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Harmful algal blooms (HABs) in freshwater ecosystems, especially of cyanobacterial species (cyanoHABs), are becoming more frequent and expanding geographically, threatening some of the world's largest and most-important water bodies, including Lake Erie in North America. HABs are the result of complex and synergistic environmental factors, though N (nitrogen) or P eutrophication is a leading cause of HABs. With global mean temperatures expected to increase an 2–5°C by 2100, cyanoHABs are predicted to increase even more, given their typically high temperature optimum. We investigated how increases in temperature and N, singly or in combination, affect the growth, food quality, and herbivory of cyanobacteria. Both algal community samples collected from Lake Erie, and isolated N-fixing and non-N-fixing cyanobacterial species, were cultured at 20, 25, or 30°C and 5, 50, 150, or 250 µM added N (as NO₃), and then analyzed for content of photosynthetic pigments. Both temperature and N affected algae, there were temperature x N interactions, and the effect of N and temperature depended on sampling date and the presence/absence of herbivores. In general, increases in N or temperature favored growth of cyanobacteria over green algae and diatoms, especially when both N
and temperature were increased and herbivores were present, and, surprisingly, both N-fixing and non-N-fixing cyanobacterial species responded strongly to N at high temperatures. Cyanobacteria (but not green algae and diatom) growth increased with temperature, especially from 25-to-30°C, but N increased cyanobacterial growth primarily only at 30°C and only on the early sampling dates (Aug & Sept, but not Oct). Herbivores often increased cyanobacterial, but decreased green algal and diatom, growth. These results indicate that global warming and N eutrophication will increase cyanoHABs in the future in western Lake Erie, and surprisingly, both N-fixing and non-N-fixing cyanobacteria may increase. Thus, in the face of global warming, it will be important to minimize N inputs into Lake Erie to help prevent cyanoHABs.
This thesis is dedicated to my family especially my mom and dad for their inspiration and love. Thank you for being in my every thick and thin. My life is all for you guys.
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List of Abbreviations

ANOVA ..................... Analysis of variance

Chl ......................... Chlorophyll

d ......................... Day
DMSO ........................ Dimethyl Sulfoxide

Fig ......................... Figure

h ............................ Hour
HAB .......................... Harmful Algal Blooms

IPCC ........................ Intergovernmental Panel on Climate Change

L ......................... Liter

n ............................ Number of replicates
N ............................... Nitrogen

P ............................. Phosphorous
PC ............................ Phycocyanin

SE .......................... Standard error

Temp ........................ Temperature
List of Symbols

+ ........................................... With
- ........................................... Without

µg/L ........................................ Microgram per liter
µM ........................................... Micromole

$ ................................................ Dollars

°C ................................................ Measure of Temperature

U/mL ......................................... Units per milliliter
Chapter 1

Introduction

Harmful algal blooms (HABs) in freshwater ecosystems, especially of cyanobacterial species, have been a major concern worldwide (Edmondson 1993; Paerl and Huisman 2008). Cyanobacterial HABs (cyanoHABs) are expanding geographically and now threaten the ecological integrity and sustainability of some of the world's largest and most-important water bodies, including: Lake Victoria, Africa; Lake Erie, US–Canada; Lake Okeechobee, Florida, USA; Lake Kasumigaura, Japan (Havens et al. 2001); Lake Taihu, China (Qin et al. 2010); the Baltic Sea in Northern Europe (Conley et al. 2009a); and the Caspian Sea in West Asia (Paerl and Huisman 2008). CyanoHABs have negative health impacts and cause serious economic losses to affected waters. In the United States alone, cyanoHABs result in losses of recreational, drinking, and agricultural water resources that are worth $2 billion annually (Dodds et al. 2009).

