Load, not loading: External nutrient loading impact on cyanobacteria and cyanotoxins

Owen Gallagher[1]*, Rebecca L. North*[1]

[1]*University of Missouri-Columbia,*

Abstract:

Eutrophication is the process in which excessive nutrients enter a body of water resulting in a rapid growth in population and density of phytoplankton. One type of phytoplankton made more concentrated during eutrophication is cyanobacteria. Some cyanobacteria can produce cyanotoxins. Cyanotoxins have many negative impacts on both aquatic ecosystems and human health. The objective of this research is to determine the effects chronic and episodic nutrient loading have on cyanobacteria and the resultant cyanotoxin concentrations. We simulated chronic loading by adding small amounts of nitrogen (N) and phosphorus (P) over a six-day period in a nutrient simulation experiment. Episodic loading was simulated by adding a large spike of nutrients to lake water on only the first day of the nine-day experiment. At the end of the experiment, we analyzed sample water for pH, microcystin and cylindrospermopsin concentrations, chlorophyll-a and phycocyanin as proxies for phytoplankton and cyanobacteria respectively, N and P concentrations, and suspended solids. Our experiment showed that there was no significant differences found between treatments testing episodic and chronic loading in the production of cyanobacteria or cyanotoxins for any of the test sites. Additions of N and P increased the nutrient load causing cyanobacteria and cyanotoxin production, however the form of nutrient loading was not significant. This research is relevant to better understand how cyanobacteria respond to different nutrient loading mechanisms and the possible effects climate change and the associated increases in episodic nutrient loading could have on bodies of freshwater.

Introduction:

Cyanotoxins are increasing globally in freshwater lakes due to events such as eutrophication: the process in which excess nutrients enter aquatic ecosystems. As a result of these excess nutrient inputs, phytoplankton experience a rapid growth in population and density. Excess nutrients, primarily nitrogen (N) and phosphorus (P), can enter the water body by both natural and anthropogenic causes. Anthropogenic eutrophication of lakes is commonplace and has been increasing since 1940, promoting the rate of harmful algal bloom (HAB) biomass (Bhagowati et al., 2020). Harmful algal blooms are events that can diminish water clarity, ecosystem function, and recreational activities (Pretty et al., 2003; Sunda et al., 2006). Eutrophication fuels the growth of phytoplankton and favors toxin-producing cyanobacteria over other species of cyanobacteria in lakes (Bogard et al., 2020; Davis et al., 2010). Cyanobacteria are photosynthetic bacteria that predominate HABs in freshwaters. Cyanobacteria have a variety

of mechanisms to potentially outcompete other species including: buoyancy regulation to compete for light, the ability to proliferate at higher water temperatures, nitrogen-fixation, and toxin production (Davis et al., 2009; Lürling et al., 2018; Paerl & Huisman, 2009).

Microcystin and cylindrospermopsin are secondary metabolites produced by some cyanobacteria, with microcystin being not only the largest cyanotoxin group, but the most structurally advanced as well (O'Neil et al., 2012). Both of these metabolites are hepatotoxins, meaning they damage the liver by inhibiting protein phosphates, as well as cytotoxins, neurotoxins, and possible human carcinogens (O'Neil et al., 2012; Pearson et al., 2010). While some forms of toxin producing cyanobacteria such as *Dolichospermum* are capable of nitrogen-fixation (Rapala et al., 1997), some studies have showed that cylindrospermopsin growth is limited in nitrogen limited environments (Van de Waal et al., 2014). Human fatalities have been reported when microcystin contaminated water was used for hemodialysis (Pugliese & Favero, 1998). Due to microcystin's chemical stability, these toxins are heat resistant, preventing detoxification by boiling (Chen et al., 2019). The stable nature of these toxins has been the source of "do not drink" orders on tap water supplies during bloom events (Jetoo et al., 2015). Apart from drinking water, exposure to toxic microcystin can also occur dermally, through inhalation of airborne particles**,** and orally during recreational water use, along with fish consumption (Backer et al., 2010; Zhang et al., 2009). HABs can deplete oxygen in the water column creating areas of hypoxia (oxygen deprivation) and anoxia (no oxygen) leading to the loss of aquatic organisms (Heisler et al., 2008). Microcystins themselves can cause fish kills even without oxygen depletion due to their occurrence in food webs (Chellappa et al., 2008). Microcystins bioaccumulate and tend to concentrate in the liver and muscles of fish

(Vasconcelos et al., 2013), but can also accumulate in gonads and bile (Hauser-davis et al., 2015).

Increases in storms due to climate change will saturate soils and increase nutrient runoff from wastewater and agriculture. Agricultural runoff is the main contributor to increasing bioavailable (reactive) forms of N and P in environments. N and P are considered the limiting nutrients of aquatic ecosystems, controlling the biomass of primary producers (Elser et al., 2007). A global budget approach estimates ecosystem P levels are 75% higher than pre-industrial levels and a large portion of P accumulation is located in agricultural soils creating concern due to the risk of leaching into water bodies (Bennett et al., 2001). Furthermore, the increasing demands of agricultural, industrial, and municipal systems have doubled the amount of annual reactive N, with 210 of the total 413 Tg N/yr coming from anthropogenic sources (Fowler et al., 2013).

There is ongoing debate as to how N : P ratios and quantities influence cyanobacterial growth. A long held hypothesis is the low N : P hypothesis stating that cyanobacteria are dominant in HABs due to adaptations in low N : P environments, such as nitrogen fixation (Davis et al., 2015; Smith, 2010). Multiyear whole lake experiments, long-term case histories, and mesocosm studies concluded that there is only evidence to support that reducing P inputs effectively reduces HABs (Schindler, 2012; Schindler et al., 2016). Conversely, a more recent long-term study shows the successful reduction of blooms without increasing N-fixing cyanobacteria was achieved with dual N and P reduction (Shatwell & Köhler, 2019). Shatwell & Köhler (2019) attributed the success to N reduction and state that a P-only strategy would not have been as successful as a result of the high internal P loading. Geographically diverse evidence has emerged showing five lakes responded successfully to combined N and P

reductions where N or P reductions alone were not as effective, indicating that the P-only approach is oversimplified (Paerl et al., 2016). The reduction of HABs resulting from reduced N inputs is likely because two out of the three most common microcystin producing genera (*Microcystis* and *Planktothri*x) do not fix nitrogen and rely on nitrogen inputs for growth. Some lab studies have also shown that total quantities of N and P predict cyanobacterial biomass better than N : P ratios (Downing et al., 2001; Pick & Lean, 1987). A less researched topic is the timing of N and P delivery: chronically or episodically.

Nutrients enter water bodies from runoff via two different loading mechanisms: chronic or episodic loading. These loading mechanisms determine how nutrients enter the water body and are a result of the nutrient concentration (the nutrient load), and the nutrient flow. Chronic loading occurs when nutrient loads enter a water body slowly over time through events such as erosion, runoff from seasonal rainfall, municipal effluents, and industrial water contamination. Episodic loading occurs when nutrient loads enter the water body in excess over a short time frame usually due to flooding events, saturating the soils, and causing runoff. Storms can also create episodic events due to wind mixing, bringing a pulse of nutrient rich sediment into the euphotic layer (Miller et al., 2006). There is scientific consensus that both chronic and episodic nutrient delivery are important in HAB development (Heisler et al., 2008). This paper aims to determine which loading mechanism, episodic or chronic, has the greatest effects on cyanobacteria and cyanotoxin concentrations. To address this research objective, we designed a nutrient addition experiment. Lake water was amended with P and both N and P in a mesocosm experiment. Adding nutrients slowly over a five-day period, simulating chronic loading, and larger one-time nutrient additions on the first day, simulating episodic loading, was used to test this question. Chlorophyll-a and phycocyanin were then measured in each treatment as proxies

for total phytoplankton and cyanobacteria concentrations, respectively. Microcystin and cylindrospermopsin concentrations were also measured to see if the different nutrient loading mechanisms impact the amount and type of toxins produced.

Site descriptions:

Crow Pond:

Crow Pond is a small pond located at Prairie Fork Conservation Area in Williamsburg, Missouri, USA. Located in a rural location and managed by the Missouri Department of Conservation, Crow Pond is used for teaching and experimental purposes. Crow Pond is surrounded by restored prairie, and Keswick Loam soil type surrounds the 501 meter shoreline (United States Department of Agriculture's Soil Survey 2021). In July of 2019, a glyphosate-based herbicide was sprayed on American Lotus (*Nelumbo lutea)* at a 2% rate. The average depth of the pond is ~2.24 meters and has a surface area of 12, 769.69 m^2.

Stephens Lake:

Stephens Lake is a small reservoir located in urban Columbia, Missouri, USA, inside Stephens Lake Park. 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). In 2005 a "beach" was added to the northwest section of the lake which further expanded the recreational uses of Stephens Lake on the western side of the lake. The eastern side of the lake is devoted for fishing. In the winter the lake is used for sledding and ice skating. The lake is owned by the city of Columbia, and the Missouri Department of Conservation manages the fish population. A walking

path goes around the perimeter of the lake, and there are parking lots on the western and southeastern sides of the lake. There is a highway that is located to the south of the lake, a road to the west of the lake, and a neighborhood to the north of the lake.

