and concentrations ranged from 0.5–46.7 $\mu\text{g L}^{-1}$ in the two reservoirs during the study period. Minimum chl-*a* concentrations were identified in the 0–15 °C temperature group for both reservoirs. The maximum chl-*a* concentration for Stephens Lake (46.7 $\mu\text{g L}^{-1}$) was in the 25–35 °C temperature group, but the peak chl-*a* concentration (26.4 $\mu\text{g L}^{-1}$) in Bethel Lake occurred in the 0–15 °C temperature group (Table 2.2). Chl-*a* concentrations did not vary significantly between reservoirs. There was a significant effect of temperature group, but no significant differences in chl-*a* concentrations were found in post-hoc analysis (Table 2.3, Figure 2.1). Chl-*a* concentrations were not correlated with microcystin ($r_s = 0.077$, $p = 0.62$) nor cylindrospermopsin ($r_s = 0.19$, $p = 0.53$) concentrations. Percent phycocyanin relative to chl-*a* was measured from YSI profiles and ranged from 8.2–65.5 % in the two reservoirs over the study period, indicating that cyanobacteria were present in all temperature groups (Table 2.2). The maximum percent phycocyanin relative to chl-*a* in Bethel Lake (49.2 %) occurred in the 25–35 °C temperature group and in Stephens Lake (65.5 %) in the 15–25 °C temperature group.

Mean daily mixed layer irradiance ($\bar{E}_{24}$) is the amount of light phytoplankton suspended in the mixed layer encounter over 24 hours. Extreme light deficiency occurs at irradiances $< 41.7 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ (Hecky and Guildford, 1984; Petty et al., 2020) and moderate light deficiency occurs at $< 69.4 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ (Knowlton and Jones, 2000). Reservoir $\bar{E}_{24}$ means were above the extreme light deficiency threshold in all temperature groups for both reservoirs, but below the moderate light deficiency threshold in the 0–15 °C temperature group for both reservoirs and the Stephens Lake 25–35 °C temperature group (Table 2.2). In the 0–15 °C temperature group, 76 and 100

% of individual samples at Bethel Lake and Stephens Lake, respectively, fell below the moderate light deficiency threshold. In the 25–35 °C group, 80 % of individual samples at Stephens Lake fell below the moderate light deficiency threshold. Throughout the study, $\bar{E}_{24}$ was significantly higher in Bethel Lake and in the 15–25 and 25–35 °C temperature groups than in Stephens Lake and the 0–15 °C temperature group (Table 2.3). We examined the relationship between particulate organic matter (POM) and chl-*a* to assess for photoacclimation. Phytoplankton did not exhibit photoacclimation as indicated by a positive, significant correlation between these parameters for both reservoirs throughout the study period ($p < 0.001$, $r_s = 0.54$), indicating that chl-*a* serves as a good proxy for phytoplankton biomass in these systems throughout the year.

Total nitrogen (TN) concentrations were significantly higher in Bethel than Stephens Lake during the study period, but they did not vary significantly by temperature group. TP concentrations did not vary significantly by reservoir nor temperature group (Table 2.3). We examined the TN:TP molar ratio to assess phytoplankton nutrient deficiency. We natural log transformed the TN:TP molar ratio to decrease the bias from calculating means of a ratio (Isles, 2020). Phosphorus deficiency occurs if $\ln(\text{TN:TP}) > 3.91$, N deficiency occurs at $\ln(\text{TN:TP}) < 3.00$, and when $\ln(\text{TN:TP})$ is 3.00–3.91, phytoplankton can be N or P deficient or constrained by another resource (e.g., light; (Guildford and Hecky, 2000)). The mean $\ln(\text{TN:TP})$ molar ratios were significantly greater in Bethel than Stephens Lake (Table 2.3). In Bethel Lake, the mean $\ln(\text{TN:TP})$ molar ratio exceeded the 3.91 threshold for all temperature groups, indicating P deficiency. The mean $\ln(\text{TN:TP})$ molar ratios for all Stephens Lake temperature groups fall between the N- and P- deficient thresholds (Table 2.2). Approximately 57 % of the

samples in the Stephens Lake 0–15 °C temperature group exceed the P-deficient conditions, and 20 % of the samples in the Stephens Lake 25–35 °C temperature group fall below the N-deficiency threshold.

Total suspended solids (TSS) and particulate organic matter (POM) did not vary significantly by reservoir nor temperature group. Particulate inorganic matter (PIM) was significantly higher in Bethel than Stephens Lake (Table 2.3). TSS was dominated by POM in all temperature groups in both reservoirs (Table 2.2). In Bethel Lake, the average percentage of POM relative to TSS was 54, 61, and 67 % in the 0–15, 15–25, and 25–35 °C temperature groups, respectively. In Stephens Lake, the average percentage of POM relative to TSS was 73, 81, and 70 % in the 0–15, 15–25, and 25–35 °C temperature groups, respectively. POM exceeded PIM in 60, 89, and 81 % of individual samples in Bethel Lake in the 0–15, 15–25, and 25–35 °C temperate groups, respectively, and in all individual samples from Stephens Lake.

Bethel Lake Microcystin Models

Presence of detectable microcystin was best explained by a binomial logistic generalized additive model (GAM) including surface water temperature and TN, with 49.1 % of the deviance explained (Table A.3). Surface water temperature was a strong predictor ($p < 0.001$), with the probability of microcystin detection generally increasing as surface water temperature increased, but TN did not have a significant effect (Figure 2.3). Once detected, microcystin concentrations are best explained by a log-link Gamma GAM including surface water temperature only, with 41.4 % of the deviance in microcystin concentrations explained (Table A.4). Excluding surface water temperature, variation in microcystin concentrations is explained by a combination of PIM

concentrations and stratification. This model explained 33.5 % of the deviance and both covariates were significant (Table A.5). There is a unimodal relationship with PIM, showing that the highest microcystin concentrations were identified under low and high concentrations of PIM (Figure A.1). Microcystin concentrations were higher when the reservoir was stratified (Table A.5, Figure A.1).

Bethel Lake Cyndrospermopsin Models

Cylindrospermopsin presence was best explained by a combination of surface water temperature and PIM. Both covariates were significant predictors and explained 41.9 % of the deviance (Table A.6). The relationship between cylindrospermopsin detection and surface water temperature is slightly unimodal with increased probability of detection at low temperature and at high temperatures, but not in between (Figure 2.3). The probability of cylindrospermopsin detection increased with PIM concentrations (Figure 2.3). Once detected, cylindrospermopsin concentrations were best predicted by mean daily mixed layer irradiance, $\bar{E}_{24}$, with 85.5 % of the deviance explained (Table A.7). This unimodal relationship shows that the lowest concentrations of cylindrospermopsin were at the lowest and highest levels of $\bar{E}_{24}$, and the highest concentrations were in between (Figure 2.3). When $\bar{E}_{24}$ is excluded from the models, cylindrospermopsin concentrations are predicted by surface water temperature, TN, TP, and PIM, although TN and TP were not significant factors. This model explained 96.7 % of the deviance (Table A.8). Cylindrospermopsin concentrations generally increased with surface water temperature, but slightly decreased at the highest temperatures and increased with high PIM concentrations (Figure A.2). There were only 11 samples from

Bethel Lake with detectable cylindrospermopsin, so models are limited by a small sample size. Additional sampling is necessary to verify results of these models.

Discussion

We found detectable concentrations of microcystin and cylindrospermopsin in both reservoirs in all temperature groups, with the exception that no detectable cylindrospermopsin was found in either reservoir when surface water temperatures were 15–25 °C. Peak microcystin and cylindrospermopsin concentrations were in the 0–15 °C temperature group in Stephens and Bethel Lakes, respectively. There was no relationship between either cyanotoxin and chl-*a*. In Bethel Lake, presence of detectable microcystin and microcystin concentrations were related to surface water temperature. Presence of detectable cylindrospermopsin was related to surface water temperature and PIM. Cylindrospermopsin concentrations were best explained by $\bar{E}_{24}$. Traditional paradigms indicate that the highest cyanotoxin concentrations should be found at high water temperatures and be correlated with chl-*a*. Our results challenge this paradigm by reporting the highest cyanotoxin concentrations at water temperatures from 0–15 °C, with no relationship to chl-*a*.

Seasonality of cyanotoxins and chlorophyll a

We found that microcystin concentrations were higher in the 25–35 °C temperature group than in the other two groups, but the maximum microcystin concentration (2.49 $\mu\text{g L}^{-1}$) was at 0–15 °C in November of the study period (Figure 2.2). Similarly, a year-round study on Mozingo Lake in Missouri, USA, found maximum microcystin concentrations (2.46 $\mu\text{g L}^{-1}$) in October. Maximum microcystin concentrations typically occur at surface water temperatures of 15–25 °C (Billam et al.,

2006; Martin et al., 2020; Peng et al., 2018; Sivonen and Jones, 1999; Walls et al., 2018).

In Texas, USA reservoirs, minimum microcystin concentrations are found when temperatures increased to 30°C or decreased below 10°C (Billam et al., 2006). A temperate lake in Poland had microcystin concentrations that ranged from 6.6 to 28.7 µg L⁻¹ at a water temperature of 3 °C, under ice (Wejnerowski et al., 2018). As winter cyanobacterial blooms continue to be reported (Ma et al., 2016; Üveges et al., 2012; Wejnerowski et al., 2018), we are learning about the wide thermal conditions in which microcystin concentrations occur *in situ*.

