THE ECOSYSTEM EFFECTS OF CYANOBACTERIA IN OLIGOTROPHIC LAKES

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IN OLIGOTROPHIC LAKES

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Cyanobacterial blooms pose a serious threat to the water quality of freshwater lakes because of their scums, toxins, and odors. Synergistic interactions between eutrophication and climate change may be causing cyanobacterial blooms to increase worldwide, which will have substantial consequences for aquatic food webs and nutrient concentrations in lakes. In particular, the trophic state of a lake may be an important determinant of how blooms affect ecosystem functioning. In this dissertation, I used a combination of literature reviews, field surveys, field experiments, and laboratory experiments to examine the causes and effects of cyanobacterial blooms in both oligotrophic and eutrophic freshwater lakes.

My research shows that increased nutrients are an important driver of the global increase in cyanobacterial blooms, and future climatic and hydrological conditions may interact to favor cyanobacterial dominance. My experiments with *G. echinulata*, a cyanobacterium that is increasing in the northeastern United States, demonstrate that high densities of *G. echinulata* can increase nitrogen and phosphorus concentrations in low nutrient lakes. In these systems, *G. echinulata* can play an important role structuring food webs by increasing the biomass of small-sized phytoplankton. *G. echinulata*'s interactions with phytoplankton are fairly complex,

however, as I observed that its positive effects were mediated by both trophic interactions (zooplankton biomass) and trophic state (nutrient concentrations), highlighting the context-dependency of the effect of this species on other plankton.

I found that nutrients play an important role mediating *G. echinulata*'s effects on phytoplankton: first, increasing nutrients in the water column may be the mechanism by which *G. echinulata* stimulate other phytoplankton in oligotrophic systems, and second, nutrient concentrations may alter the direction of *G. echinulata*'s effect (i.e., stimulatory or inhibitory) on other plankton. Finally, my data demonstrate that *G. echinulata* blooms in oligotrophic lakes may have important consequences for water quality. As cyanobacteria continue to increase, it is vitally important to understand how both oligotrophic and eutrophic systems will respond.

BIOGRAPHICAL SKETCH

Cayelan Christine Carey was born and grew up in upstate New York with her loving parents, Dr. David and Margot Carey, and younger brother Spencer. As a child, her parents instilled in her a discipline for work, a love of nature, and an insatiable quest to learn. Cayelan and her father spent several weeks every summer hiking and canoeing in the Saranac Lakes area of the Adirondack Mountains, where she developed a love for aquatic systems. At age 14, Cayelan read an article in the National Geographic magazine on diatoms, which motivated her to learn more about these beautiful organisms. During the next three years, she (with immense patience and support from her parents) sampled diatom communities in streams across an acid deposition gradient in the Adirondacks and worked with Dr. Sophia Passy, a diatomist at the Rensselaer Polytechnic Institute, to analyze trends in species composition. This early project convinced Cayelan to pursue a life in aquatic science that would allow her to be outside in the summer and use microscopes in the winter.

After completing high school, Cayelan moved to Hanover, New Hampshire to study Environmental and Evolutionary Biology at Dartmouth College. Her perspective on aquatic systems and ecology as a whole was shaped enormously during this time by working in Kathy Cottingham's lab, which began a mentorship and collaboration that continues today. Kathy was Cayelan's first limnological role model and set many of the benchmarks for excellence in science, writing, and teaching that Cayelan aspires to achieve.

In November 2004, a chance meeting between Cayelan and Kathleen Weathers ("Kak") at the Lake Sunapee Protective Association office in Sunapee, New

Hampshire began another pivotal mentorship that has thrived through countless adventures on five continents. Kak encouraged Cayelan to conduct an undergraduate honors thesis on *Gloeotrichia echinulata*, a cyanobacterium that was increasing in Lake Sunapee. In collaboration with the Lake Sunapee Protective Association (LSPA), Cayelan et al.'s work on *Gloeo* grew from a one-summer undergraduate project into the '*Gloeo* gang,' an NSF-funded team of researchers at several institutions in the northeastern United States. Throughout this experience, Cayelan acknowledges Kak, the LSPA, and the *Gloeo* gang for making Lake Sunapee her spiritual home that she continued to return to every summer for fieldwork during her Ph.D.

After graduating from Dartmouth in 2006, Cayelan moved to Uppsala, Sweden to study *Gloeo* as a Fulbright Fellow in the Department of Limnology at Uppsala University. While her original research plans did not transpire as planned, key collaborations with Emil Rydin and Karin Rengefors allowed Cayelan to pursue new research directions in lake sediment phosphorus chemistry and phytoplankton allelopathy. This year in Sweden, although more difficult than anticipated, broadened her research horizons in unexpected ways that continued to enrich her perspective on lakes throughout her Ph.D.

Cayelan returned back to the U.S. during the summer of 2007 and began a Ph.D. in Nelson Hairston, Jr.'s lab at Cornell. At Cornell, Cayelan received excellent training in ecology and evolutionary biology and benefited enormously from the Cornell faculty, her graduate student peers, and especially the Biogeochemistry and Environmental Biocomplexity Program. Nelson, Kathy, Kak, and Alex Flecker were

important mentors to Cayelan during three exciting and challenging years of fieldwork in New Hampshire. Throughout her entire Ph.D., Cayelan's husband, R. Quinn Thomas, provided critical technical and emotional support that greatly enriched both the quality of her research as well as her ability to persevere.

After completing her Ph.D., Cayelan will begin a one-year postdoctoral research position at the Center for Limnology at the University of Wisconsin-Madison before becoming an Assistant Professor in the Department of Biological Sciences at Virginia Tech in August 2013.

For David Mitchell Carey, for nurturing my love of science and lakes, and

For R. Quinn Thomas, for supporting me every step along the way.

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Ph.D. dissertations are the result of many years of mentorship, hard work, and support. Most of this work would not have been possible without the guidance and encouragement of the following individuals.

First and foremost, my Ph.D. committee- Nelson Hairston, Kak Weathers, Kathy Cottingham, and Alex Flecker- have been an amazing source of scientific and life wisdom. Their continuous support throughout unexplainable results, late night phone calls, hurricanes, wetsuit wranglers, cattle tank rustlers, saboteurs, and more made the difference. Almost everything I know about aquatic and ecosystem ecology is because of these four people, and their combined knowledge of ecological dynamics is mind-bogglingly impressive. I would like to thank my committee as a whole for being such inspiring mentors. All of them are exceptional role models, and I aspire to be half of the mentor to my future students that they are to me.

I would like to thank Nelson for never telling me no except for when I really needed to hear it and for his patience with my global wanderings during the past five years. Every single time I came into Nelson's office he always had a big smile, and I cannot have wished for a better Ph.D. advisor. His support through my 2010 field season and especially the last few months as I navigated post-Cornell opportunities has been phenomenal. Finally, I would like to thank him for taking the risk on admitting me into his lab and his painstaking editing of all of my manuscripts- I will forever be grateful.

I would like to thank Kak for her 'systems perspective' and always challenging

me to look at the big picture of every research question I have tackled. Perhaps more importantly, she has taught me many life lessons about the science of collaboration. Whenever in doubt, I always ask myself, "What would Kak do?" and aim to follow in her scientific footsteps. I would also like to thank her for all of the adventures we have shared worldwide- after surviving as many GLEON conferences as we have together, I consider her a dear friend in addition to advisor.

I have known Kathy longer than almost everyone, and she is why I wanted to become a limnologist. We have been through a lot together, and I thank her for her continuing support and letting me use her lab every summer to conduct this research. Kathy has always been my go-to person for experimental design, *Gloeo* questions, and statistical help, and much of this dissertation has been written through her limnological perspective, which she passed down to me.

Finally, Alex has been a great source of support during the dissertation process. He always had much more faith in me than I did in myself, and I think that he is a great example of someone who puts the 'ecologist' into 'aquatic ecologist' because of his wide knowledge of the discipline, which he generously shared.

The motivation for this work originated during my first meeting with the Lake Sunapee Protective Association (LSPA) in late autumn 2004. The trust that they put in me to help them with their *Gloeo* "problem" is the heart and foundation of this dissertation, and kept me going during my struggles because I knew that my work had a greater purpose. I also thank them for tolerating my unexplainable love of *Gloeo*, even when it represented a major water quality concern. My sincerest hope is that my research findings can be put to good use to improve and protect the Lake Sunapee

watershed. The motto of the LSPA is, "We are all its caretakers…" which I will always carry with me in spirit. I am greatly indebted to the LSPA staff and members that have welcomed me with open arms, helped me with sampling, and make up my Sunapee family: Midge and Tim Eliassen, Robert Wood, June Fichter, George and Jill Montgomery, Dave Richardson, Bonnie Lewis, Sue Godin, Kathleen Stowell, and many other LSPA members.

As mentioned in the biographical sketch, my undergraduate thesis on *Gloeo* motivated the formation of an impressive research team, the '*Gloeo* Gang.' The core of the Gang is Kathy Cottingham, Kak Weathers, and Holly Ewing, in addition to dozens of students and other collaborators. It would be shortsighted to refer to the *Gloeo* Gang as just a collaboration- this group of scientists has now worked, debated, sampled, traveled, and wrote together for several years, and has developed into a tremendous *tour de force*. All but one of the *Gloeo*-focused chapters of this dissertation are a direct result of *Gloeo* Gang activities.

I have benefited tremendously from amazing field and lab support during my dissertation. In particular, I want to commend Jennie Brentrup and Natalie Ruppertsberger, who worked as my field and lab assistants during my 2008, 2009, and 2010 field seasons. Their contributions to my research during those summers were not only essential for the completion of my experiments but also greatly improved the quality of the research. These two women went above and beyond the call of duty and at times risked their lives for this work- I will forever be impressed by their dedication and commitment to the *Gloeo* project. After spending several summers of 80-hour weeks together, I thank them for their companionship, unflagging smiles, and good

spirits while individually picking >5,000,000 *Gloeo* colonies, and bottomless strength. I have learned so much from both of you. I also want to thank the Brentrup and Ruppertsberger families for all of their support, especially during the 2010 field season.

In addition to Jennie and Natalie, I thank Danny O'Donnell, Stacy Davis, Quinn Thomas, Elizabeth Traver, Sam Fey, Andrew McGarrah, Laurie Griesinger, Craig Layne, and Aly Fiorillo for their field and laboratory help. Amy Smagula, the New Hampshire Department of Environmental Services (NH-DES) Exotic Species Program Coordinator, Jody O'Connor, the former NH State Limnologist, and Sara Steiner, the NH-DES Volunteer Lake Assessment Program Coordinator, were immensely helpful with providing long-term data and permits.

During most years of my Ph.D., I lived in New Hampshire from May to September and worked out of Kathy's lab at Dartmouth while running field surveys and experiments. During this time, I gained invaluable friends in lab mates Sam Fey, Elizabeth Traver, and Jess Trout-Haney. Thank you for welcoming me as one of your own. In addition, Mary Poulson and Craig Layne provided key logistical support at Dartmouth.

Back at Cornell, the Hairston Zoo always provided helpful ideas and constructive criticism. Most importantly, I need to thank Colleen Kearns for her spectacular scientific support. Our shared mornings of NPR, coffee, and microscopy are deeply missed. Lindsay Schaffner is one the most helpful people of all time, and I finally feel adequately prepared to move to Wisconsin after her teachings on Midwestern life. I thank Bob Johnson, Bill Schaffner, and all of my other lab mates

for their helpful suggestions and advice. The Corson Hall staff, especially Carol Damm, Patty Jordan, LuAnne Kenjerska, DeeDee Albertsman, and Brian Mlodzinski, have been amazingly helpful with grant budgeting, graduate school logistics, and overcoming computer malfunction.

In Sweden, I will forever owe a large debt to Emil Rydin, who found and took care of me when I was in a rough spot (both after a bike crash and scientifically-speaking). His friendship has resulted in a long-term and hopefully life-long collaboration on sediment phosphorus dynamics. Karin Rengefors also provided great mentorship and support, and I thank her for training me allelopathic techniques. Our days together in Lund and Skåne were some of my happiest in Sweden. Kurt Pettersson, Karin Pettersson, Stefan Bertilsson, and Lars Tranvik organized the logistics for me to conduct research and teach at the Limnology Department in Uppsala and Norr Malma field station at Lake Erken. Finally, my time in Sweden would not have been nearly so enjoyable without the dear friendship of Ina Bloch, who is my European phycological counterpart. I hope that we will have many more midsommars on the Swedish archipelago together.

A large part of my scientific life is invested in the Global Lakes Ecological Observatory Network (GLEON). During the past five years, I traveled to twenty-two countries on six continents to attend GLEON conferences, deploy buoys and monitoring systems, and serve as a representative for the network. The opportunities that GLEON has provided me have been innumerable and extraordinary, and I am so grateful to be part of this organization. In particular, I would like to thank Paul Hanson, Kathie Weathers, Tim Kratz, David Hamilton, Justin Brookes, Evelyn Gaiser,

and Bas Ibelings for being fantastically helpful and inclusive colleagues.

I owe a huge thanks to my immensely talented graduate student peers in EEB, especially Anna Forsman, Susan Cook-Patton, Danica Lombardozzi, Becky Doyle-Morin, Marita Davison, Krista Capps, and Jill Cohen for their friendship, support, and companionship. We have had many lovely and special experiences together along this Ph.D. path, and have persisted through together.

I know that my devotion to pond scum from such an early age has not always made sense to my family, but I thank them for trusting me to find my own path. My mother, brother, and godfather have helped me sample algae over the years, and I have very much enjoyed sharing my love of aquatic systems with them. I would like to thank my mother and brother for their continual love and support from afar, as well as to Jane and Frank Thomas, my amazing in-laws. A huge thanks also to my Babakian-Markel family, who have taken Quinn and me in as their own and have supported me throughout, cheered my successes, and talked me through the harder times- thank you.

Finally, the most important person I must thank is my husband Quinn, who not only supported me every step along the way but also read through many chapter drafts, provided statistical, experimental design, lab, and field help, and contributed in innumerable ways to all aspects of my Ph.D. research. Over the years he has generously given his time and resources to help the *Gloeo* project, tolerated my *Gloeo*-worship, endured my frequent absences, assisted at GLEON and LSPA meetings, conducted two weeklong lake surveys from Ithaca to Bangor, encouraged me to think outside of the aquatic box, and always made sure that I ate dinner. His persistence and commitment to my research was at times greater than my own, and his selflessness

and love epitomize our relationship. More than anything, I am so grateful and honored to travel through life with him as my partner.

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PREFACE

Background

The challenge of sustaining our freshwater resources is of global concern. Lakes represent the largest store of accessible freshwater on the planet (Gleick and Palaniappan 2010), and provide irreplaceable provisioning, regulating, supporting, and cultural services, including water for drinking, industry, and irrigation, fisheries and aquaculture, and recreation (MEA 2005, Carpenter et al. 2011). Simultaneously, freshwater lakes are experiencing unprecedented degradation as a result of habitat destruction, unsustainable use, invasive species introduction, eutrophication, and contaminants (MEA 2005, ILEC 2007, Carpenter et al. 2011). These drivers may interact synergistically with climate change to jeopardize water quality and ecosystem functioning (GLC 2004, Paerl and Scott 2010, Paerl et al. 2011). The complex nature of lentic (standing freshwater) ecosystems, which include long water retention times, non-linear responses to drivers, and varied roles within hydrological networks (ILEC 2007, Carpenter et al. 2011), lead to substantial uncertainty in the future functioning and resilience of lake ecosystems.

As a result of increased stress on lake ecosystems, especially higher temperatures and eutrophication, scientists have noted a global increase of cyanobacterial blooms, a symptom of water quality degradation (Hallegraeff 1993, Anderson et al. 2002, Paerl and Huisman 2008, Paerl and Huisman 2009, Brookes and Carey 2011, Kosten et al. 2012, Sinha et al. 2012). Cyanobacterial blooms impair aquatic ecosystem services because of their toxins, odors, scums, and negative effects on water quality (reviewed in Huisman et al. 2005, Hudnell 2008). From a public

health perspective, cyanobacteria produce toxins that kill hundreds of livestock and pets every year (Chorus and Bartram 1999), contaminate fisheries (Xie et al. 2005), trigger human illness (Chorus and Bartram 1999), and are responsible for >50 human deaths to date (Jochimsen 1998, Azevedo 2002). In addition, cyanobacteria represent major costs for water treatment, lost revenue, and decreasing property values (Dodds et al. 2009). In the United States alone, these costs can be greater than $2 billion per year (Dodds et al. 2009). Less is known about the ecological effect of blooms, but several studies have demonstrated that cyanobacteria toxins and scums can have negative effects on plankton food webs (Christoffersen et al. 1990, Christoffersen 1996, Bouvy et al. 1999, Huisman et al. 1999, Havens 2008).

Increased temperatures and eutrophication are predicted to interact to increase cyanobacterial blooms in lakes in the future (Jöhnk et al. 2008, Paerl and Huisman 2008, Paerl and Huisman 2009, Paerl and Scott 2010, Paerl et al. 2011, Kosten et al. 2012, O'Neil et al. 2012). Cyanobacteria have a number of eco-physiological adaptations, including their buoyancy and high affinity for phosphorus (Mur et al. 1999), that may be advantageous under future climate and nutrient loading scenarios. For example, unlike other phytoplankton, cyanobacteria can produce gas vesicles that allow them to access high light conditions at the water surface during periods of thermal stratification (Walsby et al. 1991, Walsby 1994). When stratification increases, buoyant cyanobacteria are not mixed below the photic zone, allowing them to form surface scums and decrease light availability for competing phytoplankton (Walsby et al. 1991, Walsby 1994). This adaptation is advantageous because increased temperatures are predicted to intensify and prolong periods of thermal

stratification (De Stasio et al. 1996, Gerten and Adrian 2002, Jöhnk et al. 2008). Similarly, if nutrient concentrations (specifically, nitrogen and phosphorus) increase in lakes, resulting in higher concentrations of phytoplankton biomass, cyanobacteria may dominate (Downing et al. 2001). High levels of phytoplankton biomass are associated with decreased light and CO_2 concentrations, which indirectly benefit cyanobacteria because they are generally superior competitors for light and CO_2 than other phytoplankton (Scheffer et al. 1997, Shapiro 1997, Hyenstrand et al. 1998). As a result of these and other adaptations, including a lower susceptibility to grazing from zooplankton (reviewed by Ibelings and Havens 2008), cyanobacteria are predicted to dominate in warmer, nutrient-rich lake conditions.

While increasing cyanobacterial blooms are typically linked to eutrophic conditions, they can also occur in oligotrophic and mesotrophic systems, where an increase in reports of some cyanobacterial species is occurring (Boyer 2008, Ernst et al. 2009, Winter et al. 2011). In particular, an increase of the cyanobacterium *Gloeotrichia echinulata* in low-nutrient lakes across the northeastern United States has been documented by lake managers, watershed organizations, and state officials (LSPA, NH-DES VLAP, ME-IWQAR 2006, 2008, 2010). *G. echinulata* is a unique cyanobacterium because of its large size (1-3 mm in diameter), complex life cycle, and nutrient storage characteristics (Barbiero 1993, Forsell 1994, Tymowski and Duthie 2000, Karlsson 2003). *G. echinulata* produces akinetes, or resting cells, in response to decreased growing conditions at the end of the summer (Khan and Schumacher 1973, Barbiero 1993, Forsell 1994, Karlsson 2003, Karlsson-Elfgren et al. 2003, Karlsson-Elfgren et al. 2004). The akinetes overwinter on the lake sediment and recruit into the

water column via gas vesicles in the late spring and summer in response to a combination of temperature, light, and chemical cues (Roelofs and Oglesby 1970, Khan and Schumacher 1973, Barbiero 1993, Karlsson-Elfgren et al. 2003, Karlsson-Elfgren et al. 2004). In addition, *G. echinulata* fixes nitrogen (Stewart 1967, Roelofs and Oglesby 1970, Vuorio et al. 2006) and can absorb luxury amounts of phosphorus on the lake sediment that are stored as polyphosphate so that it does not require any additional phosphorus to complete its life cycle when it recruits into the water column (Istvánovics et al. 1993, Pettersson et al. 1993, Tymowski and Duthie 2000). Interestingly, *Gloeotrichia* sp. exhibit some of the highest rates of phosphatase activity observed for any cyanobacterial taxon (Whitton et al. 1991), indicating that it may be well-adapted for low phosphorus conditions. *G. echinulata* also produces microcystin-LR, a toxin that can have negative effects on both zooplankton grazers and humans that swim in or drink contaminated water (Carey et al. 2007).

Because of its nitrogen fixation and phosphorus storage, *G. echinulata* may be able to increase nutrient availability for other phytoplankton in the water column after it recruits from the sediments. In at least two lakes, increases in phytoplankton have been attributed to *G. echinulata* blooms (Pitois et al. 1997, Nõges et al. 2004), which may be because *G. echinulata* leaks or releases nutrients through grazing damage, exudation, and senescence. Cyanobacteria are generally 'leaky' organisms, and release nutrients through several processes, especially in low-nutrient systems (Healey 1982, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004). However, in high-nutrient systems, where light availability may be more limiting for phytoplankton growth than nutrients, *G. echinulata* may have negative effects on phytoplankton because it forms

large surface scums that can decrease light (Liess et al. 2006).

In this dissertation, I used a combination of literature reviews, field surveys, field experiments, and laboratory experiments to examine the causes and effects of cyanobacterial blooms in both oligotrophic and eutrophic freshwater lakes. I use the term 'bloom' broadly to include any visible accumulation of biomass (including, but not limited to, surface scums), as there are many definitions of 'bloom' in both the scientific and popular literature (Smayda 1997). I first examined the literature on cyanobacterial blooms at a global scale, and then conducted surveys and experiments on *G. echinulata* at a regional and lake scale. I used *G. echinulata* as a case study to examine a nuisance cyanobacterium that may be increasing and can exert substantial ecological and public health effects.