CyanoHABs also have widespread negative ecological consequences; for example, growth, survivorship, and fecundity of freshwater zooplankton, on which fish communities are dependent, are suppressed by cyanobacteria in many ways (e.g., cyanobacteria are known to negatively affect their herbivores by either clogging their feeding apparatus (colonial algae), by being of low nutritional value, or by producing
toxins (Sarnelle 2009). Most cyanoHABs may have endotoxins (internal secondary metabolites) which poison susceptible taxa that ingest them, or they may excrete toxins or inhibitors which affect taxa that may or may not ingest them (Paerl, 2008). These interferences with feeding by cyanoHABs may have important effects on zooplankton population dynamics and species structure (Gilbert 1998). Such effects on the zooplankton may then secondarily influence organisms at lower and higher trophic levels, creating complex and wide-ranging changes in the ecosystem (Turner 1997).

As with HABs in general, cyanoHABs are the result of complex and synergistic environmental factors, rather than a single dominant variable (Reynolds 1984; Hyenstrand et al. 1998; Dokulil and Teubner 2000; Jöhnk et al. 2008; Paerl and Huisman 2008; Wilhelm and Adrian 2008); however, the relative availability of mineral nutrient resources in the aquatic environment plays a major role in structuring phytoplankton communities (Tilman et al. 1982; Harris 1986; Sommer et al. 1986). Eutrophic and poorly-flushed waters are typically associated with cyanobacterial blooms (Paerl 1988; Philipp et al. 1991; Carmichael 1994; Rapala et al. 1997; Oliver and Ganf 2000; Paerl et al. 2001). Nutrient loading rates into many freshwater ecosystems have dramatically increased, due to human population, agriculture, and industrial activities (Vitousek et al. 1997; Carpenter et al. 1998). As surface waters become enriched, particularly in phosphorus (P), cyanobacteria often dominant the phytoplankton community (Smith 1986; Trimbee and Prepas 1987; Watson et al. 1997; Paerl and Huisman 2008).

Many cyanobacteria can utilize (or “fix”) atmospheric nitrogen (N2), and thus are not dependent on other forms of available dissolved organic or inorganic N in the water. However, not all cyanobacteria are able to fix N2, and so availability of dissolved
inorganic N (e.g., NO₃, NH₄) may be equally important in the occurrence of non-N-fixing cyanobacteria blooms, such as *Microcystis* species (Codd and Poon 1988; Dokulil and Teubner 2000). For example, laboratory studies have indicated that increases in N loading result in increases the growth and toxicity of *Microcystis* species (Watanabe and Oishi 1985; Orr and Jones 1998; Codd and Poon 1988). Interestingly, N deficiency caused a decrease in *Microcystis* toxicity, whereas P deficiency had only a minor influence on toxicity (Watanabe and Oishi 1985; Codd and Poon 1988), and in the lab, toxic strains of *Microcystis* were able to outgrow non-toxic strains at high nitrogen levels (Vezie *et al.* 2002). Importantly, to date, relatively little attention has been paid to N effects on algal growth in freshwater lakes, and more attention has been given to P effects (Carpenter *et al.*, 1998, Paerl and Huisman, 2008, Dodds *et al.*, 2009).

In addition to eutrophication, human activities are causing an increase in the mean surface temperature of the Earth (IPCC 2001, 2007), with projected increases of ca. 5°C by 2100, and this increase in temperature will likely increase HABs in general, and cyanoHABs in particular. For example, as temperature increases, in general, the algal group with the highest growth rate changes from diatoms to green algae to cyanobacteria in lakes (Canale and Vogel 1974). Often in temperate freshwater environments during the warmest periods with eutrophic systems, cyanobacteria dominate phytoplankton assemblages (Paerl 1988; Paerl *et al.* 2001; Paerl and Huisman 2008; Paul 2008). Harmful cyanobacteria such as *Microcystis* have been found to have an optimal temperature for growth and photosynthesis at, or above, 25°C (Konopka and Brock 1978; Takamura *et al.* 1985; Robarts and Zohary 1987; Reynolds 2006; Jöhnk *et al.* 2008; Paerl and Huisman 2008). Furthermore, above 25°C, the cellular toxin content of multiple
genera of cyanobacteria increases with increasing temperature (Van der Westhuizen and Eloff 1985; Codd and Poon 1988; Sivonen 1990; Rapala et al. 1997). Despite the preference of many cyanobacteria species for warm temperatures, at present, there are insufficient data to confirm the belief that cyanobacterial growth is already benefiting from warmer global temperatures and will benefit even more in the future.