No detectable cylindrospermopsin was found in the 15–25 °C temperature group in either of our study reservoirs. In an urban, Chinese reservoir, peak cylindrospermopsin concentrations (8.25 µg L⁻¹) occurred when surface water temperatures were 17.6 °C (Lei et al., 2014), and in a lab study, Saker and Griffiths (Saker and Griffiths, 2000) found peak cylindrospermopsin production at 20 °C, indicating that temperature should not be a factor in our lack of detectable cylindrospermopsin in this temperature group. Light was sufficient for phytoplankton growth (Table 2.2) and the 15–25 °C temperature group had the highest percent phycocyanin relative to chl-*a* at 39.2 and 65.5 % in Bethel and Stephens Lakes, respectively. The lack of cylindrospermopsin identified in this temperature group could be because cylindrospermopsin-producing species are being outcompeted by *Microcystis sp.* in this temperature range (Lei et al., 2020). Alternatively, there could be different taxa producing cylindrospermopsin at different temperatures in these systems, one at lower temperatures and one at higher temperatures. Future studies should consider looking at phytoplankton species composition to provide further insights into this trend.

Cylindrospermopsin concentrations did not vary significantly between the 0–15 and 25–35 °C temperature groups. Studies from Germany and Italy showed that peak cylindrospermopsin concentrations occurred in the summer with increases in primary production, and in the fall with reductions in primary productivity (Messineo et al., 2008; Rzymiski and Poniedziałek, 2014; Wiedner et al., 2008). In a lab study, cylindrospermopsin production decreased above 20 °C, terminated at 35 °C, and was restored when temperatures were lowered to 25 °C (Saker and Griffiths, 2000). Blooms of the potentially toxic *R. raciborskii* occur at low temperatures (10–15 °C) in Lake Xihu, China (Jia et al., 2021). Although the toxicity of these blooms is not reported (Jia et al., 2021), it demonstrates that blooms can occur at low temperatures, outside the traditional “growing season” (Sommer et al., 2012). We found the highest concentration of cylindrospermopsin (0.11 µg L⁻¹) in the 0–15 °C temperature group, providing evidence of detectable cylindrospermopsin year-round.

Chl-*a* concentrations did not vary significantly among our different temperature groups. There is increasing evidence of cyanobacterial blooms in winter months with high chlorophyll *a* concentrations, 1.3 times higher than the highest concentration found in our study (Wejnerowski et al., 2018). This is at odds with the assumption that chl-*a* concentrations would be highest during the summer “growing season” (Sommer et al., 2012) and challenges the summer-only sampling approach typical of many research and monitoring programs. We also did not identify a relationship between chl-*a* and detectable concentrations of microcystin nor cylindrospermopsin. Chl-*a* is a commonly measured water quality parameter and can easily be measured *in situ*, so efforts have been made to create advisory levels of chl-*a* associated with increased risk of cyanotoxin

exposure (Hollister and Kreakie, 2016). Most studies that have found a positive correlation between chl-*a* and cyanotoxins have been conducted in the summer to early fall (Graham et al., 2006; Marion et al., 2012; Pip and Bowman, 2014; Rücker et al., 2007; Yuan et al., 2014). Positive correlations have been found between chl-*a* and microcystin, however, in year-round studies in China and Algeria, where surface water temperatures ranged from 3.4 to 33.7 °C and 5.6 to 27 °C, respectively (Samia et al., 2019). In contrast, a year-round study on Jordan Lake in North Carolina, USA, did not find a correlation between chl-*a* and microcystin concentrations (Wiltsie et al., 2018). These contradictory results highlight the variability in the relationship between microcystin and chl-*a* when considering year-round samples. Chl-*a* may not be a good predictor of cyanotoxin occurrence because the relationship depends on the presence of potentially toxic species of cyanobacteria dominating the phytoplankton assemblage (Loftin et al., 2016). Although the toxin concentrations we found were below United States Environmental Protection Agency recreational advisory levels of 8 µg L⁻¹ and 15 µg L⁻¹ for microcystin and cylindrospermopsin, respectively (US EPA, 2019), it shows that there can be potential cyanotoxin exposures when not expected. If peak toxin concentrations are occurring in colder temperatures and are unrelated to chl-*a*, we may need to rethink strategies for communicating cyanotoxin exposure risk. This is especially important in drinking water reservoirs, in which lower cyanotoxins concentrations are of concern.

What were the drivers of microcystin presence and absence in Bethel Lake?

In Bethel Lake, we found that the probability of detecting microcystin and the concentration of microcystin increases as surface water temperature increases. Even

though we found peak microcystin concentrations at low temperatures in this study, the trend in Bethel Lake aligns with literature that demonstrates increased microcystin concentrations with increased temperatures (Graham et al., 2006; Kokociński et al., 2011; Samia et al., 2019; Zhang et al., 2018). This could be linked to temporal autocorrelation, so we ran models excluding temperature as well. Exclusive of temperature, we found concentrations of microcystin were related to PIM and stratification. Detectable microcystin concentrations were highest at low and high PIM concentrations. This could be linked to a range of potential microcystin producers with different environmental optimal conditions. Similarly, microcystin concentrations were positively correlated with total suspended solids and turbidity in a reservoir in the southwest, USA (Subbiah et al., 2019). Detectable microcystin concentrations were higher when Bethel Lake was stratified (Figure A.1), and 59.4 % of samples with detectable microcystin were collected when the lake was stratified. *Microcystis aeruginosa*, a common potential microcystin producer, can contain gas vacuoles that help regulate buoyancy (Sivonen and Jones, 1999). This provides a competitive advantage during stable water conditions as buoyancy can help them find optimal light and nutrients (Wetzel, 2001).

What were the drivers of cylindrospermopsin presence and concentration in Bethel Lake?

We found that surface water temperature and PIM were related to cylindrospermopsin presence in Bethel Lake. Cylindrospermopsin was more likely to be detected at high temperatures, but we did not detect cylindrospermopsin in the 15–25 °C temperature group. There was an increased probability of cylindrospermopsin detection with increased PIM. Detectable cylindrospermopsin concentrations were related to $\bar{E}_{24}$.

Cylindrospermopsin concentrations were lowest at low (21.12–29.43 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$) and high (87.05–251.41 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$) levels of $\bar{E}_{24}$ in the 0–15 °C and 25–35 °C temperature groups, respectively. In a lab study of *R. raciborskii* cultures, concentrations of intra- and extra- cellular cylindrospermopsin (1.39 $\mu\text{g cylindrospermopsin } \mu\text{g chl-}a^{-1}$ and 140 μgL^{-1} , respectively) peaked at 140 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$, with significantly higher intra- or extracellular concentrations at light intensities of 140 and 75 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ than at 53, 35, or 18 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ (Dyble et al., 2006b). Exclusive of $\bar{E}_{24}$, detectable cylindrospermopsin concentrations were related to surface water temperature and PIM. Cylindrospermopsin concentrations increased with surface water temperature in the 0–15 °C temperature group, but flattened out in the 25–35 °C temperature group (Figure A.2). Cylindrospermopsin concentrations increased with increasing PIM. In contrast, a strong, negative correlation between cylindrospermopsin concentration and temperature was found in a lab study examining the effects of temperatures from 20 to 35 °C on production of cylindrospermopsin by *R. raciborskii* (Saker and Griffiths, 2000). In a lab study examining how temperatures of 16, 20, and 25 °C and light ranging from 10 to 60 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ impacted cylindrospermopsin concentrations in cultures of *Aphanizomenon flos-aquae*, there were increases in cylindrospermopsin concentrations when temperatures were held constant at 20 °C and light increased from 10 to 60 $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$, but decreases at 25 °C under the same light conditions (Preußel et al., 2009). A review on *R. raciborskii* (Jia et al., 2021) identified 8 water bodies where *R. raciborskii* has grown or bloomed at temperatures below 15 °C, and although cylindrospermopsin concentrations were not examined, these blooms identify a potential for cylindrospermopsin at low temperatures.

In a year-round study of a southwestern, USA reservoir, increases in turbidity were related to lower concentrations of cylindrospermopsin (Subbiah et al., 2019). Given the documented presence of potential cylindrospermopsin producers and the presence of detectable cylindrospermopsin at low temperatures in this study, additional studies are needed to quantify cylindrospermopsin concentrations at low temperatures and varying light levels.

Nutrients not significant drivers of cyanotoxins in Bethel Lake

In Bethel Lake, neither total nutrient concentrations nor TN:TP ratios were significantly related to cyanotoxin presence or concentration. This was unexpected because there are well documented relationships between microcystin and nitrogen. For example, microcystin concentrations have been associated with increases in nitrogen (Dai et al., 2008; Graham et al., 2004; Sivonen and Jones, 1999), different forms of nitrogen (Monchamp et al., 2014), increases in phosphorus (Bickel et al., 2000; Graham and Jones, 2009; Lee et al., 2000; Orihel et al., 2013; Rapala et al., 1997), TN:TP ratios (Graham et al., 2004; Orihel et al., 2012), nitrogen limitation (M. Kaebernick and Neilan, 2001; Rapala et al., 1997), and phosphorus limitation (Lee et al., 2000). Similarly, cylindrospermopsin concentrations have been related to increased nitrogen (Rücker et al., 2007; Wiedner et al., 2008), decreased nitrogen (Kokociński et al., 2013), different forms of nitrogen (Burford et al., 2006; Saker and Neilan, 2001), increased phosphorus (Kokociński et al., 2013), and phosphorus limitation (Bar-Yosef et al., 2010). Future year-round sampling should evaluate different forms of N and P to examine if there are temporal trends associated with the relationships between cyanotoxins and nutrients.