List of chapters

This dissertation is based on the following chapters, which are referred to in the preface by their Roman numerals.

I. Brookes, J.D.* and C.C. Carey.* 2011. Resilience to blooms. Science 334: 46-47. *Both authors contributed equally to this work.

II. Carey, C.C., B.W. Ibelings, E.P. Hoffmann, D.P. Hamilton and J.D. Brooks. 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research. 46: 1394-1407.

III. Carey, C.C., H.A. Ewing, K.L. Cottingham, K.C. Weathers, R.Q. Thomas, and J.F. Haney. Occurrence, toxicity, and potential ecological consequences of the cyanobacterium *Gloeotrichia echinulata* for low-nutrient lakes in the

northeastern United States. In review at Aquatic Ecology.

IV. Carey, C.C. and K.E. Rengefors. 2010. The cyanobacterium Gloeotrichia echinulata stimulates the division of other phytoplankton. Journal of Plankton Research 32: 1349-1354.

V. Carey, C.C., K.L. Cottingham, K.C. Weathers, J.A. Brentrup, N.M. Ruppertsberger, H.A. Ewing, and N.G. Hairston, Jr. The cyanobacterium Gloeotrichia echinulata: an ecosystem faciliator increasing nutrient availability and subsidizing plankton food webs in nutrient-limited aquatic ecosystems. To be submitted to Ecological Monographs.

VI. Carey, C.C., K.L. Cottingham, N.G. Hairston, Jr. and K.C. Weathers. Trophic state mediates the effect of a large, colonial cyanobacterium on phytoplankton dynamics. To be submitted to Limnology and Oceanography.

Aims of Dissertation

The main aims of my dissertation were to:

1) Explore the causes of increasing cyanobacterial blooms on a global scale (I and II).
2) Document the regional prevalence and toxicity of *Gloeotrichia echinulata* in the northeastern United States (III).
3) Screen *G. echinulata* for any allelopathic effects (IV).
4) Experimentally test the effect of *G. echinulata* on low-nutrient plankton food webs and nutrient concentrations at different mesocosm scales (III, V).

5) Examine how food web structure and trophic interactions mediate *G. echinulata*'s effect on plankton and nutrients (V, VI).
6) Determine how trophic state may mediate the effects of *G. echinulata* on plankton and nutrients (VI).

Short summary of chapters

Chapter I

In this Perspective, I (and a colleague) examined the relative importance of increased temperatures and nutrient concentrations (specifically, nitrogen and phosphorus) as drivers of cyanobacterial increases. Some researchers have hypothesized that these two stressors will interact synergistically to promote increases in cyanobacteria. Consequently, determining the relationship between climate change and nutrients is important for lake management. After reviewing modeling, experimental, and long-term data studies, we determined that increased nutrients are consistently a more important cause of blooms than increased temperatures. Lakes with decreased nutrient concentrations generally do not exhibit increased cyanobacterial biomass when temperatures increase, and factorial experiments demonstrate that nutrients are more important than temperature for explaining cyanobacterial increases. Nutrients and temperature may act synergistically to increase blooms, but only after a nutrient threshold has been exceeded.

As a result of these findings, we suggest that reducing nutrient loading may be the best way to decrease blooms, especially because nutrient management is tractable on a regional and decadal scale, while controlling temperatures will require global

efforts over a much longer time scale.

Chapter II

In this chapter, I (and colleagues) conducted a literature review to examine how cyanobacteria may respond to future climate and hydrological scenarios. We first analyzed climate and hydrological predictions that are important for freshwater systems (e.g., changes in precipitation) and then examined six cyanobacterial traits (the ability to grow in warmer temperatures; buoyancy; high affinity for, and ability to store phosphorus; nitrogen-fixation; and light-harvesting) that may influence how cyanobacteria respond to future climate. Cyanobacteria are a very diverse taxonomic group and possess different combinations of these traits, which will most likely result in variation in how different cyanobacterial taxa respond to climate change and hydrology.

The current scenarios predict substantial geographical variation in future climate and hydrological effects on freshwater systems, which may alter water temperature, thermal stratification, precipitation, nutrient loading, watershed runoff, and light availability. After an analysis of each eco-physiological adaptation and the variability in trait expression among cyanobacterial taxa, we predict that there may be species-specific differences among geographical regions that determine which cyanobacterial taxa dominate, but overall, cyanobacteria as a group are likely to increase in most regions in the future.

Chapter III

In my third chapter, I (and colleagues) surveyed 37 lakes over 4 years for a combined 193 observations to document the prevalence and toxicity of *Gloeotrichia echinulata* across the northeastern United States. I also conducted an *in situ* mesocosm experiment testing the effects of low densities of *G. echinulata* on nutrient concentrations, phytoplankton biomass, and zooplankton density. We found *G. echinulata* in the water column of 27 out of 37 lakes we sampled in Maine, New Hampshire, New York, and Vermont. *G. echinulata* densities were typically low (<15 colonies/L), but occasionally at scum-producing levels (250 colonies/L). *G. echinulata* colonies from the survey lakes exhibited detectable microcystin-LR concentrations ranging from 58 –7148 ng microcystin-LR/g dry weight colonies, which suggests that *G. echinulata* may pose human health risks if densities increase in the future.

Most of the colonies added to the mesocosms senesced and sank to the bottom of the mesocosms within 24 hours of addition, allowing us to examine the effects of low densities of *G. echinulata* on nutrients and plankton after a scum event. We found that senesced *G. echinulata* led to small but statistically significant increases in total nitrogen, small-sized phytoplankton biomass (<30 μm chlorophyll *a*), and zooplankton biomass and density. We hypothesize that these effects may be even more pronounced in systems that experience high densities of *G. echinulata*.

Chapter IV

In chapter IV, I (and a colleague) examined the potential allelopathic effects of *G. echinulata* on other phytoplankton with three laboratory experiments. We observed that *G. echinulata* had significant stimulatory effects in all experiments. We found that in the presence of *G. echinulata* colonies, the density of five of seven phytoplankton species from five algal Divisions increased up to 800% after 96 hours of co-incubation. In separate experiments using only the cryptophyte *Rhodomonas lacustris*, we observed that *G. echinulata*'s stimulatory effect was greatest at low densities of target cells and linearly increased with *Gloeotrichia* biomass. This study adds to the growing body of literature suggesting that cyanobacteria can have stimulatory effects on eukaryotic phytoplankton and reveals a mechanism by which *G. echinulata* may increase phytoplankton in natural systems, as observed in Chapters III and V.

Chapter V

As a result of the findings in Chapter III and IV, I (and colleagues) manipulated *G. echinulata* density in three separate experiments to determine if larger densities of *G. echinulata* could exert ecologically-relevant effects on plankton and nutrients. We also manipulated zooplankton biomass in one of the three experiments to examine if trophic interactions modulated *G. echinulata*'s effects. We added four different densities of *G. echinulata* to 50 L *in situ* bags with large zooplankton grazers absent, added 10 different densities of *G. echinulata* to 0.4 L flasks in the laboratory with large zooplankton grazers absent, and crossed the effects of *G. echinulata*

absence or presence with low or high levels of zooplankton biomass in a fully factorial experiment conducted in 800 L cattle tanks.

We found that *G. echinulata* exhibited significant positive effects on nutrients and plankton in all three experiments. High densities of *G. echinulata* significantly increased nitrogen and phosphorus concentrations relative to no-*G. echinulata* controls. When zooplankton grazers were absent or in low densities, small-sized (<30 μm) phytoplankton significantly increased on a gradient of *G. echinulata* density. At high densities of zooplankton grazers, small-sized phytoplankton biomass was higher in *G. echinulata* treatments relative to both no-*G. echinulata* controls and *G. echinulata* treatments with low densities of zooplankton, indicating that trophic interactions may alter *G. echinulata*'s effect on food webs.

Chapter VI

In my final chapter, I (and colleagues) examine how nutrient concentrations may alter *G. echinulata*'s effect on plankton and nutrients. We predicted that in low nutrient systems, *G. echinulata* facilitates phytoplankton by increasing available nutrients (e.g., by fixing nitrogen or transporting phosphorus from the sediments to the water column). For high nutrient systems, we predicted that *G. echinulata* inhibits phytoplankton by producing scums and toxins. We also hypothesized that zooplankton intensify negative effects of *G. echinulata* on phytoplankton by selectively grazing small algae. To test these hypotheses, we manipulated *G. echinulata* presence, nutrient enrichment, and zooplankton biomass in 800L cattle tank mesocosms.

We found that phytoplankton were stimulated by *G. echinulata* in low-nutrient treatments, potentially because *G. echinulata* significantly increased nitrogen and phosphorus concentrations, whereas in high-nutrient treatments, *G. echinulata* presence significantly decreased nutrients and other phytoplankton. Further, we observed significant interaction effects between trophic state and zooplankton biomass. As predicted, increasing zooplankton biomass intensified *G. echinulata*'s inhibitory effect on phytoplankton at high nutrient concentrations; however, increasing zooplankton intensified *G. echinulata*'s facilitative effect on phytoplankton at low nutrient concentrations.

Conclusions

My research shows that increased nutrients are an important driver of the global increase in cyanobacterial blooms, and future climatic and hydrological conditions may interact to favor cyanobacterial dominance in freshwater systems. My experiments with *G. echinulata*, a cyanobacterium that is increasing in the northeastern United States, demonstrate that high densities of *G. echinulata* can increase nitrogen and phosphorus concentrations in low nutrient lakes. In these systems, *G. echinulata* may play an important role structuring food webs by increasing the biomass of small-sized phytoplankton. *G. echinulata*'s interactions with phytoplankton are fairly complex, however, as we observed that its positive effects are mediated by both trophic interactions (zooplankton biomass) and trophic state (nutrient concentrations), highlighting the context-dependency of this species' effects on other plankton.

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CHAPTER ONE

RESILIENCE TO BLOOMS*

Cyanobacterial blooms (see Figure 1.1) present health risks worldwide for humans and livestock that drink or use contaminated water, and also represent substantial economic costs to communities due to water treatment, lost tourism and recreation revenue, and declining property values (Dodds et al. 2009). These explosive growths occur in fresh and marine water, and may be increasing globally. One recommendation is that water managers must address the effects of climate change when combating cyanobacterial blooms (Paerl and Huisman 2008). However, recent studies suggest that controlling nutrients may be more important in increasing aquatic ecosystem resilience to these blooms.

A number of factors may potentially contribute to an increase in blooms, primarily climate change and changing land use. Most climate change modeling scenarios predict that aquatic systems will experience increases in temperature, thermal stratification (Paerl and Huisman 2008), and water column stability, all factors that favor cyanobacteria over other phytoplankton (Jöhnk et al. 2008, Paerl and Huisman 2008). Thermal stratification leads to a greater propensity for cyanobacterial blooms, as many cyanobacteria have gas-filled vesicles that enable them to rise to the

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Figure 1.1. A cyanobacterial bloom in Lake Windermere, England, in June 2007.

Photo credit: Louise Miles, Freshwater Biological Association.

water surface and form dense blooms (Paerl and Huisman 2008, Wagner and Adrian 2009). In addition to climate change, deforestation, human and commercial animal waste, and agricultural fertilization have increased nutrient runoff into aquatic systems (Smith et al. 1999), also favoring cyanobacterial blooms.

What is the relative importance of warming temperature versus nutrient (nitrogen and phosphorus) loading in driving cyanobacterial dynamics? Many modeling studies (Elliott 2010, Markensten et al. 2010), historical data analyses (Wagner and Adrian 2009, Stich and Brinker 2010), and experimental studies (McKee et al. 2003, Moss et al. 2003) show increased nutrient concentrations as a consistently more important driver of blooms than warming temperatures. For example, in Lake Müggel (Müggelsee), Germany, cyanobacteria did not directly benefit from increased water temperatures; rather, blooms decreased as nutrient loading was reduced (Köhler et al. 2000, Wagner and Adrian 2009). Whereas some studies indicate that increasing nutrients and temperatures may exert a synergistic effect on cyanobacterial dominance (Wagner and Adrian 2009, Elliott 2010, Markensten et al. 2010), nutrient loading, notably of nitrogen and phosphorus, is the primary factor in the expansion of blooms (Conley et al. 2009).

There are several mechanisms by which increased nutrients lead to the dominance of cyanobacteria. Enclosure experiments have demonstrated that nutrient addition can increase water column thermal stratification without directly affecting water temperatures (Kumagai et al. 2000, Jones et al. 2005). The increased cyanobacterial and algal biomass resulting from nutrient loading increases light attenuation and modifies the vertical distribution of shortwave radiation. This

promotes thermal stratification and creates a more stable environment for cyanobacterial growth (Kumagai et al. 2000). Such an effect was demonstrated in Lake Constance, Germany, with both historical data analyses and modeling (Rinke et al. 2010). Buoyant cyanobacteria can outcompete phytoplankton by reducing available light for nonbuoyant phytoplankton competitors (Paerl and Huisman 2008). Hence, increased nutrients can create the stratification conditions suitable for cyanobacterial blooms in the absence of increased water temperatures (Kumagai et al. 2000).

Cyanobacterial biomass can indeed be decreased substantially by lowering nutrient inputs, despite warming temperatures, as observed in lakes Constance, Germany (Stich and Brinker 2010); Veluwe, Netherlands (Ibelings et al. 2007); and Müggel, Germany (Köhler et al. 2000, Wagner and Adrian 2009). Although decreasing nutrient loading may not completely stop the incidence of cyanobacterial blooms, it decreases cyanobacterial dominance. In addition to lowering external nutrient loads, lake managers must also take into account other factors—trophic structure, and the seasonal life cycles of plankton—that will be affected by changing climate (Carpenter and Kitchell 1993). In particular, the altered magnitude and timing of precipitation and consequent runoff events (overland flow of water from saturated soil into aquatic ecosystems) may increase nutrient loading (Jeppesen et al. 2009). Lowering nutrient inputs to soils should reduce nutrient loads to lake ecosystems, which would buffer both increased temperature and altered precipitation effects, and decrease the maximum phytoplankton biomass (Jeppesen et al. 2002), the incidence of problematic cyanobacterial blooms (Conley et al. 2009), and the subsequent heat

capture by phytoplankton within the surface layer of lakes. Returning aquatic systems to lower nutrient status will ultimately make them less vulnerable to the predicted negative impacts of global warming, particularly more cyanobacterial blooms, because phytoplankton biomass in low-nutrient lakes will generally not respond to the increased water temperatures expected from climate change (Hamilton et al. 2002, Arnott et al. 2003).

Nutrient reduction is a long-term investment. Decreased cyanobacterial biomass would be delayed due to nutrient recycling from the lake sediments and lengthy hydraulic residence time in large water-bodies (Ibelings et al. 2007). Regardless, nutrient loading is far easier to remediate at the decadal and regional scale than warming temperatures, which must be regulated on the global scale and will continue to increase through year 2100, even if greenhouse gases stabilize at year 2000 concentrations (Meehl et al. 2007). Alternatively, nutrient remediation can be implemented at the watershed scale, for which many successful engineering and policy options are available (Cooke et al. 2005). Increased temperatures, even under the best scenarios, are inevitable. However, it is not necessarily inevitable that cyanobacteria will grow to "bloom" proportions in aquatic ecosystems.

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CHAPTER TWO

ECO-PHYSIOLOGICAL ADAPTATIONS THAT FAVOUR FRESHWATER CYANOBACTERIA IN A CHANGING CLIMATE*

Abstract

Climate change scenarios predict that rivers, lakes, and reservoirs will experience increased temperatures, more intense and longer periods of thermal stratification, modified hydrology, and altered nutrient loading. These environmental drivers will have substantial effects on freshwater phytoplankton species composition and biomass, potentially favouring cyanobacteria over other phytoplankton. In this Review, we examine how several cyanobacterial eco-physiological traits, specifically, the ability to grow in warmer temperatures; buoyancy; high affinity for, and ability to store, phosphorus; nitrogen-fixation; akinete production; and efficient light harvesting, vary amongst cyanobacteria genera and may enable them to dominate in future climate scenarios. We predict that spatial variation in climate change will interact with physiological variation in cyanobacteria to create differences in the dominant cyanobacterial taxa among regions. Finally,

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we suggest that physiological traits specific to different cyanobacterial taxa may favour certain taxa over others in different regions, but overall, cyanobacteria as a group are likely to increase in most regions in the future.

1. Introduction

Cyanobacterial blooms present major challenges for the management of rivers, lakes and reservoirs. Blooms have adverse impacts on aquatic ecosystems and human health, with wide-ranging economic and ecological consequences (Hallegraeff 1993, Mur et al. 1999). The increased frequency and intensity of blooms have been attributed to anthropogenic changes, principally nutrient over-enrichment and river regulation (Anderson et al. 2002). More recently, it has been predicted that a changing climate associated with rising levels of atmospheric CO_2 will increase the occurrence of blooms (Beardall et al. 2009, Paerl and Huisman 2009, Paul 2008), or at least favour cyanobacterial dominance of phytoplankton communities (Mooij et al. 2005). Decision support trees for bloom formation (e.g., Oliver and Ganf 2000), as well as numerical model predictions that allow testing of multiple stressors (e.g., Trolle et al. 2011), suggest that there may be synergistic interactions amongst an array of environmental drivers to promote cyanobacterial blooms.

Why would cyanobacteria, and not other phytoplankton, be favoured under future climatic conditions? It is possible that cyanobacteria have several physiological characteristics that may be acting in concert to allow them to dominate in a changed climate. Alternatively, there may be physiological attributes of different

cyanobacterial taxa that may leave them vulnerable under some conditions expected with climate change. An improved understanding of the interactions amongst both the environmental drivers that are predicted to change in different regions and cyanobacterial physiology is crucial for developing management strategies to mitigate or avoid the potential of more frequent blooms under future climate scenarios (Brookes and Carey 2011, Paerl et al. 2011).

1.1 Bloom increases and effects

Cyanobacterial blooms are not a new phenomenon and have been occurring for centuries in both marine and freshwater systems (Codd et al. 1994, Fogg et al. 1973, Hayman 1992, Paerl 2008). Since the 1960s, however, there has been a dramatic global increase in the number of publications and reports of cyanobacterial blooms (Anderson et al. 2002, Carmichael 2008, Hallegraeff 1993, Hamilton et al. 2009, Paerl and Huisman 2008, Van Dolah 2000), primarily in freshwater and estuarine environments (Paerl 1988). While increased reports may to some extent be due to increased monitoring efforts (Sellner et al. 2003), there is substantial evidence that blooms are increasing not only in frequency, but also in biomass, duration, and distribution (Anderson et al. 2002, Glibert et al. 2005, Hallegraeff 1993, Smayda 1990). Furthermore, it has been hypothesised that cyanobacteria may continue to increase in response to global climate change (Mooij et al. 2005, Paerl et al. 2011, Paerl and Huisman 2009).

The proliferation of cyanobacteria can have numerous consequences. In addition to risks to human and animal health (Chorus and Bartram 1999, Ibelings and

Chorus 2007), there may also be substantial economic costs for water treatment, and losses in tourism, property values, and business (Dodds et al. 2009, Steffensen 2008). With a global distribution, escalating bloom occurrence and worldwide concern (Lundholm and Moestrup 2006), it is important to review the evidence for the likelihood of cyanobacterial increases with climate change and how this may be related to cyanobacterial eco-physiology. Cyanobacteria have an extensive evolutionary history, and fossil evidence indicates that they were abundant over 2.5 billion years ago (Summons et al. 1999), and may have emerged as early as 3.5 billion years ago (Schopf 2000). They are the earliest-known oxygen-producing organisms, and have key roles in global primary production and nitrogen-fixation (Chorus and Bartram 1999). The lengthy history and variable environmental conditions under which cyanobacteria evolved have resulted in the adaptation of some cyanobacterial taxa to extreme environments, and collectively they are widely dispersed across the globe (Badger et al. 2006). They exist across a multitude of hot, cold, alkaline, acidic and terrestrial environments, and can proliferate to be the dominant primary producers in freshwater, estuarine, and marine ecosystems (Chorus and Bartram 1999, Mur et al. 1999). Our focus in this Review is on freshwater and estuarine cyanobacteria.

2. Anticipated changes to temperatures, stratification, nutrient loading, and hydrology

Over the past century, global mean surface air temperatures have increased by $0.74 \pm 0.18\ ^{o}C$ (Trenberth et al. 2007). This warming trend is expected to continue, with higher latitudes warming more than lower latitudes (Solomon et al. 2007).

Warming will be strongest in winter for northern areas in Europe and North America and most of Asia, and warming is predicted to be stronger in summer for the southern areas of Europe and North America. Seasonal differences in South America are not projected to substantially change (Solomon et al. 2007). Of particular relevance for cyanobacterial blooms is the prediction that heat waves will become more frequent, more intense, and will last longer (Meehl et al. 2007).

Climate warming is expected to have a profound effect on the onset (earlier), strength (stronger) and duration (longer) of stratification of lakes (De Stasio et al. 1996, Peeters et al. 2002), even to the extent that some polymictic lakes may become dimictic, dimictic lakes may become warm monomictic, and monomictic lakes may become oligomictic (Gerten and Adrian 2002). Variation in temperature on a diel scale is anticipated to become smaller, since daily minimum temperatures will likely increase more strongly than daily maximum temperatures. Hence, climate warming increases the likelihood that microstratification occurring during the day will be maintained during the night (Hanson et al. 2008).

Both deep and shallow lakes are expected to exhibit increased stratification, which can have large effects on phytoplankton biomass and community structure (Pomati et al. In press). For example, the water column stability of the deep peri-alpine Lake Zurich, Switzerland has increased by more than 20 % over the past three decades (Livingstone 2003). In shallow lakes, microstratification can resist turbulent mixing to reduce the mixing depth to a shallow mixed layer near the water's surface (Denmann and Gargett 1983). Wilhelm and Adrian (2008) determined the mixing regime of shallow, polymictic Lake Müggel (Müggelsee, Germany) over a period of four years,

and found that heat waves in 2003 and 2006 resulted in extended stratified periods that lasted up to two months.