As with most ecosystems world-wide, Lake Erie (one of the Laurentian Great Lakes of North America) has experienced an increase in N levels in the last century caused by human activities, which will likely continue (Vitousek et al. 1997), and consistent with global warming predictions, has experienced recent increases in temperature and cyanoHABs (Havens et al., 2001). However, the interactive effects of increases in both temperature and inorganic N on cyanoHABs in the Great Lakes is not well understood, including effects on the dominant non-N-fixing vs. N-fixing cyanoHAB species in the system. For Microcystis aeruginosa, currently the most-important HAB species in western Lake Erie, optimal temperature for growth and photosynthesis has been found to be at or above 25°C (Konopka and Brock 1978; Takamura et al. 1985; Robarts and Zohary 1987; Reynolds 2006; Jöhnk et al. 2008; Paerl and Huisman 2008).

The overarching objective of my research was to investigate how global warming and nitrogen-enrichment in combination will influence the extent and species composition of cyanobacterial HABs in a model temperate freshwater system, western Lake Erie. To achieve this overarching objective, I addressed the following specific objectives.
➢ Determine how water temperature and N affect competition between cyanobacteria, green algae, and diatoms in multi-species assemblages of Lake Erie algae.

➢ Investigate how increases in water temperature and N, individually and in combination, affect the growth and food quality of important non-N fixing and N-fixing Lake Erie cyanobacteria species.
Chapter 2

Methods and Procedures

Lake algae sampling and lab species selection

Experiments were conducted with either: (1) natural algal community samples obtained from Lake Erie during late summer and early fall, or (2) isolates of the cyanobacterial species, *Microcystis aeruginosa* (*K.*), LB2385 and *Anabaena flos-aquae* (*B.*), LB2557 obtained from the University of Texas Culture Collection. In most summers, western Lake Erie experiences a bloom of non-N-fixing *Microcystis* spp. (primarily *M. aeruginosa*) during (mostly) July and August, when lake N (mostly NO$_3$) levels are high (*e.g.*, 200 µm), and then a second bloom of a N-fixing species (either *Anabaena* or *Aphanizomenon* spp.) under low N conditions (*e.g.*, <100 µM) occurs in the late summer or early fall.

Lake samples were collected from three sites in the western part of Lake Erie, near Toledo, OH (Fig. 1), and then mixed together in a large tank to ensure that algal diversity in experiments reflects that of the lake in general [collection sites: MB20, a shallow sediment-laden site within the Maumee River-Lake Erie transition zone; 7M, a medium-depth site just northeast of Maumee Bay on the outskirts of the river sediment plume; and, GR1, a deeper open-water sediment-free site beyond the mouth of the
Maumee River. Lake algal samples were collected during 2011 on four dates (26 Aug, 13 Sept, 28 Sept, 12 Oct). Sampling dates included late summer dates when non-N-fixing *Microcystis* spp. were dominant, and in the fall when *Microcystis* was declining and other N-fixing cyanoHAB species became more common. NO₃ levels in lake water were < 28µM on all dates, and water temperatures ranged from 20.8-24.4°C.

![Map of Algal Sampling Sites](image)

**Fig 2-1:** Algal sampling sites (MB20, 7M, GR1) in Lake Erie during summer/fall of 2011. Photo courtesy of Justin Chaffin.

**Culture methods and treatments**

After mixing, lake samples were divided into two different groups: (1) unaltered lake water that was used directly in experiments and that contained native zooplankton
(including herbivores), as well as algae, and (2) lake water that was first aerated with either \( \text{N}_2 \) or air for 24 h prior to use in experiments. \( \text{N}_2 \) aeration was used to kill algal herbivores by asphyxiation, and comparison of algal growth with or without \( \text{N}_2 \) aeration allowed for the determination of interactive effects of N, temperature, and herbivory on algal growth; air bubbling was used to determine the effect of bubbling alone on algae.