Limitations of this Study

Out of the 50 samples from Bethel Lake, 32 samples had detectable microcystin and 11 samples had detectable cylindrospermopsin. Small sample sizes can limit the power of a study, lead to an increase of Type II errors, and lead to models that are overfit. The small number of samples with detectable cyanotoxins limits the conclusions that can be drawn from our model results. Our findings identify important relationships between cyanotoxins and environmental parameters in Bethel Lake, however, additional sampling should be considered to confirm the relationships identified by our models.

What are the implications of this work?

Small reservoirs are ubiquitous globally and underrepresented in literature (Downing et al., 2006). Stephens and Bethel Lakes are important local resources for recreation. Stephens Lake supports a swimming beach and large public festivals, and both reservoirs are surrounded with walking trails and are frequented for shoreline fishing. The potential risk of cyanotoxin exposure through spray aerosols or direct contact during recreation near and on urban reservoirs highlights the importance of studying these systems in addition to frequently studied drinking water reservoirs. Winter lake processes are important and changing due to climate change. The year-round presence of detectable cyanotoxins in this study challenges the “summer only” sampling approach and highlights the need for continued year-round water quality sampling.

Acknowledgements

Special thanks to Gregory Kirchhofer of Rock Bridge High School and his Environmental Science students for their enthusiasm for the project and commitment to collecting samples from Bethel Lake. Thank you also to Nathan Waller and Owen Gallagher of the MU Soil and Water Conservation Club for collecting samples from Stephens Lake. We would like to thank members of the MU Limnology Lab for their many contributions to this project, specifically Tony Thorpe, Carol Pollard, Clare Vanderwerken, and Matthew Sauer. Thank you to Dr. Christine Spinka for statistical analysis support.

References

- Aho, K. (2020). asbio: A Collection of Statistical Tools for Biologists. Available at: <https://cran.r-project.org/package=asbio>.
- APHA (2017). *Standard methods for the examination of water and wastewater*. 23rd ed. , eds. E. W. Rice, R. B. Baird, and A. D. Eaton Washington, DC: American Public Health Association.
- Bar-Yosef, Y., Sukenik, A., Hadas, O., Viner-Mozzini, Y., and Kaplan, A. (2010). Enslavement in the water body by toxic aphanizomenon ovalisporum, inducing alkaline phosphatase in phytoplanktons. *Curr. Biol.* 20, 1557–1561. doi:10.1016/j.cub.2010.07.032.
- Bertilsson, S., Burgin, A., Carey, C. C., Fey, S. B., Grossart, H. P., Grubisic, L. M., et al. (2013). The under-ice microbiome of seasonally frozen lakes. *Limnol. Oceanogr.* 58, 1998–2012. doi:10.4319/lo.2013.58.6.1998.
- Bickel, H., Lyck, S., and Utkilen, H. (2000). Energy state and toxin content - experiments on *Microcystis aeruginosa* (Chroococcales, Cyanophyta). 39.
- Billam, M., Tang, L., Cai, Q., Mukhi, S., Guan, H., Wang, P., et al. (2006). Seasonal variations in the concentration of microcystin-LR in two lakes in Western Texas, USA. *Environ. Toxicol. Chem.* 25, 349–355. doi:10.1897/05-158R.1.
- Burford, M. A., Mcneale, K. L., and McKenzie-Smith, F. J. (2006). The role of nitrogen in promoting the toxic cyanophyte *Cylindrospermopsis raciborskii* in a subtropical water reservoir. *Freshw. Biol.* 51, 2143–2153. doi:10.1111/j.1365-2427.2006.01630.x.
- Carmichael, W. W., Azevedo, S. M. F. O., An, J. S., Molica, R. J. R., Jochimsen, E. M., Lau, S., et al. (2001). Human fatalities form cyanobacteria: Chemical and biological evidence for cyanotoxins. *Environ. Health Perspect.* 109, 663–668. doi:10.1289/ehp.01109663.
- CDC (n.d.). Cyanobacteria Blooms FAQs. Available at: https://www.cdc.gov/habs/pdf/cyanobacteria_faq.pdf [Accessed March 5, 2021].
- Crumpton, W. G., Isenhardt, T. M., and Mitchell, P. D. (1992). Nitrate and organic N analyses with second-derivative spectroscopy. *Limnol. Oceanogr.* 37, 907–913. doi:10.4319/lo.1992.37.4.0907.
- Dai, R., Liu, H., Qu, J., Zhao, X., Ru, J., and Hou, Y. (2008). Relationship of energy charge and toxin content of *Microcystis aeruginosa* in nitrogen-limited or phosphorous-limited cultures. *Toxicon* 51, 649–658. doi:10.1016/j.toxicon.2007.11.021.
- Davies, J.-M., Nowlin, W. H., and Mazumder, A. (2004). Temporal changes in nitrogen and phosphorus codeficiency of plankton in lakes of coastal and interior British Columbia. *Can. J. Fish. Aquat. Sci.* 61, 1538–1551. doi:10.1139/f04-092.
- Davis, T. W., Berry, D. L., Boyer, G. L., and Gobler, C. J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8, 715–725. doi:10.1016/j.hal.2009.02.004.

- Downing, J. A., Prairie, Y. T., Cole, J. J., Duarte, C. M., Tranvik, L. J., Striegl, R. G., et al. (2006). The global abundance and size distribution of lakes, ponds, and impoundments. *Limnol. Oceanogr.* 51, 2388–2397. doi:10.4319/lo.2006.51.5.2388.
- Drobac, D., Tokodi, N., Simeunovic, J., Baltic, V., Stanic, D., and Svircev, Z. (2013). Human exposure to cyanotoxins and their effects on health. *Arch. Ind. Hyg. Toxicol.* 64, 305–316. doi:10.2478/10004-1254-64-2013-2320.
- Dyble, J., Tester, P. A., and Litaker, R. W. (2006). Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. *African J. Mar. Sci.* 28, 309–312. doi:10.2989/18142320609504168.
- Graham, J. L., and Jones, J. R. (2009). Microcystin in Missouri reservoirs. *Lake Reserv. Manag.* 25, 253–263. doi:10.1080/07438140903143239.
- Graham, J. L., Jones, J. R., Jones, S. B., and Clevenger, T. E. (2006). Spatial and temporal dynamics of microcystin in a missouri reservoir. *Lake Reserv. Manag.* 22, 59–68. doi:10.1080/07438140609353884.
- Graham, J. L., Jones, J. R., Jones, S. B., Downing, J. A., and Clevenger, T. E. (2004). Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. *Water Res.* 38, 4395–4404. doi:10.1016/j.watres.2004.08.004.
- Graham, J. L., Loftin, K. A., Meyer, M. T., and Ziegler, A. C. (2010). Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the midwestern united states. *Environ. Sci. Technol.* 44, 7361–7368. doi:10.1021/es1008938.
- Guildford, S. J., Bootsma, H. A., Fee, E. J., and Hecky, R. E. (2000). Phytoplankton nutrient status and mean water column irradiance in lakes Malawi and Lake Superior. *Aquat. Ecosyst. Heal. Manag.* 3, 33–45. doi:10.1080/14634980008656989.
- Guildford, S. J., and Hecky, R. E. (2000). Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr.* 45, 1213–1223. doi:10.4319/lo.2000.45.6.1213.
- Hampton, S. E., Moore, M. V., Ozersky, T., Stanley, E. H., Polashenski, C. M., and Galloway, A. W. E. (2015). Heating up a cold subject: Prospects for under-ice plankton research in lakes. *J. Plankton Res.* 37, 277–284. doi:10.1093/plankt/fbv002.
- Hayes, N. M., Haig, H. A., Simpson, G. L., and Leavitt, P. R. (2020). Effects of lake warming on the seasonal risk of toxic cyanobacteria exposure. *Limnol. Oceanogr. Lett.* doi:10.1002/lo.10164.
- Hecky, R. E., and Guildford, S. J. (1984). Primary productivity of Southern Indian Lake before, during, and after impoundment and Churchill River diversion. *Can. J. Fish. Aquat. Sci.* 41, 591–604. doi:10.1139/f84-072.
- Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., et al. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 8, 3–13. doi:10.1016/j.hal.2008.08.006.
- Helsel, D. R., Hirsch, R. M., Ryberg, K. R., Archfield, S. A., and Gilroy, E. J. (2020).

