The Effect of Single Versus Dual Nutrient Control on Phytoplankton Growth Rates, Community Composition, and Microcystin Concentration in the Western Basin of Lake Erie

by

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A thesis submitted

in partial fulfillment of the requirements

for the degree of

Master of Science

School for Environment and Sustainability

August 2019

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Abstract

The primary management strategy for minimizing harmful algal blooms (HABs) in Lake Erie has been to reduce springtime loading of phosphorus (P) to the lake. However, some studies have shown that the growth rate and yield, particularly for the HABs-causing cyanobacterium *Microcystis*, also respond to the availability of dissolved inorganic nitrogen (N). This evidence is based on both observational studies that correlate bloom development with changes in N concentrations in the lake, and experiments in which P and and/or N are added at concentrations in excess of those present in the lake. The goal of this study was to determine whether a combination of reduced N and P from ambient levels in Lake Erie could limit the development of HABs more than a reduction in P concentration only. To directly test the impact of P-only versus N and P reduction on phytoplankton in the western basin of Lake Erie, we evaluated changes in growth rate, community composition, and microcystin (MC) concentration through eight bioassay experiments performed from June through October 2018, which encompassed the normal Lake Erie *Microcystis*-dominated HAB season. Our results showed that during the first five experiments covering June 25 to August 13, the –P and the –N-P treatment had similar effect, but later in the season when ambient N becomes scarce, the –N-P reductions resulted in negative growth rates for cyanobacteria whereas –P only reductions did not. During low ambient N conditions, dual nutrient reduction lowered the prevalence of cyanobacteria among the total phytoplankton community and decreased microcystin concentrations. The results presented here suggest that dual nutrient control could be an effective management strategy to decrease microcystin production during the bloom and

even possibly diminish or shorten the bloom based on creating nutrient limiting

conditions sooner in the HAB growing season.

Acknowledgements

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Introduction

Freshwater ecosystems have experienced a recent increase in the occurrence of cyanobacterial harmful algal blooms (cHABs) (Paerl et al., 2016a; Paerl and Huisman, 2009; Smith 2003). This global phenomenon has also been observed in Lake Erie, one of the Laurentian Great Lakes that serves as a source of drinking water and recreation for millions of people (Fuller et al., 2002; Munawar et al., 1989). The cHABS found in western Lake Erie are most commonly dominated by the cyanobacterium *Microcystis aeruginosa* that produces secondary metabolite cyanotoxins called microcystins (MCs) (Rinta-Kanto et al. 2005). These toxins can contaminate potable water supplies and lead to serious threats to public health (Bullerjahn et al., 2016; Pouria et al., 1998; Qin et al., 2010). The Great Lakes Water Quality Agreement (GLWQA) of 1972 identified reductions to phosphorus (P) loads as a primary goal for initially managing eutrophication in the lake (Bertram 1993; DePinto et al, 1986; Makarewicz 1985). A continued focus on P control was reported in several studies that compared inter-annual correlations between P load and total bloom biomass (Kane et al., 2014; Scavia et al., 2014; Stumpf et al., 2012) and in bioassay experiments in which P was added to lake water to examine its effect on algal growth (Saxton et al., 2012; Moon and Carrick, 2007). However, despite these research findings and the past successes in reducing P inputs compared to the levels measured in the 1960s, there has been a resurgence of cHABs in western Lake Erie over the past decade (Stumpf et al. 2016, Chaffin et al. 2008). In response to this current resurgence of cHABS, current management recommendations are set to decrease P loading from the Maumee River, a major source

of agricultural P runoff, into Lake Erie by 40% by 2025 as compared to current P loads (Annex 4 2015; Verhamme et al., 2016). This reduction in P is expected to have beneficial impacts on mitigating the harmful algal blooms that dominate the western basin of Lake Erie, but may have some limitations.

A number of factors have been proposed for explaining the recent increase in cHABs, including changes in the forms of P entering the lake from its watershed (Bertani et al., 2016), invasive mussels that recycle P (Hecky et al., 2004; Vanderploeg et al., 2001), and changes in precipitation patterns that alter the timing of P inputs (Paerl et al., 2016a, Michalak et al., 2013; Paerl and Huisman, 2009). While each of these mechanisms are supported to some extent, there is evidence that N is both a crucial limiting nutrient in freshwater eutrophication (Conley et al., 2009; Lewis and Wurtsbaugh, 2008) and may impact cyanobacterial biomass and toxin concentration (Muller and Mitrovic, 2015, Gobler et al., 2016; Newell et al., 2019). Non-N_2 fixing cyanobacteria, such as *Microcystis*, are capable of bloom formation in lakes that maintain a high N:P ratio (Paerl and Otten, 2013). Eutrophic systems, such as the western basin of Lake Erie, are prone to increased biomass production in established cHABs due to additional N inputs especially if the system contains enough autochthonous P (Paerl and Otten, 2013). When P is widely available N has the capability to constrain the growth of cyanobacteria during bloom conditions, while excess N inputs often leads to excessive algal production (Chaffin et al., 2013; Jeppesen et al., 2005; Paerl et al., 2016b; Gobler et al., 2016). Additionally, in Lake Erie dissolved inorganic nitrogen (N) is widely available through the early part of August following high spring tributary loads, and this seasonal availability plays a pivotal