Lake samples (1L replicates) were grown in 2L clear-plastic bottles covered with a sterile plug that permitted gas exchange. Samples were incubated under one of four levels of N (5, 50, 150, or 250 \( \mu \text{M} \) of added \( \text{NO}_3 \)) and one of three levels of temperature (20, 25, or 30\(^\circ\)C) (4 replicates per treatment combination). N levels in western Lake Erie typically range from \textit{ca.} 250 \( \mu \text{M} \) during the spring runoff and early summer, to as low as 5 \( \mu \text{M} \) in the fall, and the algal community starts to shift from non-N-fixers to N-fixers between 50 and 150 \( \mu \text{M} \) N (Chaffin \textit{et. al.} 2010). The three temperature treatments represent three different scenarios: 20\(^\circ\)C = typical contemporary early-summer water temperature; 25\(^\circ\)C = typical contemporary late-summer water temperature; and 30\(^\circ\)C represents maximum summer water temperature under a future global-warming scenario of +5\(^\circ\)C. All samples were grown in controlled-environment chambers for 11 days, under a 14-h photoperiod of 80 \mu\text{mol m}^{-2} \text{s}^{-1} \text{PAR}.

Isolated strains of \textit{M. aeruginosa} and \textit{A. flos-aquae} were first cultured in autoclaved 1L jars containing 250 mL of complete WC nutrient medium (Guillard and Lorenzen 1972) [1 mM \text{NaNO}_3, 250\mu\text{M} \text{CaCl}_2, 150\mu\text{M} \text{MgSO}_4, 50\mu\text{M} \text{K}_2\text{HPO}_4, 11.7\mu\text{M Fe-EDTA, 0.9}\mu\text{M MnCl}_2, 0.08\mu\text{M ZnSO}_4, 0.05\mu\text{M CoCl}_2, 0.04 \mu\text{M CuSO}_4, 10\mu\text{M H}_3\text{BO}_3, and 0.0037\mu\text{M (NH}_4)_6\text{Mo}_7\text{O}_{24}; \text{and TES buffer (115 mg/L)}], under the same growth chamber settings as for lake samples, but at 27\(^\circ\)C. Jars were capped with filter
paper to allow gas exchange, but prevent algal contamination. After 7 days of growth, cultures were examined under a light microscope to ensure absence of algal contamination, and these stocks were used for the experiment. Replicates of a single stock of each species were grown for 11 days at the N and temperature levels as above, for the lake-algae experiment (i.e., all nutrients but N were provided as for the stocks).

**Pigment content**

Algae were harvested on GF/C filters, and then immediately stored at -70°C. Samples were analyzed for content of chlorophyll *a* (as a measure of total algal growth), chlorophyll *b* (green algal growth), chlorophyll *c* (diatom growth), and phycocyanins (cyanobacteria). Chlorophyll content was determined spectrophotometrically, after extraction with DMSO and dark incubation at 65°C, using formulas and absorbance wavelengths in Barnes *et al.* (1991) for chl *a* & *b*) and Seely *et al.* (1972) for chl *c*. Following the procedures of Chaffin *et al.* (2010) and equations of Furuki (2010) and Sampath and Neefus (2007), phycocyanin content was determined after sonication (to break cells) for 15 min at 4°C in 0.1M phosphate buffer (pH 6.8) (model 1510, Bransonic). Samples were then incubated for 1 h at 4°C and centrifuged for 10 min at 3,800g. Phycocyanin concentration of samples was determined by fluorescence (model 10-AU fluorometer with P/N 10-305 filters, Turner Design). Pigment contents are expressed as final minus initial levels, and hence are growth rates over the 11d interval used for all experiments and trials used in the study.
Herbivore removal with N\textsubscript{2} and Chitinase