Under future scenarios of modified hydrology, it is expected that nutrient loading will be increasingly variable, with regional differences. Across almost all regions, precipitation variability will increase in the future, and when precipitation events do occur, their intensity will be increased (Parry et al. 2007, Solomon et al. 2007). Warmer climates are predicted to experience higher precipitation extremes, and droughts will increase at low latitudes and mid-latitude continental interiors due to both decreased precipitation and increased evapotranspiration (Parry et al. 2007, Jeppesen et al. 2007). By the end of the 21st century, the distribution of the global land surface in extreme drought is predicted to increase from 1 - 3 % at the present day to 30 % (Burke et al. 2006). Similarly, the incidence of extreme droughts every 100 years and mean drought duration are expected to increase by factors of two and six, respectively, by the 2090s (Burke et al. 2006).

Opposite to low-latitude regions, temperate and high-latitude regions are predicted to experience increased mean precipitation and the highest increase in precipitation intensity, primarily in the winters (Meehl et al. 2005, Parry et al. 2007). Runoff in the high latitudes of North America and Eurasia is expected to increase by 10 - 40 % by 2050, whereas runoff will decrease in the Mediterranean, southern Africa, western USA, and northern Mexico by 10 - 30 %. Cumulatively, however, the areas with decreased runoff will expand between the late 20th century and 2050 (Milly et al. 2002). Taken altogether, these changes are likely to have large effects on nutrient

delivery to lakes, reservoirs and rivers as rainfall distribution and runoff characteristics change.

In response to these climatic changes, many lake physical and chemical characteristics will change, potentially synergistically, which will subsequently affect phytoplankton communities (e.g., Pomati et al., In Press). While there are large regional differences in the expected climatic changes, we expect that in most scenarios different cyanobacterial taxa will likely be able to dominate under increasingly variable conditions.

3. Cyanobacterial evolution and adaptations

Cyanobacteria possess a range of uncommon and highly-adaptable eco-physiological traits (Litchman et al. 2010). These traits, which can be specific at the genus level, include: 1) the ability to grow in warmer temperatures; 2) buoyancy, due to gas vesicle production; 3) high affinity for, and ability to store, phosphorus; 4) nitrogen-fixation; 5) akinete production and associated life history characteristics; and 6) light capture at low intensities and a range of wavelengths. Cyanobacteria possess many other important eco-physiological traits (e.g., toxin production), however, we chose to focus on the traits listed above because we predict that these cyanobacterial characteristics may allow adaptation specific to the climate changes that we expect in future conditions (and that we are already experiencing). These climate changes include higher temperatures (Parry et al. 2007), stronger and longer periods of stratification (Jeppesen et al. 2007), and modified hydrology (i.e., changed flows,

more intense storms but reduced frequency, which will create sporadic nutrient delivery; Jeppesen et al. 2007, Jeppesen et al. 2011).

Below, we evaluate how different traits may interact with a changing climate to allow cyanobacteria to dominate aquatic systems. Cyanobacteria are a diverse group, ranging in size from unicellular picoplankton to multicellular macroscopic colonies. Consequently, no cyanobacterium possesses all of the traits listed above, so the response of cyanobacteria to changes in the environment will likely vary greatly among genera, which makes it harder to generalise the expected outcome of different climate change scenarios. Similarly, different geographical regions are predicted to experience varying climatic changes, which will affect the environmental drivers that interact with cyanobacterial physiological traits. Finally, there are several other factors that contribute to the success of cyanobacteria in freshwater systems, such as grazing resistance, that may be affected by climate change. Cyanobacterial taxa are considered to be poor quality food for zooplankton grazers (e.g., *Daphnia* sp.) because of the morphology of their filaments and colonies, which clog filtering appendages (Arnold 1971, Lampert 1982, 1987), their toxins (Fulton and Paerl 1987, Lampert 1981, 1982), and because they lack certain required fatty acids, sterols, and nutrients (Ahlgren et al. 1990, Brett et al. 2006, Gulati and Demott 1997, Holm and Shapiro 1984). The effect of climate change on grazing resistance falls outside the scope of this paper, but we refer interested readers to reviews by Visser et al. (2005) or Ibelings and Havens (2008).

3.1 Direct and indirect effects of increasing temperatures

Temperature is an all-pervasive environmental parameter that affects the metabolism, growth, reproduction, and survival of living organisms, as well as the interactions among species (Ibelings et al. 2011, Kingsolver 2009). Warmer temperatures will also result in a number of indirect effects (Dale et al. 2006), including increasing stratification and enhanced internal nutrient loading, that are likely to favour at least some cyanobacterial taxa (Boyd and Doney 2002, De Stasio et al. 1996).

With an increase in water temperatures to values approaching physiological optima for a wide range of phytoplankton species, more phytoplankton will grow and replicate faster, at least until warming raises the water temperature beyond the optimal temperature for growth. Optimal temperatures and the degree to which growth rate increases with temperature, as determined by the rate of change in the rate-limiting anabolic process, differ greatly between phytoplankton species. As a consequence, climate warming will result in shifts in phytoplankton community composition (e.g., Winder et al. 2008), including shifts between cyanobacteria. Reynolds (1989, 2006) compiled literature data on the effect of temperature on phytoplankton growth under controlled conditions. The temperature at which maximum replication rates occurred for cyanobacteria varied from just over 20 ^{o}C for *Aphanizomenon flos-aquae* and *Planktothrix agardhii*, to 28 ^{o}C for *Microcystis aeruginosa*, and even 41 ^{o}C for *Synechococcus* sp. (Reynolds 1989, 2006). The rate of acceleration, commonly measured as Q_{10} (acceleration over a 10 ^{o}C step, generally 10 – 20 ^{o}C) for *Synechococcus* sp. was ~2.6, whereas for *M. aeruginosa* it was ~9.6, the highest value

recorded for all of the cyanobacterial or eukaryotic phytoplankton species in the assembled data. Based upon these data, *M. aeruginosa* will have a clear physiological advantage over other phytoplankton when water temperatures increase above 20 ^{o}C. For larger phytoplankton with a low surface area to volume ratio, which includes many bloom-forming, colonial cyanobacterial genera (e.g., *Anabaena*, *Aphanizomenon*), temperature dependence of growth tends to be controlled more by nutrient uptake and rates of intracellular assimilation than by photosynthetic rates (Foy et al. 1976, Konopka and Brock 1978, Reynolds 2006).

In contrast to the data presented above from Reynolds (2006), Lürling et al. (In press) tested - but rejected - the hypothesis that cyanobacteria have higher optimum growth temperatures and higher growth rates at their optimum temperature when compared to chlorophytes (green algae). Lürling et al. (In press) ran a controlled experiment with eight cyanobacteria (including *M. aeruginosa*, *Cylindrospermopsis raciborskii*, and *P. agardhii*) and eight green algae at six different temperatures (20 - 35 °C), and found no significant difference in optimum temperatures for growth between the two taxonomic groups (optimum temperatures for both were ~29 ^{o}C). However, while the green algae grew faster at the lowest experimental temperature (20 ^{o}C), the mean growth rates at the optimal temperature (29 ^{o}C) were not significantly different, indicating that the cyanobacteria benefited more than the chlorophytes from an increase in temperature. Nevertheless, the data also suggest that increasing temperatures, at least in regards to their effect on replication rates, do not offer cyanobacteria a clear advantage over their competitors, even at their optimal growth temperature. Data from Lürling et al. (In press) also provide a warning against

simplification, as there were considerable differences in response to temperature increases among different cyanobacterial species and even among strains. For example, Lürling et al. (In press) found that the Q_{10} for *M. aeruginosa* CYA140 was ~4.3, while the Q_{10} of *M. aeruginosa* PCC7941 was only ~2.1 (calculations by the authors based upon data in Lürling et al., In press). The former value was the highest Q_{10} of all phytoplankton tested, lending support to Reynolds (2006) that *M. aeruginosa* exhibits an exceptionally high Q_{10}. The latter value, however, is well within the range for most cyanobacteria (1.8 – 4.3), and even chlorophytes (1.1 – 3.7; Lürling et al. In press). Finally, it is important to realise that both datasets (Reynolds 2006, Lürling et al., In press) were obtained under laboratory conditions where nutrients and light were saturating for growth. Light-limited growth rates of *P. agardhii*, for example, have been shown to be independent of temperature (Post et al. 1985, Robarts and Zohary 1987).

In natural systems, it has been shown that warmer water temperatures do favour cyanobacterial dominance in phytoplankton communities (Kosten et al. 2012). Similarly, it has been proposed that warmer temperatures will mean earlier and longer potential bloom periods, as well as lead to possible range expansions (Dale et al. 2006, Moore et al. 2008, Wiedner et al. 2007). If the direct effects of warming on cyanobacteria are limited, as suggested in the above paragraph (i.e., in regards to replication rates and Q_{10} values), consequently, indirect effects must underlie the observations on increased occurrence of cyanobacterial blooms with ongoing climate warming (Paerl and Huisman 2008).

In the literature there appear to be conflicting opinions about the relative importance of the direct versus indirect effects of lake warming on cyanobacteria (direct effects examine differences in Q_{10} and replication rates, while indirect effects focus on how temperatures modify the environment in ways that indirectly affect cyanobacteria; e.g., via stratification). Jöhnk et al. (2008) presented model output showing that increased water temperatures favour cyanobacteria directly through increased growth rates. Paerl and Huisman (2008) reached a similar conclusion on the basis of temperature-dependent growth rates from the literature: while the growth rates of most eukaryotic taxa decline at temperatures exceeding 20 ^{o}C, cyanobacterial growth rates for many taxa, including *M. aeruginosa* and *P. agardhii*, continue to increase. Others posit that cyanobacteria may only benefit indirectly from temperature increases, especially from enhanced water column stability, and not the direct effects of climate warming (Wagner and Adrian 2009). Contrary to their expectations, Moss et al. (2003) found that an increase in temperatures of 3 °C above ambient conditions did not result in an increase in cyanobacteria in mesocosm experiments, perhaps because the mesocosms allowed only for the direct, and not indirect, effects of lake warming on cyanobacterial dominance. More work is clearly needed to determine the relative importance of the indirect versus direct effects of increased temperatures on cyanobacteria in freshwater systems.

3.2 Buoyancy

Many species of planktonic cyanobacteria produce gas vesicles, which provide buoyancy and allow access to well-lit surface waters (Walsby 1994). This buoyancy

can be offset with ballast arising from photosynthetic carbohydrate production and other cell constituents (Utkilen et al. 1985). The regulation of buoyancy, which allows migration in stratified lakes between illuminated surface waters and nutrient-rich bottom waters (Ganf and Oliver 1982), occurs as cells accumulate carbohydrates when exposed to light and respire these products of photosynthesis in the dark (Kromkamp and Walsby 1990). Under prolonged irradiance, gas vesicles may collapse under turgor pressure (Kinsman et al. 1991).

As described above, changes in climate, such as increased temperatures, prolonged droughts, and longer water residence times (De Stasio et al. 1996), are predicted to promote cyanobacterial bloom establishment by increasing the strength and duration of stratification (Boyd and Doney 2002, Paerl and Huisman 2008). Furthermore, increased nutrient loading from storm events can synergistically interact with climate-driven effects to increase stratification, further strengthening the competitive advantage of buoyancy-regulating cyanobacteria (Jones et al. 2005). Rinke et al. (2010) demonstrated that the increased phytoplankton biomass resulting from eutrophic conditions increased light attenuation and surface temperatures relative to oligotrophic conditions, thereby strengthening stratification. Thus, buoyant cyanobacteria can themselves modify their environment to promote further blooms by increasing water temperatures and stratification (Kumagai et al. 2000, Rinke et al. 2010).

The buoyancy strategies of cyanobacteria, which are a function of gas vesicle volume, the rate of change in dense cellular constituents, and colony size, play a significant role in their ability to dominate their habitats. *Microcystis* sp. can form

large colonies that float rapidly to the surface, but as with *Anabaena* sp., these colonies can be mixed through the surface mixed layer with wind (Bormans et al. 1999, Brookes et al. 1999, Brookes et al. 2002, Ibelings et al. 1991). Hence, these two cyanobacterial genera may be well adapted to a regime with stronger stratification and a reduced mixing depth. *Microcystis* sp. and *Anabaena* sp.'s fast flotation velocity (Walsby et al. 1991) allows them to efficiently track the near-surface mixed layer (Humphries and Lyne 1988), as demonstrated by Ibelings et al. (1991) in the Dutch lake Vinkeveen. If there is increased stratification and reduced turbulence, then *Microcystis* sp. colonies will be larger and their buoyancy will be enhanced during vertical migration cycles (O'Brien et al. 2004). Another cyanobacterium, *Planktothrix rubescens*, maintains its vertical position in part because its small filaments have a low sinking or floating velocity (Walsby 2005), and also because the tight coupling between carbohydrate accumulation and gas vesicle-mediated buoyancy maintains filaments close to the depth supporting neutral buoyancy (Walsby et al. 2004). Jacquet et al. (2005) suggested that the recent increase of *P. rubescens* in pre-alpine lakes undergoing re-oligotrophication (Ernst et al. 2009) may be because the cyanobacterium can take advantage of the earlier onset of stratification caused by increased temperatures.

Motile or buoyant species may also be able to combine light harvesting near the surface with uptake of nutrients in the hypolimnion. According to Bormans et al. (1999), there is little evidence that buoyant cyanobacteria are capable of exploiting these spatially separated resources. However, Wagner and Adrian (2009) argue that in relatively shallow lakes, such as Lake Müggelsee (maximum depth = 8 m),

cyanobacterial migration is sufficient to allow access to the hypolimnion where nutrient concentrations are elevated. Ganf and Oliver (1982) also found that *M. aeruginosa* and *Anabaena spiroides* were able to migrate 12 m to access light and nutrients, despite substantial density barriers. Hence, fast-migrating genera (e.g., *Microcystis*, *Anabaena*) may benefit from the climate-induced strengthening of stratification by gaining a competitive advantage over other non-migrating or slow-migrating phytoplankton. While increased stratification is predicted to favour buoyancy-regulating cyanobacteria in comparison to non-buoyant algae in most environmental conditions (Huisman et al. 2004), Wagner and Adrian (2009) found that certain thresholds needed to be exceeded for bloom-forming cyanobacteria (especially *Aphanizomenon, Anabaena, and Microcystis* sp.) to dominate: stratification periods in Lake Müggelsee needed to be longer than 3 weeks and exhibit a Schmidt stability index exceeding 44 g cm^{-2} within a critical total phosphorus (TP) range of 70 - 215 μg L^{-1}.

The short-term buoyancy response to light, where carbohydrate is accumulated and respired, is nested within a longer-term response that is a function of both the cyanobacterium's previous nutrient and light history (Brookes and Ganf 2001) and these resources' effects on gas vesicle production and cell metabolism. The rate of gas vesicle production relative to growth, which dilutes the gas vesicle pool per cell, can decrease as nitrogen becomes limiting (Brookes and Ganf 2001, Klemer 1978, Klemer et al. 1982). Konopka et al. (1987) showed that gas vesicle volume increased in phosphate-limited *Aphanizomenon flos-aquae* but that the filaments remained non-buoyant while P-limitation persisted. Similarly, Brookes et al. (2000) observed

considerably fewer gas vesicles in P-limited *M. aeruginosa* cells relative to P-replete cells.

In contrast to the reduction in buoyancy that accompanies gas vesicle dilution in nitrogen-limited cultures, nitrogen-replete *M. aeruginosa* colonies can show persistent buoyancy (Brookes and Ganf 2001). There are several examples in which a proportion of cyanobacterial cells maintained at high light failed to lose buoyancy (Walsby et al. 1989), for which excess nutrients was invoked as the mechanism maintaining persistent buoyancy (Brookes et al. 1999). Eutrophic conditions in freshwater ecosystems, which are expected to occur more frequently in regions that will experience increased nutrient loading and changed hydrology, could lead to 'over-buoyancy' and surface accumulations of cyanobacteria.

Gas vesicle strength is related to the depth of the water body in which the cyanobacteria are found. For example, *P. rubescens* from Lake Zurich (maximum depth = 143 m) has narrower and stronger gas vesicles than found in any other freshwater cyanobacteria (Bright and Walsby 1999), which may have evolved to withstand the high hydrostatic pressures experienced during deep winter mixing. Thus, deep mixing may select for species with strong gas vesicles able to withstand deep mixing without collapsing. Modifications to the mixing regime that may occur if winters become milder and turnover less frequent (Gerten and Adrian 2002) could potentially open up new habitat for species with weaker gas vesicles that were previously outcompeted. Similarly, if lakes experience dramatic decreases in water levels under future scenarios of modified hydrology and drought, cyanobacteria with weaker gas vesicles may proliferate.

Decreasing water temperature in the autumn is a central factor in the loss of buoyancy and resultant sinking of many cyanobacteria to the sediments (Visser et al. 2005). The temperature at which *Microcystis* sp. colonies have been observed to lose buoyancy has been shown to range from 12 - 18 ^{o}C (Visser et al. 1995 and references therein). The loss of buoyancy as water temperatures decrease is not due to the weakening and collapse of gas vesicles, but rather to the accumulation of carbohydrate ballast, caused by a difference in temperature sensitivity between respiration and photosynthesis (Thomas and Walsby 1986). Warmer waters in autumn may delay the sinking of cyanobacteria that have formed blooms, but it is likely to be a strongly strain-dependent effect, as demonstrated by the wide range of temperatures that triggered *Microcystis* sp. sinking (Visser et al. 1995). Experiments with *M. aeruginosa* in water that was warmed from 15 ^{o}C to 20 and 28 ^{o}C demonstrated that buoyancy became constitutive at higher temperatures, with cells remaining buoyant throughout the light period (Kromkamp et al. 1988). Hence, lake warming may result in additional buoyancy that sustains prolonged blooms.

The high degree of variability in catchment hydrology, nutrient loading and hydrodynamics that lakes will experience makes it difficult to predict how the buoyancy of different cyanobacterial groups will be impacted by climate change. However, general conclusions can be made. Increased stratification will favour the fast-migrating buoyant cyanobacteria because decreased mixing will allow the cyanobacteria that reach the water surface first to shade and outcompete the slower-migrating and non-buoyant cyanobacteria. As nutrient loading and stratification increase, there will tend to be a shift towards buoyant species that can access both the

well-lit surface waters and hypolimnetic nutrient pool during periods of mixing. High nocturnal temperatures will act to maintain a shallower surface mixed layer, causing cells to not mix as deeply during night due to wind, which will enable them greater access to light during day. These changes may shift the balance between competing cyanobacteria. For example, in Lake Ijsselmeer, The Netherlands, *Microcystis* is commonly the dominant cyanobacterial genus unless summers are exceptionally warm and stable, at which point *P. agardhii* dominates because of shallow mixing over extended periods (Ibelings 1992). Higher water temperatures persisting into autumn may mean that the loss of buoyancy and mass-sedimentation of cyanobacterial populations that normally occurs will be delayed.

3.3 Luxury phosphorus uptake and storage

In many freshwater systems, phosphorus (P) is a limiting nutrient (Schindler 1974, 1977, Schindler et al. 2008). Cyanobacteria, however, have been shown to overcome this limitation by at least two mechanisms: they produce phosphatases, enzymes that hydrolyse phosphate from organic solutes that then can be taken up (Coleman 1992) and they have the ability to sequester luxury P intracellularly as polyphosphate (reviewed in Healey 1982). Luxury P uptake into storage can increase the P cell quota from 0.2 - 0.4 % of ash-free dry mass to ≥ 3 % of ash-free dry mass, almost 8-16 times the minimum quota (Reynolds 2006). As a result, cyanobacteria can theoretically double three to four times without having to uptake any additional P (Reynolds 2006), which provides a large competitive advantage in P-limiting environments.

Different cyanobacterial genera vary in their ability to access organic P with phosphatases and to store luxury P. Whitton et al. (1991) found significant differences among 50 cyanobacterial strains (10 genera) in their ability to access P from various organic molecules. For example, the Rivulariaceae tested (*Calothrix, Dichothrix*, and *Gloeotrichia*) produced significantly higher P yields than filamentous non-Rivulariaceae (*Anabaena*, *Fischerella, Lyngbya,* and *Tolypothrix*) from most organic molecules. *Gloeotrichia*, in particular, exhibited significantly higher extracellular phosphomonoesterase (PMEase) activity than any other genus tested (Whitton et al. 1991). Similar differences in luxury P storage ability may exist among cyanobacteria, which could favour certain taxa over others during periods of P deficiency. For example, *Anacystis* may be less able to take up luxury P than *Anabaena*, *Plectonema*, or *Synechococcus* (Healey 1982).

Paradoxically, cyanobacteria are able to dominate in both low and high P conditions. In low nutrient conditions, cyanobacteria's high affinity for P allows them to outcompete other phytoplankton (e.g., Posselt et al. 2009). Increased P results in higher concentrations of phytoplankton biomass, which is the best predictor for cyanobacterial dominance in lakes (Downing et al. 2001). Cyanobacteria benefit indirectly from high phytoplankton conditions, potentially because of the low light and CO_2 concentrations that result from high levels of production (reviewed in Hyenstrand et al. 1998). Cyanobacteria are superior competitors for light (but with noted differences among taxa, see section 3.6), and can create higher turbidity per unit P than any other phytoplankton group, thereby excluding their competitors (Scheffer et al. 1997). At low CO_2 levels, cyanobacteria can become dominant because they

generally have better CO_2 uptake kinetics than other phytoplankton (Shapiro 1997). As a result, cyanobacteria can reduce CO_2 concentrations to levels that allow them to persist but exclude other phytoplankton (Shapiro 1997). Finally, when phytoplankton are high, buoyant cyanobacteria can shade out competitors by forming scums (see sections 3.2 and 3.6 on buoyancy and photoacclimation, respectively), further strengthening their dominance at high P conditions.