- Statistical methods in water resources: U.S. Geological Survey Techniques and Methods, book 4, chapter A3.* doi:<https://doi.org/10.3133/tm4a3>.
- Hollister, J. W., and Kreakie, B. J. (2016). Associations between chlorophyll a and various microcystin-LR health advisory concentrations. *F1000Research* 5, 151. doi:10.12688/f1000research.7955.1.
- Isles, P. D. F. (2020). The misuse of ratios in ecological stoichiometry. *Ecology* 101, 1–7. doi:10.1002/ecy.3153.
- Jia, N., Wang, Y., Guan, Y., Chen, Y., Li, R., and Yu, G. (2021). Occurrence of *Raphidiopsis raciborskii* blooms in cool waters: Synergistic effects of nitrogen availability and ecotypes with adaptation to low temperature. *Environ. Pollut.* 270, 116070. doi:10.1016/j.envpol.2020.116070.
- Jones, J. R., Knowlton, M. F., Obrecht, D. V., and Graham, J. L. (2011). Temperature and oxygen in missouri reservoirs. *Lake Reserv. Manag.* 27, 173–182. doi:10.1080/07438141.2011.583713.
- Jones, J. R., Obrecht, D. V., Perkins, B. D., Knowlton, M. F., Thorpe, A. P., Watanabe, S., et al. (2008). Nutrients, seston, and transparency of missouri reservoirs and oxbow lakes: an analysis of regional limnology. *Lake Reserv. Manag.* 24, 155–180. doi:10.1080/07438140809354058.
- Kaebernick, M., and Neilan, B. A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.* 35, 1–9.
- Knowlton, M. F. (1984). Flow-through micocuvette for fluorometric determination of chlorophyll. *Water Resour. Bull.* 20, 795–799. doi:10.1111/j.1752-1688.1973.tb05876.x.
- Knowlton, M. F., and Jones, J. R. (2000). Non-algal seston, light, nutrients and chlorophyll in missouri reservoirs. *Lake Reserv. Manag.* 16, 322–332. doi:10.1080/07438140009354239.
- Koenings, J. P., and Edmundson, J. A. (1991). Secchi disk and photometer estimates of light regimes in Alaskan lakes: Effects of yellow color and turbidity. *Limnol. Oceanogr.* 36, 91–105. doi:10.4319/lo.1991.36.1.0091.
- Kokociński, M., Mankiewicz-Boczek, J., Jurczak, T., Spoof, L., Meriluoto, J., Rejmonczyk, E., et al. (2013). *Aphanizomenon gracile* (Nostocales), a cylindrospermopsin-producing cyanobacterium in Polish lakes. *Environ. Sci. Pollut. Res.* 20, 5243–5264. doi:10.1007/s11356-012-1426-7.
- Kokociński, M., Stefaniak, K., Izydorczyk, K., Jurczak, T., Mankiewicz-Boczek, J., and Soinen, J. (2011). Temporal variation in microcystin production by *Planktothrix agardhii* (Gomont) *Anagnostidis* and *Komárek* (Cyanobacteria, Oscillatoriales) in a temperate lake. *Ann. Limnol. - Int. J. Limnol.* 47, 363–371. doi:10.1051/limn/2011046.
- Lee, S. J., Jang, M., Kim, H., Yoon, B., and Oh, H. (2000). Variation of microcystin content of *Microcystis aeruginosa* relative to medium N : P ratio and growth stage. 323–329.
- Lei, L., Dai, J., Lin, Q., and Peng, L. (2020). Competitive dominance of *Microcystis aeruginosa* against *Raphidiopsis raciborskii* Is s train- And temperature-dependent.

- Knowl. Manag. Aquat. Ecosyst.* 2020-Janua. doi:10.1051/kmae/2020023.
- Lei, L., Peng, L., Huang, X., and Han, B. P. (2014). Occurrence and dominance of *Cylindrospermopsis raciborskii* and dissolved cylindrospermopsin in urban reservoirs used for drinking water supply, South China. *Environ. Monit. Assess.* 186, 3079–3090. doi:10.1007/s10661-013-3602-8.
- Loftin, K. A., Graham, J. L., Hilborn, E. D., Lehmann, S. C., Meyer, M. T., Dietze, J. E., et al. (2016). Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. *Harmful Algae* 56, 77–90. doi:10.1016/j.hal.2016.04.001.
- Ma, J., Qin, B., Paerl, H. W., Brookes, J. D., Hall, N. S., Shi, K., et al. (2016). The persistence of cyanobacterial (*Microcystis* spp.) blooms throughout winter in Lake Taihu, China. *Limnol. Oceanogr.* 61, 711–722. doi:10.1002/lno.10246.
- Magnuson, J. J., Robertson, D. M., Benson, B. J., Wynne, R. H., Livingstone, D. M., Arai, T., et al. (2000). Historical trends in lake and river ice cover in the northern hemisphere. *Science* (80-.). 289, 1743–1746. doi:10.1126/science.289.5485.1743.
- Marion, J. W., Lee, J., Wilkins, J. R., Lemeshow, S., Lee, C., Waletzko, E. J., et al. (2012). In vivo phycocyanin fluorescence as a potential rapid screening tool for predicting elevated microcystin concentrations at eutrophic lakes. *Environ. Sci. Technol.* 46, 4523–4531. doi:10.1021/es203962u.
- Martin, R. M., Moniruzzaman, M., Stark, G. F., Gann, E. R., Derminio, D. S., Wei, B., et al. (2020). Episodic Decrease in Temperature Increases *mcy* Gene Transcription and Cellular Microcystin in Continuous Cultures of *Microcystis aeruginosa* PCC 7806. *Front. Microbiol.* 11, 1–15. doi:10.3389/fmicb.2020.601864.
- Messineo, V., Melchiorre, S., Di Corcia, A., Gallo, P., and Bruno, M. (2008). Seasonal Succession of *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* Blooms with Cylindrospermopsin Occurrence in the Volcanic Lake Albano, Central Italy. *Environ. Toxicol.* 25, 18–27. doi:10.1002/tox.20469.
- Monchamp, M., Pick, F. R., Beisner, B. E., and Maranger, R. (2014). Nitrogen Forms Influence Microcystin Concentration and Composition via Changes in Cyanobacterial Community Structure. *PLoS One* 9. doi:10.1371/journal.pone.0085573.
- O'Reilly, C. M., Rowley, R. J., Schneider, P., Lenters, J. D., McIntyre, P. B., and Kraemer, B. M. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophys. Res. Lett.*, 1–9. doi:10.1002/2015GL066235. Received.
- Orihel, D. M., Bird, D. F., Brylinsky, M., Chen, H., Donald, D. B., Huang, D. Y., et al. (2012). High microcystin concentrations occur only at low nitrogen-to-phosphorus ratios in nutrient-rich Canadian lakes. *Can. J. Fish. Aquat. Sci.* 69, 1457–1462. doi:10.1139/F2012-088.
- Orihel, D. M., Hadas, O., Pinkas, R., Viner-Mozzini, Y., and Sukenik, A. (2013). Internal nutrient loading may increase microcystin concentrations in freshwater lakes by promoting growth of *Microcystis* populations. *Ann. Limnol. - Int. J. Limnol.* 49, 225–235. doi:10.1051/limn/2013052.
- Paerl, H. W., and Huisman, J. (2008). Blooms Like It Hot. *Science* (80-.). 320, 57–58.

doi:10.1126/science.1155398.

- Peng, G., Martin, R. M., Dearth, S. P., Sun, X., Boyer, G. L., Campagna, S. R., et al. (2018). Seasonally Relevant Cool Temperatures Interact with N Chemistry to Increase Microcystins Produced in Lab Cultures of *Microcystis aeruginosa* NIES-843. *Environ. Sci. Technol.* 52, 4127–4136. doi:10.1021/acs.est.7b06532.
- Pernica, P., North, R. L., and Baulch, H. M. (2017). In the cold light of day: the potential importance of under-ice convective mixed layers to primary producers. *Int. Waters* 7, 138–150. doi:10.1080/20442041.2017.1296627.
- Petty, E. L., Obrecht, D. V., and North, R. L. (2020). Filling in the Flyover Zone: High Phosphorus in Midwestern (USA) Reservoirs Results in High Phytoplankton Biomass but Not High Primary Productivity. *Front. Environ. Sci.* 8. doi:doi:10.3389/fenvs.2020.00111 ORIGINAL.
- Pip, E., and Bowman, L. (2014). Microcystin and Algal Chlorophyll in Relation to Nearshore Nutrient Concentrations in Lake Winnipeg, Canada. *Environ. Pollut.* 3, 36–47. doi:10.5539/ep.v3n2p36.
- Plaas, H. E., and Paerl, H. W. (2021). Toxic Cyanobacteria: A Growing Threat to Water and Air Quality. *Environ. Sci. Technol.* 55, 44–64. doi:10.1021/acs.est.0c06653.
- Preußel, K., Wessel, G., Fastner, J., and Chorus, I. (2009). Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. *Harmful Algae* 8, 645–650. doi:10.1016/j.hal.2008.10.009.
- Rapala, J., Sivonen, K., Lyra, C., and Niemelä, S. I. (1997). Variation of microcystins in *Anabaena* spp as a function of growth stimuli. *Appl. Environ. Microbiol.* 63, 2206–2212.
- Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I., and Wiedner, C. (2007). Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. *Toxicon* 50, 800–809. doi:10.1016/j.toxicon.2007.06.019.
- Rzymiski, P., and Poniedziałek, B. (2014). In search of environmental role of cylindrospermopsin: A review on global distribution and ecology of its producers. *Water Res.* 66, 320–337. doi:10.1016/j.watres.2014.08.029.
- Saker, M. L., and Griffiths, D. J. (2000). Seven isolates of *Cylindrospermopsis raciborskii* S-I (Beyan) [T (ml-I)], [W. 39, 349–354.
- Saker, M. L., and Neilan, B. A. (2001). Varied Diazotrophies, Morphologies, and Toxicities of Genetically Similar Isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from Northern Australia. *Appl. Environ. Microbiol.* 67, 1839–1845. doi:10.1128/AEM.67.4.1839-1845.2001.
- Samia, C., Tarek, M., Zohra, G. F., Hassen, T., and Mourad, B. (2019). Dynamic of filamentous cyanobacteria in the dam Ain Zada (North of Algeria). *J. Ecol. Eng.* 20, 97–110. doi:10.12911/22998993/105335.
- Sartory, D. P., and Grobbelaar, J. U. (1984). Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114, 177–187. doi:10.1007/BF00031869.