Pike Lake

Pike Lake is an approximately 4 km long and 250 m wide oxbow lake located in Pike Lake Provincial Park, 30 km southwest of Saskatoon, Saskatchewan, Canada, within the South Saskatchewan River (SSR) floodplain. Pike Lake is in the Moist Mixed Grassland Ecoregion, a region heavily influenced by agricultural activities and is nearly 80% cultivated (Floate & Shorthouse, 2010). There is crop production in close proximity to the southern shoreline and within 200 meters along the eastern shoreline. The lake experiences prolonged ice cover with the mean winter air temperatures of −10.2 °C, and mean summer temperature of 15.5 °C (Government of Canada, 2019). Annually, the average precipitation in the region is 356 mm per year (Government of Canada, 2019). Historically (until 1958), the SSR water rejuvenated the lake during periods of seasonal flooding. However, the Gardiner Dam construction led to controlled water levels in the river, curtailing this process (Government of Saskatchewan, 2020). Today, the water levels in Pike Lake are maintained and controlled manually within the park by pumping water from the SSR; water depth is typically no greater than 3 m (Saskatchewan Environment 2019). The lake is a hub for recreational activities, with many people visiting the park throughout the summer months. Single-family dwellings are scattered along the western shoreline. A public pool is open at the south end of the park providing an alternate recreational venue when water quality is poor. There was a visible bloom on August 13th 2020, the day water samples were collected from Pike Lake.

Lake 3

Lake 3 is a man-made lake at Fort Whyte Alive in Winnipeg, Manitoba, Canada that was established in the 1970s following the previous land occupation of a clay quarry mine. The 0.1 km^2 lake is part of a closed hydrologic system with no aerators surrounded by flat topography and was selected for its presence of algal blooms.

Table 1: Lake site descriptions. Redfields ratio based on TN : TP mass ratio values found in Table 2

Lake	Longitude	Latitude	Country	Mixing Depth (m)	Lake Depth (m)	KD	Trophic Status	TN:TP Ratio	% Cyano-bacteria
Stephens	38.95061	-92.30695	USA	4.8	5.7	1.3188	Eutrophic	14.25	0.28
Crow	38.89367	-91.73759	USA	2.5	3.9	0.4907	Hypereutr-ophic	24.12	11.90
Pike	51.9033176	-106.8172187	Canada	2.4	2.4	0.9987	Eutrophic	37.87	NA
Lake 3	49.816978	-97.228789	Canada	NA	5.1	1.7242	Eutrophic	NA	1.80

Table 2: Initial parameters, with soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), and cylindrospermopsin (CYN)

Site	Chlorophyll (mg/L)	Microcystin (mg/L)	Phycocyanin (m/L)	SRP (mg/L)	TP (mg/L)	TN (mg/L)	CYN (mg/L)
Stephen's Lake	0.02	0.09	0.05	0.00	0.04	0.65	0.04
Crow Pond	0.09	0.81	9.42	0.00	0.02	0.42	0.00
Pike Lake	0.01	2.32	*NA*	0.03	0.03	1.17	0.02
Lake 3	0.02	0.26	0.42	*NA*	*NA*	*NA*	0.04

Methods

Field Methods

166 The collection of data and samples took place near the deepest portion of each water
167 body. A Secchi disk was used on the shady side of the boat to determine the depth of water
168 visibility. A cosine corrected underwater quantum sensor (LI-192, Li-Cor Biosciences, Lincoln,
169 Nebraska, United States) was used to measure photosynthetically active radiation (PAR) down
170 the water column at 0.25 meter increments starting just below the surface. A Yellow Springs
171 Instruments EXO3 multi-parameter sonde (Yellow Springs, Ohio, United States) was used to
172 record temperature (0.001 °C resolution with an accuracy of ± 0.01 °C), dissolved oxygen (0.01
173 mg L^{-1}), depth with a resolution of 0.001 m, phycocyanin, and chlorophyll-a readings every
174 second.
175 Water was collected at Stephens Lake and Crow Pond from below the surface to 1 meter
176 above the mixing depth determined by the observed thermocline measured on the YSI, where
177 temperature decreased one °C per meter. Pike Lake collected water from a depth of
178 approximately one meter below the surface, and Lake 3 collected water from approximately 0.25
179 meters below the surface. After collection, water was taken to be immediately processed for
180 experimental set-up.
181 Mixing depth was determined by graphing the change in temperature with the change in
182 depth of the water column and determining a change of one °C per meter. The vertical
183 attenuation coefficient (K_d) was determined by graphing the regression line of the PAR readings
184 to the depth (Petty et al., 2020).
185 Samples from Stephens Lake and Crow Pond were collected August 28th 2020 and
186 September 11th 2020, respectively. At the time of collection for Stephens Lake, a visible surface
187 bloom was present. Pike Lake samples were collected on August 13th 2020 during a visible
188 surface bloom event. Samples were collected on Lake 3 on August 25th 2020.

Experimental Methods

After collection, water was mixed to ensure homogeneity, and raw lake water was set aside for analysis of the initial lake conditions. Lake water was then divided in triplicate into fifteen, four-liter cubitainers and labeled with the treatment they would receive.

The experiment was conducted in triplicate using additions of nitrate in the form of KNO_3^-, and phosphate in the form of $KH_2PO_4^{-3}$. On day zero (day of collection), three cubitainers were treated to reach a final concentration of 3.402 mg/L $KH_2PO_4^{-3}$, and three cubitainers were treated to reach a final concentration of 3.402 mg/L $KH_2PO_4^{-3}$ and 0.126 mg/L KNO_3^-. These six cubitainers act as the episodic additions of nutrients, which were only added on day zero of the experiment. Chronic treatments were treated everyday over a six day period (day zero through five) to achieve the same final concentrations in the cubitainers. Lastly, three cubitainers were treated with deionized water to ensure all volumes were the same. These cubitainers acted as our controls (Figure 1).

The experiment lasted ten days, with subsamples taken during day six for pH, chlorophyll-a, phycocyanin, microcystin, and cylindrospermopsin concentrations. The experiment ended on day nine. Cubitainers were incubated outside in cattle tanks where they floated freely (Figure 1). HOBOs were placed in each cattle tank to continuously record the temperature in each tank during the course of our experiment. Photosynthetically active radiation (PAR) readings were taken using a Licor in an attempt to make the light intensity similar to the intensity observed during collection. Each cattle tank had five cubitainers floating in them, one from each treatment (Figure 1). On day nine, water was processed and frozen for future analysis.

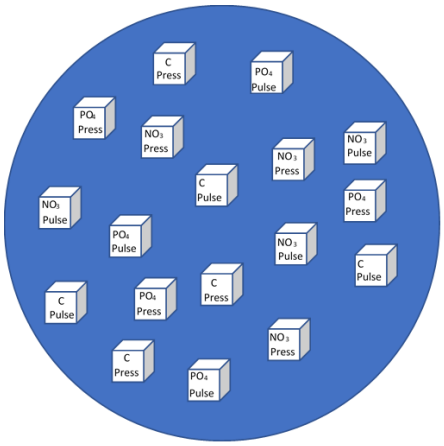

Figure 1: Positioning of free floating cubitainers in outdoor incubator cattle tanks where: press= chronic loading, pulse= episodic loading, C= Control, PO_4 = P only additions, and NO_3= N+P additions.

Laboratory Methods

Initial water conditions were tested on day zero for pH, chlorophyll-a, phycocyanin, microcystin, cylindrospermopsin, total suspended solids (TSS) including both volatile (VSS) and nonvolatile suspended solids (NVSS), nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphorus (SRP), total nitrogen (TN), and total phosphorus (TP).

Chlorophyll-a was measured by filtering lake water through 0.7 µm GFF filter paper and placing filters in the freezer with desiccant until ready for analysis. To analyze these samples, filters were placed into glass vials and treated with 90% ethanol, placed in a hot water bath for twenty minutes, and incubated overnight. This process was done in a dark room to limit light exposure. A Cary Eclipse Fluorescence Spectrophotometer, Agilent Technologies was used to collect chlorophyll readings (detection limit total chlorophyll: 0.3 µg/L; detection limit pheophytin corrected chlorophyll-a: 0.7 µg/L) and acidified readings were taken to correct for pheophytin (detection limit: 0.8 µg/L) in accordance to Knowlton (1984) and Sartory & Grobbelaar (1984).

Phycocyanin was measured by filtering lake water through 0.7 µm GFF filter paper and placing filters in the freezer with desiccant until ready for analysis. Phycocyanin was extracted

from the filters using sonication with known quantities of a 0.1 M sodium phosphate buffer. A Cary Eclipse Fluorescence Spectrophotometer, Agilent Technologies, and a Cary 60 UV_VIS Spectrophotometer were used to analyze filters with a detection limit of 1.0 µg/L.

Microcystin and cylindrospermopsin samples underwent three freeze/thaw cycles to burst cells, releasing stored cyanotoxins. Samples were then filtered using 0.45 μm glass fiber filters and analyzed using an Abraxis LLC ELISA test kit. Microcystin had a detection limit of 0.10 µg/L and cylindrospermopsin had a detection limit of 0.04 µg/L.

TSS samples were collected by filtering water and drying the 1.5 µm pore filter papers in an oven at 105 °C until filters were completely dry. Filters were then weighed to determine the total weight of both organic (VSS) and inorganic (NVSS) matter. Once weighed, filters were placed inside of a 550 °C muffle furnace for twenty minutes to burn away all organic matter. Filters were then weighed to determine the mass of the inorganic matter. The weight of the NVSS was subtracted from the weight after the first drying process to determine the amount of VSS.