As described above, the hydrology of low-latitude and continental mid-latitude regions will be characterised by oscillating periods of drought and flooding by the end of the 21st century. When the associated effects of warmer temperatures are also taken into account, we expect that low-latitude and continental mid-latitude inland waters will experience greater thermal stratification, lower water levels during drought periods, less ice cover, and pulsed nutrient loads (Jeppesen et al. 2007, Parry et al. 2007). During long periods of stratification, P limitation in the epilimnion will increase while the hypolimnion may experience anoxia and consequently increased P concentrations due to internal recycling from the sediments (Nürnberg 1984, 1988, Nürnberg et al. 1986). Cyanobacteria may be able to overcome the epilimnetic P limitation during stratification due to their internal nutrient storage (Istvánovics et al. 1993, Pettersson et al. 1993), while phytoplankton without luxury P uptake may not be able to persist during low nutrient periods. For example, *Gloeotrichia echinulata* is able to outcompete other phytoplankton in nutrient-limited conditions because it absorbs additional P in the sediments so as to not require any additional P uptake for its own metabolism or reproduction after it recruits into the water column (Istvánovics et al. 1993). *G. echinulata*'s P uptake and storage may explain why the

cyanobacterium is able to dominate nutrient-limited lakes across the northeastern U.S. (Carey et al. 2008). Despite lower precipitation overall in low to mid-latitudes, high-intensity episodic storm events are predicted to increase in these regions (Parry et al. 2007). Because of their anticipated increased severity (Parry et al. 2007), these storms may be more effective at triggering mixing, which could result in the release of large concentrations of hypolimnetic P to the epilimnion (Søndergaard et al. 2003). The release of hypolimnetic P during mixing events caused by storms may also be coupled with large external loads of P entering lakes through surface runoff (Jeppesen et al. 2007). During these periods of increased nutrient loading due to storms, we expect that the high levels of P will lead to higher primary production and may favour cyanobacteria, as seen with anthropogenic eutrophication. We predict that small cyanobacteria with large surface area to volume ratios and high nutrient uptake rates may especially benefit (Finkel et al. 2009). Finally, lower water levels during drought periods and less ice cover may also promote cyanobacteria because lower lake levels may concentrate and increase nutrient concentrations, and longer ice-free periods extend the cyanobacterial growing season (Peeters et al. 2007).

In temperate and high-latitude regions, which are predicted to experience increased mean precipitation and the greatest increase in precipitation intensity, cyanobacteria may also dominate. Jeppesen et al. (2007) found that in Danish lakes, the higher external P loads caused by runoff would be greater than the predicted water volume increase, resulting in net higher nutrient concentrations in aquatic ecosystems. The increased precipitation intensity in winter will increase flows, erosion, and nutrient delivery in the spring while lower flows in the summer will compound the

stratification and drought conditions described above (Jeppesen et al. 2007). These three drivers- increased nutrient concentrations, greater erosion and discharge due to high precipitation intensity, and summer drought- are all predicted to favour cyanobacteria with rapid nutrient uptake and P luxury storage.

3.4 Nitrogen-fixation

Nitrogen-fixation (N-fixation) is a physiological adaptation of some species of cyanobacteria that can provide them with a competitive advantage when available sources of N in the water column are strongly depleted (Oliver and Ganf 2000, Wood et al. 2010). Dissolved inorganic forms of N, primarily nitrate and ammonium, are preferentially assimilated by phytoplankton, although dissolved organic N has occasionally been shown to constitute an additional component of N nutrition for some cyanobacteria (Berman 1997). Utilisation of gaseous N_2 via N-fixation is energetically expensive because of its requirement to both break the triple bond linking N_2 molecules during the formation of ammonium and to maintain the nitrogenase enzyme essential to catalyse the reaction. However, energetic investments in N-fixation and maintenance of heterocysts in freshwater N-fixing cyanobacteria may be offset by the competitive advantage provided to them in severely N-deficient environments (Oliver and Ganf 2000). In freshwater environments, N-fixation is generally accomplished by heterocysts, specialised cells that prevent the incursion of oxygen from surrounding water and neighbouring vegetative cells, which would otherwise inactivate the activity of nitrogenase. Heterocystous cyanobacteria occur in pelagic freshwater and brackish environments but rarely in the ocean (Paerl 1996).

Non-heterocystous strategies of N-fixation are best known in the marine cyanobacteria *Trichodesmium* sp. (Berman-Frank et al. 2003) but can also occur in freshwater and brackish cyanobacteria by temporal separation of N-fixation. For example, nitrogenase activity at night in some species of *Lyngbya* can be separated from the oxygen-producing photosynthetic activity that would otherwise inactivate nitrogenase during the day (Stal et al. 2010). Species of *Lyngbya* are known to form toxic blooms in marine or brackish-water environments and some species (e.g., *Lyngbya wollei*) also form large benthic mats that can be dislodged and form surface blooms in lake environments, often in association with storm-driven mixing (Bridgeman and Penamon 2010).

Like many other physiological processes specific to cyanobacteria, there are several hypotheses about how increases in water temperature will affect rates of N-fixation *in vivo* (e.g., Miyamato et al. 1979). In a warmer climate, enzymatically-controlled processes such as N-fixation might be expected to increase at a rate approximating cyanobacterial growth rate responses to temperature (i.e., a Q_{10} of $\geq$ 1.8; Reynolds 2006). Staal et al. (2003) showed that Q_{10} values for N-fixation of heterocystous strains of *Nodularia spumigena* and *Anabaena* sp., inferred from nitrogenase activity rates, were indeed > 1.8 and commonly close to 2.0 in the light. They found for 21 different heterocystous species that rates of N-fixation in the light were 2.5 - 4 times higher in the light than in the dark, and that there was substantially greater temperature dependence of N-fixation in the light (Staal et al. 2003). Further work is required to understand if, and the extent to which, temperature dependence of N-fixation may enhance the competitiveness of this group of cyanobacteria in a

warmer climate. There are also a number of indirect effects of temperature on N-fixation. For example, an increase in water temperature will reduce the solubility of oxygen and nitrogen in water, and may result in adaptation of the heterocyst by decreasing cell wall thickness and permeability (Staal et al. 2003). This response has been hypothesised to reduce the competitiveness of heterocystous over non-heterocystous cyanobacteria, but is probably unlikely to supplant the dominance of heterocystous N-fixing cyanobacteria in freshwater and brackish environments.

The availability of dissolved inorganic N is critical to the occurrence of N-fixation, both for heterocystous (e.g., *Anabaena, Aphanizomenon*, and *Planktothrix*) and non-heterocystous genera (e.g., *Lyngbya*). Heterocysts have been shown to differentiate rapidly *in vivo* as nitrate concentrations decrease below ~30 μg L^{-1} (Agawin et al. 2007) and their proliferation has also been shown to precede the rapid increase in vegetative cells associated with blooms, when the relative abundance of heterocysts can decrease rapidly (Wood et al. 2010). Changes in N loading therefore need to be considered when assessing changes in N-fixation under a future climate. Nitrogen loading from lake catchments may potentially either increase or decrease with climate change, driven primarily by geographic heterogeneity of rainfall and temperature-induced changes in soil and vegetation dynamics (Jeppesen et al. 2007), but it will also be strongly influenced by human activities relating to changes in land use and intensification in cultivated catchments (Jeppesen et al. 2011). In this context it is relevant to consider that human activities have already profoundly altered the global N cycle through massive escalation of N-fixation and application of synthetic N fertilisers for both crop production for human food and pasture production to support

greater numbers of domesticated animals (Vitousek et al. 1997). Increases in N loading are not only specific to cultivated catchments, however, because with more than twice the amount of reactive N circulating in the biosphere due to human activities, atmospheric N deposition has alleviated N-limitation of phytoplankton in lakes that would otherwise be largely unaffected by human activities (Elser et al. 2010). Greater availability of inorganic N species arising from increases in atmospheric and terrestrial inputs and storm-driven N loading to freshwater systems could reduce the occurrence of N-fixation.

Despite the increase in N loading from cultivation and atmospheric deposition in many catchments, there is not yet, to the best of our knowledge, any evidence that a link exists between N loading and changes in occurrence of N-fixing cyanobacteria. Several factors may offset the expected net alleviation of N-limitation by increased anthropogenic N loading. For example, within lakes there can be changes in the way that N is transformed and utilised, including observed increases in N losses due to denitrification as N loads increase in association with greater percentages of the catchment in pasture (Bruesewitz et al. 2011). Climate change is likely to increase the duration of water column stratification, which may favour N-fixing, buoyancy-regulating cyanobacteria as inorganic N-species are depleted from surface waters over extended growing seasons (Jeppesen et al. 2011). Thus, the interplay of nutrient availability and increases in water temperature will be critical to the future occurrence and proliferation of N-fixing cyanobacteria.

Considerations of how N-fixation may be altered by climate change could be assisted with models of N-fixation that explicitly include water temperature. The

models that currently exist (e.g., Hense and Beckmann 2006, Howarth et al. 1999, Levine and Lewis 1987, Stal and Walsby 1998) tend to be more specifically targeted to a species level and are based primarily on substrate limitation (e.g., by light and nitrogen). These models offer limited insight into the complexity of N-fixation in natural ecosystems. Most other models of cyanobacteria populations have been directed either at non N-fixing cyanobacteria (Robson and Hamilton 2004) or have not explicitly included N-fixation (e.g., Howard et al. 1996). The complexity involved in modelling N-fixation, even at the scale of chemostats (Agawin et al. 2007), indicates that challenges remain to incorporating the major processes relevant to N-fixation in models that operate at the lake ecosystem scale, including a need for fundamental process information on N-fixation in different species of cyanobacteria at different temperatures and N concentrations.

3.5 Akinete production and life cycle attributes

Some taxa within the Nostocaceae, Rivulariaceae and Stigonemataceae families of cyanobacteria (which include, but are not limited to, the genera of *Anabaena*, *Cylindrospermopsis*, *Gloeotrichia*, and *Nodularia*) can produce akinetes, or thick-walled dormant cells (Nichols and Adams 1982). Akinete differentiation typically occurs during unfavourable growth conditions and is triggered by changes in light, nutrients, temperature and potentially desiccation (reviewed in Kaplan-Levy et al. 2010). Akinete metabolism is very low or undetectable, allowing the cells to survive in bottom sediments for extended periods of time until germination occurs under favourable growth conditions (Adams and Duggan 1999). Akinete development

may ensure the long-term survival of cyanobacterial populations (Whitton 1987), as akinetes can survive temperatures up to 55 ^{o}C (Yamamoto 1976), and are viable up to 64 and potentially > 100 years after deposition (Livingstone and Jaworski 1980, Wood et al. 2009) and after desiccation on land for six winter months (Forsell 1998). Akinete germination can be activated by increasing light, temperatures, nutrients, or dissolved oxygen (or a combination of these factors; Kaplan-Levy et al. 2010).

Under future climate scenarios, cyanobacteria that produce akinetes may have an advantage in withstanding increasingly variable conditions. For example, cyanobacterial populations that form akinetes could be better adapted to intermittent nutrient availability that may be caused by altered precipitation regimes and changes in light intensity and quality due to increased turbidity from storm-induced erosion. In regions that will experience increased drought conditions, akinete-forming cyanobacteria can potentially persist even if water bodies dry up seasonally (Paerl et al. 2011). In general, cyanobacteria exhibit high tolerance to desiccation by producing polyhydroxy saccharides that protect cellular macromolecules from denaturation (Potts 1994), allowing them to survive alternating drought and wet conditions.

Microcystis sp. do not form akinetes; rather, they overwinter in a vegetative state (Verspagen et al. 2005). Successful overwintering is crucial for *Microcystis* sp. because the sediment provides an inoculum for population growth in the following spring. Model simulations indicate that *Microcystis* sp. blooms may be reduced by as much as 50% if the size of the benthic inoculum is reduced (Verspagen et al. 2005). A study by Brunberg and Blomqvist (2002) on overwintering under different environmental conditions indicates that a reduction in the snow cover on ice-covered

lakes would reduce overwintering *Microcystis* sp. populations. We expect that warmer temperatures may increase *Microcystis* sp. recruitment from the sediments, as has been observed for other cyanobacterial taxa (e.g., Karlsson-Elfgren et al. 2004), and may allow *Microcystis* sp. to overwinter in the water column. However, Verspagen et al. (2006) found that increasing temperatures also increase *Microcystis* sp. mortality rate, and overwintering in the water column may increase the light and temperature fluctuations cells are exposed to, decreasing their survival (Brunberg and Blomqvist 2002). Consequently, decreasing ice cover and rising temperatures in temperate and high-latitude regions may potentially favour other, akinete-forming, cyanobacterial genera over *Microcystis*.

3.6 Photosynthesis

In this section, we explore photosynthesis and photoacclimation in cyanobacteria and whether the direct and indirect effects of climate change may affect the competition for light between cyanobacteria and their phytoplankton competitors. Cyanobacteria are reputed to be strong competitors for light due to their accessory pigmentation and the structural organisation of their light-harvesting antenna (e.g., Osborne and Raven 1986). Laboratory experiments demonstrated that the picoplanktonic cyanobacterium *Synechocystis* sp. attenuated light to lower levels than any other tested species and readily outcompeted other phytoplankton species when light was limiting (Passarge et al. 2006). However, Huisman et al. (1999) found that *Microcystis* sp. were not particularly strong competitors for light in a well-mixed environment, as did Reynolds (2006), who found that *Microcystis* sp. had the poorest

light efficiency (by examining the steepness of the initial slope of growth vs. irradiance curves) of 19 phytoplankton species tested. The differences in light interception among different species were mostly due to morphological characteristics: slender, attenuated forms – like that of filamentous cyanobacterial species – were superior to large colonial species at harvesting light (Reynolds 2006). Huisman et al. (1999) concluded that the observed dominance of cyanobacteria in eutrophic waters cannot be explained (solely) by competition for light.

Overall, we hypothesise that the indirect effects of climate warming (i.e., the strengthening stability of the water column) will have a bigger impact on the competition for light among cyanobacterial taxa and with other phytoplankton than the direct effect of warmer water temperatures. The ability of phytoplankton to acclimate to higher water temperatures and photosynthesise is dependent on changes in the fluidity of the thylakoid membranes, which occurs by changing the lipid composition of the membranes (Herrero and Flores 2008). Increased temperatures as a result of climate change may affect photosynthetic electron transport in cyanobacterial photosystems, photoacclimation, and the maximum rates of photosynthesis (e.g., Post et al. 1985, Wu et al. 2009). As a result, cyanobacterial photosynthesis may not actually be affected by warmer temperatures during bloom periods or other times when photosynthetic rates are limited because temperature does not have a large effect on the overall efficiency of photosynthesis (Post et al. 1985).

By comparison, the indirect effect of stronger thermal stability on photosynthesis may alter dominance among cyanobacterial taxa and other phytoplankton. Field observations show that when mixing is strong and buoyant

phytoplankton do not have a competitive advantage, *Microcystis* sp. are outcompeted by superior competitors for light such as *P. agardhii* (see Scheffer et al. 1997) or *C. raciborskii* (O'Brien et al. 2009, Wu et al. 2009). *P. agardhii* has a higher affinity for light than *Microcystis* sp. and if its biomass is sufficiently concentrated during bloom periods, it can shade out competitors, including *Microcystis* sp. (Scheffer et al. 1997). If climate warming stimulates blooms, thereby reducing light availability in the water column, a genus such as *Planktothrix* would benefit. *Microcystis*, however, is adapted to alternating periods of mixing and quiescence and is highly buoyant, potentially favouring the genera under climate scenarios of increased water column stability. When mixing subsides, buoyant colonies can rapidly float up into the near-surface mixed layer and benefit from enhanced access to light. This short-term benefit may have long-term consequences, however, because the time spent at or near the lake surface determines the risk of photoinhibition (described below). Consequently, *Microcystis* may face a trade-off in lakes with strongly enhanced water column stability.

Photoacclimation is the phenotypic adjustment to changes in the availability of light, most notably up or downsize regulation of cellular pigment contents, but also in electron chain components or Calvin cycle enzymes (Falkowski and Laroche 1991, MacIntyre et al. 2002). The role of photoacclimation is not just to maximise the rate of photosynthetic carbon assimilation, but also to protect the cells against damage from an excess of energy (Schagerl and Mueller 2006, Zonneveld 1998). *Synechococcus* sp., for example, are able to grow under full sunlight but only when acclimated gradually to the extreme conditions (MacIntyre et al. 2002). Photoinhibition of

photosynthesis will occur if cells are exposed to an irradiance level that is much higher than what they were acclimated to, causing light stress if irradiance is in excess to what can be used directly in photosynthesis (Powles 1984). Under these conditions, safe dissipation of the excess excitation energy is required to protect the photosystems from long-term damage (Niyogi 2000).

Surface bloom formation by buoyant cyanobacteria after a period of intensive mixing will dramatically increase irradiance and can cause severe photoinhibition, even in nutrient-replete cells (Ibelings 1996, Ibelings and Maberly 1998). The effect of photoinhibition can be extremely detrimental because cyanobacteria synthesise new gas vesicles during periods of deep mixing (and ensuing low irradiance; Konopka et al. 1987). Once mixing subsides, the now over-buoyant colonies can no longer reduce their buoyancy and will become trapped at the surface (Walsby et al. 1991). Photoinhibition sets in quickly and can induce long-lasting cellular damage (Abeliovich and Shilo 1992, Zohary and Pais-Madeira 1990). Ibelings et al. (1994) directly compared *M. aeruginosa* and the green alga *Scenedesmus protuberans* to high (and fluctuating) irradiance, mimicking natural light regimes in lakes. They found that buoyant *M. aeruginosa* was more sensitive to photoinhibition than its green algal competitor. Photoinhibition that is invoked promptly protects cells from potentially much more damaging effects, i.e., it is a mechanism for the long-term protection of photosystem 2 (PS2; Oquist et al. 1992). Photoinhibition that is quickly activated may protect PS2, but during relatively short periods of high irradiance under wind-mixed conditions, *M. aeruginosa* apparently depresses its rate of photosynthesis while *S. protuberans* maintains uninhibited photosynthesis. It appears that *M. aeruginosa* is not

as well adapted to fluctuating light as its eukaryotic competitors and is thus unable to benefit from the saturating irradiance levels that are temporarily available when mixing takes cells to the upper layers of the water column.

Others have also found that mixing not only prevents surface bloom formation but also arrests the growth of bloom-forming species (e.g., Reynolds et al. 1983). This re-emphasises the dependence of buoyant *Microcystis* sp. on a water column that is at least partially stable, enabling the colonies to be maintained in a shallow near-surface mixed layer where light fluctuations are reduced and where irradiance levels are usually consistently high. In a changing climate with stronger and longer periods of water column stability, the buoyancy-dependent niche of *Microcystis* sp. would be strengthened, even in relatively shallow lakes. However, if stability gets too strong, and mixing subsides, *Microcystis* sp. biomass would accumulate in surface scums, possibly causing population losses.

The combination of extreme conditions – high irradiance, depleted carbon, and elevated temperatures (Ibelings 1996, Ibelings and Maberly 1998) - makes it likely that scums should be considered as net loss factors for buoyant bloom-forming genera, which are costly for slow growing specialists like *Microcystis.* Finally, whether lake warming would indeed promote blooms of taxa like *Microcystis* sp. may critically depend on the degree of water column stability and lake morphometry.

4. Conclusion

Incorporating cyanobacterial physiology into bloom predictions is essential to understand how freshwater and brackish cyanobacteria will respond to climate

changes. Fundamentally, cyanobacteria are an extremely diverse group with different sets of traits, and will respond to different aspects of climate change (e.g., increased stratification, altered nutrient availability). For example, *Microcystis* sp. do not fix N but have a relatively high Q_{10} (however, see discussion in this paper), while *Anabaena* sp. fix N but have a lower Q_{10}, and both genera have efficient buoyancy regulation and migration in stable water columns. We would then predict that in warmer waters *Microcystis* may be favoured, but that its competitive advantage may be compromised if N becomes limiting due to altered nutrient delivery. There will most likely also be regional differences in which cyanobacterial taxa dominate, depending on how future climate, hydrology, and nutrient loading vary geographically. Taken together, however, we believe that trade-offs in cyanobacterial physiology among species will overall promote cyanobacterial dominance over other phytoplankton in most future climate scenarios.

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CHAPTER THREE

OCCURRENCE, TOXICITY, AND POTENTIAL ECOLOGICAL CONSEQUENCES OF THE CYANOBACTERIUM *GLOEOTRICHIA ECHINULATA* FOR LOW-NUTRIENT LAKES IN THE NORTHEASTERN UNITED STATES*

Abstract

To date, most research on cyanobacterial blooms has focused on high-nutrient, not low-nutrient, lakes. We investigated reports of the cyanobacterium *Gloeotrichia echinulata* in lakes with low concentrations of nitrogen and phosphorus across the northeastern United States by surveying selected oligotrophic and mesotrophic lakes during four summers. *G. echinulata* is a large (1 – 3 mm diameter) colonial cyanobacterium that may have substantial effects on low-nutrient lakes used for drinking water and recreation because it has the potential to fix nitrogen, transport phosphorus from the lake sediments into the water column, and produce the toxin microcystin-LR. We found *G. echinulata* in the water column of 27 out of 37 lakes we sampled in Maine, New Hampshire, New York, and Vermont. *G. echinulata* densities were typically low (<5 colonies L^{-1}), but occasionally at surface scum-

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producing levels (up to 250 colonies L^{-1}). *G. echinulata* colonies from the survey lakes exhibited detectable microcystin-LR concentrations ranging from 58 – 7148 ng microcystin-LR g^{-1} dry weight colonies. Our data suggest that the microcystin-LR concentrations attributable to *G. echinulata* may pose human health risks if *G. echinulata* densities increase to bloom levels observed in eutrophic systems. To determine the effect of low-level *G. echinulata* densities on oligotrophic lakes, we added *G. echinulata* colonies to *in situ* mesocosms at densities similar to what we observed in the lake survey. We found that there were small but statistically significant increases in total nitrogen, small (<30 μm) phytoplankton biomass, and zooplankton biomass and density after the senescence of the colonies. Additionally, mesocosms with senesced *G. echinulata* had a significantly higher proportion of total phosphorus samples above the method detection limit than no-*G. echinulata* controls. We expect that these effects may be more pronounced in lakes exhibiting higher densities of *G. echinulata*.