In preliminary experiments, we analyzed the utility of both N\textsubscript{2} bubbling and the chitin degrading enzyme, chitinase, in killing zooplankton; N\textsubscript{2} by asphyxiation and chitinase by digestion of the chitin-based exoskeleton or mouth parts of zooplankton. In an initial experiment, a non-sterile culture of an unidentified green-algal *Chlorella* species from Lake Erie, contaminated with various rotifers, was (1) aerated for 20 h with N\textsubscript{2} and then incubated in low light at room temperature for 4 d, or (2) incubated with various levels of chitinase (0-500 activity units/mL = U/mL) for 5 d; samples were then inspected microscopically for zooplankton, and no zooplankton were observed in N\textsubscript{2}-treated or chitinase-treated samples > 50 U/mL (results not shown). In a second confirmatory experiment, lake water containing a mix of algal species and zooplankton was bubbled with either air or N\textsubscript{2} (or nothing) for 7 d, and zooplankton density of these samples (n = 4 per treatment) was determined microscopically on day 0, 1, and 7. For chitinase treatments, samples (n = 4) were incubated with 0, 50, 100, or 300 U/mL of purified chitinase (P5205S, New England Biolabs), and inspected for zooplankton density on day 1, 3, and 7.

Statistical Analysis

Results were analyzed statistically using “analysis of variance” (ANOVA), followed by Tukey’s post-hoc test when appropriate (JMP 8 software). A “P value” of 0.05 or less was considered statistically significant and values between 0.05 and 0.1 are reported as marginally significant. For the first lake-algae sampling date, ANOVA was conducted separately for each pigment, using temperature and N as the main factors (and
their interaction). For the remaining lake-algae sampling dates, ANOVA was first conducted for each pigment using temperature, N, date, and ±herbivores as main factors (with all possible interactions); as either date or herbivore had some significant effects (alone or in combination with N or temperature) for all pigments, individual ANOVAs, followed by Tukey’s, were then conducted for each date and herbivore combination (i.e., each panel within the figures). For the isolated species experiment, ANOVAs were conducted for each species separately.
Chapter 3

Results

Removal of algal herbivores with N₂ or chitinase

In preliminary experiments, we analyzed the utility of both N₂ bubbling and chitinase in killing zooplankton, and hence algal herbivores. In water samples collected from western Lake Erie, containing natural algal and zooplankton communities, both N₂ bubbling and chitinase were effective in killing zooplankton (mostly copepods, *Daphnia* spp., and rotifers) over the 7d duration of the experiment (Figs 2 and 3). Similar results were observed for an unidentified *Chlorella* species isolated from Lake Erie, contaminated with various rotifer species (not shown). For subsequent experiments wherein effects of herbivory are included, N₂ bubbling was used to remove herbivores, as the cost of chitinase with large volumes of samples is prohibitive.

Experiment 1- lake algae samples

In 2011, when water samples were collected from western Lake Erie and used in the laboratory incubation experiment, natural algal communities were typically diverse and the relative abundance of major algal taxa varied with sampling date (Fig 4). On 26
Aug, the algal community was dominated by cyanobacteria (especially *M. aeruginosa*, based on microscopic examination), but on the other sampling dates, diatoms (especially *Fragilaria* and *Aulacoseira* spp.) were abundant too; green algae were only abundant on 13 Sept.

In an initial trial laboratory incubation of lake water samples collected on 26 Aug, both N and temperature had significant effects (P<0.015) on cyanobacterial growth (measured as chl *a*), and there were marginally-significant interactive effects of N and temperature (P=0.0765); N and temperature effects on green algae (chl *b*) and diatoms (chl *c*) were not significant (Fig 5). In general, algal growth tended to increase with increasing temperature and N, with maximum growth for all algal types at 30°C and 250µM N, and N saturation occurring at lower N levels at 20 and 25°C.