NO_3 and NH_4 samples were analyzed in duplicates using a Lachat QuikChem Flow Injection Analyzer Hach, Loveland, Colorado, United States. This analysis followed a modified Lachat method for NO_3 (10-107-04-1-B/C0) with a detection limit of 0.36 µmol/L, and a NH_4 method (10-107-06-1-K) with a detection limit of 0.71 µmol/L. A Fisher Scientific Accument AE 150 pH reader was used to collect pH readings. TP and TDP were analyzed in triplicate using a spectrophotometer. TDP samples were filtered using glass fiber filters with a 0.7 µm pore size. This analysis followed an ascorbic acid colorimetric method followed by a digestion period in accordance to APHA (2017). Detection limits for TP and TDP 0.03 µm/L. TN was analyzed in

triplicate using a second derivative spectroscopy method with a detection limit of 2.50 μm/L (Petty et al., 2020).

Statistical Methods

Statistical analysis was done using the software R. A non-parametric Friedman test was performed to view significance between lake sites. Analysis was then done of on the individual lake sites using a pairwise permutation post-hoc test to determine which sample treatments were significant using a p-value of <0.05 as significant.

Results

Initial Parameters

Each lake was determined to be N or P limited by examining the TN:TP ratio of the initial lake water, in accordance to Redfields ratio (Redfield, 1934). Redfields ratio states that a mass ratio greater than fifteen is P limiting, and less than seven is N limiting. Using this ratio, Pike Lake and Crow Pond were determined to be P limited, while Stephens Lake was determined to be between N and P limitation values (Table 1). These ratios help identify how nutrient additions will perform under the varying lake environments, as P limited water bodies would be assumed to respond differently to additions of P than N limited water bodies would.

Along with identifying the nutrient limitations of each water body, lake trophic status was determined by viewing the chlorophyll-a values of the initial lake water and comparing them to values presented in Jones et al. (2008; Table 3). Crow Pond displayed a tropic status of hypereutrophic, while all other water bodies were eutrophic.

Table 3: Trophic status guidelines based on chlorophyll values given by Jones et al. (2008). Trophic status of each water body is given based on the average of chlorophyll values collected from the initial lake water on day 0 before nutrient additions were added.

Trophic State	Criterion (µg/L)	Lake	Value (µg/L)	Trophic State
Oligotrophic	<3	Pike Lake	10.2	Eutrophic
Mesotrophic	≥ 3-9	Stephen's Lake	18.1	Eutrophic
Eutrophic	≥ 9-40	Lake 3	12.0	Eutrophic
Hypereutrophic	≥ 40	Crow Pond	55.9	Hypereutrophic

Day Zero to Day Nine

Triplicate day nine controls were averaged for each site and compared to initial conditions of chlorophyll-a, phycocyanin, and microcystin, for each lake site (Figures 2-4). Initial conditions remained similar to the day nine average for all parameters with the exception of Crow Pond chlorophyll-a levels (Figure 2), and Pike Lake microcystin levels (Figure 4) which saw a decrease from day zero to day nine.

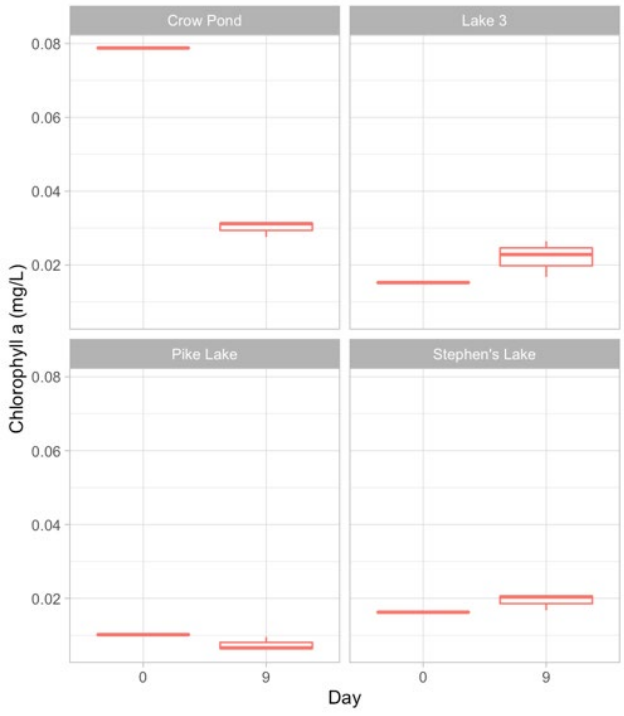

Figure 2: Initial chlorophyll-a concentrations compared to average (*n*= 3) of day 9 controls.

290

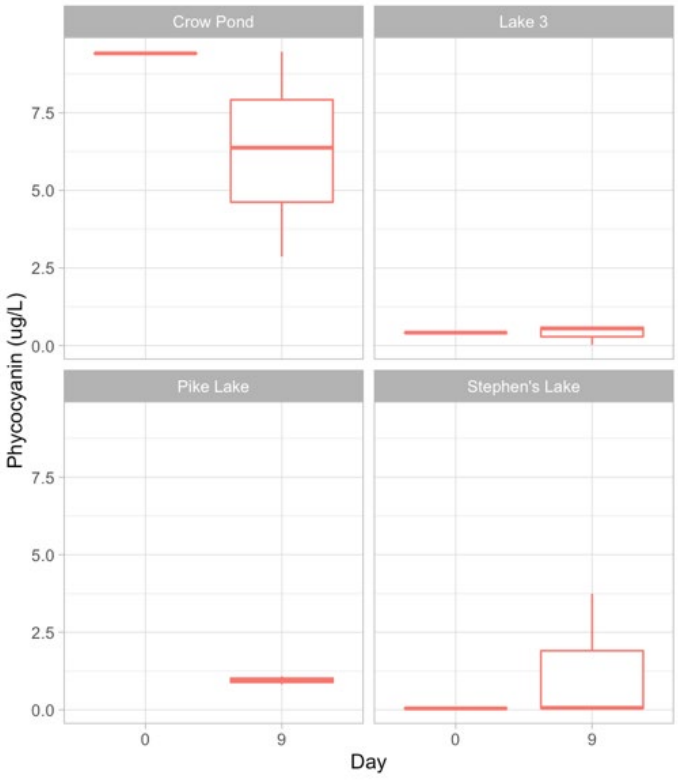

Figure 3: Initial phycocyanin concentrations compared to average of day nine controls measured in mg/L. Pike Lake did not have an initial phycocyanin reading.

291

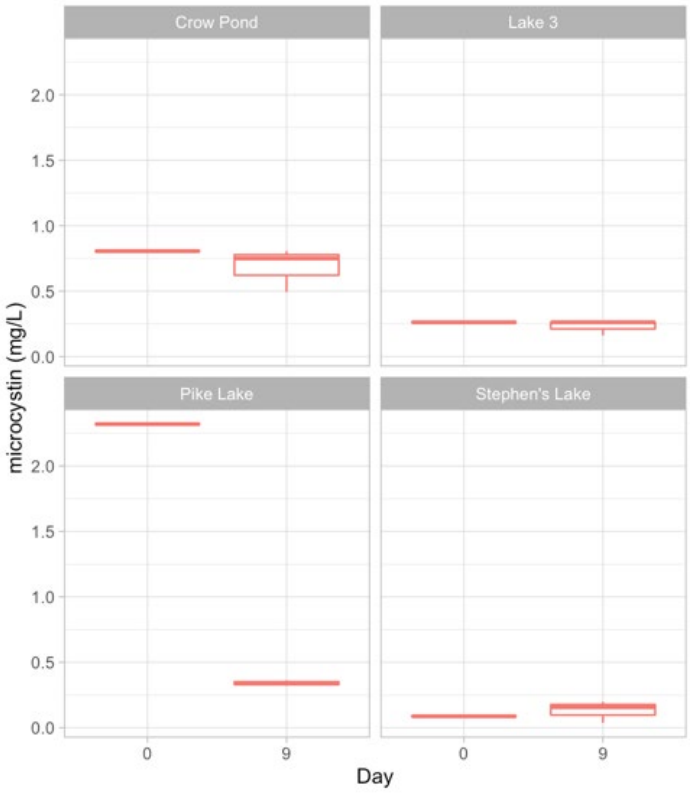

Figure 4: Initial microcystin concentrations compared to average of day nine controls measured in mg/L.

292

It is important to note that each sample site had different initial chlorophyll-a, phycocyanin, and microcystin concentrations (Table 2). These starting points should be kept in mind when comparing the different sample sites, as some sites had higher concentrations of toxins and or cyanobacteria at the initiation of the experiment (i.e., Crow Pond). Crow Pond saw a decline in chlorophyll-a readings from initial lake water to the average of the controls on day nine (Figure2). This could be due to insufficient light during incubation. When collected, Li-Cor readings showed that the surface light readings during time of collection was 167.6 $\mu molm^{-2}sec^{-1}$, and when water was set out to incubate on day zero was 124.29 $\mu molm^{-2}sec^{-1}$. Furthermore, the nine days of incubation were rainy which could have led to insufficient light for algal growth. Additionally, temperature data indicates that there was around a 2.73 °C decrease in temperatures in the incubation tanks compared to at the time of collection for Crow Pond. In the incubation tanks, temperature was recorded using HOBOS once an hour every hour throughout each of the three tanks creating an average of 21.43 °C for all time spent in incubation. The time of collection on the other hand recorded a water temperature of 24.19 °C.