Introduction

Cyanobacterial blooms in freshwater lakes have long been considered problematic because of their toxins, foul odors, and harmful effects on aquatic food webs (Paerl 1988, Paerl et al. 2001). Blooms are typically associated with eutrophic lakes, yet phytoplankton records indicate that cyanobacteria can also bloom and form scums in oligotrophic and mesotrophic lakes (Downing et al. 2001, Padisak et al. 2003, Lepisto et al. 2005, Galvao et al. 2008, Ernst et al. 2009, Vareli et al. 2009). Cyanobacterial blooms in low-nutrient systems have not engendered much discussion

among limnologists (e.g., there is no text on the topic in Wetzel 2001 or Kalff 2002), despite the fact that cyanobacterial blooms in a water column characterized by low nutrients is puzzling.

In the past three decades, scientists have observed an increase in cyanobacterial blooms in eutrophic (Hallegraeff 1993, Van Dolah 2000, Anderson et al. 2002, Paerl and Huisman 2008, Paerl and Huisman 2009) as well as oligotrophic and mesotrophic systems (Boyer 2008, Ernst et al. 2009, Winter et al. 2011), highlighting the importance of understanding bloom dynamics, especially in lakes used for drinking water, irrigation, and recreation. Although many of the recent blooms in the oligotrophic and mesotrophic lakes are attributed to increasing nutrient concentrations (e.g., Winter et al. 2011), their water column nutrient concentrations still meet the established criteria for oligotrophic (mean summer epilimnion total phosphorus (TP) concentration $< 10\ \mu g\ L^{-1}$) or mesotrophic ($10\ \mu g\ L^{-1} \leq TP \leq 30\ \mu g\ L^{-1}$) systems (Nürnberg 1996). Understanding the effects of cyanobacterial blooms in these systems is important for understanding changes in lake ecosystem functioning, as well as determining whether there are potential consequences to human health.

Gloeotrichia echinulata (J.E. Smith) P. Richter 1894, a nitrogen-fixing cyanobacterium that forms large (1-3 mm diameter) filamentous colonies, may be increasing in low-nutrient systems in the northeastern United States and Canada (Carey et al. 2008, 2009, Winter et al. 2011). Monitoring data from watershed and state organizations indicate that *G. echinulata* was not common in the past few decades in lakes in the northeastern U.S. (AWI, LSPA, ME-DEP, NH-DES-VLAP, VT-DEC-VLMP). However, the number of reports of *G. echinulata* blooms is

increasing (ME-IWQAR 2006, 2008, 2010, NH-DES-VLAP). Because phytoplankton monitoring in the northeastern U.S. is limited both spatially and temporally, the distribution of lakes in which *G. echinulata* is present and its abundance in the water column are unknown.

G. echinulata has been well-studied in high-nutrient systems (e.g., Barbiero 1993, Jacobsen 1994, Karlsson-Elfgren et al. 2003), but until recently much less was known about *G. echinulata* dynamics in low-nutrient systems. The cause of its increase in northeastern U.S. nutrient-poor lakes is uncertain. However, higher temperatures (Karlsson-Elfgren et al. 2004) or increasing phosphorus (P) concentrations in the lake sediment, perhaps linked to watershed development (Carey et al. 2009), may be implicated. Regardless of the cause, many of the systems that are now experiencing increased *G. echinulata* densities have not exhibited high levels of cyanobacteria in the recent past, raising the question of what consequences *G. echinulata* might have for water quality.

G. echinulata has several physiological attributes that may cause it to have significant effects on ecosystem functioning in low-nutrient lakes. First, *G. echinulata* has a meroplanktonic life history in which akinetes (dormant cells) are formed that overwinter on the lake sediment (Roelofs and Oglesby 1970, Karlsson 2003). In response to increased light and temperatures, the akinetes germinate and grow on the lake sediment, take up luxury concentrations of P from pore water, and then recruit into the water column via gas vesicles, translocating stored P with them (Carr and Whitton 1982, Istvánovics et al. 1993, Pettersson et al. 1993, Tymowski and Duthie 2000). In eutrophic Lake Erken, Sweden and Green Lake, Washington, U.S., *G.*

echinulata recruitment from the sediments can contribute up to two-thirds of the total summer internal P load (Barbiero and Welch 1992, Istvánovics et al. 1993). Second, *G. echinulata* is able to fix atmospheric nitrogen (N; Stewart 1967, Roelofs and Oglesby 1970, Carr and Whitton 1982). Some of *G. echinulata*'s fixed N and stored P may become available to other phytoplankton in the water column (Pitois et al. 1997, Nõges et al. 2004, Fey et al. 2010). Third, *G. echinulata* produces a low concentration of microcystin-LR (MC-LR; Carey et al. 2007), which can have adverse effects on phytoplankton (e.g., Christoffersen 1996, Kearns and Hunter 2001), macrophytes (Pflugmacher 2002, Romanowska-Duda and Tarczynska 2002), zooplankton (Fulton and Paerl 1987, DeMott et al. 1991, Rohrlack et al. 2001, Rohrlack et al. 2005), and fish (Malbrouck and Kestemont 2006, El Ghazali et al. 2010), as well as humans, livestock, and pets (Miura et al. 1991, Jochimsen et al. 1998, Wiegand and Pflugmacher 2005, Hernández et al. 2009). *G. echinulata* is also known to cause skin irritation for humans that swim in contaminated waters (Backer 2002, Serediak and Huynh 2011). Because the concentration of MC-LR in *G. echinulata* has only been reported from one lake (Carey et al. 2007), even low concentrations of microcystins can exert substantial negative effects on food webs (reviewed in Babica et al. 2006), it is important to determine *G. echinulata*'s toxin production in other systems.

To examine the distribution, abundance, and MC-LR concentrations of *G. echinulata* in the northeastern U.S., we conducted an initial survey of *G. echinulata* in low-nutrient lakes in summer 2006 and continued sampling some of those lakes and others in 2008, 2009, and 2010. We measured *G. echinulata* MC-LR concentrations from a subset of these lakes in 2008. To examine the effects of the *G. echinulata*

densities that we observed in the lake survey on nutrients (N and P) and food web structure, we manipulated *G. echinulata* densities in mesocosms in a low-nutrient lake that recently has begun exhibiting surface scums for the first time in its recent monitoring history. We hypothesized that *G. echinulata* would increase nutrient concentrations in the water column by releasing fixed N and stored P through leakage, senescence, or grazing (as has been observed for other cyanobacteria; Healey 1982, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004), which in turn would stimulate other phytoplankton and indirectly increase zooplankton density and biomass.

Methods

Northeastern U.S. Lake Survey

In summer 2006, we conducted an initial survey of 14 lakes in Maine and New Hampshire. We targeted low-nutrient lakes that had incidental reports of increased cyanobacteria (LSPA, ME-DEP, NH-DES-VLAP) as well as nearby lakes that were logistically feasible to sample. In summer 2008, we re-sampled many of the lakes in the 2006 survey and expanded our survey to sample additional lakes in Maine, New Hampshire, New York, and Vermont. We continued sampling a subset of the 2006 and 2008 lakes in 2009 and 2010. In total, we sampled 37 lakes across four years for a combined 193 observations from June to September (Table 3.1), the summer period in which *G. echinulata* has been observed in the water column in other lakes (Barbiero 1993, Karlsson-Elfgren et al. 2003).

At each lake, we sampled the littoral zone near its state-designated boat launch and noted lake conditions (e.g., if a scum was a present). We sampled *G. echinulata*

surface density by collecting plankton from the top 1 m of the water column with an 80 μm plankton net and always used the same site for repeat samplings to compare *G. echinulata* densities over time. When the water level was low, the resulting vertical plankton tow was <1 m, but given the distributed pattern of the colonies in the water column at nearly all observations (i.e., absence of a scum), we have no evidence that these lower volume collections were biased with respect to measures of colonies per L. We also collected water samples in 250 mL acid-washed polyethylene bottles from 0.5 m depth for total N (TN) and TP analysis in 2006 and 2008. *G. echinulata* samples were immediately preserved with Lugol's solution, and nutrient samples were frozen until they were processed in the laboratory. We analyzed TN samples with spectrophotometric methods after a basic persulfate digestion (Crumpton et al. 1992) and TP samples were analyzed colorimetrically according to Van Velholden and Mannaerts (1987) with an acidic persulfate digestion. Method detection limits for the nutrient samples were 74 μg L^{-1} for TN and 7.8 μg L^{-1} for TP.

We counted *G. echinulata* colonies with dissecting microscopes to determine *G. echinulata* densities (colonies L^{-1}). Following the procedures of previous studies (e.g., Barbiero 1993, Istvánovics et al. 1993, Pettersson et al. 1993, Forsell and Pettersson 1995, Tymowski and Duthie 2000, Hyenstrand et al. 2001, Karlsson-Elfgren et al. 2003, Eiler et al. 2006), we enumerated colonies instead of filaments because colonies were the 'natural unit' of *G. echinulata* biomass that we observed in the net samples (following Cottingham et al. 1998). We evaluated a colony as a central core with a mucilaginous sheath surrounded by vegetative cells (Karlsson 2003).

To determine if there was a time of the summer consistently associated with high *G. echinulata* densities across sites, we analyzed differences in *G. echinulata* density among months and 10-day periods with one-way Welch ANOVA (which accounted for unequal variance) in JMP (v. 8.0). We chose 10 days as an appropriate interval because *G. echinulata* akinetes typically take <12 days for germination and recruitment (Karlsson 2003). We repeated the analysis for all ten possible 10-day groupings of sampling days throughout the summer (i.e., the first 10-day period of the first grouping was June 1 – June 10, the first 10-day of the second grouping was June 2 – June 11, etc.) to ensure that our groupings did not bias our analysis. Significance (α) was initially set at $p \leq 0.05$, and a sequential Bonferroni procedure was performed to adjust α for multiple comparisons (Hochberg 1988).

In August 2008, we collected *G. echinulata* colonies to determine their (microcystin-LR) MC-LR concentration from the 18 sample lakes that exhibited sufficient colony biovolume for analysis. From each lake, we used a dissecting microscope to isolate two samples of 100 colonies each, thoroughly rinsed the colonies ten times with reverse osmosis water, and estimated the mean colony biovolume from radius measurements of 25 different colonies. We froze these samples and transported them to the Center for Freshwater Biology Analytical Laboratory at the University of New Hampshire for analysis by enzyme-linked immunosorbent assay (ELISA). Suspensions of *G. echinulata* were treated with three freeze-thaw cycles followed by sonification to disrupt the cells and release microcystins. Immediately before analysis, the samples were passed through a 13 mm, 0.2 μm Whatman PTFE syringe filter to remove particulates (Sasner et al. 2001).

ELISA analyses were performed using instructions for Microcystin 96-Well-Plate Kits (EnviroLogix, Portland, ME), with a method detection limit of 2.5 pg MC-LR mL^{-1}. We calculated the mean MC-LR concentration attributable to *G. echinulata* (i.e., the MC-LR concentration within colonies, which may be potentially released to the water column) in each survey lake using the equation:

$$\left(\frac{\mu g\ MC-LR}{L}\right)=\left(\frac{ng\ MC-LR}{g}\right)\times\left(\frac{g}{mL}\right)\times\left(\frac{mL}{colony}\right)\times\left(\frac{colony}{L}\right)\times\left(\frac{0.001\ \mu g}{1\ ng}\right) \quad \text{eqn. 3.1}$$

where μg MC-LR L^{-1} refers to the mean water column MC-LR concentration attributable to *G. echinulata*, ng MC-LR g^{-1} is the mean colonial MC-LR concentration determined by ELISA, g mL^{-1} is the specific gravity of an individual *G. echinulata* colony (assumed to be 1 g mL^{-1}; Reynolds 2006), mL $colony^{-1}$ is the mean colony biovolume determined by 25 radius measurements, and colony L^{-1} is the mean water column *G. echinulata* density in each lake. We repeated the calculations for the minimum and maximum MC-LR concentration attributable to *G. echinulata* using the minimum and maximum values of MC-LR g^{-1} colonies, colony biovolume, and water column *G. echinulata* density for each lake.

Mesocosm Experiment

In summer 2009, we deployed *in situ* mesocosms in Burkehaven Cove, Lake Sunapee, New Hampshire, U.S. (43°24'N, 72°20'W) to examine the effects of the low densities of *G. echinulata* colonies we observed in the lake survey on N and P concentrations, phytoplankton biomass (as chlorophyll *a*), and zooplankton biomass and density. Lake Sunapee is a large (16.55 km^2 total surface area) oligotrophic lake

with a 25-year mean TP concentration in open water during summer of 5.2 ± 2.3 (1 SD) μg L^{-1} (NH-DES-VLAP). *G. echinulata* has been present in the water column of Lake Sunapee every summer since 2003, occasionally producing scums (Carey et al. 2008).

We suspended clear polyethylene mesocosms from four 4.9 m-long wooden floating frames in the littoral zone. Each mesocosm was 1.2 m in diameter, 1.5 m deep, and open to the sediment, for a volume of ~1500 L. The mesocosms were deployed so that they enclosed the water column as it existed at the study site and thus contained the resident phytoplankton and zooplankton communities at the time of installation. We maintained the mesocosms' cylindrical shape using two 1 m diameter hoops, one attached to the wooden frames at the top and the other embedded 15 cm into the sediments and secured with plastic stakes and weights to prevent horizontal water exchange. We were careful to not disturb the internal mesocosm sediment during deployment. The tops of the enclosures were covered with mesh (~1.5 mm) to prevent disturbance from waterfowl and were situated 0.2 m above the water level to prevent lake water from over-topping the rims of the mesocosms.

We randomly assigned four *G. echinulata* density treatments (with four replicates each, n = 16 total) to the mesocosms, and blocked the treatments by wooden frame. The treatments consisted of four densities of *G. echinulata*: 0 *G. echinulata* colonies L^{-1}, and approximately 2×, 4×, and 12× the ambient colonies L^{-1} in Lake Sunapee (hereafter, referred to as the no-*G. echinulata* control and low, medium, and high *G. echinulata* treatments). We maintained the no-*G. echinulata* control treatment by removing visible *G. echinulata* colonies with dip nets before *G. echinulata* addition

and on each sampling day.

We also monitored two sites adjacent to the frames (1× ambient, hereafter, referred to as the lake reference treatment) in the littoral zone of Burkehaven Cove. These lake reference replicates outside the exclosures were excluded from the statistical analyses to maintain a balanced design; they were used to provide a "check" on mesocosm effects on response variables.

One day before the experiment began, we collected known aliquots of *G. echinulata* colonies from Herrick Cove, a bay in the northeastern part of Lake Sunapee, using a plankton net (100 μm mesh). We then allocated the aliquots to the low, medium, and high treatments as multiples of the ambient density. We transported all colonies back to the lab in clear bottles in coolers, rinsed the colonies three times with GF/C Whatman-filtered lake water, and removed any adhered plankton and debris with microscalpels and probes. We discarded colonies that were missing trichomes or were not buoyant and kept the treatments in incubators at 20^{o}C and on a light-dark cycle that approximated natural lake conditions until we added the colonies to the mesocosms. We observed less than 1% *G. echinulata* senescence (indicated by the lack of trichomes and loss of buoyancy) during this ~48 h period. The bottles were brought in coolers to Lake Sunapee the morning of the pre-*G. echinulata* sampling and remained in the shade onshore with their caps off. Immediately after the initial pre-treatment sampling was finished, the *G. echinulata* colonies were added to the mesocosms to create the bloom treatments (8 August: day of year 220).

Upon addition, the colonies formed scums on the water surface, which subsided within a few days and sank to the sediment. As a result of the senescence of the

colonies, the inferences from the mesocosm experiment were limited to examining the effects of different densities of senesced, not live, *G. echinulata* colonies. To maintain our treatments, we applied a second addition of *G. echinulata* colonies (collected and processed as described above) to the mesocosoms on day of year 228 (16 August). Again, the colonies added to the mesocosms rapidly senesced. We calculated the number of colonies added to the mesocosms in each addition by counting preserved samples of added colony treatments and dividing by the volume of each mesocosm.

We sampled the mesocosms and two littoral sites adjacent to the frames 24 h after the initial *G. echinulata* addition, and every 4 d afterward for 12 d. On each sampling day, we measured dissolved oxygen and temperature at the water's surface and at 0.5 m depth with a 556 MPS meter (YSI, Yellow Springs, Ohio, USA). With an integrated tube sampler (0.8 m long, 5.1 cm diameter), we collected ~8 L of water by sampling 1.6 L each from 5 locations within mesocosm, which were pooled in a clean, rinsed bucket. We retained 1 L of this pooled water for chlorophyll *a* analyses and 500 mL for nutrient analyses, and returned the unused water to the mesocosm. On every other sampling day after *G. echinulata* addition, we filtered 15 L of water through a 80 μm mesh net to measure *G. echinulata* and zooplankton densities; this sample was immediately preserved with 70% ethanol and filtered water was returned to the mesocosms. In all, we removed <1% of a mesocosm's volume on each sampling day.

We processed the chlorophyll samples in the laboratory immediately after field collection. We measured both total and <30 μm chlorophyll *a* (pre-filtered through Nitex mesh) by vacuum-filtering each sample onto Whatman GF/C filters, extracting

them in methanol, and determining the chlorophyll *a* concentration on a Turner TD-700 fluorometer (TurnerDesigns, Sunnyvale, California, USA) according to Arar and Collins (1997). As *G. echinulata* colonies in northeastern U.S. lakes are typically 1 – 3 mm in diameter (Carey et al. 2008), they are excluded from the <30 μm chlorophyll *a* fraction, which represents a size fraction of phytoplankton that most zooplankton can generally graze (Cottingham 1996). It is possible that the 30 μm mesh inadvertently removed some of the <30 μm phytoplankton, which would suggest that our <30 μm chlorophyll *a* concentrations may be conservative.

We analyzed nutrients and plankton according to standard procedures. We saved whole-water samples for TN and TP analyses; filtered water through Whatman GF/F filters for NH_4^+, NO_3^-, and soluble reactive P (SRP) analyses; retained the filter for particulate P analysis; and froze all nutrient samples until analysis. All P fractions (total, particulate, and SRP) and TN samples were analyzed as described above for the lake survey. Nitrate and ammonium samples were analyzed on a Lachat QuikChem 8000 (Lachat Instruments, Loveland, Colorado, USA) according to QuikChem Phenate method #10-107-106-1-J and QuikChem Cadmium Reduction method #10-107-04-1-A, respectively. Method detection limits for the soluble nutrient samples were 7.8 μg L^{-1} for SRP and 9.7 μg L^{-1} for NO_3^--N and NH_4^+-N. We counted *G. echinulata* colonies as described above, and counted zooplankton to genus on a dissecting microscope. We calculated zooplankton biomass using established length-mass regressions (Bottrell et al. 1976, Downing and Rigler 1984). To avoid bias, we estimated log-transformed weights individually from each log-transformed length and retransformed them to original units before calculating the mean weight and size of a

taxon (Bird and Prairie 1985).

We determined the effects of *G. echinulata* treatments on nutrients, chlorophyll, and zooplankton density and biomass using repeated measures (RM) one-way ANOVA in both SAS PROC GLM and PROC MIXED (SAS version 9.2, SAS Institute, North Carolina; Wolfinger and Chang 1999). We report the PROC GLM results here because in almost all cases the GLM statistics, which were Greenhouse-Geisser-corrected to meet assumptions of compound symmetry and sphericity (Quinn and Keough 2002), were more conservative than the PROC MIXED results. We compared the differences between the high *G. echinulata* density treatment and the no-*G. echinulata* control treatment on all of the response variables with a linear contrast and assessed significance (α) at $p \leq 0.05$. We found that more than half of the TP concentrations we measured in the mesocosms were below the limit of detection, which prevented the use of RM ANOVA to compare treatment effects. For TP only, we calculated the proportion of samples collected for each mesocosm after the first *G. echinulata* addition that was above method detection limit. We then analyzed the effect of *G. echinulata* treatment on the proportions with a non-parametric median test in JMP (v. 8.0).