- Sharma, S., Blagrove, K., Magnuson, J. J., Reilly, C. M. O., Oliver, S., Batt, R. D., et al. (2019). Widespread loss of lake ice around the Northern Hemisphere in a warming world. *Nat. Clim. Chang.* 9, 227–231. doi:10.1038/s41558-018-0393-5.
- Silsbe, G. M., and Malkin, S. Y. (2015). phytotools: Phytoplankton Production Tools.
- Sinha, R., Pearson, L. A., Davis, T. W., Burford, M. A., Orr, P. T., and Neilan, B. A. (2012). Increased incidence of *Cylindrospermopsis raciborskii* in temperate zones - Is climate change responsible? *Water Res.* 46, 1408–1419. doi:10.1016/j.watres.2011.12.019.
- Sivonen, K., and Jones, G. (1999). “Cyanobacterial toxins,” in *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*, 41–111. doi:10.1046/j.1365-2427.2003.01107.x.
- Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., et al. (2012). Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession. *Annu. Rev. Ecol. Evol. Syst.* 43, 429–448. doi:10.1146/annurev-ecolsys-110411-160251.
- Subbiah, S., Karnjanapiboonwong, A., Maul, J. D., Wang, D., and Anderson, T. A. (2019). Monitoring cyanobacterial toxins in a large reservoir: Relationships with water quality parameters. *PeerJ* 2019. doi:10.7717/peerj.7305.
- Taranu, Z. E., Gregory-Eaves, I., Steele, R. J., Beaulieu, M., and Legendre, P. (2017). Predicting microcystin concentrations in lakes and reservoirs at a continental scale: A new framework for modelling an important health risk factor. *Glob. Ecol. Biogeogr.* 26, 625–637. doi:10.1111/geb.12569.
- Trevino-Garrison, I., Dement, J., Ahmed, F. S., Haines-Lieber, P., Langer, T., Ménager, H., et al. (2015). Human illnesses and animal deaths associated with freshwater harmful algal blooms—Kansas. *Toxins (Basel)*. 7, 353–366. doi:10.3390/toxins7020353.
- US EPA (n.d.). Harmful Algal Blooms. Available at: <https://www.epa.gov/nutrientpollution/harmful-algal-blooms#main-content> [Accessed May 3, 2021].
- US EPA (2019). Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. doi:EPA 822-F-19-001.
- Üveges, V., Tapolczai, K., Krienitz, L., and Padisák, J. (2012). Photosynthetic characteristics and physiological plasticity of an *Aphanizomenon flos-aquae* (Cyanobacteria, Nostocaceae) winter bloom in a deep oligo-mesotrophic lake (Lake Stechlin, Germany). *Hydrobiologia* 698, 263–272. doi:10.1007/s10750-012-1103-3.
- Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., et al. (2016). How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* 54, 145–159. doi:10.1016/j.hal.2015.12.006.
- Walls, J. T., Wyatt, K. H., Doll, J. C., Rubenstein, E. M., and Rober, A. R. (2018). Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Sci. Total Environ.* 610–611, 786–795. doi:10.1016/j.scitotenv.2017.08.149.
- Wejnerowski, Ł., Rzymiski, P., Kokociński, M., and Meriluoto, J. (2018). The structure

- and toxicity of winter cyanobacterial bloom in a eutrophic lake of the temperate zone. *Ecotoxicology* 27, 752–760. doi:10.1007/s10646-018-1957-x.
- Wetzel, R. G. (2001). *Limnology: Lake and River Ecosystems*. Third. Academic Press.
- Wiedner, C., Rücker, J., Fastner, J., Chorus, I., and Nixdorf, B. (2008). Seasonal dynamics of cylindrospermopsin and cyanobacteria in two German lakes. *Toxicon* 52, 677–686. doi:10.1016/j.toxicon.2008.07.017.
- Wiedner, C., Visser, P. M., Fastner, J., Metcalf, J. S., Mur, L. R., Metcalf, J. S., et al. (2003). Effects of Light on the Microcystin Content of Microcystis Strain PCC 7806. *Appl. Environ. Microbiol.* 69, 1475–1481. doi:10.1128/aem.69.3.1475-1481.2003.
- Wiltsie, D., Schnetzer, A., Green, J., Borgh, M. Vander, and Fensin, E. (2018). Algal blooms and cyanotoxins in Jordan Lake, North Carolina. *Toxins (Basel)*. 10. doi:10.3390/toxins10020092.
- Winslow, L., Read, J., Woolway, R., Brentrup, J., Leach, T., Zward, J., et al. (2017). rLakeAnalyzer: Lake Physics Tools.
- Wood, R. (2016). Acute animal and human poisonings from cyanotoxin exposure - A review of the literature. *Environ. Int.* 91, 276–282. doi:10.1016/j.envint.2016.02.026.
- Wood, S. A., and Stirling, D. J. (2003). First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand. *New Zeal. J. Mar. Freshw. Res.* 37, 821–828. doi:10.1080/00288330.2003.9517211.
- Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc.* 73, 3–36.
- Yuan, L. L., Pollard, A. I., Pather, S., Oliver, J. L., and D’Anglada, L. (2014). Managing microcystin: Identifying national-scale thresholds for total nitrogen and chlorophyll a. *Freshw. Biol.* 59, 1970–1981. doi:10.1111/fwb.12400.
- Zhang, L., Liu, J., Zhang, D., Luo, L., Liao, Q., Yuan, L., et al. (2018). Seasonal and Spatial Variations of Microcystins and Their Relationships with Physiochemical and Biological Factors in Poyang Lake. *Environ. Sci. Pollut. Res.* 25, 6300–6307. doi:10.13227/j.hjkx.201708227.
- Zhou, L., Hai, Y., and Kun, C. (2002). Relationship Between Microcystin in Drinking Water and Colorectal Cancer. *Biomed. Environ. Sci.* 15, 166–171.

Tables

Table 2.1. Characteristics of the reservoirs sampled during the study period. Trophic status was determined from chl-*a*, TN and TP concentrations based on the classification thresholds defined by Jones et al. (2008). Watershed land-use/land-cover classifications consist of: U, urban; F, forested; Ag-P, pasture.

Reservoir	Coordinates	Surface	Max	Trophic	Mixing	Reservoir	Watershed	Watershed Land		
		Area	Depth	Status	Pattern	Purpose	Area (ha)	Use (%)		
		(ha)	(m)					U	F	Ag-P
Bethel Lake	38.9012, - 92.3421	2.4	4.2	Eutrophic	Warm Monomictic	Angling	5,716	60.2	24.4	11.2
Stephens Lake	38.9507, - 92.3068	4.9	5.0	Eutrophic	Warm Monomictic	Recreation/ Angling	216	87.0	12.0	0.8

Table 2.2. Limnological parameters for Bethel and Stephens Lakes during the study period. Values are means and ranges (minimum – maximum) for samples collected at three different temperature groups.

	Bethel Lake			Stephens Lake		
	0–15 °C <i>n</i> = 25	15–25 °C <i>n</i> = 9	25–35 °C <i>n</i> = 16	0–15 °C <i>n</i> = 7	15–25 °C <i>n</i> = 1	25–35 °C <i>n</i> = 5
Physical Parameters						
Surface Water Temperature (°C)	8.30 (4.10 – 14.90)	20.57 (15.09 – 24.93)	28.65 (25.13 – 32.19)	7.47 (3.68 – 12.59)	22.43	26.65 (25.42 – 28.85)
Mean daily mixed layer irradiance, $\bar{E}_{24}$ ($\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$)	57.78 (19.06 – 205.46)	189.02 (42.30 – 407.61)	233.98 (87.05 – 346.56)	45.67 (32.39 – 67.46)	104.40	62.28 (51.34 – 77.09)
Stratification (% sample days stratified)	8.0	66.7	93.8	-	-	-
Chemical Parameters						
Total Nitrogen, TN ($\mu\text{mol L}^{-1}$)	89.28 (32.36 – 204.14)	74.52 (36.64 – 155.13)	65.16 (45.68 – 91.36)	50.42 (42.35 – 62.46)	48.54	50.80 (34.74 – 73.52)
Total Phosphorus, TP ($\mu\text{mol L}^{-1}$)	1.47 (0.83 – 2.20)	1.32 (0.71 – 1.75)	1.13 (0.69 – 1.57)	1.05 (0.82 – 1.39)	1.32	1.82 (1.57 – 2.01)
ln(Total Nitrogen:Total Phosphorus), (molar ratio)	3.98 (3.20 – 4.81)	3.94 (3.24 – 4.60)	4.05 (3.69 – 4.34)	3.88 (3.61 – 4.19)	3.60	3.30 (2.96 – 3.60)
Biological Parameters						
Microcystin, MC ($\mu\text{g L}^{-1}$)	0.15 (BDL – 0.31)	0.18 (BDL – 0.39)	0.32 (BDL – 0.77)	0.70 (BDL – 2.49)	0.16	0.60 (0.15 – 2.04)
Samples above detection limit (%)	44.0	77.8	87.5	85.7	100.0	100.0
Cylindrospermopsin, CYL ($\mu\text{g L}^{-1}$)	0.07 (BDL – 0.11)	BDL	0.07 (BDL – 0.10)	0.06 (BDL – 0.06)	BDL	0.05 (BDL – 0.05)
Samples above detection limit (%)	16.0	-	43.8	14.3	-	20.0
Chlorophyll- <i>a</i> , Chl- <i>a</i> ($\mu\text{g L}^{-1}$)	11.9 (0.5 – 26.4)	12.2 (3.1 – 25.9)	14.4 (2.8 – 26.0)	8.3 (1.3 – 19.7)	19.1	23.7 (10.9 – 46.7)
Pheophytin, Pheo ($\mu\text{g L}^{-1}$)	11.9	11.2	6.0	1.0	11.6	9.7