Percent Cyanobacteria

Calculations were done to determine the percentage of cyanobacteria observed. Phycocyanin, which acts as a proxy for cyanobacteria, was divided by the uncorrected chlorophyll-a values for each treatment to derive percentage cyanobacteria. When examining the initial lake water conditions for Stephens Lake and Crow Pond it was found that of the chlorophyll-a measured, 0.28 % and 11.90 % was cyanobacteria respectively (Table 1). On day nine of the experiment, the percent cyanobacteria in Stephens Lake increased in all of the treatments when compared to the initial conditions (Table 4). Crow Pond saw an increase in the

316 percent cyanobacteria in the control and chronic P addition only, and Lake 3 saw an increase in
317 cyanobacteria in all treatments besides episodic P (Table 4). Pike Lake saw a decrease in the
318 percent cyanobacteria when treatments were compared to the day nine control (Table 4).
319 Comparing episodic and chronic treatments in Stephens Lake showed that the episodic addition
320 of P increased the percent cyanobacteria more than the chronic addition of P, however the
321 opposite was true when comparing chronic and episodic N+P for Stephens Lake. For Crow
322 Pond, no difference was observed between chronic and episodic N+P additions in the percentage
323 of cyanobacteria while chronic P accounted for a 15.96 % increase in the percent cyanobacteria
324 than the episodic addition of P (Table 4). In Pike Lake the percentage cyanobacteria in both
325 episodic treatments were slightly more than in their chronic counterparts, with episodic P
326 increasing the percent cyanobacteria by 2.54 % more than the chronic P, and episodic N+P
327 creating only a 0.23 % increase in the percentage of cyanobacteria than the chronic N+P (Table
328 4). Counter to Pike Lake, Lake 3 saw an increase in the percent cyanobacteria in both of the
329 chronic treatments when compared to the episodic treatments (Table 4).
330
331 *All Sites*
332 Sample triplicates from day nine were averaged to view significance between each site.
333 Phycocyanin is used as a proxy to show the total amount of cyanobacteria, while chlorophyll-a
334 acts as a proxy for total phytoplankton concentrations. Analyzing chlorophyll-a as a function of
335 site showed that chlorophyll-a concentrations in Pike Lake and Lake 3 were found to be similar
336 (p=0.896), and chlorophyll-a in Stephens Lake and Crow Pond were found to be similar
337 (p=0.265). Analyzing phycocyanin concentrations as a function of site showed that phycocyanin
338 concentrations in Stephens Lake were significantly different from Crow Pond (p= 0.007), Lake 3

(p=0.007), and Pike Lake (p=0.006). Microcystin analysis as a function of sight showed that levels of microcystin were similar between Crow Pond and Pike Lake (p=0.576). Cylindrospermopsin analysis as a function of site showed that, similar to microcystin, cylindrospermopsin concentrations were similar between Crow Pond and Pike Lake (p=0.189). These comparisons based on site help show how different sites react differently, or similarly, to one another. These results indicate that at the end of our experiment sites respond differently to nutrient additions, possibly due to different initial conditions. To better understand these results, analysis on a lake-by-lake basis was performed to see how each lake responded to the different nutrient additions.

Stephens Lake

A pairwise permutation post hoc test was used to compare day nine samples based on nutrient additions among each site. For chlorophyll-a in Stephens Lake, all treatments significantly increased when compared to the controls (Table 5). Chronic additions of P were not found to be significant when compared to episodic additions of P, or to either chronic or episodic additions of N+P. Likewise, chronic and episodic additions of N+P were not found to significantly affect the chlorophyll-a concentration when compared to each other, or when compared to either addition of P. Phycocyanin analysis showed that all treatments significantly increased the amount of phycocyanin compared to the controls. Episodic and chronic N+P additions, and chronic P additions were significantly lower in phycocyanin concentrations when compared to episodic P phycocyanin concentration (Table 5). Microcystin analysis showed that there was no significance between any of the treatments. Cylindrospermopsin analysis that the controls were significantly lower than episodic and chronic additions of P (Table 5).

Crow Pond

For chlorophyll-a concentrations in Crow Pond, both episodic and chronic additions of N+P were significantly higher than those of the controls (Table 5, Figure 5). Chlorophyll-a concentrations created by the episodic N+P addition was also significantly higher than the concentrations of the episodic P addition (Table 5, Figure 5). No significance was observed in phycocyanin concentrations between any of the nutrient additions (Table 5, Figure 6). Microcystin analysis showed that episodic N+P microcystin concentrations were significantly higher when compared to the control, chronic P, and episodic P additions (Table 5, Figure 7). No significance in cylindrospermopsin concentrations for Crow Pond were observed between nutrient additions (Table 5, Figure 8).

Pike Lake

Chlorophyll-a concentrations in Pike Lake were significantly lower in the control when compared to episodic N+P and chronic N+P additions (Table 5, Figure 5). Chlorophyll-a concentrations in both the episodic N+P and chronic N+P additions were also significantly higher than both the chronic and episodic additions of P (Table 5, Figure 5). For phycocyanin, the episodic N+P treatment had significantly higher concentrations that the control, chronic P, and episodic P additions (Table 5, Figure 6). Microcystin analysis showed that chronic N+P additions had significantly higher microcystin concentrations then the control, chronic P, and episodic P additions (Table 5, Figure 7). When a non-parametric Friedman test was performed for cylindrospermopsin to find global significance, no significance in cylindrospermopsin concentrations were observed between sample treatments. However, performing a pairwise permutation post-hoc test to view significance between treatments, the episodic addition of P was

shown to be significantly higher in cylindrospermopsin concentrations than the control (Table 5, Figure 8).

Lake 3

Lake 3 showed no significance difference in chlorophyll-a concentrations between any of the treatments (Table 5, Figure 5). For phycocyanin concentrations, the episodic N+P addition had significantly higher phycocyanin concentrations than the control, episodic P, and chronic P additions (Table 5, Figure 6). Phycocyanin concentrations were also significantly higher in the chronic addition of N+P than in then control, episodic P, and chronic P treatments (Table 5, Figure 6). The chronic P addition also had significantly higher concentrations of phycocyanin than that of the episodic P treatment (Table 5, Figure 6). Microcystin analysis showed that the chronic N+P additions had significantly higher microcystin concentrations than the control and episodic P treatments (Table 5, Figure 7). Cylindrospermopsin concentrations were not found to be significantly different among any of the treatments (Table 4, Figure 8).

Table 4: Day nine treatments shown. TN : TP values are based on mass ratio values. % Cyanobacteria was found by dividing phycocyanin by uncorrected chlorophyll-a.

Lake	Treatment	TN : TP	% Cyanobacteria
Stephens Lake	Control	14.75	7.49
Stephens Lake	Chronic P	5.97	45.28
Stephens Lake	Chronic N+P	11.17	65.91
Stephens Lake	Episodic P	7.58	153.82
Stephens Lake	Episodic N+P	8.82	23.98
Crow Pond	Control	12.43	20.4
Crow Pond	Chronic P	5.65	17.46
Crow Pond	Chronic N+P	4.98	0
Crow Pond	Episodic P	2.95	1.5
Crow Pond	Episodic N+P	7.4	0
Pike Lake	Control	252.46	19.22

Pike Lake	Chronic P	23.83	10.14
Pike Lake	Chronic N+P	20.06	5.3
Pike Lake	Episodic P	136.09	12.68
Pike Lake	Episodic N+P	36.65	5.53
Lake 3	Control	NA	1.98
Lake 3	Chronic P	NA	14.3
Lake 3	Chronic N+P	NA	157.83
Lake 3	Episodic P	NA	0.79
Lake 3	Episodic N+P	NA	93.28

400

401 **Table 5**: Significance values between different treatments in each water body determined by a pairwise permutation

402 post-hoc test. Bolded comparisons represent treatments significantly different from each other at a $p<0.05$ level.

Lake	Parameter	Comparison	Stat	P Value
Stephens Lake	Chlorophyll-a	**Control - Chronic P**	-2.098	0.036
Stephens Lake	Chlorophyll-a	**Control - Episodic P**	-2.103	0.035
Stephens Lake	Chlorophyll-a	**Control -Chronic N+P**	-2.222	0.026
Stephens Lake	Chlorophyll-a	**Control - Episodic N+P**	-2.031	0.042
Stephens Lake	Chlorophyll-a	Chronic P - Episodic P	0.103	0.918
Stephens Lake	Chlorophyll-a	Chronic P - Chronic N+P	0.504	0.615
Stephens Lake	Chlorophyll-a	Chronic P -Episodic N+P	0.223	0.824
Stephens Lake	Chlorophyll-a	Episodic P - Chronic N+P	0.389	0.697
Stephens Lake	Chlorophyll-a	Episodic P - Episodic N+P	0.134	0.893
Stephens Lake	Chlorophyll-a	Chronic N+P - Episodic N+P	-0.152	0.88
Stephens Lake	Phycocyanin	**Control - Chronic P**	-2.145	0.032
Stephens Lake	Phycocyanin	**Control - Episodic P**	-2.218	0.027
Stephens Lake	Phycocyanin	**Control -Chronic N+P**	-1.972	0.049
Stephens Lake	Phycocyanin	**Control - Episodic N+P**	-1.996	0.046