On subsequent sampling dates (13 Sept, 28 Sept, 12 Oct), lake water samples used in laboratory incubations were either used “as is” (*i.e.*, + zooplankton, including herbivores) or were first bubbled with N2 to kill herbivores. For the total algal community, measured as chl *a*, there were statistically-significant effects of N, temperature, herbivores, and sampling date on algal growth, and many instances of significant interactive effects (P<0.05) of these factors on algae (Fig 6). For example, growth was greater at 30°C vs. 25 and 20°C (for which growth was similar; all dates), but N affected growth only at 30°C, and the effect of N depended on date (*e.g.*, increasing growth with N seen only on 13 Sept). Removal of herbivores tended to increase algal growth early in the season, but had little effect later in Oct, and herbivores affected the response to N on 28 Sept. Lastly, growth was greater early in the season, compared to 12 Oct.
For green algae, measured as chl b, there were statistically-significant effects of temperature, herbivores, and sampling date on algal growth (P<0.034), but the effect of N was only marginally significant (P=0.0935), and there were only two instances of significant interactive effects (P<0.05) of these factors on algae (temp x date, temp x herb x date) (Fig 7). For example, temperature had little effect on green algal growth early in the season, but growth decreased at high temperature late in the season, and the absence of herbivores resulted in a positive response to temperature on 28 Sept, but resulted in a negative response on 12 Oct.

For diatoms, measured as chl c, there were statistically-significant effects of N and herbivores on algal growth (P<0.031), but the effect of temperature was only marginally significant (P=0.0935), there was no effect of sampling date, and there were only three instances of significant interactive effects (P<0.05) of these factors on algae (temp x date, N x date, temp x herb x date) (Fig 8). For example, temperature had little effect on diatom growth, except for a trend of decreasing growth with increasing temperature on 12 Oct (±herbivores) and a trend of increasing growth with temperature on 28 Sept (-herbivores only). Effects of increasing N were mostly evident only at 30°C (excluding 28 Sept, -herbivores), where N tended to increase growth and N benefits tended to saturate at 50µM N.

For cyanobacteria, measured as phycocyanins, there were statistically-significant effects of N, temperature, herbivores, and sampling date on algal growth (P<0.0001), and all of the interactive effects of these main factors were significant (P<0.05), excluding for two instances (temperature x N x herbivore, temperature x N x herbivore x date) (Fig 9). In general, growth was greater at 30 vs. 25 and 20°C (which did not differ), excluding 28
Sept when herbivores were absent. Growth typically increased with N early in the season, but not on 12 Oct, and the N response tended to saturate by 50-150µM N (except for 28 Sept, -herbivores). The presence of herbivores tended to increase cyanobacterial growth, suggesting that herbivores consumed competing algae from other taxa, and the removal of competitors made additional resources available to cyanobacteria, thereby increasing their growth. Lastly, cyanobacterial growth was greater early in the season (especially 28 Sept.), and decreased later in the season.