	(1.3 – 38.9)	(1.9 – 29.4)	(2.8 – 19.1)	(0.1 – 1.8)		(2.1 – 20.8)
Phycocyanin (% Phycocyanin relative to Chl- <i>a</i> , from YSI)	24.4 ¹	39.2 ¹	27.7 ⁴	8.2 ¹	65.5 ¹	13.9 ²
			(19.3 – 49.2)			(2.9 – 25.0)
Total Suspended Solids, TSS (mg L ⁻¹)	9.8	8.5	9.6	4.8	9.0	8.0
	(5.2 – 22.7)	(2.0 – 22.5)	(2.6 – 53.9)	(2.9 – 6.8)		(7.1 – 10.2)
Particulate Inorganic Matter, PIM (mg L ⁻¹)	5.1	3.7	4.7	1.3	1.7	2.6
	(1.8 – 16.4)	(0.8 – 13.5)	(0.5 – 44.9)	(0.6 – 2.6)		(1.0 – 4.7)
Particulate Organic Matter, POM (mg L ⁻¹)	4.9	4.9	4.9	3.5	7.3	5.5
	(2.6 – 9.0)	(1.2 – 9.0)	(1.3 – 14.5)	(2.1 – 4.9)		(4.4 – 6.1)

BDL indicates value is below the detection limit specified in the methods. Superscripts denote n values that differed from those reported in column headers.

Table 2.3. Results of the two-factor non-parametric Brunner-Dette-Munk (BDM) test for each parameter.

				Reservoir		Temperature Group		
	Effect	<i>F</i> -value	<i>p</i> -value	Bethel Lake	Stephens Lake	0–15 °C	15–25 °C	25–35 °C
Physical Parameters								
Ē₂₄	Reservoir	F_{1,14} = 10.250	0.0066	a	b			
	TempGroup	F_{2,14} = 20.034	0.0001			a	b	b
	Reservoir:TempGroup	F_{2,14} = 5.477	0.0208					
Stratification	Reservoir	F_{1,11} = 32.408	0.0001	a	b			
	TempGroup	F_{1,11} = 11.867	0.0029			a	b	b
	Reservoir :TempGroup	F_{1,11} = 7.598	0.012					
Chemical Parameters								
TN	Reservoir	F_{1,4} = 13.738	0.0245	a	b			
	TempGroup	F_{2,4} = 1.289	0.3673					
	Reservoir :TempGroup	F_{2,4} = 0.155	0.8341					
TP	Reservoir	F_{1,2} = 1.120	0.3986					

ln(TN:TP)	TempGroup	$F_{1,2} = 2.867$	0.2411				
	Reservoir :TempGroup	$F_{1,2} = 15.779$	0.0564				
	Reservoir	$F_{1,3} = 25.071$	0.0225	a		b	
	TempGroup	$F_{2,3} = 1.772$	0.3150				
	Reservoir :TempGroup	$F_{2,3} = 4.384$	0.1480				
Biological Parameters							
Microcystin	Reservoir	$F_{1,13} = 2.253$	0.1579				
	TempGroup	$F_{2,13} = 10.531$	0.0025		a	a	b
	Reservoir :TempGroup	$F_{2,13} = 3.842$	0.0537				
Cylindrospermopsin	Reservoir	$F_{1,4} = 3.625$	0.1221				
	TempGroup	$F_{1,4} = 3.641$	0.1187				
	Reservoir :TempGroup	$F_{1,4} = 0.563$	0.5552				
Chlorophyll a	Reservoir	$F_{1,10} = 0.161$	0.6971				
	TempGroup	$F_{2,10} = 4.492$	0.0434*				
	Reservoir :TempGroup	$F_{2,10} = 1.534$	0.2620				

Pheophytin	Reservoir	$F_{1,9} = 2.900$	0.1223		
	TempGroup	$F_{2,9} = 0.993$	0.4035		
	Reservoir :TempGroup	$F_{2,9} = 4.438$	0.0462		
TSS	Reservoir	$F_{1,7} = 0.598$	0.4650		
	TempGroup	$F_{2,7} = 0.318$	0.7085		
	Reservoir :TempGroup	$F_{2,7} = 8.416$	0.0155		
PIM	Reservoir	$F_{1,5} = 7.472$	0.0339	a	b
	TempGroup	$F_{1,5} = 0.114$	8.8678		
	Reservoir :TempGroup	$F_{1,5} = 5.422$	0.0479		
POM	Reservoir	$F_{1,9} = 0.043$	0.8411		
	TempGroup	$F_{2,9} = 1.644$	0.2463		
	Reservoir :TempGroup	$F_{2,9} = 2.176$	0.1733		

Parameters were tested for the effects of Reservoir and Temperature Group. When factors were statistically significant ($p < 0.05$), we computed all possible pairwise Wilcoxon rank-sum tests of the factor of interest after subtracting the medians of the secondary factor to adjust the data for differences attributed to the secondary factor. Differences between factor levels are indicated by different lowercase letters. The same letters indicate no significant difference between factor levels. *No significant difference among Temperature Groups was identified during post-hoc analysis.

Figures

Figure 2.1. Boxplots comparing (A) chlorophyll *a*, (B) microcystin, and (C) cylindrospermopsin concentrations by temperature group for Bethel and Stephens Lake. Temperature was divided into three groups based on preliminary data visualization that identified a pattern of detectable cylindrospermopsin at lower and higher surface water temperatures. No detectable cylindrospermopsin was found in the 15–25 °C temperature group. The solid line within each box represents the median, and the top and bottom hinges represent the 75th and 25th percentiles. Whiskers depict values the smallest and largest

values within 1.5 times the interquartile range (IQR). Points identify values outside 1.5 times the IQR. Similar lowercase letters indicate no significant difference between temperature groups (Table 2.3).

Figure 2.2. Scatterplots depicting the distribution of (A) microcystin and (B) cylindrospermopsin concentrations by month throughout the study period. Dashed lines represent microcystin ($0.10 \mu\text{g L}^{-1}$) and cylindrospermopsin ($0.04 \mu\text{g L}^{-1}$) method detection limits. .

Figure 2.3. GAM partial effect splines (solid line) with 95% confidence area (shaded area) for Bethel Lake cyanotoxin models. Subplots A, B, and C represent microcystin models and D, E, and F represent cylindrospermopsin models. **(A)** surface water temperature and **(B)** TN represent the best fit model predicting the probability of detectable microcystin, explaining 49.1% of the deviance. TN is not a significant driver. **(C)** surface water temperature is the best fit model predicting concentrations of microcystin,

once detected, and explains 41.4% of the deviance. **(D)** surface water temperature and **(E)** PIM best explains the probability of detectable cylindrospermopsin and explains 41.9% of the deviance. **(F)** $\bar{E}_{24}$ explains 85.5% of the deviance in cylindrospermopsin concentrations once detected. Summary model statistics are shown in Tables A.3, A.4, A.6 and A.7.

Chapter 3: Reservoir Observer Student Scientists: A High School Community

Science Program for Year-Round Water Quality Monitoring

Cyanobacterial blooms are an ongoing threat to water quality worldwide. They can form dense, mucilaginous surface scums, produce taste and odor compounds, and deplete waters of oxygen. Some species of cyanobacteria can produce toxic secondary metabolites that are dangerous to humans and animals. Cyanobacterial blooms can damage local economies through increased drinking water treatment costs, decreased property values, and lost revenue from lake and reservoir closures (Figure 3.1).

Although cyanobacterial blooms are commonly found in the summer and early fall, there have been occasional observations in the winter (Wejnerowski et al., 2018). There is a recognized knowledge gap regarding winter lake processes. From the few available year-round studies, we know winter cyanobacterial blooms can produce toxins. One year-round study found concentrations of the cyanotoxins microcystin and anatoxin did not vary significantly by season (Wiltsie et al., 2018). Satellite remote sensing imagery indicates that we may be underestimating the frequency of winter cyanobacterial blooms (Coffer et al., 2021). Many water quality monitoring programs focus on collecting samples during the summer “growing” season, but we need to know what is happening year-round to effectively manage lakes and reservoirs and protect human health. Running a year-round monitoring program can be logistically complicated with potential winter safety issues (Block et al., 2019), high costs associated with frequent visits to sampling sites, and lack of available personnel to collect and analyze a high volume of samples. The Reservoir Observer Student Scientists (ROSS) program was

created as a community science initiative to ease these complications and engage students in water quality monitoring.

What is the Reservoir Observer Student Scientists (ROSS) Program?