Stephens Lake	Phycocyanin	**Chronic P - Episodic P**	-2.177	0.029
Stephens Lake	Phycocyanin	Chronic P - Chronic N+P	-0.864	0.388
Stephens Lake	Phycocyanin	Chronic P -Episodic N+P	1.819	0.069
Stephens Lake	Phycocyanin	**Episodic P - Chronic N+P**	2.022	0.043
Stephens Lake	Phycocyanin	**Episodic P - Episodic N+P**	2.202	0.028
Stephens Lake	Phycocyanin	Chronic N+P - Episodic N+P	1.666	0.096
Stephens Lake	Microcystin	Control - Chronic P	-0.511	0.609
Stephens Lake	Microcystin	Control - Episodic P	0.532	0.595
Stephens Lake	Microcystin	Control -Chronic N+P	0.151	0.88
Stephens Lake	Microcystin	Control - Episodic N+P	-1.344	0.179
Stephens Lake	Microcystin	Chronic P - Episodic P	1.077	0.282
Stephens Lake	Microcystin	Chronic P - Chronic N+P	0.814	0.416
Stephens Lake	Microcystin	Chronic P -Episodic N+P	-0.904	0.366
Stephens Lake	Microcystin	Episodic P - Chronic N+P	-1.096	0.273
Stephens Lake	Microcystin	Episodic P - Episodic N+P	-1.95	0.051
Stephens Lake	Microcystin	Chronic N+P - Episodic N+P	-1.929	0.054
Stephens Lake	Cylindrospermopsin	**Control - Chronic P**	-2.134	0.033
Stephens Lake	Cylindrospermopsin	**Control - Episodic P**	-2.08	0.038
Stephens Lake	Cylindrospermopsin	Control -Chronic N+P	-1.89	0.059
Stephens Lake	Cylindrospermopsin	Control - Episodic N+P	0.254	0.8
Stephens Lake	Cylindrospermopsin	Chronic P - Episodic P	0.472	0.637
Stephens Lake	Cylindrospermopsin	Chronic P - Chronic N+P	1.849	0.064
Stephens Lake	Cylindrospermopsin	Chronic P -Episodic N+P	1.947	0.052

Stephens Lake	Cylindrospermopsin	Episodic P - Chronic N+P	1.659	0.097
Stephens Lake	Cylindrospermopsin	Episodic P - Episodic N+P	1.879	0.06
Stephens Lake	Cylindrospermopsin	Chronic N+P - Episodic N+P	1.473	0.141
Crow Pond	Chlorophyll-a	Control - Chronic P	-1.382	0.167
Crow Pond	Chlorophyll-a	Control - Episodic P	-1.215	0.224
Crow Pond	Chlorophyll-a	**Control -Chronic N+P**	-2.183	0.029
Crow Pond	Chlorophyll-a	**Control - Episodic N+P**	-2.175	0.03
Crow Pond	Chlorophyll-a	Chronic P - Episodic P	0.849	0.396
Crow Pond	Chlorophyll-a	Chronic P - Chronic N+P	-0.905	0.366
Crow Pond	Chlorophyll-a	Chronic P -Episodic N+P	-1.438	0.151
Crow Pond	Chlorophyll-a	Episodic P - Chronic N+P	-1.9	0.057
Crow Pond	Chlorophyll-a	**Episodic P - Episodic N+P**	-2.023	0.043
Crow Pond	Chlorophyll-a	Chronic N+P - Episodic N+P	-1.521	0.128
Crow Pond	Phycocyanin	Control - Chronic P	-0.692	0.489
Crow Pond	Phycocyanin	Control - Episodic P	1.805	0.071
Crow Pond	Phycocyanin	Control -Chronic N+P	1.908	0.056
Crow Pond	Phycocyanin	Control - Episodic N+P	1.908	0.056
Crow Pond	Phycocyanin	Chronic P - Episodic P	1.056	0.291
Crow Pond	Phycocyanin	Chronic P - Chronic N+P	1.096	0.273
Crow Pond	Phycocyanin	Chronic P -Episodic N+P	1.096	0.273
Crow Pond	Phycocyanin	Episodic P - Chronic N+P	1	0.317
Crow Pond	Phycocyanin	Episodic P - Episodic N+P	1	0.317
Crow Pond	Phycocyanin	Chronic N+P - Episodic N+P	NA	NA
Crow Pond	Microcystin	Control - Chronic P	1.135	0.256
Crow Pond	Microcystin	Control - Episodic P	1.48	0.139
Crow Pond	Microcystin	Control -Chronic N+P	-1.506	0.132
Crow Pond	Microcystin	**Control - Episodic N+P**	-2.095	0.036
Crow Pond	Microcystin	Chronic P - Episodic P	0.477	0.633
Crow Pond	Microcystin	Chronic P - Chronic N+P	-1.829	0.067
Crow Pond	Microcystin	**Chronic P -Episodic N+P**	-2.168	0.03
Crow Pond	Microcystin	Episodic P - Chronic N+P	-1.924	0.054
Crow Pond	Microcystin	**Episodic P - Episodic N+P**	-2.205	0.027

Crow Pond	Microcystin	Chronic N+P - Episodic N+P	-1.537	0.124
Crow Pond	Cylindrospermopsin	Control – Chronic P	-0.775	0.439
Crow Pond	Cylindrospermopsin	Control – Episodic P	-1.166	0.244
Crow Pond	Cylindrospermopsin	Control – Chronic N+P	-0.123	0.902
Crow Pond	Cylindrospermopsin	Control – Episodic N+P	-1.905	0.057
Crow Pond	Cylindrospermopsin	Chronic P – Episodic P	-0.033	0.974
Crow Pond	Cylindrospermopsin	Chronic P – Episodic P	0.451	0.652
Crow Pond	Cylindrospermopsin	Chronic P – Episodic N+P	-1.592	0.111
Crow Pond	Cylindrospermopsin	Episodic P – Chronic N+P	0.605	0.546
Crow Pond	Cylindrospermopsin	Episodic P – Episodic N+P	-1.777	0.076
Crow Pond	Cylindrospermopsin	Chronic N+P – Episodic N+P	-1.501	0.134
Pike Lake	Chlorophyll-a	Control - Chronic P	-1.201	0.23
Pike Lake	Chlorophyll-a	Control - Episodic P	0.178	0.859
Pike Lake	Chlorophyll-a	**Control -Chronic N+P**	-2.215	0.027
Pike Lake	Chlorophyll-a	**Control - Episodic N+P**	-2.126	0.033
Pike Lake	Chlorophyll-a	Chronic P - Episodic P	1.156	0.248
Pike Lake	Chlorophyll-a	**Chronic P - Chronic N+P**	-2.215	0.027
Pike Lake	Chlorophyll-a	**Chronic P -Episodic N+P**	-2.118	0.034
Pike Lake	Chlorophyll-a	**Episodic P - Chronic N+P**	-2.214	0.027
Pike Lake	Chlorophyll-a	**Episodic P - Episodic N+P**	-2.124	0.034
Pike Lake	Chlorophyll-a	Chronic N+P - Episodic N+P	1.855	0.064
Pike Lake	Phycocyanin	Control - Chronic P	0.645	0.519
Pike Lake	Phycocyanin	Control - Episodic P	0.927	0.354
Pike Lake	Phycocyanin	Control -Chronic N+P	-1.854	0.064
Pike Lake	Phycocyanin	**Control - Episodic N+P**	-2.026	0.043
Pike Lake	Phycocyanin	Chronic P - Episodic P	0.128	0.898
Pike Lake	Phycocyanin	Chronic P - Chronic N+P	-1.87	0.062
Pike Lake	Phycocyanin	**Chronic P -Episodic N+P**	-1.979	0.048
Pike Lake	Phycocyanin	Episodic P - Chronic N+P	-1.886	0.059
Pike Lake	Phycocyanin	**Episodic P - Episodic N+P**	-2.028	0.043
Pike Lake	Phycocyanin	Chronic N+P - Episodic N+P	1.306	0.192
Pike Lake	Microcystin	Control - Chronic P	0.415	0.678

Pike Lake	Microcystin	Control - Episodic P	-1.876	0.061
Pike Lake	Microcystin	**Control -Chronic N+P**	-2.115	0.034
Pike Lake	Microcystin	Control - Episodic N+P	-1.69	0.091
Pike Lake	Microcystin	Chronic P - Episodic P	-1.3	0.194
Pike Lake	Microcystin	**Chronic P - Chronic N+P**	-2.113	0.035
Pike Lake	Microcystin	Chronic P -Episodic N+P	-1.696	0.09
Pike Lake	Microcystin	**Episodic P - Chronic N+P**	-2.105	0.035
Pike Lake	Microcystin	Episodic P - Episodic N+P	-1.665	0.096
Pike Lake	Microcystin	Chronic N+P - Episodic N+P	-0.425	0.671
Pike Lake	Cylindrospermopsin	Control - Chronic P	0.19	0.85
Pike Lake	Cylindrospermopsin	**Control - Episodic P**	2.048	0.041
Pike Lake	Cylindrospermopsin	Control -Chronic N+P	-0.539	0.59
Pike Lake	Cylindrospermopsin	Control - Episodic N+P	1.613	0.107
Pike Lake	Cylindrospermopsin	Chronic P - Episodic P	1.103	0.27
Pike Lake	Cylindrospermopsin	Chronic P - Chronic N+P	-0.443	0.658
Pike Lake	Cylindrospermopsin	Chronic P -Episodic N+P	0.798	0.425
Pike Lake	Cylindrospermopsin	Episodic P - Chronic N+P	-1.85	0.064
Pike Lake	Cylindrospermopsin	Episodic P - Episodic N+P	0.905	0.366
Pike Lake	Cylindrospermopsin	Chronic N+P - Episodic N+P	1.381	0.167
Lake 3	Chlorophyll-a	Control - Chronic P	1.086	0.278
Lake 3	Chlorophyll-a	Control - Episodic P	0.134	0.893
Lake 3	Chlorophyll-a	Control -Chronic N+P	-1.702	0.089
Lake 3	Chlorophyll-a	Control - Episodic N+P	-0.35	0.726
Lake 3	Chlorophyll-a	Chronic P - Episodic P	-0.962	0.336
Lake 3	Chlorophyll-a	Chronic P - Chronic N+P	-1.862	0.063
Lake 3	Chlorophyll-a	Chronic P -Episodic N+P	-1.303	0.193
Lake 3	Chlorophyll-a	Episodic P - Chronic N+P	-1.696	0.09
Lake 3	Chlorophyll-a	Episodic P - Episodic N+P	-0.46	0.646
Lake 3	Chlorophyll-a	Chronic N+P - Episodic N+P	1.67	0.095
Lake 3	Phycocyanin	Control - Chronic P	-1.934	0.053
Lake 3	Phycocyanin	Control - Episodic P	0.307	0.759
Lake 3	Phycocyanin	**Control -Chronic N+P**	-2.008	0.045
Lake 3	Phycocyanin	**Control - Episodic N+P**	-2.161	0.031
Lake 3	Phycocyanin	**Chronic P - Episodic P**	1.988	0.047