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role in bloom duration (Gobler et al. 2016). Numerous studies have shown that N and P provided in unison yield greater biomass concentrations compared to single nutrient amendments (Elser et al., 2007) and multiple bioassay experiments have shown a positive response to N additions in Lake Erie (Chaffin et al., 2018; Newell et al., 2019). Studies have indicated that phytoplankton in Lake Erie, *Microcystis* in particular, are responsive to the availability of nitrogen in the lake (Chaffin et al., 2018, Newell et al., 2019). Bloom development over the course of the season is coincident with a reduction in dissolved inorganic N concentration (Chaffin et al., 2013; Jankowiak et al., 2019). Many researchers have advocated for management of both P and N to alleviate eutrophication and cyanobacterial blooms due to the importance of N in bloom ecology (Paerl et al., 2016; Cotner 2016); however, others recommend a P-only reduction to reduce lake eutrophication (Schindler et al., 2016, Baker et al., 2019). Based on these more recent studies and a broader examination of nutrient effects, it is apparent that the reduction of both N and P in management practices has the potential to be more effective in constraining biomass than P-only reductions.

In addition to acting as a secondary limiting nutrient for growth of HABs, nitrogen also impacts the production of microcystin by cyanobacteria. Evidence has suggested that N availability and form is linked to toxin production (Orr et al., 1998, Newell et al., 2019), where N has been documented to regulate the *mcy* genes dedicated to the synthesis of the cyanobacterial hepatotoxin, MC (Harke and Gobler, 2013; Harke and Gobler, 2015; Davis et al, 2015, Oullette et al., 2006). In *Microcystis*, the transcription of N uptake and MC production genes are both controlled by the same

mechanism of the NtcA (global nitrogen regulator) transcription factor, suggesting that N metabolism coincides with microcystin synthesis (Pimentel and Giani, 2014, Harke and Gobler, 2013). Under N-depleted conditions, *Microcystis* allocates its N toward cell functions required for survival and growth (Harke and Gobler, 2013). Multiple studies have emphasized the importance of N in controlling microcystin levels and the proportion of potentially toxin-producing cells in Lake Erie cHABS (Jankowiak et al., 2019; Chaffin et al., 2018; Gobler et al., 2016), and studies by Davis et al. (2015) and Donald et al. (2011) have found a greater increase in microcystin concentrations in response to N than to P additions. The reduction of both N and P in management practices has the capability to be more effective in reducing toxin production, as *Microcystis* is unlikely to produce toxins under low N conditions. One of the challenges for clearly understanding the potential benefits of managing both N and P, is that most previous work regarding dual-nutrient control has focused on nutrient addition experiments, versus responses to direct N or P reductions. This latter type of evaluation can provide critical information to inform potential outcomes of policy-driven reductions.

Our overarching goal for this study was to determine if reductions of both P and N, compared to P-only, has a greater capability to decrease the growth rate, toxin concentrations and prevalence of *Microcystis* among the phytoplankton community in western Lake Erie when ambient concentrations of N are low as seen in the end of August and September. To address this goal, we tested three hypotheses: 1) Dual nutrient reductions are required to reduce growth rates in comparison to single nutrient control when ambient N concentrations are low; 2) Dual nutrient reduction will decrease the

abundance of cyanobacteria within the phytoplankton community, particularly when N is low; and 3) Dual nutrient control will reduce toxin concentration, biomass normalized toxicity, and proportion of mcyE containing cyanobacteria when compared to single or no nutrient control. Our experiments were performed with intact phytoplankton communities from the western basin of Lake Erie in order to identify the role of single and dual nutrient reductions on cyanobacterial growth rates, community prevalence, and MC production.

Methodology

        In order to test our hypotheses during different stages of the bloom, we collected water on eight occasions in 2018 from pre-bloom (June) to post-bloom season (October). Lake sampling dates were June 25, July 16, July 23, July 31, August 13, August 27, September 11, and October 1. We collected water from the NOAA Great Lakes Environmental Research Laboratories monitoring site WE2 (41° 43.4619 -83° 22.2134) located in the western basin approximately 14.5 kilometers northeast of the Maumee River mouth and 15 kilometers west of the municipal water intake for the city of Toledo, Ohio. Selection of site WE2 was based on using a location known to be influenced by Maumee River effluent and the availability of real-time nutrient data from a continuous monitoring buoy. For each sampling event between 50 and 60 L of lake water were collected via peristaltic pump at 1-meter below the water surface and stored in dark insulated containers. Once at the laboratory (approximately 4 hours after collection), the carboys were inverted 10 times to ensure water was well mixed. A series of dilution experiments was conducted to determine the impacts of phosphorus-only reductions and dual-nutrient control in Lake Erie (Paerl et al., 2016b). Our treatments were -N-P, -P, Ambient, and +N+P. Ambient levels refer to the nutrient concentrations as determined by in-situ WE2 buoy data at noon (12:00pm) of that day. To dilute nutrients to below ambient concentrations, lake water was homogenized with Hard Water Mussel Media (HWMM) and then amended with treatment specific nutrient amendments. HWMM is a salt solution that reflects the major ion chemistry of Lake Erie and contains no major nutrients (Chapra et al, 2012). Each mesocosm was replicated three times in each experiment using 4-L polycarbonate bottles.