ROSS is a novel water quality monitoring program that teaches high school students about limnology and trains students to collect year-round water samples. At the heart of the ROSS program is education. Our goal is to teach the next generation of scientists and decision makers about their local lake or reservoir and empower them to understand the impacts of water quality in their community. We create a customized presentation for each class that we partner with, and teach students about their local lake or reservoir, including its history, purpose, major uses, and how watershed use may impact the system. We also teach the students basic limnological principles, identify the problems associated with cyanobacterial blooms, and explain how we can use physical and biological parameters to monitor water quality.

After students have learned about water quality, we provide hands-on water sampling training. The University of Missouri (MU) Limnology Laboratory supplies all necessary equipment. We take the students to their lake or reservoir, pick out a shoreline sampling location, and teach them how to collect a surface water grab sample. Students also learn how to take high quality field notes by properly recording date and time information, making observations about water conditions, documenting ice cover or anything unusual, and noting weather conditions.

Back in the classroom “lab,” we teach students how to process the water for preservation and analysis. We provide hands-on training for filtering water and distributing the sample into the appropriate bottles for future analysis, all while teaching

students how to prevent contamination of the sample bottles (Figures 3.2 and 3.3). Students learn the importance of correctly labeling bottles and recording pertinent notes. We explain how to store samples for proper preservation, and we make sure all students are comfortable with the process. Samples are analyzed by the MU Limnology Lab for total nitrogen, total phosphorus, nitrate, ammonium, chlorophyll-a, phycocyanin, total suspended solids, microcystin, cylindrospermopsin, and phytoplankton identification.

Approximately quarterly, we visit the students to pick up the samples and to sample side-by-side with the students. Side-by-side sampling allows us to evaluate the quality of the data collected by the students. Over the last three years of the ROSS program, we have found no significant difference between mean values for samples collected by trained lab technicians and high school students for total nitrogen, total phosphorus, chlorophyll-a, microcystin, nor cylindrospermopsin using a Kruskal-Wallis test at the 5 % confidence level. Students are able to collect and process water samples that lead to high quality data.

From its inception in the fall of 2017 through the spring term of 2021, approximately 300 students at 4 different high schools have been part of the ROSS program. Student outcomes of the ROSS program are assessed through a survey before and after their participation. Post-survey results indicate that students correctly answered 76 % of the questions about limnology. We also asked students open-ended questions about what they liked about the ROSS program and to list one thing they learned from participation in the program. Select student responses are illustrated in Figures 3.4 and 3.5. Once the samples have been analyzed at the MU Limnology Lab, data is shared with

the teachers to incorporate into their lessons so students can learn about data analysis and visualization.

ROSS Student Success

ROSS participants from Maize High School in Maize, Kansas were inspired by learning about cyanobacterial blooms in Cheney Reservoir, their local drinking water reservoir, to take action against cyanobacterial blooms. These students were semi-finalists in the 2018 Lexus EcoChallenge and were awarded \$10,000 in grants and scholarships for their research. They also deployed their own thermistor chain in Cheney Reservoir to study stratification patterns. They used the temperature data they collected and cyanotoxin data we provided from their samples and presented this research at the 2019 Great Plains Limnology Conference, winning an award for Best Undergraduate Presentation. These ROSS participants also traveled to other high schools in Kansas to teach Future Farmers of America groups about cyanobacterial blooms so the next generation of farmers could understand the importance of nutrient management to help prevent blooms.

ROSS Research Questions

Data from the ROSS Program is invaluable in helping to answer important research questions. This data will help us understand how cyanotoxin concentrations vary throughout the year and how the presence and concentration of cyanotoxins relate to physical and chemical drivers. A research article with findings from two of the ROSS study systems is currently in submission. Data from the paper has been published and is available online (Kinzinger and North, 2021). As the ROSS program continues to collect more data, we will be able to analyze the annual variability in cyanotoxin presence and

concentration and determine if and how drivers of cyanotoxins change throughout seasons. Collecting year-round water quality data is vital for understanding the seasonal risks of cyanotoxins in recreational and drinking water systems.

Vision for the Future

Community science programs, like ROSS, are invaluable for both participants and scientists. Students can gain a deeper understanding of- and desire to care about- their local natural resources through engaging in research. Participation in community science also makes science attainable to students and shows them that their contributions are important towards advancing scientific knowledge. By sharing data with students, they are able to learn how to ask questions, form hypotheses, and explore how the data can be used to inform their questions. The collaboration between students and scientists also teaches students valuable skills in working with others.

Scientists are able to educate students about their research topic and collect a high volume of data that would otherwise be unattainable. The ability of scientists to collect samples or record observations is often limited by time and distance. By partnering with community scientists, data can be gathered at a high frequency from sites far away from their home base. Community science programs have a tremendous potential to educate students and make important contributions to research.

Conclusions

The ROSS Program teaches high school students about limnology and water quality issues their local lake or reservoir may be facing. Students gain hands-on experience collecting real-world data that will contribute to expanding our knowledge regarding year-round aquatic processes. This community science monitoring program is a

successful way to educate the next generation of thinkers while also collecting important data.

Acknowledgements

Thank you to Gregory Kirchhofer, Amy Hammett, Mike Richards, Chaps Wilcke, and all of the students who have participated in ROSS. We are grateful for your support of this project and continued commitment to collecting samples. Thank you also to Dr. Christine Li for creating the student surveys. Special thanks to Dan Downing for his confidence in this project and to the North Central Region Water Network for funding.

References

- Block, B. D., Denfeld, B. A., Stockwell, J. D., Flaim, G., Grossart, H. P. F., Knoll, L. B., et al. 2019. The unique methodological challenges of winter limnology. *Limnol. Oceanogr. Methods* 17, 42–57. doi:10.1002/lom3.10295.
- Coffer, M. M., Schaeffer, B. A., Salls, W. B., Urquhart, E., Loftin, K. A., Stumpf, R. P., et al. 2021. Satellite remote sensing to assess cyanobacterial bloom frequency across the United States at multiple spatial scales. *Ecol. Indic.* 128, 107822. doi:10.1016/j.ecolind.2021.107822.
- Kinzinger, E. C., and North, R. 2021. Water Quality Data from Bethel and Stephens Lakes, Columbia, Missouri 2017-2019 ver 1. doi:doi:10.6073/pasta/5f29f3e04e78922879fcca5932ddc54f.
- Wejnerowski, Ł., Rzymiski, P., Kokociński, M., and Meriluoto, J. 2018. The structure and toxicity of winter cyanobacterial bloom in a eutrophic lake of the temperate zone. *Ecotoxicology* 27, 752–760. doi:10.1007/s10646-018-1957-x.
- Wiltsie, D., Schnetzer, A., Green, J., Borgh, M. Vander, and Fensin, E. 2018. Algal blooms and cyanotoxins in Jordan Lake, North Carolina. *Toxins*. 10. doi:10.3390/toxins10020092.

Figures

Figure 3.1. One our study systems, Stephens Lake, closed due to a cyanobacterial bloom in Fall 2018.

Figure 3.2. Students learn how to filter samples for chlorophyll-*a*

Figure 3.3. Students process a water sample collected from Cheney Reservoir in Maize, Kansas.

Figure 3.4. Selected student responses to the prompt: “What do you like about the ROSS project?”

Figure 3.5. Selected student responses to the prompt: “Please list one topic that you learned from the ROSS project.”

Chapter 4: Overall Conclusions

Conclusions

The objective of this thesis was to implement a community science program to assess the year-round presence, concentration, and environmental drivers of cyanotoxins in two urban reservoirs. I found that cyanotoxins were present year-round, algal biomass did not vary significantly at different temperature groups, concentrations of cyanotoxins were not associated with algal biomass, and cyanotoxin presence and concentration was related to physical water quality parameters. Samples were collected through the ROSS program, which educated high school students about limnology and water quality in their local reservoirs.

Chapter 2 Summary and Significance

This chapter adds to the body of literature assessing year-round water quality and is currently in review at Limnology and Oceanography Letters. The main conclusions of this chapter are that concentrations of microcystin and cylindrospermopsin were detectable year-round in Bethel and Stephens Lakes and that physical water quality parameters were the drivers of cyanotoxin presence and concentration in Bethel Lake. There was no significant difference in chlorophyll-*a* among the different water temperature groups, and there was no relationship between chlorophyll-*a* and detectable concentrations of microcystin nor cylindrospermopsin. There are few year-round field studies available, so this chapter contributes valuable information towards understanding the occurrence of cyanotoxins in the winter months. This chapter highlights the need for continued year-round sampling on additional systems to study seasonal cyanotoxin trends and drivers.

This study would have benefited from a higher volume of samples equally distributed across all temperature groups and both lakes. If I had an additional year of sampling at Stephens Lake, my data set would have been more robust. This would have allowed for me to run the analysis of environmental drivers of cyanotoxin presence and absence for data from both lakes, instead of just for Bethel. Then the results could have been compared to each other to determine how lake-specific my findings are or if both lakes had similar drivers.

Phytoplankton samples were collected as part of my dataset, but at the time of analysis, funding for identification and enumeration was not available. Knowing which taxa were present at different water temperatures would provide valuable information regarding if the same toxin producers were present at different temperature extremes or if toxin producers at cool temperatures were different than those at warmer temperatures. It is worth noting, however, that even with this data it may be challenging to draw connections between specific tax and cyanotoxins presence and concentration.