Lake 3	Phycocyanin	**Chronic P - Chronic N+P**	-1.976	0.048
Lake 3	Phycocyanin	**Chronic P -Episodic N+P**	-2.14	0.032
Lake 3	Phycocyanin	**Episodic P - Chronic N+P**	-2.01	0.044
Lake 3	Phycocyanin	**Episodic P - Episodic N+P**	-2.163	0.031
Lake 3	Phycocyanin	Chronic N+P - Episodic N+P	1.309	0.19
Lake 3	Microcystin	Control - Chronic P	0.363	0.717
Lake 3	Microcystin	Control - Episodic P	1.197	0.231
Lake 3	Microcystin	Control -Chronic N+P	-2.032	0.042
Lake 3	Microcystin	Control - Episodic N+P	-1.753	0.08
Lake 3	Microcystin	Chronic P - Episodic P	0.5	0.617
Lake 3	Microcystin	Chronic P - Chronic N+P	-1.917	0.055
Lake 3	Microcystin	Chronic P -Episodic N+P	-1.694	0.09
Lake 3	Microcystin	**Episodic P - Chronic N+P**	-2.133	0.033
Lake 3	Microcystin	Episodic P - Episodic N+P	-1.941	0.052
Lake 3	Microcystin	Chronic N+P - Episodic N+P	0.474	0.636
Lake 3	Cylindrospermopsin	Control - Chronic P	-0.714	0.475
Lake 3	Cylindrospermopsin	Control - Episodic P	-0.877	0.38
Lake 3	Cylindrospermopsin	Control -Chronic N+P	-0.793	0.428
Lake 3	Cylindrospermopsin	Control - Episodic N+P	-0.921	0.357
Lake 3	Cylindrospermopsin	Chronic P - Episodic P	0.339	0.734
Lake 3	Cylindrospermopsin	Chronic P - Chronic N+P	-0.519	0.604
Lake 3	Cylindrospermopsin	Chronic P -Episodic N+P	-0.413	0.68
Lake 3	Cylindrospermopsin	Episodic P - Chronic N+P	-0.662	0.508
Lake 3	Cylindrospermopsin	Episodic P - Episodic N+P	-0.693	0.488
Lake 3	Cylindrospermopsin	Chronic N+P - Episodic N+P	0.256	0.798

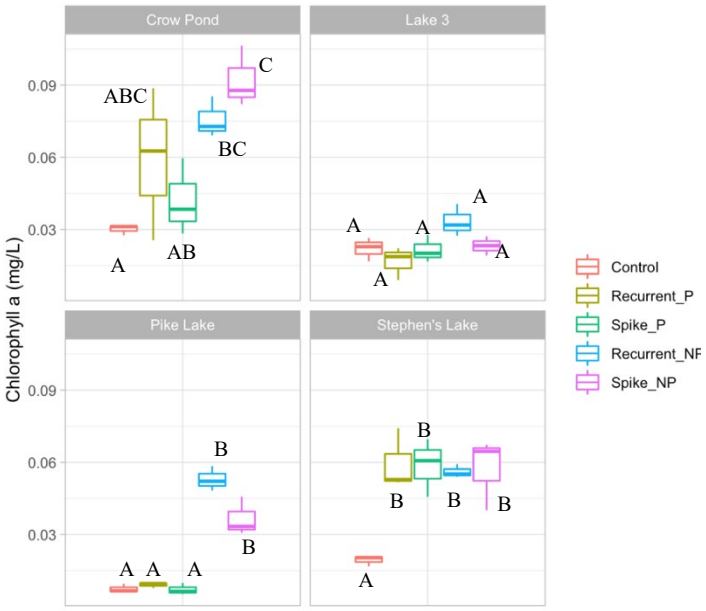

Figure 5: Chlorophyll-a concentrations for day nine. Where "Recurrent" = Chronic and "Spike" = Episodic. Treatments with the same letter indicate that they are not significantly different from one another (Table 5)

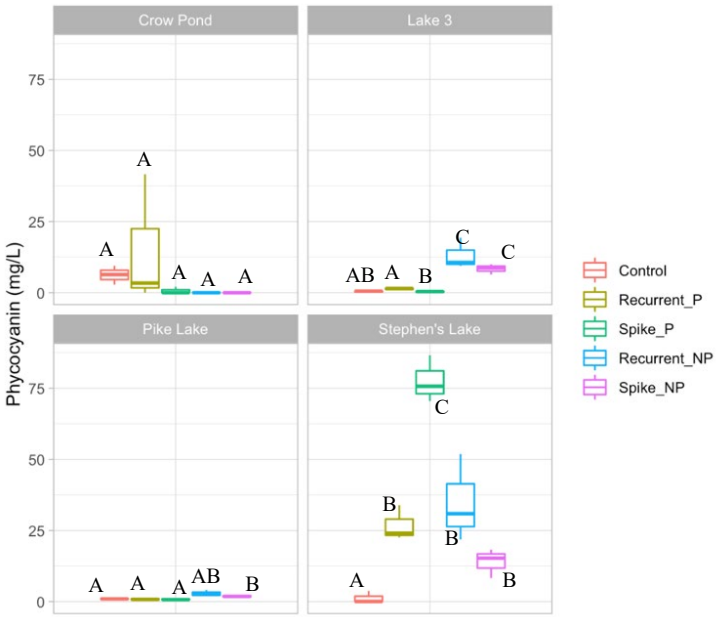

Figure 6: Note the scale is spread out to show Stephen's Lake concentrations of phycocyanin. Where "Recurrent" = Chronic and "Spike" = Episodic. Treatments with the same (Table 5).

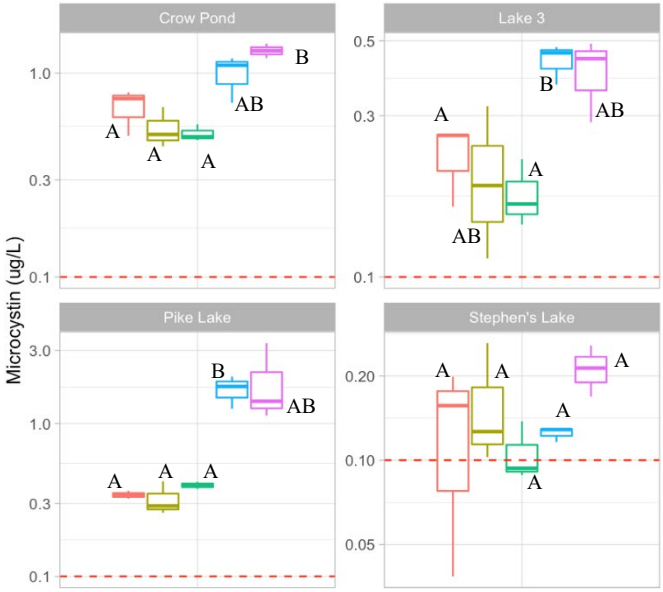

Figure 7: Day nine microcystin concentrations. Where "Recurrent" = Chronic and "Spike" = Episodic. Treatments with the same letter indicate that they are not significantly different from one another. The red line indicates the detection limit.

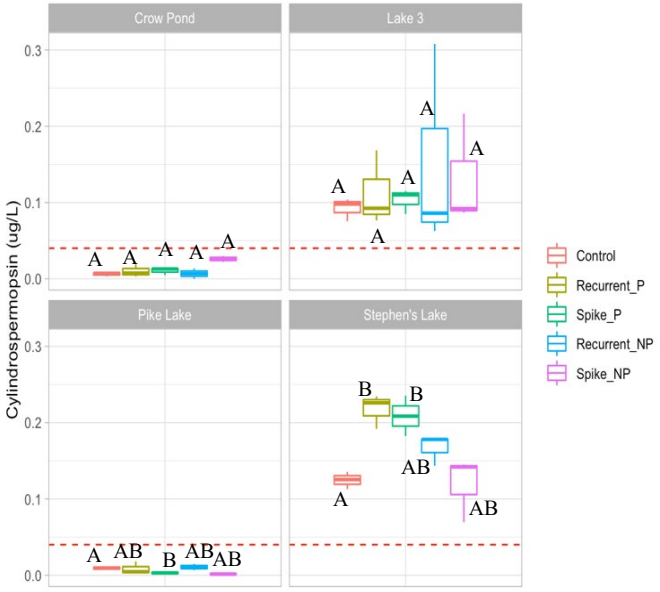

Figure 8: Day nine cylindrospermopsin concentrations. Where "Recurrent" = Chronic and "Spike" = Episodic. Treatments with the same letter indicate that they are not significantly different from one another. Red line indicates the detection limit.

Discussion

What does the data show us?