For our first treatment, -N-P, no additional nutrients were added following the 40% dilution with HWMM and represents a total 40% reduction of nutrients and biomass. For our second treatment, -P, N is added back to ambient levels and represents a 40% reduction of phosphorus and biomass. For our third treatment, Ambient, N and P were added back to ambient levels. If phosphorus levels were not detected via buoy sensors, then 1.5 ug/l of phosphorus was added to represent a typical low-level concentration observed by laboratory analysis of the weekly NOAA-GLERL monitoring. For our fourth treatment, +N+P, N and P were added back to match ambient levels, and then spiked with additional N and P to represent the nutrient conditions under the influence of spring loads (2mg/L N and 20ug/L P). If phosphorus levels were not detected, then the final concentration of P in the +N+P treatment was brought to 21.5 ug/L. The mesocosm bottles were placed in an insulated incubation tank with moving water controlled by two pumps oriented to create a whirlpool effect and maintain bottle movement (Rowe et al., 2016). This water movement was designed to reduce *Microcystis* colony settling and promote gentle mixing with the treatment bottle. The tanks were temperature controlled within 1°C of the measured Lake Erie water temperatures at station WE2 at noon (12:00pm) of the collection day by a recirculating temperature Bath (Cole-Parmer Instrument Company). We used a neutral density filter to cut light intensity to 50% of surface irradiance to represent the light intensity that algal cells would undergo at approximately 1 meter in depth (sampling depth). An RDR Solo temperature sensor was placed in the tank to monitor tank temperature.

Response variables identified include size-fractionated chlorophyll, fluorometry, CHN, dissolved organic nutrients, particulate phosphorus, pH, qPCR (total cyano-16s and toxin *mcyE*), and particulate microcystins. On Day 0 (T_0), Day 3 (T_3), and Day 7 (T_7) the entire range of parameters were collected. Day 0 was collected from the homogenized treatment water following initial dilution. Experiment 1 ran for 9 days and the midpoint was collected on Day 4, to maintain consistency across all experiments T_3 and T_7 will refer to Day 4 and Day 9 respectively regarding experiment Day 3 and Day 7 data was collected from each individual bottle around 9am local time.

Biomass Concentrations

Size-fractionated chlorophyll was analyzed by filtering two replicate subsamples (approximately 150 mL) at T_0, T_3, and T_7 through 53 µm Nitex screen or a Whatman GF/F filter (Bowers 1980; Vanderploeg et al. 2001). Screens and filters were frozen and later extracted with N,N-dimethylformamide (DMF) and analyzed fluorometrically (Speziale et al. 1984) using a Turner Designs 10-AU fluorometer. Total chlorophyll was obtained from the sum of the two size fractions. A benchtop Fluoroprobe (bbe Moldaenke, Series 3) was used to identify algal group-specific fluorescence. The phytoplankton group concentrations were allocated from the total fluorescent concentration to a spectral algal class due to its fluorescence spectrum (Catherine et al., 2012, Chaffin et al. 2013). In addition to a full range of parameters collected on T_0, T_3, and T_7, daily fluorometry data was collected at 9am. Particulate carbon (C) and N were determined by flash combustion method using a Carlos Erba EA1110 configured for CHN. We estimated exponential growth rates during days 0-3 for both in vivo

fluorescence and extracted chlorophyll using the following equation:

$$\frac{(ln(biomass_3) - ln(biomass_0))}{t_3 - t_0}$$

Particulate Microcystins (MCYs)

  Particulate MCYs were determined from filtered mesocosm samples. Samples underwent three freeze/thaw cycles, QuikLyse Cell Lysis (Abraxis #529911QL), and were quantified using a microcystin/nodularin-specific enzyme-linked immunosorbent assay (ELISA) (Abraxis #520011; Fischer et al. 2001).

Nutrient Concentrations

  Concentrations of nitrate, ammonium, and dissolved reactive P were quantified with a Seal I AA3 continuous segmented flow analyzer (SEAL Analytical Inc., Mequon, WI) using standard U.S. EPA methods (EPA 353.1, 354.1, 350.1, and 365.1, respectively). Particulate P from filtered mesocosm samples was determined by persulfate digestion adapted from Menzel and Corwin (1965), followed by the ascorbic acid molybdenum blue method (Strickland and Parsons 1972).

qPCR (total cyano-16s and toxin mcyE)

  Estimates of total cyanobacterial cell concentrations and the proportion of *mcyE* containing cyanobacteria within the treatments were determined using quantitative polymerase chain reaction (qPCR). Toxin concentration is the overall concentration of particulate microcystins and was determined for all experiments except for the Oct 1 experiment. Biomass normalized toxicity refers to particulate microcystins normalized to cyanobacterial fluorescence and was determined for all experiments except for the Oct 1 experiment. Toxin concentration was normalized to cyanobacterial fluorescence in order

to get a more accurate prediction of potential toxin production within the cyanobacterial population. The proportion of *mcyE* containing cyanobacteria refers to the number of *mcyE* gene copies normalized to the number of Cyano-16S gene copies at T7 and was determined for all experiments except for the June 26 and Oct 1 experiments. For quantification, total cellular nucleic acids were extracted from filtered mesocosm samples using the Qiagen DNeasy Blood and Tissue Kit, adding a lysate homogenization step (QiaShredder spin-column) prior to DNA purification. The quantity and quality of nucleic acids were determined using a NanoDrop Lite Spectrophotometer (Thermo Scientific). DNA extract was frozen at -80 °C until analysis. Two Cyanobacteria-specific genetic targets were used during this study, the 16S-Cyano rRNA gene (16S rDNA) and the mcyE gene. Targeting the 16S rRNA gene allows for the quantification of the abundance of total Microcystis population within the mesocosm. The mcyE gene is found within the microcystin synthetase gene cluster and is one of the genes responsible for the production of microcystin (Genuario et al., 2010; Tillet et al.,2000). *mcyE* is only found in potentially-toxic strains of *Microcystis* and allows for the quantification of toxin potential in the mesocosm. qPCR was executed using an Applied Biosystems 7500 Fast Instrument using TaqMan labeled probes (Applied Biosystems) and Cyanobacteria-specific mcyE and 16S-Cyanobacteria rDNA primers. For amplification of the both the 16S and mcyE gene targets, the cycling conditions were for 95 °C for 2 minutes for initial denaturation, followed by 40 cycles of 95 °C for 15 seconds for denaturation and 60 °C for 30 seconds for annealing-extension.