Results

Northeastern U.S. Lake survey

We observed *G. echinulata* in low-nutrient lakes throughout the northeastern U.S. during multiple years (Figures 3.1, 3.2, and 3.3). In 2006, all 13 of the Maine

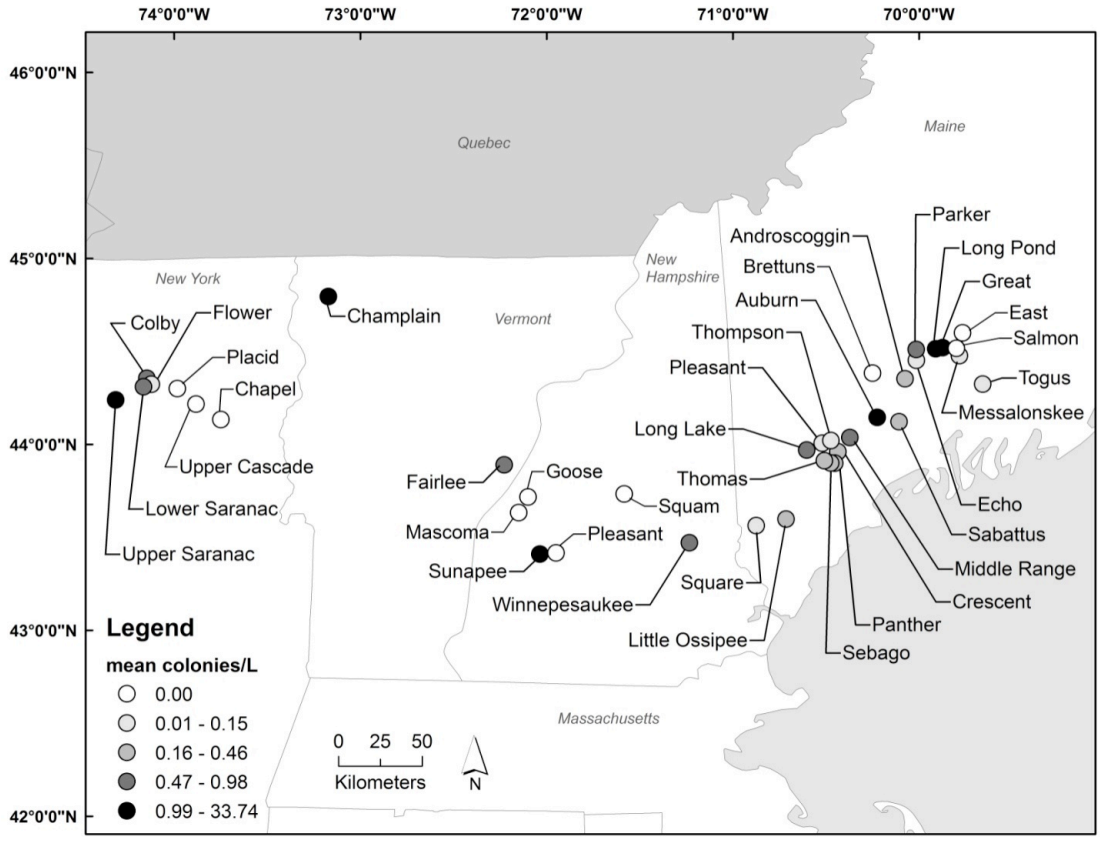

Figure 3.1. Map of the 37 lakes in the northeastern U.S. sampled during surveys in 2006 – 2010, with the mean *G. echinulata* density from all observations for each lake color-coded by density range. The observed densities were divided into quartiles with white representing no observed *G. echinulata* colonies and black representing the highest observed densities. Lake descriptions with water quality parameters are given in Table 3.1.

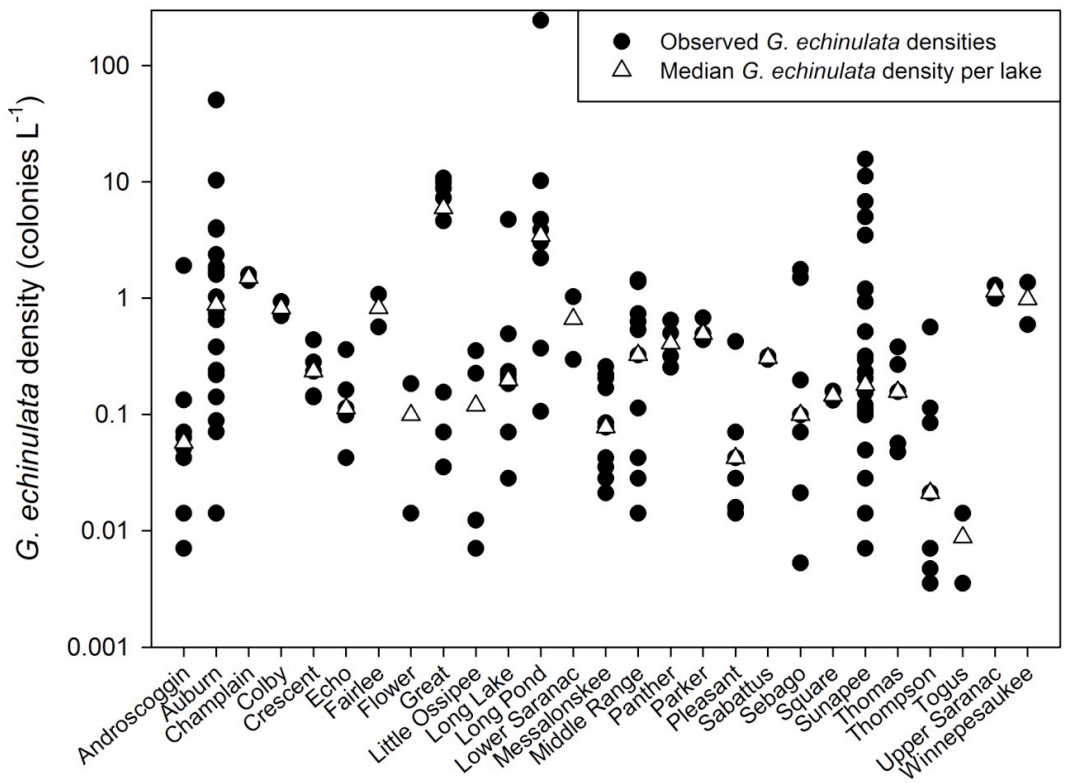

Figure 3.2. Individual observations of *G. echinulata* water column density (colonies L^{-1}) for each lake (filled circles) and median densities for each lake (open triangle) during our surveys in 2006 – 2010. Note that the y-axis is on a logarithmic scale.

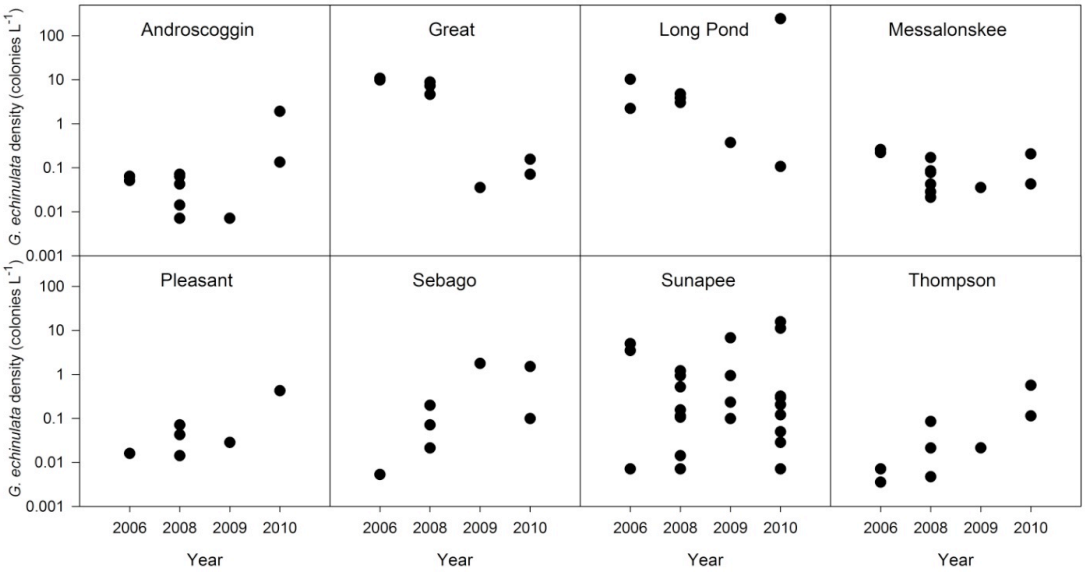

Figure 3.3. Individual observations of *G. echinulata* density (colonies L^{-1}), shown by year for all eight lakes sampled every year of the study. Note that the y-axis is on a logarithmic scale.

Table 3.1. Summary geographical information and water quality parameters for each of our study lakes in the northeastern U.S., grouped by whether or not *G. echinulata* colonies were observed in the lake on sampling visits. Mean summer total nitrogen, total phosphorus, and chlorophyll *a* concentrations and pH values for 2006-2010 (our study period) are listed with 1 standard deviation, with the number of samples in parentheses. Asterisks (*) denote means that include 2000-2010 data, crosses (†) denote means that include 1988-2010 data, and Ref. refers to the origin of the data as described in the footnote.

Lake	Latitude	Longitude	U.S. State	Total nitrogen ($\mu g\ L^{-1}$)	Total phosphorus ($\mu g\ L^{-1}$)	Chlorophyll *a* ($\mu g\ L^{-1}$)	pH	Number of *G. echinulata* samples	Number of sample years	Ref.
Lakes that exhibited G. echinulata on sampling visits										
Androscoggin	N 44°21′	W 70°4′	Maine	260 (1)	16 ± 13 (51)	4.7 ± 1.3 (3)	7.1 ± 0.2 (2)	11	4	1,2
Auburn	N 44°9′	W 70°13′	Maine	.	7 ± 1 (5)	3.2 ± 0.8 (5)	7.3 (1)	20	3	2
Champlain-St. Albans Bay	N 44°48′	W 73°10′	Vermont, New York	390 ± 83 (64)	28 ± 9 (70)	9.3 ± 7.1 (75)	8.4 ± 0.4 (58)	2	1	3
Colby	N 44°21′	W 74°9′	New York	245 (1)	14 ± 4 (20)	4.5 ± 2.8 (20)	7.4 ± 0.3 (20)	2	1	1,4
Crescent	N 43°57′	W 70°28′	Maine	125 (1)	11 ± 4 (3)	3.2 (1)	7.3 (1)	5	2	1,2
Echo	N 44°27′	W 70°1′	Maine	200 (1)	5 ± 2 (3)	2.5 ± 0.1 (2)*	7.3 (1)	6	1	1,2
Fairlee	N 43°53′	W 72°14′	Vermont	160 (1)	17 ± 5 (46)	4.9 ± 2.8 (92)	.	2	1	1,5
Flower	N 44°19′	W 74°8′	New York	405 (1)	19 ± 3 (5)	6.0 ± 1.3 (5)	6.6 ± 0.1 (5)	2	1	1,4
Great	N 44°31′	W 69°52′	Maine	320 (1)	12 ± 5 (7)	5.6 ± 1.4 (3)	7.1 (1)	8	4	1,2
Little Ossipee	N 43°36′	W 70°43′	Maine	160 (1)	6 ± 3 (12)	2.8 (1)	6.9 (1)	4	2	1,2
Long Lake	N 43°58′	W 70°36′	Maine	155 (1)	7 ± 2 (101)	2.9 ± 0.7 (71)	6.8 ± 0.1 (59)	9	3	1,2
Long Pond	N 44°31′	W 69°55′	Maine	180 (1)	9 ± 3 (21)	4.6 ± 1.5 (7)	7.1 ± 0.1 (5)	8	4	1,2
Lower Saranac	N 44°19′	W 74°10′	New York	170 (1)	16 ± 3 (3)	4.9 ± 1.1 (13)	6.9 ± 0.3 (13)	2	1	1,4
Messalonskee	N 44°29′	W 69°47′	Maine	185 ± 28 (2)	13 ± 8 (22)	4.7 ± 0.6 (4)	7.2 ± 0.1 (3)	11	4	1,2
Middle Range	N 44°2′	W 70°22′	Maine	145 (1)	8 ± 2 (8)	3.9 ± 1.2 (6)	7.0 ± 0.3 (5)	11	3	1,2
Panther	N 43°54′	W 70°28′	Maine	140 (1)	5 ± 4 (2)*	2.5 ± 0.1 (2)*	7.2 (1)	4	2	1,2
Parker	N 44°31′	W 70°1′	Maine	155 (1)	8 ± 3 (6)	3.0 ± 0.8 (4)*	7.2 (1)	3	1	1,2
Pleasant	N 44°0′	W 70°31′	Maine	.	6 ± 3 (6)	3.7 ± 0.1 (2)	7.0 ± 0.1 (2)	9	4	2
Sabattus	N 44°7′	W 70°6′	Maine	.	50 ± 15 (18)	27.5 ± 21.5 (16)	7.2 ± 0.3 (10)	2	1	2
Sebago	N 43°54′	W 70°28′	Maine	165 (1)	4 ± 1 (2)	1.6 ± 0 (2)*	7.1 ± 0 (2)	8	4	1,2
Square	N 43°34′	W 70°52′	Maine	.	8 ± 3 (4)	2.0 (1)	7.2 (1)	2	1	2
Sunapee	N 43°25′	W 72°2′	New Hampshire	170 ± 41 (427)	6 ± 1 (15)	2.0 ± 0.5 (15)	6.6 ± 0 (3)	30	4	1,6,7

Thomas	N 43°55′	W 70°30′	Maine	165 (1)	15 ± 8 (4)*	2.5 ± 0.3 (2)*	7.4 ± 0 (2)*	5	2	1,2
Thompson	N 44°1′	W 70°28′	Maine	145 (1)	5 ± 2 (16)	3.7 ± 5.2 (16)	6.8 ± 0.2 (11)	11	4	1,2
Togus	N 44°19′	W 69°39′	Maine	.	16 ± 14 (5)	15.8 ± 17.3 (2)	7.3 (1)*	2	1	2
Upper Saranac	N 44°14′	W 74°19′	New York	170 (1)	14 ± 5 (34)	4.6 ± 2.2 (34)	7.3 ± 0.5 (33)	2	1	1,8
Winnipesaukee	N 43°28′	W 71°14′	New Hampshire	250 (1)	5 ± 0 (9)	1.4 (1)	7.1 (1)	2	1	1,9
Lakes that did not exhibit G. echinulata on sampling visits										
Brettuns	N 44°23′	W 70°15′	Maine	.	9 ± 5 (5)*	3.9 ± 2.5 (2)*	7.2 ± 0 (2)	1	1	2
Chapel	N 44°8′	W 73°45′	New York	230 (1)	7 ± 3 (8)*	2.3 ± 1.2 (8)*	6.7 ± 0.5 (8)*	1	1	1,4
East	N 44°36′	W 69°46′	Maine	.	16 ± 5 (98)	9.7 ± 12.9 (83)	7.1 ± 0 (2)*	1	1	2
Goose	N 43°43′	W 72°6′	New Hampshire	.	7 (1)	13 (1)	5.1 (1)	1	1	9
Mascoma	N 43°38′	W 72°9′	New Hampshire	.	10 ± 4 (3)	3.9 ± 0.5 (3)	7.0 ± 0.3 (3)	1	1	9
Placid	N 44°18′	W 73°59′	New York	230 (1)	6 ± 1 (2)	.	.	1	1	1,10
Pleasant	N 43°25′	W 71°57′	New Hampshire	.	5 ± 1 (14)	4.1 ± 2.6 (14)	6.4 ± 0.4 (10)	1	1	9
Salmon	N 44°31′	W 69°48′	Maine	.	21 ± 31 (49)	6.6 ± 1.0 (4)	7.7 ± 0.3 (3)	1	1	2
Squam	N 43°44′	W 71°35′	New Hampshire	.	5 ± 3 (6)†	2.9 ± 1.6 (6)†	6.7 ± 0.3 (6)†	1	1	9
Upper Cascade	N 44°13′	W 73°53′	New York	240 (1)	8 ± 7 (2)	.	7.3 (1)	1	1	1,10, 11

[1]Total nitrogen (TN): our measurements; [2]Chlorophyll *a*, total phosphorus (TP), and pH: KB; [3]Chlorophyll *a*, TN, TP, and pH: VT-DEC-LC; [4]Chlorophyll *a*, TP, and pH: AWI; [5]Chlorophyll *a* and TP: VT-DEC-VLMP; [6]Chlorophyll *a* and TP: NH-DES-VLAP; [7]pH: NH-DES-LWQR; [8]Chlorophyll *a*, TP, and pH: USLA; [9]Chlorophyll *a*, TP, and pH: NH-DES-LWQR; [10]TP: our measurements; [11]Chlorophyll *a* and pH: ALSC

lakes we sampled (Androscoggin, Crescent, Great, Little Ossipee, Long Pond, Messalonskee, Panther, Pleasant, Sebago, Square, Thomas, Thompson, and Togus; Table 3.1) and the single New Hampshire lake (Sunapee) exhibited low, but non-zero, *G. echinulata* water column densities (ranging from 0.004 – 11 colonies L^{-1}).

In 2008, we sampled all of the lakes surveyed in 2006 (except for Togus) and found that the lakes again all exhibited non-zero *G. echinulata* concentrations. In addition, we observed non-zero, and some quite high (up to 51 colonies L^{-1} in Auburn), densities in five additional Maine lakes (Auburn, Echo, Long Lake, Middle Range, and Parker), one additional New Hampshire lake (Winnipesaukee), four additional New York lakes (Colby, Flower, Lower Saranac, and Upper Saranac), and two additional Vermont lakes (Champlain and Fairlee). In 2008, we also visited ten more lakes– three in Maine (Brettuns, East, and Salmon), four in New Hampshire (Goose, Mascoma, Pleasant, and Squam), and three in New York (Chapel, Upper Cascade, and Placid)– but did not observe any visible *G. echinulata* in their water columns. Those lakes were only visited once, and were not visited again in future years. In 2009 and 2010, we resampled a subset of the lakes that exhibited *G. echinulata* when surveyed in 2006 and 2008 as well as Sabattus Pond (Maine). *G. echinulata* was present in the water column on at least one sampling date during both 2009 and 2010 for all of the lakes visited, and densities ranged from 0.007 colonies L^{-1} (Androscoggin) to 250 colonies L^{-1} (Long Pond), which exhibited a surface scum.

Overall, *G. echinulata* was present in the water column in 174 out of 183 samples from the 27 lakes that exhibited *G. echinulata* on at least one sampling date. During the four years of surveys, we observed a mean non-zero density of 2.8 ± 19 *G.*

echinulata colonies L^{-1} (± 1 S.D.) and a median density of 0.21 colonies L^{-1}. For all lakes combined, we did not observe a significant difference in *G. echinulata* density among years (Welch one-way ANOVA, $F_{3,179} = 0.54$, $p = 0.66$) or months (Welch one-way ANOVA, $F_{2,180} = 2.11$, $p = 0.13$). However, when we partitioned the summer sampling months into 10-day periods, we found that samples at the end of August consistently exhibited higher *G. echinulata* densities than any other period during the summer, regardless of how the 10-day groupings were chosen (seven out of ten 10-day groupings exhibited significant differences: Welch one-way ANOVA, $F_{7,175} > 3.68$, Bonferroni-corrected $p < 0.01$; for the three non-significant groupings: $F_{7,175} \leq 2.58$, $p \geq 0.05$).

In 2008, *G. echinulata* colonies exhibited low, but detectable, MC-LR concentrations in every lake where *G. echinulata* was in sufficient density to collect a sample for MC-LR analysis (Figure 3.4). *G. echinulata* MC-LR concentrations varied considerably among lakes, ranging from a low in Great Pond of 58.5 ± 4.2 ng MC-LR g^{-1} d.w. (± 1 S.D.) to 7148.1 ± 1521.5 ng MC-LR g^{-1} d.w. in Panther Pond (Table 3.2). When the ranges of *G. echinulata* water column densities and biovolumes we measured for each lake were taken into account, the estimated mean concentration of MC-LR in the water column attributable to *G echinulata* colonies was between 4 and 7 orders of magnitude below the 1 µg MC-LR L^{-1} World Health Organization drinking water guideline (WHO 1998; Table 3.2). The maximum MC-LR concentrations for each lake, as determined by the upper bound of the observed MC-LR concentration, *G. echinulata* colonial biovolume, and water column *G. echinulata* density, were at least 3 orders of magnitude below the WHO guideline.

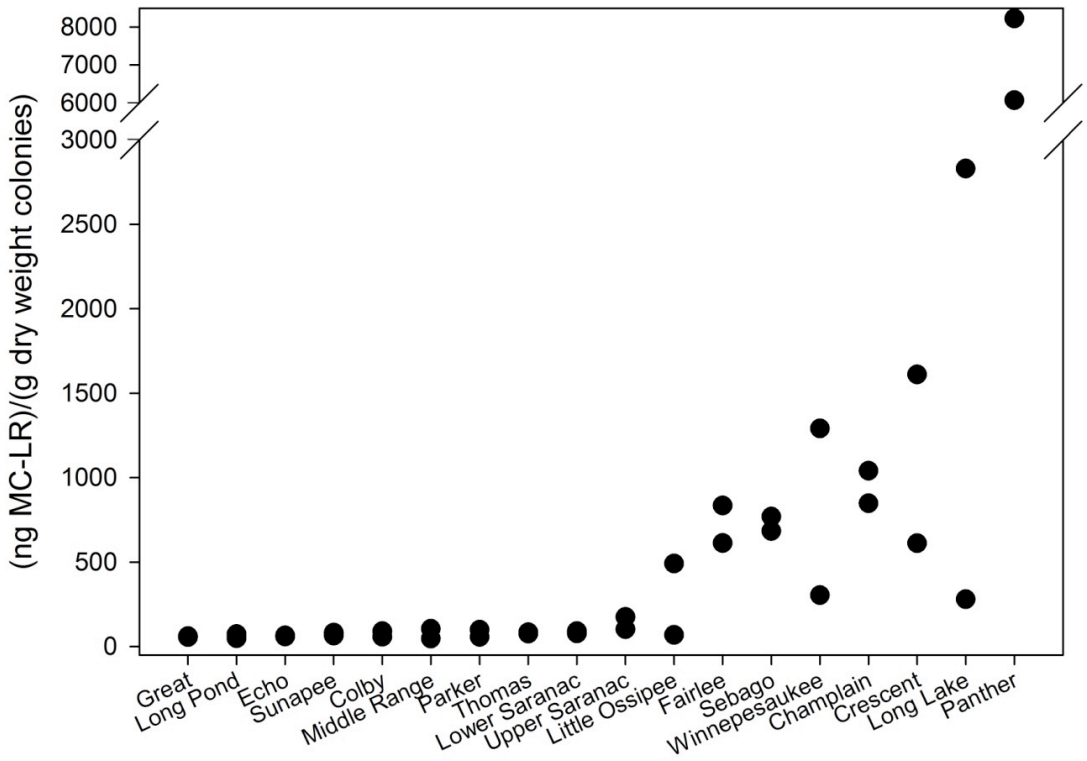

Figure 3.4. The microcystin-LR (MC-LR) concentration (ng MC-LR g^{-1} dry weight colonies) from survey lakes (n = 2 measurements per lake) sampled in August 2008. The lakes are listed in order of increasing mean MC-LR concentrations. Note the break between 3000 and 6000 ng MC-LR g^{-1} dry weight.