At the end of the experiment it was determined that between like nutrient additions, P or N+P, only phycocyanin saw any significant differences between the external loading mechanisms in Stephens Lake and Lake 3. Phycocyanin concentrations in Stephens Lake were significantly higher in the episodic addition of P compared to the chronic addition of P (Table 5, Figure 6). Conversely, in Lake 3 the episodic addition of P created significantly higher phycocyanin concentrations compared to the chronic addition of P. Besides these two instances, the external loading mechanism alone did not significantly increase or decrease the concentration of any of the other parameters. Despite a lack of significance in the loading mechanism used, there was significance among different treatments (P vs N+P) as can be seen in Table 5 and Figures 2-5. Episodic additions of N+P in Crow Pond had a significantly higher chlorophyll-a concentration than the episodic addition of P (Table 4, Figure 5). Pike Lake chlorophyll-a concentrations were significantly higher in both the additions of N+P compared to all the other treatments (Table 5, Figure 5). Phycocyanin significance, which was measured as a proxy for cyanobacteria concentrations, showed that both N+P additions were significantly lower in phycocyanin concentrations than both of the P only treatments for Crow Pond. Pike Lake on the other hand showed that the episodic addition of N+P had significantly higher phycocyanin values compared to both P only additions. Microcystin analysis showed that the episodic addition of N+P created significantly higher microcystin concentrations than both the chronic and episodic P treatments. The microcystin concentration of the chronic N+P addition in Pike Lake was significantly higher than both the chronic and episodic P treatments. Similarly, the chronic addition of N+P in Lake 3 had significantly higher concentrations of microcystin than the

episodic P addition. This data shows that in some cases additions of P alone impacted the concentration of the different parameters differently than the additions of N+P. However, with exception of the Stephens Lake and Lake 3 chronic and episodic P additions, loading mechanism (chronic vs episodic) did not significantly impact the concentrations of any of the parameters tested. These results indicate that differences in external loading mechanisms do not cause differences in cyanobacteria concentrations, as they do not experience major changes in growth when nutrients enter the water body through episodic means, or when nutrients are added chronically to the water body. These results suggest that while nutrient loads matter, changes in external loading mechanisms are insignificant in the growth of cyanobacterial blooms and their toxicity.

Day 0 to Day Nine Change

Chlorophyll-a readings were taken of the initial lake water and compared to the average of controls on day nine of our experiment (Figure 6). Pike Lake and Crow Pond both saw a decrease in chlorophyll-a when comparing initial conditions to day nine. Pike Lake saw a decrease of -0.0027 mg/L while Crow Pond saw a larger drop of -0.49 mg/L. Crow Pond also saw a small decrease in phycocyanin; no initial phycocyanin reading was taken for Pike Lake. This decline could have been caused by insufficient light during the experiment. During collection of Crow Pond, light readings were 43.31 $\mu molm^{-2}sec^{-}1$greater during the time of collection than at the time of incubation which may have hindered photosynthesis. Temperature also could have played a factor, as average temperature in the incubation tanks was measured 2.73 °C lower than the temperature measured during collection. Additionally, only one water sample was used to determine to chlorophyll-a concentration for the initial lake water. This one value may represent an outlier and may not be representative of the entire water body.

Microcystin also saw a decline in Pike Lake from 2.36 μg/L to 0.34 μg/L. Microcystin is a nitrogen rich secondary metabolite (Van de Waal et. al , 2014), and thus needs amble amounts of nitrogen in order to form; examining Redfields TN:TP ratio for the controls on day nine showed that Pike Lake moved from a ratio of 37.87 (Table 1) to 19.22 (Table 4). This 49 % decrease in Redfields ratio may explain the decline in microcystin in Pike Lake observed through the experiment.

Additional downsides to our experiment are that it only lasted over a nine day period. These nine days may not have allowed for changes in population dynamics of different phytoplankton phyla (Heisler et al., 2008). Another downside is grazers. Zooplankton were observed in at least two of the four lakes during filtering in day nine, Stephens Lake and Crow Pond. These zooplankton can graze on phytoplankton, altering which form of phytoplankton may be dominant as some phytoplankton are better food sources than others.

Are Our Results Consistent with Literature?

Our results indicate that nutrients entering the water body chronically or episodically do not significantly affect the rates or toxicity of cyanobacteria dominant harmful algal blooms. In other words, as long as factors such as light, water temperature, and nutrients are sufficiently available for cyanobacteria, the cyanobacteria will grow. In an experiment examining how increases in temperature and nutrients simulating climate change affect cyanobacteria growth in urban water bodies, increasing temperature favored cyanobacteria growth over other eukaryotic phytoplankton, but a pulse of nutrients N and P in the form of $NaNO_3$ and K_2HPO_4 had a much greater effect (Lürling et al., 2018). While our experiment did experience in increase in phytoplankton growth shown by the significant increases in chlorophyll-a (Table 5) Lürling et al., (2018) only tested episodic additions of nutrients. Episodic loading is predicted to increase in

478 the future as temperatures continue to rise (Lürling et al., 2018; O'Neil et al., 2012). The increase
479 in temperatures increase evaporation rates and lead to both longer periods of drought, and more
480 intense and frequent storms (Carey et al., 2012; Paerl & Huisman, 2009). Our study has shown
481 that episodic nutrient entry is not significantly different from chronic entry, which disputes
482 Lürling et al., (2018) claim that episodic specific events will increase the prevelance of
483 cyanobacteria blooms. A review by Heisler et al. (2008) stated the idea that both chronic and
484 episodic nutrient loading will promote the formation of cyanobacterial algal blooms. While no
485 studies examining the effects of both chronic and episodic nutrient loading was given in the
486 review, Heisler et al. (2008) gave case studies where both chronic and episodic loading were able
487 to promote HABs. Our experiment is in support with this review, as both chronic and episodic
488 additions supported the growth of cyanobacteria and cyanotoxins.

489 ***Conclusions***

490 Studies have shown that increasing temperatures increase the prevelence of harmful
491 cyanobacteria (Davis et al., 2009). The presence of cyanobacteria may be influenced on local
492 scales by changes in temperature, precipitation, and land use (Taranu et al., 2017). Toxin-
493 producing cyanobacteria tend to outcompete non-toxin producing cyanobacteria in higher
494 temperatures, and limiting temperature rise could prove important for reducing the occurrences
495 and toxicity of HABs (Davis et al., 2009; O'Neil et al., 2012). As flooding events increase as a
496 result of global climate change, so will cyanobacterial blooms (Carey et al., 2012). Our study has
497 shown that blooms will not increase with flooding due to shift from chronic nutrient loading to
498 an episodic nutrient loading pathway specifically, but only because it will allow for greater
499 amounts of nutrients to enter water body. Increases in impermeable surfaces and anthropogenic
500 caused climate change will possibly increase the load of nutrients entering the water. In this

study the form of nutrient deliever was found to insignificant for the growth of cyanobacteria and their associated toxins microcystin and cylindrospermopsin, however, the idea that a larger nutrient load could enter the water body may be significant as cyanobacteria and the cyanotoxins will respond to the load (concentration times flow) rather than the change in chronic or episodic nutrient loading. The best way to protect our water bodies may not be found in the water, but may be found on the land by using more sustainable agricultural practices that allow for less nutrient runoff. Therefore, management and mitigation practices should not focus on reducing the episodic nutrient loading mechanism but rather the size of the nutrient load able to be deposited into a water body.

Citation list

APHA (2017). Standard Methods for the Examination of Water and Wastewater, 23 Edn, eds E. W. Rice, R. B. Baird, and A. D. Eaton (Washington, DC: American Public Health Association).

Backer, L. C., McNeel, S. V., Barber, T., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, T. B., Nierenberg, K., Aubel, M., LePrell, R., Chapman, A., Foss, A., Corum, S., Hill, V. R., Kieszak, S. M., & Cheng, Y. S. (2010). Recreational exposure to microcystins during algal blooms in two California lakes. *Toxicon*, *55*(5), 909–921. https://doi.org/10.1016/j.toxicon.2009.07.006

Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impact on erodable phosphorus and eutrophication: A global perspective. *BioScience*, *51*(3), 227–234. https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Bhagowati, B., Talukdar, B., & Ahamad, K. U. (2020). *Lake Eutrophication: Causes, Concerns and Remedial Measures*. Springer Singapore. https://doi.org/10.1007/978-981-32-9771-5_12

Bogard, M. J., Vogt, R. J., Hayes, N. M., & Leavitt, P. R. (2020). Unabated Nitrogen Pollution Favors Growth of Toxic Cyanobacteria over Chlorophytes in Most Hypereutrophic Lakes. *Environmental Science and Technology*, *54*(6), 3219–3227. https://doi.org/10.1021/acs.est.9b06299

Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P., & Brookes, J. D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research*, *46*(5), 1394–1407. https://doi.org/10.1016/j.watres.2011.12.016

Chellappa, N. T., Chellappa, S. L., & Chellappa, S. (2008). Harmful phytoplankton blooms and fish mortality in a eutrophicated reservoir of Northeast Brazil. *Brazilian Archives of Biology and Technology*, *51*(4), 833–841. https://doi.org/10.1590/S1516-89132008000400022

Chen, J., Bian, R., Li, J., Qiu, L., Lu, B., & Ouyang, X. (2019). Chronic exposure to microcystin-LR reduces thyroid hormone levels by activating p38/MAPK and MEK/ERK signal pathway. *Ecotoxicology and Environmental Safety*, *173*(January), 142–148. https://doi.org/10.1016/j.ecoenv.2019.02.024