Statistical Analyses

For each experiment, a one-way analysis of variance (ANOVA) was used for each treatment to examine the effects of total fluorescence and cyanobacterial growth rate, particulate toxins, particulate MCs normalized to cyanobacterial fluorescence, *mcyE* gene abundance normalized to Cyanobacterial-16S gene abundance, and their interaction on the dependent variables. Phytoplankton community composition was tested via ANOVA and log transformed to meet assumptions. A Tukey post-hoc test to determine the significance of interaction between treatment types. All statistical analyses were performed using R version 3.4.3 (R Core Team 2017).

Results

The initial physio-chemical water quality conditions and phytoplankton biomass concentrations at site WE2 including temperature, turbidity, soluble reactive phosphorus, nitrate, ammonium, extracted phycocyanin, and extracted chlorophyll-*a* are given in Table 1 for each sampling event. Across the experimental sampling dates ambient surface water temperature ranged from 19.3 to 26.7° C, and the concentrations of phytoplankton biomass (estimated by extracted chlorophyll-a and phycocyanin) indicate HAB development occurred by the July 31st sampling event, persisted throughout August and then declined during September and October. Dissolved inorganic nutrient concentrations (NH_4 ($\mu g\ L^{-1}$), NO_3 ($mg\ L^{-1}$), and SRP ($\mu g\ L^{-1}$)) showed expected levels of seasonal decline related to phytoplankton accumulation and biological assimilation (Table 1, Figure 1). Elevated concentrations during the June 25th and September 11th experiment reflect both changes in biomass and a response to rain events and higher inputs from the Maumee River as noted by water chemistry changes at the monitoring buoy. Figure 2 shows that fluorescence of the phytoplankton increased rapidly over the first 3-4 days of each experiment, then decelerated or decreased. These time-series of fluorescence also indicate that the treatments had varying effects based on time of year and initial ambient conditions. In the beginning of the season, all treatments except the +N+P treatment shared similar trends, however; by the end of the season (August 27, Sept 11, and Oct 1 experiments) the -N-P treatment resulted in much lower biomass yields compared to the other treatments (Figure 2). This pattern of lower yields within the -N-P treatment coincides with the DIN concentration in the lake (Figure 1). The +N+P

treatment always resulted in higher yields of biomass, except for the October 1

experiment where the +N+P, Ambient, and -P treatments trended similarly (Figure 1).

We hypothesized that phosphorus reduction would reduce growth rates of

cyanobacteria and that reducing N and P would result in lower growth rates that just P

reduction alone. Growth rates were determined for each treatment for all eight

experiments from T_0 to T_3. This time interval was selected because growth rates in the

ambient and nutrient reduction treatments all became negative during the T3 to T7

interval. Overall, the -P only treatment did not have a significant impact on the growth

rate of the total phytoplankton or cyanobacteria, and rates were only slightly lower than

the ambient treatment for five of eight experiments (Figure 2).   During August and

September however, the -N-P treatment resulted in significantly lower growth rates of

cyanobacteria compared to the -P, ambient and elevated nutrient treatments. In fact, dual

nutrient decreases resulted in negative growth rates for cyanobacteria in experiments 6

(Aug 30, p = 8.85e-7) and 7 (Sept 14, p = 0.0005), even when the other treatments

maintained positive growth rates. This pattern of reduced and even negative growth rates

within the -N-P treatment coincides with low DIN concentration in the lake (Figure 1).

While the nutrient reduction treatments had variable effects on growth rates, the elevated

nutrient treatment consistently resulted in higher growth rates for both cyanobacteria and

the total community (p<0.05 for experiments 3-7).

We hypothesized that dual nutrient control would decrease the abundance of

cyanobacteria among the phytoplankton community. Table 2 shows that early in the

season (from experiment 1 through 5), all the treatments except the +N+P treatment had

similar yield responses. More specifically in experiments 3 through 7, cyanobacteria accounted for the majority (54.94% to 73.49%) of the phytoplankton community at T_0. By T_3, cyanobacteria accounted for less than half of the community on average (7.40% to 50.91%) and by T_7 cyanobacteria accounted for approximately 28.77% of the community. Differences among treatment effects become more obvious by the end of the season and the greatest reduction in cyanobacterial abundance was observed in the -N-P treatment (Table 2). This pattern of reduced cyanobacteria abundance within the -N-P treatment coincides with the reduced DIN concentration in the lake (Figure 1). Dual nutrient control reduced the abundance of cyanobacteria within the entire phytoplankton community compared to the other treatments (Table 2). Experiment 6 (Aug 30, p = 2.2e-16) had an initial cyanobacterial abundance of 8.26 (ug/L) and dual nutrient control (-N-P) reduced the final cyanobacterial yield to 4.84 (ug/L) compared to a final concentration of 9.44 (ug/L) in the -P treatment, and 18.63 (ug/L) in the +N+P treatment (Table 2). Dual nutrient control also reduced cyanobacterial abundance in experiment 7 (Sept 14, p = 1.884e-09) to 5.53 (ug/L) in the -N-P treatment compared to 8.87 (ug/L) in the -P treatment, compared to the initial cyanobacterial yield of 6.97 (ug/L) (Table 2).