Table 3.2. The mean *G. echinulata* colony biovolumes (mm^3; n = 25 for each lake), microcystin-LR (ng MC-LR g^{-1} dry weight; n = 2 for each lake) concentrations in *G. echinulata* colonies, and the estimated mean MC-LR concentration attributable to *G. echinulata* in the water column (μg MC-LR L^{-1}; n = 2 for each lake), calculated with the mean, minimum, and maximum *G. echinulata* MC-LR concentrations, colony biovolumes, and *G. echinulata* water column densities (see Methods). For reference, the World Health Organization guideline for drinking water is 1 μg MC-LR L^{-1} (WHO 1998). Asterisks (*) denote lakes that are public drinking water sources.

Lake	Mean *G. echinulata* colony biovolume (mm^3), with the range of observed biovolumes in parentheses	*G. echinulata* MC-LR concentration (ng MC-LR g^{-1} d.w.)	Mean lake MC-LR concentration attributable to *G. echinulata* (μg MC-LR L^{-1})	Range of lake MC-LR concentrations attributable to *G. echinulata* (μg MC-LR L^{-1})	
				Minimum	Maximum
Champlain*	0.047 (0.011 – 0.15)	944.0	6.7×10^{-5}	1.4×10^{-5}	2.5×10^{-4}
Colby*	0.038 (0.011 – 0.18)	74.3	2.3×10^{-6}	4.7×10^{-7}	1.5×10^{-5}
Crescent	0.082 (0.022 – 0.20)	1111.5	3.9×10^{-5}	1.9×10^{-6}	4.3×10^{-4}
Echo	0.075 (0.0059 – 0.41)	62.1	1.4×10^{-6}	1.5×10^{-8}	2.9×10^{-5}
Fairlee*	0.011 (0.0032 – 0.028)	723.5	6.4×10^{-6}	1.1×10^{-6}	2.6×10^{-5}
Great	0.038 (0.0084 – 0.14)	58.5	1.4×10^{-5}	6.6×10^{-9}	1.2×10^{-4}
Little Ossipee	0.026 (0.0039 – 0.13)	279.6	1.1×10^{-6}	1.9×10^{-9}	2.3×10^{-5}
Long Lake	0.045 (0.0099 – 0.18)	1554.6	6.2×10^{-5}	2.3×10^{-7}	2.4×10^{-3}
Long Pond	0.032 (0.0059 – 0.085)	61.3	6.8×10^{-5}	3.1×10^{-8}	1.5×10^{-3}
Lower Saranac	0.042 (0.0039 – 0.18)	84.3	2.3×10^{-6}	9.1×10^{-8}	1.7×10^{-5}
Middle Range	0.021 (0.0084 – 0.052)	75.9	1.3×10^{-6}	5.5×10^{-9}	2.3×10^{-5}
Panther	0.030 (0.0084 – 0.21)	7148.1	1.5×10^{-4}	1.3×10^{-5}	2.7×10^{-3}
Parker*	0.039 (0.0059 – 0.15)	78.3	2.5×10^{-6}	1.7×10^{-7}	2.0×10^{-5}
Sebago*	0.035 (0.015 – 0.16)	726.0	3.8×10^{-6}	5.5×10^{-8}	7.3×10^{-5}
Sunapee*	0.34 (0.16 – 0.76)	73.2	4.1×10^{-5}	7.2×10^{-8}	9.7×10^{-4}
Thomas	0.045 (0.0071 – 0.092)	80.3	7.4×10^{-7}	2.6×10^{-8}	3.0×10^{-6}
Upper Saranac*	0.060 (0.011 – 0.33)	138.8	9.6×10^{-6}	1.2×10^{-6}	7.4×10^{-5}
Winnipesaukee*	0.029 (0.0048 – 0.11)	797.6	2.3×10^{-5}	8.8×10^{-7}	2.0×10^{-4}

Mesocosm Experiment

At the beginning of the experiment (before *G. echinulata* addition), we observed no significant differences in *G. echinulata* density, total and <30 μm chlorophyll *a*, particulate P, TN, aggregate zooplankton density and biomass, and cladoceran, copepod, and rotifer density among *G. echinulata* treatments (one-way ANOVA, all $p > 0.11$). NO_3^-, NH_4^+, and SRP concentrations throughout the experiment were below the limit of detection; more than half of the TP concentrations were below the limit of detection.

Our treatments increased *G. echinulata* density ($p = 0.0009$, Figure 3.5; see Table 3.3 for mesocosm statistical results), with a peak density in the high treatment of ~15 colonies L^{-1}; however, most colonies died soon after the two additions. Senesced colonies in the high treatment significantly increased TN concentrations and zooplankton biomass and density relative to the no-*G. echinulata* control (treatment effects $p \leq 0.05$; linear contrasts $p \leq 0.03$; Figure 3.5), but we did not observe significant treatment effects for total chlorophyll *a*, <30 μm chlorophyll *a*, and particulate P ($p > 0.05$). There were, however, significant effects of time on both chlorophyll fractions, zooplankton biomass and density, and particulate P ($p \leq 0.048$), and a significant contrast between the no-*G. echinulata* control and high treatment for <30 μm chlorophyll *a* ($p = 0.047$). The increase in zooplankton density was primarily driven by increases in cladocerans and rotifers (linear contrasts between the no-*G. echinulata* control and high treatment: cladocerans, $p = 0.007$; rotifers, $p = 0.03$; copepods, $p = 0.07$). For TP, we found that senesced *G. echinulata* colonies significantly increased the proportion of TP samples above the detection limit (non-

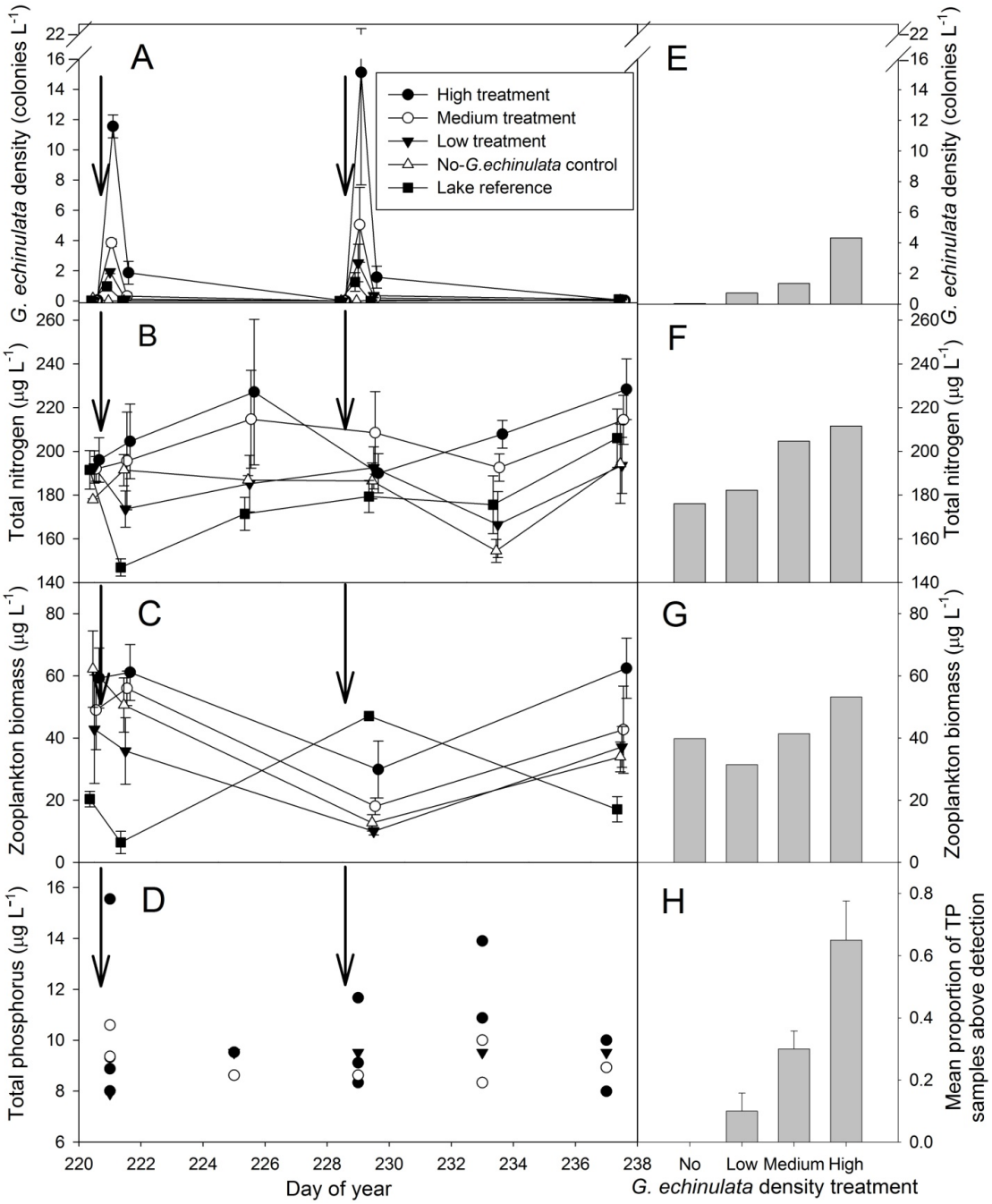

Figure 3.5. Panel A: *G. echinulata* density (colonies L^{-1} ± 1 S.E.) in the mesocosm treatments during the experiment. The high, medium, low, and no-*G. echinulata* control treatments were manipulated experimentally within the mesocosms; the lake reference treatment refers to the ambient *G. echinulata* density in the lake outside the

mesocosms. The arrows indicate the days *G. echinulata* colonies were added to the mesocosms. Note the break between 16 and 22 colonies L^{-1}. Panels B and C: The total nitrogen and zooplankton biomass concentrations of the *G. echinulata* treatments, respectively (± 1 S.E.). Panel D: Total phosphorus concentrations measured in the *G. echinulata* treatments that were above the method detection limit. Panels E, F, and G: The mean *G. echinulata* density, total nitrogen concentration, and zooplankton biomass concentration observed, in the no-*G. echinulata* controls, low, medium, and high treatments, respectively, throughout the experiment. Panel H. The mean (± 1 S.E.) proportion of total phosphorus (TP) samples that were above the method detection limit in the no-*G. echinulata* controls, low, medium, and high treatments, respectively, throughout the experiment.

Table 3.3. Statistical results from the repeated measures ANOVA testing the effects of four densities of *G. echinulata* (approximately 0×, 2×, 4×, and 12× ambient colonies L^{-1}) on chlorophyll, nutrients, and zooplankton in the mesocosm experiment. The planned treatment contrast was between the no-*G. echinulata* control (0×) and high *G. echinulata* density (12×) treatments. DF denotes degrees of freedom. Effects with p-values ≤0.05 are in bold.

Response Variable	Repeated Measures ANOVA test	DF	F-statistic	p-value
G. echinulata density	**Treatment**	3,10	12.88	0.0009
	Planned treatment contrast	1,10	31.44	0.0002
	Time	2,20	2.18	0.16
	Treatment*Time	6,20	1.48	0.27
Total chlorophyll *a*	Treatment	3,12	1.18	0.36
	Planned treatment contrast	1,12	3.54	0.089
	Time	4,48	16.34	<0.0001
	Treatment*Time	12,48	1.44	0.22
<30 μm chlorophyll *a*	Treatment	3,12	2.16	0.15
	Planned treatment contrast	1,12	4.88	0.047
	Time	4,48	12.83	<0.0001
	Treatment*Time	12,48	0.90	0.55
Particulate phosphorus	Treatment	3,7	1.42	0.31
	Planned treatment contrast	1,7	1.92	0.21
	Time	4,28	4.10	0.048
	Treatment*Time	12,28	0.88	0.52
Total nitrogen	**Treatment**	3,10	25.57	<0.0001
	Planned treatment contrast	1,10	45.99	<0.0001
	Time	4,40	1.35	0.28
	Treatment*Time	12,40	0.48	0.86
Zooplankton density	**Treatment**	3,12	3.41	0.05
	Planned treatment contrast	1,12	7.10	0.02
	Time	2,24	4.26	0.04
	Treatment*Time	6,24	0.41	0.82
Zooplankton biomass	**Treatment**	3,12	3.43	0.05
	Planned treatment contrast	1,12	5.81	0.03
	Time	2,24	23.80	<0.0001
	Treatment*Time	6,24	0.44	0.81
Cladoceran density	**Treatment**	3,12	12.83	0.0005
	Planned treatment contrast	1,12	20.35	0.007
	Time	2,24	23.64	<0.0001
	Treatment*Time	6,24	0.85	0.53
Copepod density	Treatment	3,12	1.59	0.24
	Planned treatment contrast	1,12	4.06	0.07
	Time	2,24	13.53	0.0003
	Treatment*Time	6,24	0.46	0.80
Rotifer density	Treatment	3,12	2.99	0.07
	Planned treatment contrast	1,12	5.96	0.03
	Time	2,24	2.83	0.10
	Treatment*Time	6,24	0.43	0.80

parametric median test, $\chi^2 = 12.50$, DF = 3, p = 0.006). Mesocosms in the high *G. echinulata* treatment exhibited 65% (± 13%, 1 S.E.) of their TP samples above the method detection limit, significantly higher than the 0% (± 0%) of the TP samples in the no-*G. echinulata* treatments that were above the method detection limit (non-parametric comparisons for all pairs using the Dunn method for joint ranking, p = 0.007).

Discussion

G. echinulata in northeastern U.S. lakes

Our survey data indicate that *G. echinulata* may be widespread in lakes throughout the northeastern U.S., corresponding to reports from state officials and watershed groups (LSPA, ME-DEP, NH-DES-VLAP). Strikingly, all but one (Sabattus) of the 27 lakes in which we found *G. echinulata* would be characterized as low-nutrient (Nürnberg 1996): 14 were oligotrophic (TP <10 $\mu g\ L^{-1}$) and 12 were mesotrophic (TP ≤30 $\mu g\ L^{-1}$). Most research on cyanobacteria, including the majority of the published studies on *G. echinulata* (e.g., Barbiero 1993, Jacobsen 1994, Karlsson-Elfgren et al. 2003), is from high-nutrient systems, though there is evidence from paleoecological work that *G. echinulata* may be a common species early in the eutrophication process (Bunting et al. 2007). While a randomized sampling of lakes is needed to determine the relative abundance of *G. echinulata* at the landscape scale, our data suggest that *G. echinulata* may be more common in low-nutrient systems in the northeastern U.S. than previously thought.

Twenty-seven of the 37 lakes we sampled had *G. echinulata* colonies in their water column. Given the limited data provided by our 'snapshot' sampling, we are unable to determine why *G. echinulata* was present in some lakes but not others. The 10 lakes we visited that did not have detectable *G. echinulata* were not significantly different from the lakes in which *G. echinulata* occurred in water column nutrient concentrations, chlorophyll *a*, and pH (Welch t-test, all $t \leq 1.80$, $p \geq 0.09$; see Table 3.1). It is possible that *G. echinulata* may be dispersal-limited as has been observed for other large colonial cyanobacteria (Reynolds 2006). Alternatively, *G. echinulata* may have been present in the 10 lakes in which we did not find colonies, but in densities too low to detect with our procedures or because we were unable to observe them in the part of the lake or the particular day that we sampled. For example, we found no *G. echinulata* in the water column in Androscoggin, Pleasant, Sebago, Sunapee, and Thompson on at least one sampling visit, even though these lakes had detectable *G. echinulata* densities on other dates.

We found substantial within- and among-lake variation in *G. echinulata* densities (Figure 3.2). This heterogeneity may exist because both recruitment from the sediments and currents that drive redistribution in the water column are spatially and temporally variable. *G. echinulata* surface populations are heavily subsidized (up to 50%) by recruitment from the sediments, which has been shown to be extremely variable both temporally (over a summer) and spatially (at different sites) within the same lake (Barbiero and Welch 1992, Barbiero 1993, Forsell and Pettersson 1995, Karlsson-Elfgren et al. 2005, Carey et al. 2008). The combination of factors that drive recruitment is still unknown, but a number of factors may be important, including

sediment P (Carey et al. 2008, 2009), nitrate (Chang 1979), light (Roelofs and Oglesby 1970, Barbiero 1993, Forsell and Pettersson 1995, Karlsson-Elfgren et al. 2004), temperature (Barbiero 1993, Forsell and Pettersson 1995, Karlsson-Elfgren et al. 2004), dissolved oxygen (Barbiero 1993), sediment bioturbation (Pierson 1992, Karlsson-Elfgren et al. 2004), and depth (Karlsson-Elfgren et al. 2004). Each of these factors may vary considerably among lakes during the summer. Hence, it is not surprising that *G. echinulata* surface concentrations in neighboring lakes are so variable.

In spite of this variability, however, *G. echinulata* densities across all lakes were significantly higher in late August than any other time of year, which may be coincident with a regional cue, such as changes in light or temperature. This finding is in contrast to *G. echinulata* dynamics in eutrophic systems in Estonia, Sweden, and Washington (U.S.), where peak *G. echinulata* densities are observed earlier in the summer (e.g., Barbiero 1993, Karlsson-Elfgren et al. 2003, Nõges et al. 2004, Karlsson-Elfgren et al. 2005).

Although the variability we observed in *G. echinulata* densities may have been amplified by sampling only the littoral zone in one part of each lake, *G. echinulata* densities within many of the lakes showed less variation year-to-year within the same lake than among lakes (Figure 3.3). For example, lakes that typically exhibited water column *G. echinulata* densities >1 colony L^{-1} consistently exhibited higher than median *G. echinulata* densities in other years (e.g., Long Pond, Sunapee; Figure 3.3), despite being sampled at different times. This may be due to a substantially larger akinete pool in those lakes' sediments (Pettersson et al. 1993, Forsell 1998); a shallow

lake bathymetry (Karlsson-Elfgren et al. 2003, Karlsson-Elfgren et al. 2004); an organic, non-rocky lake sediment substrate (Carey et al. 2008); or other persistent environmental conditions that may promote higher recruitment rates. Similarly, lakes with low *G. echinulata* water column densities (e.g., Messalonskee, Pleasant, Thompson) consistently exhibited lower densities every year (Figure 3.3).

G. echinulata's microcystin-LR concentrations and possible toxic effects

All of the *G. echinulata* samples tested exhibited detectable concentrations of MC-LR. We observed a large range of colonial MC-LR concentrations among lakes (Figure 3.4), which may be because our data only represented samples collected on one day, rather than an integrated sample of colonies collected throughout the summer. However, the mean MC-LR concentration for Lake Sunapee samples collected in August 2008 (73.2 ± 11.8 μg MC-LR g^{-1} dry weight colonies, ± 1 S.D.) was similar to the mean concentration in August - September 2005 (97.07 ± 7.78 μg MC-LR g^{-1} dry weight colonies; Carey et al. 2007). MC-LR production in other cyanobacteria can vary depending on light (Utkilen and Gjolme 1992, Wiedner et al. 2003, Tonk et al. 2005), temperature (van der Westhuizen and Eloff 1985), nutrients (Kotak et al. 2000, Lee et al. 2000, Downing et al. 2005), pH (Eloff and van der Westhuizen 1981), and other environmental conditions, causing large variation in MC-LR concentrations spatially and temporally within the same system (e.g., Makarewicz et al. 2009). These factors may be important drivers of among-lake variation in MC-LR, although we did not observe any correlations between nutrients (TN and TP) and

MC-LR concentrations in our survey lakes (untransformed, log-log, and Spearman's rank correlations: $r \leq 0.17$, $p \geq 0.48$).

Our calculations of the MC-LR concentration in lake water attributable to *G. echinulata*, which take into account the range of measured colonial MC-LR concentrations, *G. echinulata* biovolumes, and *G. echinulata* surface densities, indicate that *G. echinulata*'s MC-LR concentrations are at least two orders of magnitude below levels that affect food webs. However, an increase in *G. echinulata* densities to the maximum level observed in this study (250 colonies L^{-1}) could result in MC-LR concentrations that are associated with ecological effects if those toxins are released to the water column. For example, exposure to low levels of MC-LR (0.1 - 0.5 μg MC-LR L^{-1}) over short time periods (24 h) inhibited growth and photosynthesis for macroalgal, emergent macrophyte, and submerged macrophyte species (Pflugmacher 2002). Similarly, Pietsch et al. (2001) found that photosynthesis by the green alga *Scenedesmus armatus* was inhibited after exposure to 0.25 μg MC-LR L^{-1} for 1 h. Since blooms of *G. echinulata* colonies can last for >2 weeks (Karlsson-Elfgren et al. 2003), it is possible that macrophytes and algae in northeastern lakes could be exposed to MC-LR for longer periods if the MC-LR is released from *G. echinulata* colonies to the water column.

Low concentrations of MC-LR may also affect higher trophic levels. While there are few studies examining the effect of low MC-LR levels on food webs as a whole, Kotak et al. (1996) surveyed phytoplankton, macroinvertebrates, zooplankton and fish for three years in four Canadian lakes where maximum water column MC-LR concentrations during the sample period were ≤0.34 μg L^{-1} and mean concentrations

were less than half that (~0.12 μg L^{-1}). They found that all food web levels except for fish exhibited detectable concentrations of MC-LR accumulated in biomass, and that low MC-LR concentrations may be linked to shifts in zooplankton community structure (Kotak et al. 1996). We hypothesize that *G. echinulata*'s MC-LR production could have similar effects on plankton in northeastern U.S. lakes.