Davis, T. W., Berry, D. L., Boyer, G. L., & 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*(5), 715–725. https://doi.org/10.1016/j.hal.2009.02.004

Davis, T. W., Bullerjahn, G. S., Tuttle, T., McKay, R. M., & Watson, S. B. (2015). Effects of Increasing Nitrogen and Phosphorus Concentrations on Phytoplankton Community Growth and Toxicity During Planktothrix Blooms in Sandusky Bay, Lake Erie. *Environmental Science & Technology*, *49*(12), 7197–7207. https://doi.org/10.1021/acs.est.5b00799

Davis, T. W., Harke, M. J., Marcoval, M. A., Goleski, J., Orano-dawson, C., Berry, D. L., & Gobler, C. J. (2010). *Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of Microcystis during cyanobacterial blooms*. *61*, 149–162. https://doi.org/10.3354/ame01445

Downing, J. A., Watson, S. B., & McCauley, E. (2001). Predicting cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(10), 1905–1908. https://doi.org/10.1139/f01-143

566 Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H.,
567 Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of
568 nitrogen and phosphorus limitation of primary producers in freshwater, marine and
569 terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. https://doi.org/10.1111/j.1461-
570 0248.2007.01113.x

571 Floate, K., and J. D. Shorthouse. 2010. Ecoregions of Canada's prairie grasslands. Arthropods of
572 canadian grasslands (volume 1): Ecology and interactions in grassland habitats, 1(volume
573 1). 53–81.

574 Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., Sheppard, L. J., Jenkins,
575 A., Grizzetti, B., Galloway, N., Vitousek, P., Leach, A., Bouwman, A. F., Butterbach-bahl,
576 K., Dentener, F., Stevenson, D., Amann, M., Voss, M., & Fowler, D. (2013). *The global*
577 *nitrogen cycle in the twenty- first century*.

578 Government of Canada. 2012. Guidelines for Canadian recreational water quality – third edition.
579 https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-
580 canadian-recreational-water-quality-third-edition/guidelines-canadian-recreational-water-
581 quality-third-edition-page-11.html (accessed 10 February 2021)

582 Government of Canada. 2019. Station results: historical data.
583 https://climate.weather.gc.ca/historical_data/search_historic_data_stations_e.html?search
584 Type=stnName&timeframe=1&txtStationName=saskatoon&searchMethod=contains&op
585 tLimit=yearRange&StartYear=1901&EndYear=2020&Year=2020&Month=2&Day=4&s
586 elRowPerPage=25 (accessed 10 February 2021)

587 Government of Canada. 2021. Guidelines for Canadian drinking water quality: guideline

technical document- cyanobacterial toxins. https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-cyanobacterial-toxins-document.html (accessed 11 February 2021)

Government of Saskatchewan. 2019. Saskatchewan recreational water sampling results. https://publications.saskatchewan.ca/api/v1/products/102039/formats/112845/download (accessed 16 October 2020)

Hauser-davis, R. A., Teixeira, R., Cavalcanti, R., Gonzalo, E., Rojas, A., Wagner, A., Guarino, S., & Lourenço, R. (2015). Ecotoxicology and Environmental Safety Accumulation and toxic effects of microcystin in tilapia (Oreochromis niloticus) from an eutrophic Brazilian lagoon. *Ecotoxicology and Environmental Safety*, *112*, 132–136. https://doi.org/10.1016/j.ecoenv.2014.10.036

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. A., Stoecker, D. K., & Suddleson, M. (2008). *Eutrophication and harmful algal blooms : A scientific consensus*. *8*, 3–13. https://doi.org/10.1016/j.hal.2008.08.006

Jetoo, S., Grover, V. I., & Krantzberg, G. (2015). *The Toledo Drinking Water Advisory: Suggested Application of the Water Safety Planning Approach*. 9787–9808. https://doi.org/10.3390/su7089787

Jones, J. R., Obrecht, D. V., Perkins, B. D., Knowlton, M. F., Thorpe, A. P., Watanabe, S., & Bacon, R. R. (2008). Nutrients, seston, and transparency of missouri reservoirs and oxbow

lakes: An analysis of regional limnology. *Lake and Reservoir Management*, *24*(2), 155–180. https://doi.org/10.1080/07438140809354058

Knowlton, M. F. (1984). FLOW-THROUGH MICROCUVETTE FOR FLUOROMETRIC DETERMINATION OF CHLOROPHYLL1. *JAWRA Journal of the American Water Resources Association*, *20*(5), 795–799. https://doi.org/https://doi.org/10.1111/j.1752-1688.1984.tb04763.x

Lürling, M., Mello, M. M., van Oosterhout, F., Domis, L. de S., & Marinho, M. M. (2018). Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. *Frontiers in Microbiology*, *9*(AUG), 1–14. https://doi.org/10.3389/fmicb.2018.01851

Miller, W. D., Harding, L. W., & Adolf, J. E. (2006). Hurricane Isabel generated an unusual fall bloom in Chesapeake Bay. *Geophysical Research Letters*, *33*(6), 2–5. https://doi.org/10.1029/2005GL025658

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Paerl, H. W., & Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, *1*(1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x

Paerl, H. W., Scott, J. T., Mccarthy, M. J., Newell, S. E., Gardner, W. S., Havens, K. E., Ho, D. K., Wilhelm, S. W., & Wurtsbaugh, W. A. (2016). *It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream*

Ecosystems. https://doi.org/10.1021/acs.est.6b02575

Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., & Neilan, B. (2010). On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. In *Marine Drugs* (Vol. 8, Issue 5). https://doi.org/10.3390/md8051650

Petty, E. L., Obrecht, D. V., & 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. *Frontiers in Environmental Science*, *8*(July). https://doi.org/10.3389/fenvs.2020.00111

Pick, F. R., & Lean, D. R. S. (1987). The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 425–434. https://doi.org/10.1080/00288330.1987.9516238

Pretty, J. N., Mason, C. F., Nedwell, D. B., Hine, R. E., Leaf, S., & Dils, R. (2003). Environmental costs of freshwater eutrophication in England and Wales. *Environmental Science and Technology*, *37*(2), 201–208. https://doi.org/10.1021/es020793k

Pugliese, G., & Favero, M. S. (1998). Liver Failure and Death After Exposure to Microcystins at Hemodialysis Center. *Infection Control & Hospital Epidemiology*, *19*(6), 465–465. https://doi.org/10.1017/s0195941700087890

Rapala, J., Sivonen, K., Lyra, C., & Niemelä, S. I. (1997). Variation of microcystins, cyanobacterial hepatotoxins, in Anabaena spp. as a function of growth stimuli. *Applied and Environmental Microbiology*, *63*(6), 2206–2212. https://doi.org/10.1128/aem.63.6.2206-2212.1997

Redfield, A. C. (1934). On the Proportions of Organic Derivatives in Sea Water and Their Relation to the Composition of Plankton. *University Press of Liverpool, James Johnstone Memorial Volume*, 1767–192.

Sartory, D. P., & Grobbelaar, J. U. (1984). Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, *114*(3), 177–187. https://doi.org/10.1007/BF00031869

Schindler, D. W. (2012). The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1746), 4322–4333. https://doi.org/10.1098/rspb.2012.1032

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science and Technology*, *50*(17), 8923–8929. https://doi.org/10.1021/acs.est.6b02204

Shatwell, T., & Köhler, J. (2019). *Decreased nitrogen loading controls summer cyanobacterial blooms without promoting nitrogen- fi xing taxa : Long-term response of a shallow lake*. 166–178. https://doi.org/10.1002/lno.11002

Smith, V. H. (2010). *Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton Published by : American Association for the Advancement of Science Stable URL : http://www.jstor.org/stable/1691193 Low Nitrogen to Phosphorus Ratios Favor Dominan*. *221*(4611), 669–671.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: http://websoilsurvey.sc.egov.usda.gov/. Accessed [April/23/2021].

675 Sunda, W. G., Graneli, E., & Gobler, C. J. (2006). Positive feedback and the development and
676 persistence of ecosystem disruptive algal blooms. *Journal of Phycology*, *42*(5), 963–974.
677 https://doi.org/10.1111/j.1529-8817.2006.00261.x

678 Taranu, Z. E., Gregory-Eaves, I., Steele, R. J., Beaulieu, M., & Legendre, P. (2017). Predicting
679 microcystin concentrations in lakes and reservoirs at a continental scale: A new framework
680 for modelling an important health risk factor. *Global Ecology and Biogeography*, *26*(6),
681 625–637. https://doi.org/10.1111/geb.12569

682 Van de Waal, D. B., Smith, V. H., Declerck, S. A. J., Stam, E. C. M., & Elser, J. J. (2014).
683 Stoichiometric regulation of phytoplankton toxins. *Ecology Letters*, *17*(6), 736–742.
684 https://doi.org/10.1111/ele.12280

685 Vasconcelos, J. F., Barbosa, J. E. L., Lira, W., & Azevedo, S. M. F. O. (2013). Microcystin
686 bioaccumulation can cause potential mutagenic effects in farm fish. *Egyptian Journal of*
687 *Aquatic Research*, *39*(3), 185–192. https://doi.org/10.1016/j.ejar.2013.11.002

688 Zhang, D., Xie, P., Liu, Y., & Qiu, T. (2009). Transfer, distribution and bioaccumulation of
689 microcystins in the aquatic food web in Lake Taihu, China, with potential risks to human
690 health. *Science of the Total Environment*, *407*(7), 2191–2199.
691 https://doi.org/10.1016/j.scitotenv.2008.12.039

692