We hypothesized that dual nutrient control will reduce toxin concentration, through both a reduction in how much toxin was made for a given amount of cyanobacterial biomass, as well as, the proportion of potential toxin producing cells containing the *mcy*E marker when compared to single or no nutrient control. Figure 4 shows that dual nutrient control decreased toxins (panel 1) in experiment 3 (July 26, p = 7.41e-05) and 5 (Aug 16, p = 1.6e-07) and decreased the amount of biomass-normalized

toxin concentration (panel 2) in experiment 3 (July 26, p = 4.69e-09). Microcystin

concentration and toxin potential (mcyE (copies/ml) / 16s (copies/ml)) trends with overall

biomass concentration. Toxin production declines over the bloom season from mid-July

to October, where non-toxic strains of *Microcystis* were able to more efficiently grow

with decreasing nutrients and toxic strains are more likely to be nutrient limited (Chaffin

et al., 2018). This is seen in Figure 4, where an increase in nutrients increased toxins

(panel 1) in experiments 3 (July 26, p = 6.73e-06), 4 (Aug 3, p = .0107) and 6 (Aug 30, p

= 0.000153).

Discussion

In total eight dilution experiments were conducted and demonstrated varying treatment effects based on time of year. In the beginning of the season (June to mid-August), all treatments except the +N+P treatment trended similarly and displayed little treatment effect for biomass, cyanobacterial prevalence, and toxin production. This lack of effect in the nutrient reduction treatments is likely ascribed to the exposure history and availability of nutrients within the lake water (Table 1, Figure 1). The exact timing and magnitude of response would depend on the status of the internal pools of N and P that should also reflect this seasonal exposure history. By the end of the season treatment effects become more obvious, with the -N-P yielding less biomass and MC than the -P only or Ambient treatments. These results help identify under which nutrient conditions it might be possible to establish N limited effects on growth and toxin concentrations. In our experiments, this response occurred at ambient nitrate concentration of approximately 0.06 µg L^{-1} (Table 1, Figure 1). The finding that the +N+P treatment almost always resulted in higher yields of biomass, community prevalence, and toxin production, as increased biomass concentration of phytoplankton is expected and provides a meaningful reminder of the negative outcome of having nutrient concentrations present at these high spring-time levels when cyanobacterial species are likely to be dominant. Our results showed that dual nutrient reduction resulted in negative growth rates (i.e. mortality) for cyanobacteria in multiple experiments, even when the other treatments maintained positive growth rates. This finding suggests that dual nutrient reductions are, in fact, required to reduce growth rates in comparison to single or no nutrient control under given conditions. Excess nutrients seen in pulse events, as depicted by the +N+P treatment,

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often resulted in high growth rates for both cyanobacteria and total community and support the capability of cyanobacteria to exploit excess nutrients seen in storm events. Furthermore, dual nutrient control also decreased the abundance of cyanobacteria among the phytoplankton community. For most of the experiments (3 through 7), cyanobacteria accounted for the majority (54.94% to 73.49%) of the initial phytoplankton community. Our fluorescent biomass data shows that green algae dominated (>95%) in experiments 1 and 2, and no dominance of any algal group was seen in experiment 8 (Supplemental Data) and can explain the different yield responses between treatments. Overall, cyanobacterial abundance in the -N-P treatment was often lower than in any of the other treatments, with +N+P yielding the greatest abundance. Furthermore, in experiments 6 and 7 dual nutrient control (-N-P) reduced the final cyanobacterial abundance when compared to the initial yield, while all other treatments saw increased abundance when compared to T_0 (initial abundance). These reductions in abundance could potentially be explained by allowing for nutrient-uptake efficient algal species, such as green algae, to outcompete cyanobacteria under nutrient replete conditions. However, it is much more likely that by reducing both N and P when DINs (mg/L) are low in ambient lake conditions, cyanobacterial abundance is limited.

In the western basin of Lake Erie, nitrogen availability is higher during bloom initiation in early July and declines throughout the summer, reaching minimum concentration in September or October (Chaffin et al., 2011, 2013; Gobler et al., 2016). Studies suggest that microcystin concentrations increase when nitrate concentration and other environmental conditions such as water temperature are conducive to

cyanobacterial growth, thusly higher concentrations of cyanotoxins are expected during the mid-summer (i.e. higher production) (Chaffin et al., 2018; Gobler et al., 2016; Horst et al., 2014). Within the dilution experiments, toxin concentration and the proportion of *mcyE* bearing cyanobacteria was attributed to overall biomass concentration. This seasonal pattern is expected and has been noted in previous studies (Obenour et al., 2014). Furthermore, biomass normalized toxin concentrations declined over the course of the season, suggesting that non-toxic strains of *Microcystis* seemed to grow better than toxin-capable strains during reduced nutrient conditions. A shift in the gene pool from toxic to non-toxic strains is not uncommon and has been seen in previous studies within Lake Erie (Davis et al., 2015) and within other eutrophic bodies of water (Briand et al., 2009, 2008; Davis et al., 2009 and 2010; Sabart et al., 2010). Reducing the availability of both nitrogen and phosphorus could lead to a faster decline in the internal pool of nutrients within the phytoplankton cell. Reducing the amount of intracellular nutrients available to be allocated to processes by which toxins are produced may have led to a reduction in toxicity of a bloom. The results presented here suggest that dual nutrient control has the capability to decrease toxicity, production, and toxin potential within blooms at a more significant pace than with single nutrient control alone.