Chapter 3 Summary and Significance

This chapter describes the Reservoir Observer Student Scientist (ROSS) program. ROSS is an innovative, year-round water quality monitoring program that I managed as part of my thesis work. I communicated with extension representatives and teachers to find participants for the project, created and gave presentations to students and their teachers, organized and implemented hands-on sampling and processing training for the students, and maintained a supply inventory for the project, in addition to performing laboratory analyses. I showed that students collected high quality data, and that the ROSS program is an effective model for collecting high-frequency, year-round data. It was

inspiring to see some of the students start their own water quality research and work to educate their peers about cyanobacterial blooms. The implementation of the ROSS program not only contributes valuable data about cyanotoxin occurrence, but also educates and empowers high school students to be knowledgeable about their local water resources.

One challenging aspect of the ROSS program is finding volunteers to continue weekly sampling during summer. Students are out of school for the summer months, so we are reliant on a keen student volunteering to collect samples. For local reservoirs, an MU Limnology Lab technician can conduct the weekly sampling. Elsewhere, a willing community member could step in absence of a student volunteer. Volunteers could be found through collaboration with local extension representatives, master naturalist groups, or other existing community science programs. Another solution is to conduct the ROSS program on lakes and reservoirs with existing state summer monitoring. Many states have consistent summer monitoring programs and provide data to the public. The data obtained by the state during the summer would supplement data collected by students during the school year.

Looking Ahead

Climate change is one of the greatest threats to water quality and requires additional research to understand impacts on our freshwater resources (O'Neil et al., 2012). As climate change continues to result in warmer summer water temperatures (O'Reilly et al., 2015) and reduced ice cover (Sharma et al., 2019), our dimictic lakes could shift to a warm monomictic mixing pattern (Livingstone, 2008). Given that the winter and shoulder seasons of these lakes and reservoirs are changing and winter

processes set the stage for following seasons (Hampton et al., 2017), we need to understand what is happening in winter months to better predict future change. We know from this current study that cyanotoxins can persist year-round in our warm monomictic study systems, so winter processes are important. Taking this study one step further by comparing lakes and reservoirs that exhibit different mixing patterns with varying levels of ice cover will provide insight on how lakes and reservoirs may respond to climate-induced shorter winters.

This thesis shows that a community science program like ROSS can successfully collect large volumes of high-quality, year-round data. The ROSS program is a template that can be used by other researchers to greatly expand sampling efforts when otherwise impeded by staff or travel constraints. The education component of the ROSS program can have far-reaching impacts. Teaching the next generation of students about water quality can help them to be stewards of their local water resources.

References

- Hampton, S. E., Galloway, A. W. E., Powers, S. M., Ozersky, T., Woo, K. H., Batt, R. D., et al. (2017). Ecology under lake ice. *Ecol. Lett.* 20, 98–111. doi:10.1111/ele.12699.
- Livingstone, D. M. (2008). Review paper A change of climate provokes a change of paradigm: Taking leave of two tacit assumptions about physical lake forcing. *Int. Rev. Hydrobiol.*, 404–414. doi:10.1002/iroh.200811061.
- O’Neil, J. M., Davis, T. W., Burford, M. A., and Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14, 313–334. doi:10.1016/j.hal.2011.10.027.
- O’Reilly, C. M., Rowley, R. J., Schneider, P., Lenters, J. D., McIntyre, P. B., and Kraemer, B. M. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophys. Res. Lett.*, 1–9. doi:10.1002/2015GL066235. Received.
- Sharma, S., Blagrove, K., Magnuson, J. J., Reilly, C. M. O., Oliver, S., Batt, R. D., et al. (2019). Widespread loss of lake ice around the Northern Hemisphere in a warming world. *Nat. Clim. Chang.* 9, 227–231. doi:10.1038/s41558-018-0393-5.

Appendix

Supplemental Information

Table A.1. Kruskal-Wallis test to evaluate the difference between samples collected by the MU Limnology lab and volunteers.

Parameter	Chi square	<i>p</i> -value	n	<i>df</i>
Chl- <i>a</i>	0.429	0.5127	6	1
TN	0.6	0.4389	4	1
TP	0.6	0.4389	4	1

Table A.2. Correlation matrix of environmental variables.

r_s															
	mcpresence	cylpresence	MC	Cyl	TN	TP	CHLA	PHEO	TSS	POM	PIM	Temp	E24	TNTP	PHEOCHLA
mcpresence															
cylpresence	0.29														
MC	0.81	0.23													
Cyl	0.28	0.97	0.23												
TN	0.48	0.26	0.24	0.3											
TP	-0.01	0.11	-0.21	0.18	0.49										
CHLA	0.13	0.04	0.11	0.06	0.56	0.37									
PHEO	0.09	-0.03	-0.09	-0.03	0.63	0.48	0.55								
TSS	-0.1	0.31	-0.24	0.35	0.41	0.61	0.18	0.47							
POM	0.12	0.11	0.02	0.16	0.51	0.45	0.54	0.48	0.67						
PIM	-0.11	0.38	-0.28	0.43	0.25	0.57	-0.13	0.26	0.82	0.3					
Temp	0.47	0.44	0.63	0.42	0.03	-0.33	0.25	-0.25	-0.28	0.06	-0.43				
E24	0.36	0.06	0.56	0.06	-0.29	-0.43	-0.09	-0.62	-0.6	-0.29	-0.57	0.75			
TNTP	0.53	0.08	0.4	0.06	0.68	-0.18	0.28	0.37	0.01	0.19	-0.1	0.13	-0.19		
PHEOCHLA	0.09	-0.03	-0.09	-0.03	0.63	0.48	0.55	1	0.47	0.48	0.26	-0.25	-0.62	0.37	
POMCHLA	-0.14	-0.02	-0.24	-0.04	-0.3	-0.07	-0.77	-0.26	0.24	0.02	0.34	-0.35	-0.18	-0.18	-0.26
p															
mcpresence															

cylpresence	0.015														
MC	0	0.0566													
Cyl	0.0194	0	0.0612												
TN	0	0.0303	0.0503	0.0126											
TP	0.967	0.3785	0.0908	0.155	0										
CHLA	0.2861	0.7661	0.3928	0.6515	0	0.0018									
PHEO	0.4642	0.8263	0.4601	0.8299	0	0	0								
TSS	0.4611	0.0139	0.0699	0.0051	0.0012	0	0.164	0.0001							
POM	0.3518	0.3911	0.8582	0.1983	0	0.0001	0	0	0						
PIM	0.4017	0.0024	0.0292	0.0005	0.0517	0	0.2969	0.0391	0	0.0165					
Temp	0.0001	0.0003	0	0.0005	0.7954	0.0074	0.0463	0.0423	0.0289	0.6153	0.0006				
E24	0.0063	0.6664	0	0.6607	0.0275	0.0009	0.4842	0	0	0.0282	0	0			
TNTP	0	0.5131	0.0011	0.636	0	0.134	0.0223	0.0023	0.9298	0.124	0.4344	0.3002	0.1794		
PHEOCHLA	0.4642	0.8263	0.4601	0.8299	0	0	0	0	0.0001	0	0.0391	0.0423	0	0.0023	
POMCHLA	0.2557	0.8971	0.0571	0.718	0.0147	0.5782	0	0.0324	0.0549	0.8534	0.0058	0.0044	0.1658	0.1597	0.0324

Table A.3. GAM output for MC presence in Bethel Lake (binomial model, full dataset).

Smooth terms	Ref edf	edf	Chi. sq	<i>p</i> -value
TN	4.12	3.26	7.71	0.11
Temp	1	1	11.57	<0.001
Deviance explained: 49.1%				

Table A.4. GAM output for MC concentration in Bethel Lake (log-link Gamma model, detects only).

Smooth term	Ref edf	edf	F statistic	<i>p</i> -value
Temp	4.389	3.554	4.7	<0.01
Deviance explained: 41.4%				

Table A.5. GAM output for MC concentration in Bethel Lake, excluding Temp (log-link Gamma model, detects only).

Smooth term	Ref edf	edf	F statistic	<i>p</i> -value
PIM	3.45	2.74	2.79	<0.05
Parametric coefficients	Estimate	Std. Error		<i>p</i> -value
Mixed	-1.70	0.19		
Stratified	0.530	0.26		<0.05
Deviance explained: 33.5%				

Table A.6. GAM output for CYL presence in Bethel Lake (binomial model, full dataset).

Smooth terms	Ref edf	edf	Chi. sq	<i>p</i> -value
Temp	3.32	2.64	10.57	<0.05
PIM	1	1	9.13	<0.01
Deviance explained: 41.9%				

Table A.7. GAM output for CYL concentration in Bethel Lake (log-link Gamma model, detects only).

Smooth term	Ref edf	edf	F statistic	<i>p</i> -value
$\bar{E}_{24}$	4.54	3.74	13.11	<0.01
Deviance explained: 85.5%				

Table A.8. GAM output for CYL concentration in Bethel Lake, excluding $\bar{E}_{24}$ (log-link Gamma model, detects only).

Smooth term	Ref edf	edf	F statistic	<i>p</i> -value
Temp	2.8	2.48	13.09	<0.01
TN	2.96	2.82	4.41	0.08
TP	1	1	1.58	0.26
PIM	1	1	24.43	<0.01
Deviance explained: 96.7%

Figure A.1. GAM partial effect splines (solid line) with 95% confidence area (shaded area) for Bethel Lake detectable microcystin concentrations, exclusive of surface water temperature

Figure A.2. GAM partial effect splines (solid line) with 95% confidence area (shaded area) for Bethel Lake detectable cylindrospermopsin concentrations, exclusive of $\bar{E}_{24}$.