**Experiment 2- isolated cyanobacterial species**

Growth of pure cultures of non-N-fixing *Microcystis* and N-fixing *Anabaena* both increased with temperature (P<0.0001), and as expected, *Microcystis* growth increased with N (P<0.0001), saturating by 150µM N at all temperatures (Fig 10). Interestingly, growth of *Anabaena* did not increase with N at 20 and 25°C, but did increase with N at 30°C, indicating a decrease in N acquisition by N-fixation and increased acquisition of N as NO₃, and suggesting that N-fixation may be damaged at 30°C.
Fig 3-1: Effects of N₂ or air bubbling on the concentration of living zooplankton in water samples collected from Lake Erie and used in experiments. Replicate samples (n = 4) were bubbled for 24 h with N₂, air, or nothing (control), and then incubated at room temperature and low light for 7 d, during which the concentration of zooplankton was monitored microscopically.
Fig 3-2: Effects of chitinase treatment on the concentration of living zooplankton in water samples collected from Lake Erie and used in experiments. Replicate samples (n = 4) were incubated with purified chitinase (0, 50, 100, or 300 activity units/mL) at room temperature and low light for 7 d, during which the concentration of zooplankton was monitored microscopically.
Fig 3-3: Composition of the 2011 algal community in water samples from Lake Erie used in experiments. Concentrations of the major taxa are expressed in levels of taxa-specific photosynthetic pigments (chl $b$ for green algae, phycocyanins for cyanobacteria, chl $c$ for diatoms, determined by fluorometry (model Fluoroprobe, Series 3, bbe moldaenke, Germany). Samples were integrated composites of 3 different sampling sites.
Fig 3-4: Effect of temperature and N on the growth of algae collected from western Lake Erie on 26 Aug 2011. Growth (final minus initial pigment concentration) of the total algal community (measured as chl a) vs. green algae (measured as chl b) or diatoms (measured as chl c) was determined after incubation for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250μM added N (NO₃⁻). Results are means±1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters (α = 0.05).
Fig 3-5: Effects of temperature, N, and herbivory on the growth of algae collected from western Lake Erie on 13 Sept, 28 Sept, and 12 Oct (2011). Growth of the total algal community was measured as chl a (final minus initial pigment concentration) after incubation for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250 μM added N (NO₃). Replicate samples were aerated for 24 h with N₂ prior to N and temperature treatments to kill herbivores. Results are means±1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters (α = 0.05).
Fig 3-6: Effects of temperature, N, and herbivory on the growth of algae collected from western Lake Erie on 13 Sept, 28 Sept, and 12 Oct (2011). Growth of the green algal community was measured as chl $b$ (final minus initial pigment concentration) after for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250µM added N (NO$_3$). Replicate samples were aerated for 24 h with N$_2$ prior to N and temperature treatments to kill herbivores. Results are means±1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters ($\alpha = 0.05$).
Fig 3-7: Effects of temperature, N, and herbivory on the growth of algae collected from western Lake Erie on 13 Sept, 28 Sept, and 12 Oct (2011). Growth of the diatom algal community was measured as chl c (final minus initial pigment concentration) after incubation for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250µM added N (NO₃). Replicate samples were aerated for 24 h with N₂ prior to N and temperature treatments to kill herbivores. Results are means+1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters (α = 0.05).
Fig 3-8: Effects of temperature, N, and herbivory on the growth of algae collected from western Lake Erie on 13 Sept, 28 Sept, and 12 Oct (2011). Growth of the cyanobacterial algal community was measured as phycocyanin (final minus initial pigment concentration) after incubation for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250μM added N (NO₃). Replicate samples were aerated for 24 h with N₂ prior to N and temperature treatments to kill herbivores. Results are means±1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters (α = 0.05).
Fig 3-9: Effect of temperature and N on the growth of isolated non-N-fixing (*M. aeruginosa*) and N-fixing (*A. flos-aquae*) cyanobacterial species that are dominant in western Lake Erie. Growth was measured as chl $a$ (final minus initial pigment concentration) after incubation for 11 d at 20, 25, or 30°C and 5, 50, 150, or 250µM added N (NO$_3$). Results are means±1 SE, n=4. Letters above bars indicate results from Tukey’s test, wherein significant differences are indicated by different letters ($\alpha = 0.05$).
Chapter 4

Discussion

The overarching objective of this research was to investigate how global warming and N eutrophication in combination will influence the extent and species composition of cyanobacterial HABs in a model temperate freshwater system, western Lake Erie. Harmful algal blooms in freshwater ecosystems, especially of cyanobacterial species, have been a major concern worldwide, as they cause serious ecological and economic problems (Turner 1997; Dodds et al. 2009). Both temperature and N availability are key controls on HABs, and HABs are expected to become more common and severe with increases in temperatures and N caused by human-driven global warming and alterations in the global N cycle (Paerl and Huisman 2008). Though many past studies have examined effects of temperature and N individually on HAB species, including cyanobacteria (Tilman et al. 1982; Paerl et al. 2001; Reynolds 2006; Jöhnk et al. 2008), few studies have examined the interactive effects of N and temperature (Havens et al. 2001). Further, few studies have considered the impact of temperature and N on the relative performance of N- and non-N-fixing cyanoHABs. Increases in N eutrophication should favor non-N over N-fixing cyanoHABs, unless temperature has different effects on N₂ fixation compared to metabolism of other forms of N, etc., since N fixation is
energetically more expensive. There have also been few studies that have examined how N and temperature interact with herbivory to affect algal growth (Gilbert 1998; Tillmanns et al. 2008). In the present study, N and temperature affected the growth of cyanobacteria and other major algal taxa, there were interactive effects of N and temperature, and the effects of N and temperature were influenced by the presence/absence of herbivores, sampling date, and whether cyanobacteria were N-fixers or not. In general, increases in N or temperature favored growth of cyanobacteria over green algae and diatoms, especially when both N and temperature were increased and herbivores were present, and, surprisingly, both N-fixing and non-N-fixing cyanobacterial species responded strongly to N at high temperatures.