The overall results of this study showed that cyanobacterial blooms biomass and toxin concentration can be restricted under dual nutrient limitations when ambient levels of N are low. These results suggest that management strategies for reducing the extent, duration and toxicity of cHABs in Lake Erie would be most effective if they considered controlling both nitrogen and phosphorus. The reduction in N and P applied in our

bioassay treatments was able to drive cyanobacterial growth negative while P only reductions maintained positive growth. P only reductions did, however, result in lower growth rates than the ambient treatment. As such, any additional management decisions made in addition to Annex 4 options should consider the impact of relying on P-only reductions, and the potential benefits of implementing targets for both N and P reductions. It is important to note that because the watershed of the western basin is 86% agricultural land, the actions taken to address P runoff will also likely contribute to a reduction in N runoff. However, it is still vital to understand the effects of limiting phosphorus loads without limiting nitrogen loads in the western basin of Lake Erie. Our conclusions suggest that both P and N reductions would be more effective to mitigate cHABs in Lake Erie. While current best management practices are focused on P mitigation in the Great Lakes basin, managers must be aware of the short-term implications of not managing N loading as our study clearly indicates that for much of the growing season, N inputs may significantly impact bloom growth, composition, and toxicity. Finally, we recommend that more research needs to be conducted to better understand the nutrient dynamics that cause cyanobacterium like *Microcystis* to undergo co-limitation during the peak (August) of the growing season and to determine how N runoff is affected with the current P restrictions.

Tables and Figures

Table 1. Ambient conditions at sampling site WE2 at the time of initial collection. Data displayed on July 31 was collected on July 30, a sampling miscommunication pushed back the experiment start date. Ambient conditions were not determined on July 31.

	Experiment Start Date	Temperature (°C)	Turbidity (NTU)	SRP ($\mu g\ L^{-1}$)	NH_4 ($\mu g\ L^{-1}$)	NO_3 (mg L^{-1})	Extracted PC ($\mu g\ L^{-1}$)	Extracted Chl-*a* ($\mu g\ L^{-1}$)
1	June 25	22.1	28.4	24.4	131.1	1.3760	0.2	4.9
2	July 16	26.7	3.5	3.3	25.0	0.6515	4.4	6.9
3	July 23	24.0	3.7	3.7	7.6	0.6765	3.1	9.4
4	July 31*	24.4	9.2	2.1	3.0	0.7030	18.9	25.6
5	Aug 13	26.0	7.2	2.1	3.8	0.3715	15.7	19.4
6	Aug 27	24.4	9.0	2.2	3.6	0.0565	18.5	25.6
7	Sept 11	21.8	47.2	17.8	33.0	0.0645	5.4	17.0
8	Oct 1	19.3	7.6	13.6	9.2	0.1850	2.4	13.5

Table 2. The yield of cyanobacterial fluorescence (ug/L) at initial collection following dilution (T_0) and T_3 averaged by treatment, demonstrating change in cyanobacteria abundance.

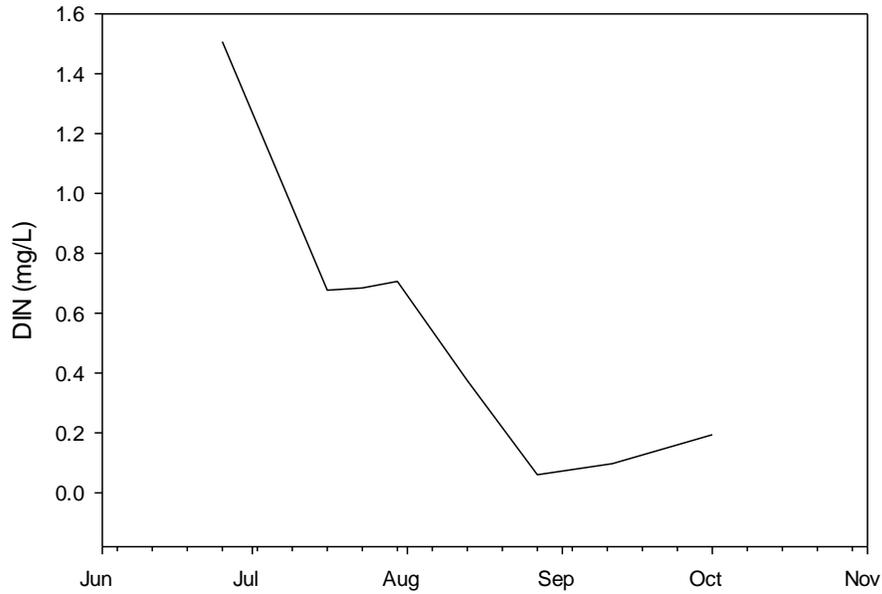| Experiment	Cyanobacterial Fluorescence Biomass (ug/L)				
	Initial	-N-P	-P	Ambient	+N+P
1	1.092	4.291	4.221	4.128	
2	2.325	2.812	3.084	2.978	
3	3.169	4.095	4.073	4.544	8.428
4	5.827	6.378	6.673	7.252	16.102
5	6.534	7.193	7.102	7.966	16.253
6	8.257	4.844	9.439	9.127	18.632
7	6.971	5.529	8.874	8.657	9.170
8	1.856	2.858	3.179	2.954	3.062