In the case of temperature alone, for all sampling dates, total algal community growth was highest at 30°C, and similar between 20 and 25°C, and this same temperature response was observed for by cyanobacteria, but not for green algae and diatoms. Notably, temperature responses were dependent on sampling date: e.g., little temperature response in green algae and diatoms on the early sampling dates (26 Aug, 13 Sept, 28 Sept.), but a negative response to increasing temperature on the last sampling date (12 Oct). Further, the response to temperature was sometimes modified by the presence/absence of herbivores: e.g., in green algae and diatoms, no temperature response with herbivores, but a positive response to temperature on 28 Sept, and a stronger negative response to high temperatures without herbivores on 12 Oct. Hence, any future increases in water temperature with global warming are likely to increase growth of cyanobacteria, relative to green algae and diatoms, in western Lake Erie, especially late in the summer and fall.
In the case of N alone, total algal community growth tended to increase with increasing N only at higher temperatures and on the early sampling dates (26 Aug, 13 Sept), and tended to decrease with increasing N on the last sampling date (12 Oct). For the major algal taxa examined (cyanobacteria, green algae, diatoms), positive N responses also tended to be observed primarily at the warmer temperatures and on the earlier sampling dates. Herbivores affected the response to N only in cyanobacteria (28 Sept). Importantly, positive responses of algal growth to N generally were generally saturated by 50-150µM added N, which is below maximum N levels observed in western Lake Erie (typically in spring and early summer), but are above the low N levels typically observed in the late summer and fall, when cyanobacterial blooms usually occur (Chaffin et al. 2010). Further, *M. aeruginosa* growth responded positively to added N at all growth temperatures examined, and *A. flos-aquae* did so at 30°C. As noted before, the positive response to N in *M. aeruginosa* was expected, but the positive response in *A. flos-aquae* was not, and indicates a shift from acquisition of N via N fixation exclusively at lower temperatures to acquisition of N as NO₃ at higher temperatures, which may be driven by heat effects on N fixation. Nitrogenase, the enzyme that catalyzes N fixation, was relatively sensitive to high temperatures (e.g., by 39°C) in the heterocysts of N-fixing cyanobacteria (*Anabaena* and *Nostoc* spp.) (Compaore and Stal, 2010). Hence, any additional N eutrophication of western Lake Erie is likely to fuel increases in cyanoHABs, and may interact with temperature to affect the relative success of N-fixing and non-N-fixing cyanobacteria (e.g., increased N without increased temperature could favor *M. aeruginosa* over *A. flos-aquae*, but the reverse might happen with increased N and temperature).
Effects of N and temperature on algae will likely have indirect interactive effects on algal herbivores, and these herbivore effects will then have cascading effects on other trophic levels and the entire aquatic ecosystem. For example, in this study, total algal growth tended to be higher in the absence of herbivores, and cyanobacterial growth tended to be higher in the presence of herbivores. Further, there were often statistically-significant interactive effects of herbivores with other main factors, especially for total algal and cyanobacterial growth. Given that cyanobacteria are typically not the preferred food of most algal herbivores (Sarnelle 2009), then any factor that increases growth of cyanobacteria relative to other major algal taxa, will impact zooplankton community structure (Davis, 2011). Both N and temperature affect the toxicity of cyanobacteria, including M. aeruginosa (Song et al. 1998; Jacoby et al. 2000; Sekadende et al. 2005; Wu et al. 2006), and this will affect other trophic levels (Davis, 2011). Further, both N and temperature can be expected to influence the food quality of algae in other ways, and this will then impact the zooplankton community. For example, increases in N typically increase the protein content of algae (Ananadhi Padmanabhan et al. 2010), while higher-than-optimal temperatures usually decrease the content of non-structural carbohydrates (due to increased respiration) and alter the composition of cell lipids (e.g., increasing fatty acid length and saturation) (Hochachka and Somero 2002).
References


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