Figure 1. The concentration of Dissolved Inorganic Nitrogen (NO$_3$ (mg/L) and NH$_4$ (mg/L)) in ambient lake water at site WE2 from June to October in 2018, where DIN (mg/L) concentrations are high at the early part of the season and decline as the season progresses.
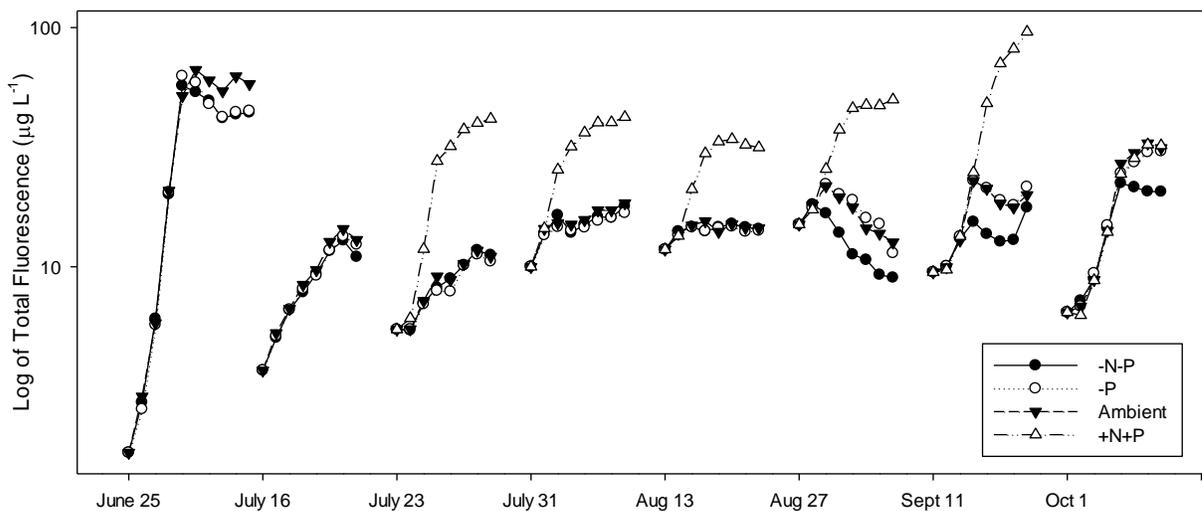
Figure 2. Total fluorescence (µg/L) on a log scale over the course of the 2018 experimental field season (June – October) separated by treatment type from experiments 1 through 8. Each grouping of lines represents a different experiment. Experiments on June 25 (Exp 1) and July 16 (Exp 2) did not have a +N+P treatment.
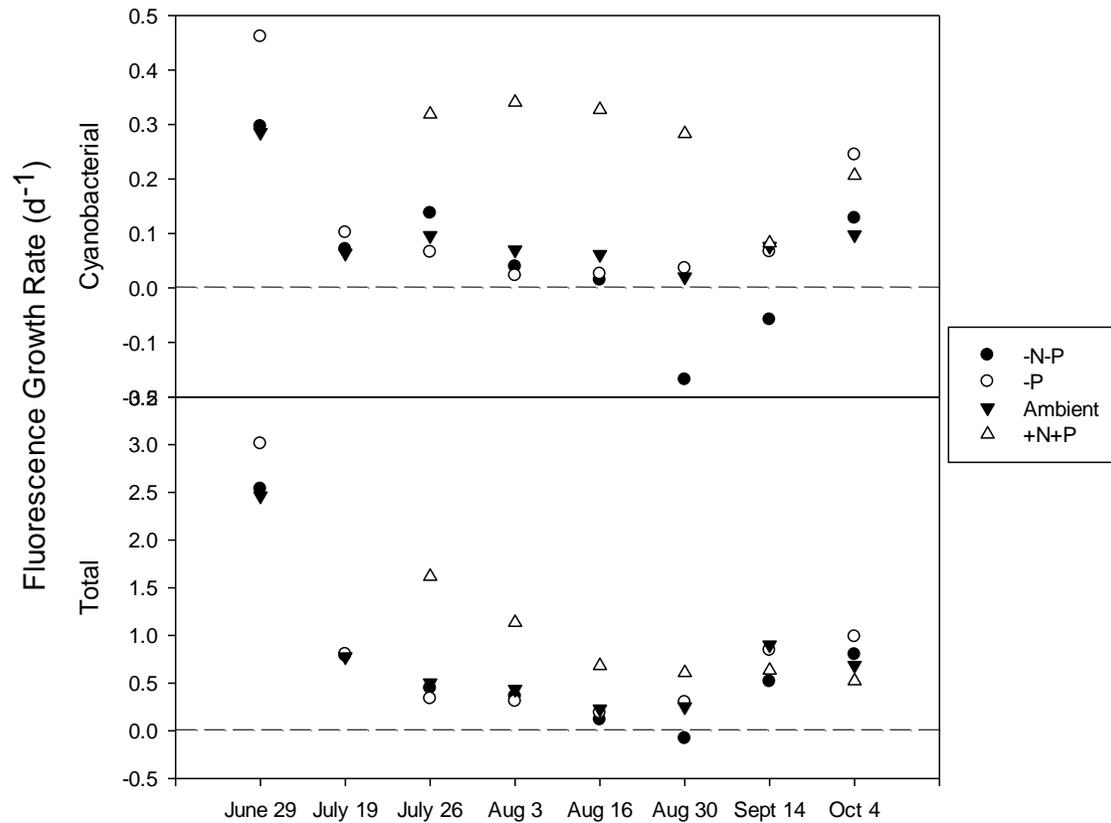
Figure 3. Fluorescence growth rate on day 3 (exponential growth phase) throughout the 2018 field season (June – October) for both the total and cyanobacterial communities. The top panel depicts cyanobacterial growth rate and the bottom panel depicts total community growth rate. Cyanobacterial growth rates became negative in experiments 6 (Aug 30, $p = 8.85e\text{-}7$) and 7 (Sept 14, $p = 0.000456$).
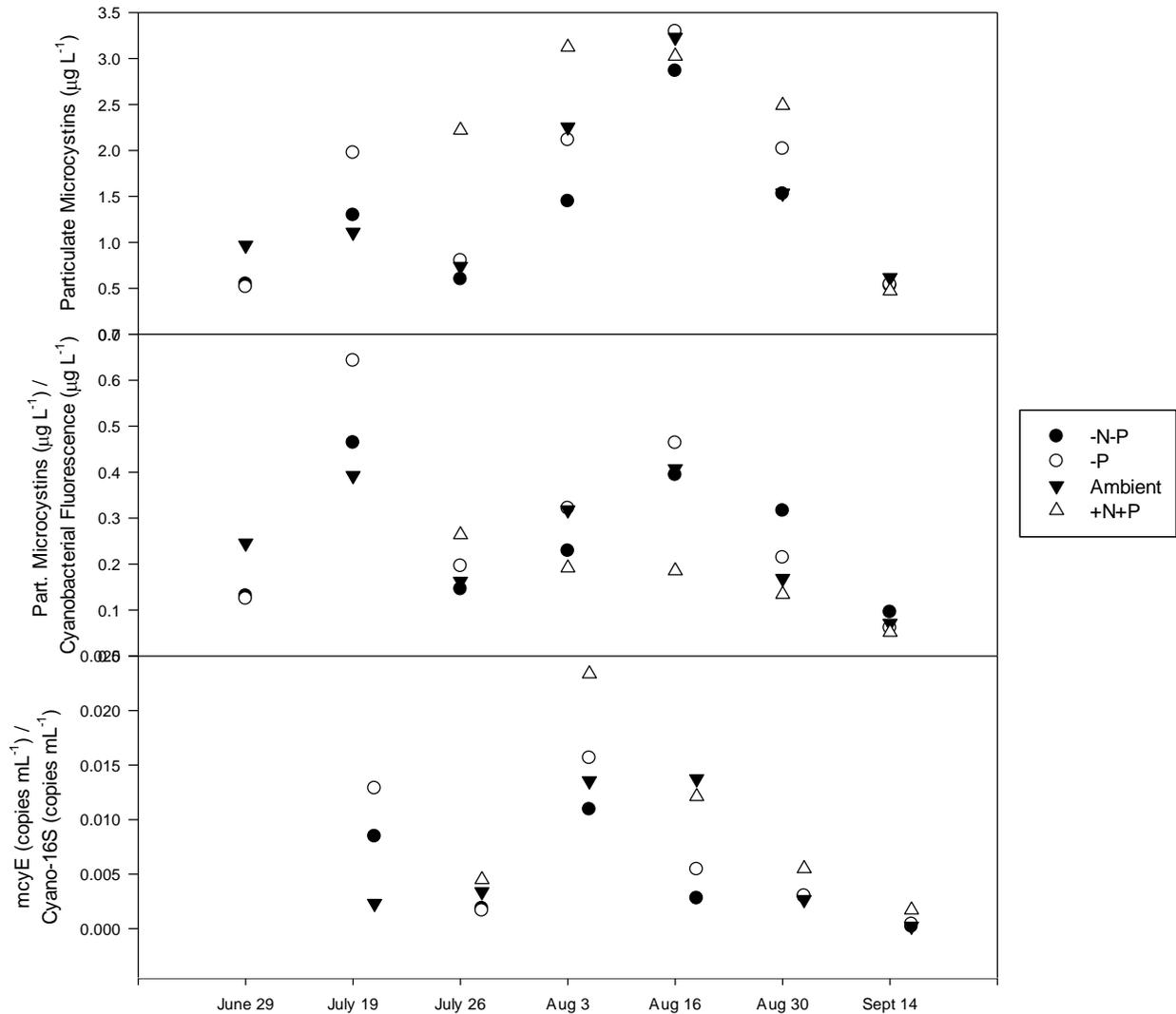
Figure 4. Panel 1 depicts toxin concentration, as determined by the concentration of particulate microcystins (ug/L) at T_3 for experiments 1 through 7 over the course of the 2018 experimental field season. Panel 2 depicts biomass normalized toxicity, as determined by the concentration of particulate microcystins (ug/L) normalized to cyanobacterial fluorescence (ug/L) at day 3 for each experiment over the course of the 2018 experimental field season. Panel 3 depicts proportion of *mcyE* bearing cyanobacteria, as determined by the number of *mcyE* gene (copies/mL) normalized to Cyanobacterial-16S gene (copies/mL) at day 7 for each experiment over the course of the 2018 experimental field season. Toxin concentration and biomass normalized toxicity data were not determined for experiment 8. The proportion of *mcyE* bearing cyanobacteria data was not determined for experiment 1 or 8.

Data Availability Statement

The entire dataset for this research and experiments will be archived and publicly available on Deep Blue under the file Baer_Mikayla_Thesis_SupplementalData.

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