While the MC-LR concentrations in northeastern lakes attributable to *G. echinulata* could approach levels that affect food webs, current *G. echinulata* densities are low enough that it is unlikely that there is a toxicity concern for drinking water quality. However, if *G. echinulata* densities in the northeastern U.S. increased while the MC-LR concentrations per colony stayed constant, MC-LR attributable to *G. echinulata* could become a public health concern. For example, if densities increased to the bloom levels observed in Lake Erken, Sweden (5000 colonies/L; Eiler et al. 2006), three lakes (Crescent, Panther, and Long Lake) would exhibit MC-LR concentrations exceeding the World Health Organization drinking water guideline of 1 μg L^{-1}, and 8 lakes would exhibit MC-LR concentrations between 0.1 and 1.0 μg L^{-1} (Champlain, Echo, Fairlee, Little Ossipee, Sebago, Sunapee, Upper Saranac, and Winnipesaukee). If *G. echinulata* densities in Panther Pond increased to the maximum density we observed in Long Pond (250 colonies L^{-1}), Panther MC-LR concentrations could be as high as 0.4 μg L^{-1}. It is important to note that these estimates are based on littoral *G. echinulata* densities and may not represent pelagic MC-LR concentrations attributable to *G. echinulata* because wind and currents can accumulate colonies in downwind coves, as has been found for other cyanobacteria (Wynne et al. 2011).

Even at current *G. echinulata* surface densities, MC-LR concentrations attributable to *G. echinulata* should be monitored. There have been reports of swimmers developing rashes after exposure to low levels of *G. echinulata* (Backer 2002, Serediak and Huynh 2011), suggesting that *G. echinulata* may be a recreational nuisance even at low densities. All of our survey lakes are used for swimming or other recreation and nine of the 27 lakes in which we observed *G. echinulata* are public or commercial drinking water sources (FTP 2008, JBS 2008, LCA, LCBP, ME-DWP, NH-DWSPP, USLA). Given that the long-term effects of chronic exposure to low-level microcystins is unknown (WHO 1998), we recommend monitoring MC-LR concentrations in any lake that exhibits visible surface densities of *G. echinulata*.

The effects of G. echinulata on nutrients and plankton

In the mesocosm experiment, we were unable to measure the effect of live *G. echinulata* colonies on nutrients and plankton food webs because the colonies senesced soon after addition to the mesocosms. However, these circumstances provided the opportunity to directly test whether *G. echinulata* colonies undergoing senescence influence nutrients, phytoplankton, and zooplankton in the water column. We found that senesced *G. echinulata* colonies had significant effects on nutrients and plankton, even at the low densities tested. The *G. echinulata* densities used in the experiment were similar to densities observed in the lake survey, indicating that senesced *G. echinulata* may exert similar effects on nutrients and food webs in the survey lakes.

Karlsson-Elfgren et al. (2005) also observed senescence of *G. echinulata* colonies soon after addition to mesocosms in mid- to late-summer in Lake Erken, Sweden. In our mesocosms, *G. echinulata* colonies may have died shortly after addition because the colonies were collected from Lake Sunapee in mid-August, after *G. echinulata* had been present in the water column for several weeks, and it is possible that the colonies we used were older and more sensitive to the stress of collection and cleaning. *G. echinulata* colonies have successfully been transplanted into *in situ* mesocosm enclosures where they lived in the water column for several weeks after addition (e.g., Hyenstrand et al. 2001), though those colonies were collected at the beginning of the summer, soon after *G. echinulata* began recruiting into the water column.

Our data suggest that low densities of senesced *G. echinulata* may significantly increase total nutrient concentrations. TN significantly increased in our high *G. echinulata* density treatment relative to no-*G. echinulata* controls, although the difference in concentrations was close to our limit of detection. We also observed that the high *G. echinulata* treatment significantly increased the proportion of TP samples above the method detection limit. Assuming that each colony contains 0.081 μg P (Pettersson et al. 1993) and that almost all of this P is released to the water column, the increase in TP associated with the high *G. echinulata* treatment would be just above the method detection limit, and hence the inability to detect a TP response in the low and medium treatments is unsurprising. In lieu of any published data on colony N content, we estimated the TN concentration to be 0.58 μg N colony^{-1} by multiplying the P concentration by the Redfield ratio (7.2 by atomic mass; Redfield 1934). Using

that estimate, the increase in TN observed in the high treatment after the first and second addition of *G. echinulata* senesced (31 ± 35 μg TN L^{-1} and 32 ± 14 μg TN L^{-1}, respectively) is consistent with the amount of TN potentially released by *G. echinulata* colonies. Soluble N and P concentrations were below method detection limits but presumably the senesced *G. echinulata* colonies released nutrients that other phytoplankton accessed and used rapidly: in general, live cyanobacteria 'leak' nutrients, especially in oligotrophic conditions (Healey 1982, Ray and Bagchi 2001, Wetzel 2001), which could have been taken up immediately by other phytoplankton. It is highly probable that the senesced colonies in the mesocosms released more nutrients and at a higher rate than live colonies could.

The small but significant increase in zooplankton biomass and density in the presence of *G. echinulata* in the mesocosm experiment suggests that low densities of senesced *G. echinulata* may affect higher trophic levels. Cladoceran and rotifer densities were 760% and 240% greater in the high *G. echinulata* treatment than in the no-*G. echinulata* control. Senesced *G. echinulata* may have directly increased cladocerans and rotifers by providing a food source (*G. echinulata* trichomes) that zooplankton could graze (Fey et al. 2010) or indirectly by increasing small phytoplankton. We observed a significant, but small (22%), increase of <30 μm chlorophyll *a* (the small, non-*G. echinulata* fraction of phytoplankton) in the high *G. echinulata* density treatment relative to the control, indicating that small-sized phytoplankton likely increased in response to senesced *G. echinulata* colonies. It is possible that the <30 μm chlorophyll *a* fraction did not increase any more than

observed because any stimulated phytoplankton growth was immediately grazed by zooplankton.

Conclusions

Phytoplankton records from monitoring organizations indicate that *G. echinulata* has not been common over the past decade or longer in lakes in the northeastern U.S. (AWI, AWSD, LSPA, ME-DEP, NH-DES-VLAP, VT-DEC-VLMP), but may now be increasing (ME-IWQAR 2006, 2008, 2010, NH-DES-VLAP). For example, in-depth phytoplankton monitoring in five of our Maine lakes (Androscoggin, Great, Long Pond, Messalonskee, and Sebago) during 1971 – 1973 did not find *G. echinulata* (Davis et al. 1978). *G. echinulata*'s large colonies (up to 3 mm in diameter) are easy to see, so we expect that limnological monitoring would have detected the species if it had been present in the water column. Our survey data indicate that some of our survey lakes may be exhibiting increasing *G. echinulata* densities (Figure 3.3), and more temporally extensive monitoring at several sites in a lake would allow us to determine whether those are real trends.

Our data provide strong evidence that cyanobacteria *can* reach nuisance concentrations (e.g., up to 250 colonies L^{-1}) in low-nutrient lakes, systems in which cyanobacterial blooms are not traditionally studied. Cyanobacterial toxicity is primarily assessed in eutrophic lakes (Chorus and Bartram 1999); however, *G. echinulata*'s abundance in low-nutrient systems indicates the need for monitoring MC-LR concentrations in oligotrophic and mesotrophic lakes. We found that *G. echinulata* persists in the same lakes from year to year when they are sampled in

different months (Figure 3.3). This finding suggests that once *G. echinulata* is established in a low-nutrient lake, it may become a consistent part of the phytoplankton assemblage. In addition, our data suggest that senesced colonies may provide a nutrient subsidy to the water column. As such, the cyanobacterium's MC-LR production and nutrient characteristics may allow it to exert substantial effects on food webs and ecosystem functioning if *G. echinulata* densities increase in low-nutrient northeastern U.S. lakes.

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CHAPTER FOUR

THE CYANOBACTERIUM *GLOEOTRICHIA ECHINULATA* STIMULATES THE GROWTH OF OTHER PHYTOPLANKTON*

Abstract

We tested the effect of the cyanobacterium *Gloeotrichia echinulata* on a diverse array of phytoplankton. We found that *Gloeotrichia* increased the growth rates of five of seven phytoplankton species up to 620% in comparison to a medium-only control after 96 hours.

Introduction

Biochemical interactions, either inhibitory or stimulatory, have gained attention from phycologists for their importance in structuring plankton communities (reviewed in Gross 2003, Legrand et al. 2003), by altering plankton succession, competition, and bloom formation (Keating 1977, Rengefors and Legrand 2001). Although most research conducted on the biochemical impacts of cyanobacterial blooms indicates that they negatively affect other phytoplankton by triggering cellular

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paralysis or inhibiting photosynthesis, enzyme production or nucleic acid synthesis (Gross 2003, Leflaive and Ten-Hage 2007), a growing number of studies indicate that cyanobacteria can also stimulate the growth and division of other phytoplankton in both laboratory and field settings (Keating 1977, Mohamed 2002, Suikkanen et al. 2005, Karjalainen et al. 2007). As the incidence of cyanobacterial blooms increases worldwide due to eutrophication and climate change (Hallegraeff 1993, Paerl and Huisman 2008), understanding the effects of cyanobacteria on aquatic food webs is essential for predicting changes in water quality and ecosystem services.

One cyanobacterial species that may substantially affect lake ecosystems is the colonial nitrogen-fixer *Gloeotrichia echinulata* (J.E. Smith) P. Richter (Carey et al. 2008). *Gloeotrichia echinulata* (hereafter, *Gloeotrichia*) is a large (1-3 mm diameter) filamentous cyanobacterium that forms surface scums in summer and produces the hepatotoxin microcystin-LR (Carey et al. 2007). Although *Gloeotrichia* has historically been observed in meso-eutrophic and eutrophic lakes (Karlsson-Elfgren et al. 2003), it has recently been found blooming in oligo- to mesotrophic lakes throughout the northeastern United States that have no recent (>30 yrs) record of previous *Gloeotrichia* blooms (Carey et al. 2008, 2009). In at least some of these low-nutrient lakes, *Gloeotrichia* blooms are sufficiently dense to cause lake management concerns (Carey et al. 2008). Thus, understanding the factors that enable *Gloeotrichia* to dominate plankton assemblages is interesting ecologically and important for lake management in both oligotrophic and eutrophic lakes.

In this study, we conducted three laboratory experiments to examine the effect of *Gloeotrichia* on other phytoplankton taxa. A multi-clonal culture of *Gloeotrichia*

was obtained from akinetes isolated from Lake Erken, Sweden in winter 2007 and spring 2008 (Karlsson 2003). Lake Erken has experienced *Gloeotrichia* blooms for several decades, and its sediments contain up to 7800 akinetes cm^{-3} in the littoral zone (Forsell 1998). Seven target species were studied, including cultures of the cryptophyte, *Rhodomonas lacustris* NIVA 8/82; three species of cyanobacteria: *Anabaena circinalis* NIVA-CYA 82, *Aphanizomenon* cf. *gracile* NIVA-CYA 338, and *Microcystis aeruginosa* PCC 7806; and one diatom, monospecific *Cyclotella* sp. (Kütz.) Brèb. NIVA-CYA 20, all obtained from the Norsk Institutt for Vannforskning (NIVA), Norway. In addition, one chrysophyte, *Synura petersenii* Korsh CCAP 960/3, and one dinoflagellate, *Peridinium inconspicuum* Lemmermann CCAP (Dinophyceae), were obtained from the Culture Collection of Algae and Protozoa (CCAP) in the United Kingdom. These species (hereafter denoted by their genus) have been observed to co-occur with *Gloeotrichia* in at least two separate lakes, Lake Sunapee (USA) and Lake Erken (Sweden) (Lake Erken database, unpublished data; Lake Sunapee Protective Association, unpublished data). None of these target cultures had been isolated from Lake Erken and so had not co-evolved with the Lake Erken *Gloeotrichia*.

Prior to the experiments, *Gloeotrichia* colonies and stock cultures were grown in modified WC-medium (MWC) (Guillard and Lorenzen 1972) for a minimum of 14 days at $20^{o}C$ at 20 μmol photons $m^{-2}s^{-1}$ with a 14:10 light:dark cycle (Rengefors and Legrand 2007). We measured the incident light with a LI-COR (LI-250A) light meter placed adjacent to the microdishes and assume that a high proportion, if not all, of the incident light reached the phytoplankton because the microdish lids were transparent.

We reproduced these temperature, light intensity, and photoperiod conditions in all experiments. We harvested only one mature *Gloeotrichia* colony from each germinated akinete to maintain multi-clonal *Gloeotrichia* cultures. We transferred these colonies from their germination cultures to new MWC medium for incubation prior to an experiment once they became mature (to synchronize the age of the colonies used in the experiments). We determined colony maturity by the formation of a central core consisting of terminal heterocytes, germinated akinetes in spore sheaths, and vegetative cells (Karlsson 2003). The target species used in the experiments were obtained from MWC stock solutions in exponential growth phase.

First, we tested the allelopathic effect of *Gloeotrichia* on the phytoplankton taxa listed above. To compare the responses of the seven target species, biovolume equivalents for each species were used that corresponded to 10,000 *Rhodomonas* cells mL^{-1} (Table 4.1), calculated according to Blomqvist and Herlitz (1996).

We examined the effect of three treatments (live *Gloeotrichia* colonies, *Gloeotrichia* cell-free filtrate, and a medium-only control) on each target species in 24-well Nunclon^{TM} microdishes (2 mL final volume in all treatments), except for the cyanobacterial species, where only the effects of live *Gloeotrichia* colonies and a medium-only control were tested. This sterile microdish set-up has been used in several studies examining phytoplankton interactions in laboratory settings (e.g., Rengefors and Legrand 2001, 2007). The *Gloeotrichia* cell-free filtrate was collected from MWC medium incubated with *Gloeotrichia* colonies for 1 week at a density of 100 *Gloeotrichia* colonies L^{-1} (~100 mg L^{-1}), within the range of bloom densities

Table 4.1. Biovolumes (μm^3, calculated from measuring >10 different cells of each species) and concentrations of seven different autotrophic target species used in Experiment 1. Biovolumes and standard errors were calculated according to Blomqvist and Herlitz (1996).

Species	Cell biovolume ± 1 S.E. (μm^3)	Target concentration (cells mL^{-1})
Anabaena circinalis	22 ± 5	6818
Aphanizomenon cf. *gracile*	35 ± 7	4220
Cyclotella sp.	438 ± 105	342
Microcystis aeruginosa	13 ± 3	11,540
Peridinium inconspicuum	1,161 ± 53	200
Rhodomonas lacustris	15 ± 2	10,000
Synura petersenii	185 ± 47	810

observed in nature (Carey et al. 2007), before filtration with GF/F (0.7 μm pore size) Whatman filters. We initiated the treatments immediately after the target cells were placed in the microdishes. All of the live *Gloeotrichia* addition treatments received 2 mL of fresh MWC medium and one *Gloeotrichia* colony of similar biomass (~1000 μg), except for the *Peridinium* wells, which received three colonies. The biomass of an individual *Peridinium* cell was considerably higher than the biomass of the other target species; consequently, to match biomass equivalents with the other species yet still ensure adequate encounter rates, we increased the number of *Gloeotrichia* colonies and *Peridinium* cells mL^{-1}. Each treatment by species combination had 4 replicates, which were terminated by adding 25 μL of Lugol's solution to each well at 96 h. We counted cells directly in wells at 400X with an inverted light microscope (Nikon Eclipse TS100).

We analyzed the differences in growth rate, *r,* among treatments over the 96 h period with a one-way ANOVA separately for each species because of the unbalanced design. We calculated growth rate with the equation: $r = [\ln(n_1) - \ln(n_0)]/[t_1 - t_0]$ where n is cell density and t is time. Statistical analyses were conducted in JMP (v. 7.0, SAS Institute, 2007). Finally, we used a Tukey's test ($\alpha = 0.05$) to analyze differences in target species growth rate among the treatments.

Gloeotrichia colonies significantly stimulated the growth rate of five of the seven target species relative to the medium-only control: *Anabaena*, *Cyclotella* sp., *Microcystis*, *Peridinium,* and *Rhodomonas* all increased (Table 4.2, Figure 4.1). The

Table 4.2. One-way ANOVA tests of treatment (*Gloeotrichia echinulata* live colonies, filtrate, and medium-only control) of growth rate for each target taxon over the 96 h experiment.

Species	DF	F ratio	P-value
Anabaena circinalis	1,6	9.03	0.02
Aphanizomenon cf. *gracile*	1,6	4.00	0.09
Cyclotella sp.	2,9	95.05	<0.0001
Microcystis aeruginosa	1,6	28.45	0.002
Peridinium inconspicuum	2,9	5.15	0.03
Rhodomonas lacustris	2,9	75.92	<0.0001
Synura petersenii	2,9	1.15	0.35

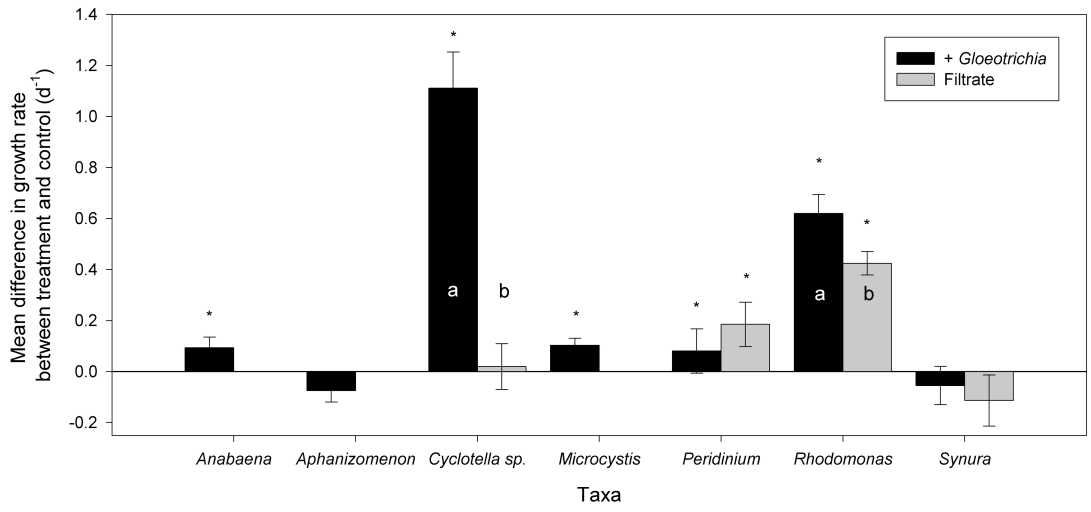

Figure 4.1. The mean difference in growth rate (d^{-1}) between treatments of *Gloeotrichia echinulata* or filtrate and the medium-only control for seven phytoplankton species after 96 h (n = 4). Error bars represent 1 S.E. Asterisks represent treatments significantly different from the control, and letters represent significant differences between the two treatments (Tukey's test).

effect of *Gloeotrichia* was considerable for some species: growth rate in the *Gloeotrichia* treatment for *Rhodomonas* and *Cyclotella* sp. was 620% and 150% greater, respectively, than in the corresponding control at 96 hours. *Anabaena*, *Microcystis*, and *Peridinium* each exhibited a 24-53% greater growth rate in the *Gloeotrichia* treatment than the control after 96 hours. In addition, target species growth rate was substantially greater in the *Gloeotrichia* filtrate treatments for *Peridinium* and *Rhodomonas* than in their corresponding medium-only controls (100% and 420%, respectively); but not for *Cyclotella* sp. or *Synura,* the two other species that received filtrate treatments. For *Aphanizomenon* and *Synura*, there were no significant differences in growth rate among treatments ($p > 0.09$). We were unable to detect any negative effects of *Gloeotrichia* on any of the target species, as would be expected if *Gloeotrichia* produced inhibitory compounds.

Second, we tested the effect of live *Gloeotrichia* on five densities of *Rhodomonas*: 500; 1,000; 10,000; 20,000; and 40,000 cells mL^{-1}. We chose to use *Rhodomonas* because of its documented sensitivity to allelochemicals (Rengefors and Legrand 2001, 2007). We established initial *Rhodomonas* densities from dilutions of the stock cultures, with standard deviations <1%. We exposed each density to three treatments (*Gloeotrichia*, *Gloeotrichia* cell-free filtrate, and a medium-only control) with four replicates each in 2 mL, 24-well Nunclon microdishes. We used four microdishes for the experiment, with each microdish containing one replicate of every treatment × density combination. The live *Gloeotrichia* treatment consisted of 2 live non-clonal colonies of similar biomass (~1000 μg) with 2 mL of fresh medium. The

experiment ran 45 h and cells were counted as described above. We analyzed the mean difference in *Rhodomonas* growth rate among treatments with a one-way ANOVA.

With all growth rate data grouped together, regardless of initial *Rhodomonas* density, there were significant differences among treatments in *Rhodomonas*' growth rate over the experimental period (Figure 4.2, one-way ANOVA, $F_{2,57} = 6.46$, $p = 0.003$): *Rhodomonas* growth rates were significantly higher after exposure to *Gloeotrichia* filtrate than to *Gloeotrichia* colonies (Tukey's test). The effect of *Gloeotrichia* filtrate on *Rhodomonas* growth rate compared to the control growth rate was significantly greater at lower initial densities of *Rhodomonas* (one-way ANOVA, $F_{4,15} = 6.38$, $p = 0.003$).

We observed a stronger stimulatory effect of the filtrate than *Gloeotrichia* colony treatment on *Rhodomonas* (10,000 cells mL^{-1}) in the multi-density experiment and vice versa in the multi-species experiment. The filtrate treatment effect (*i.e.*, the difference in growth rates between the filtrate and the control) was very similar between experiments: 0.41 ± 0.08 d^{-1} in the multi-density experiment and 0.42 ± 0.05 d^{-1} in the multi-species experiment. The *Gloeotrichia* treatment effect was more variable between experiments: we observed an effect of 0.12 ± 0.05 d^{-1} in the multi-density experiment and 0.62 ± 0.07 d^{-1} in the multi-species experiment. We hypothesize that the differences in growth rate may be due to the varying physiological state of the *Gloeotrichia* in our separate experiments, which were started several days apart. It is possible that the *Gloeotrichia* colonies were providing less of a stimulatory effect in the multi-density experiment than in the multi-species

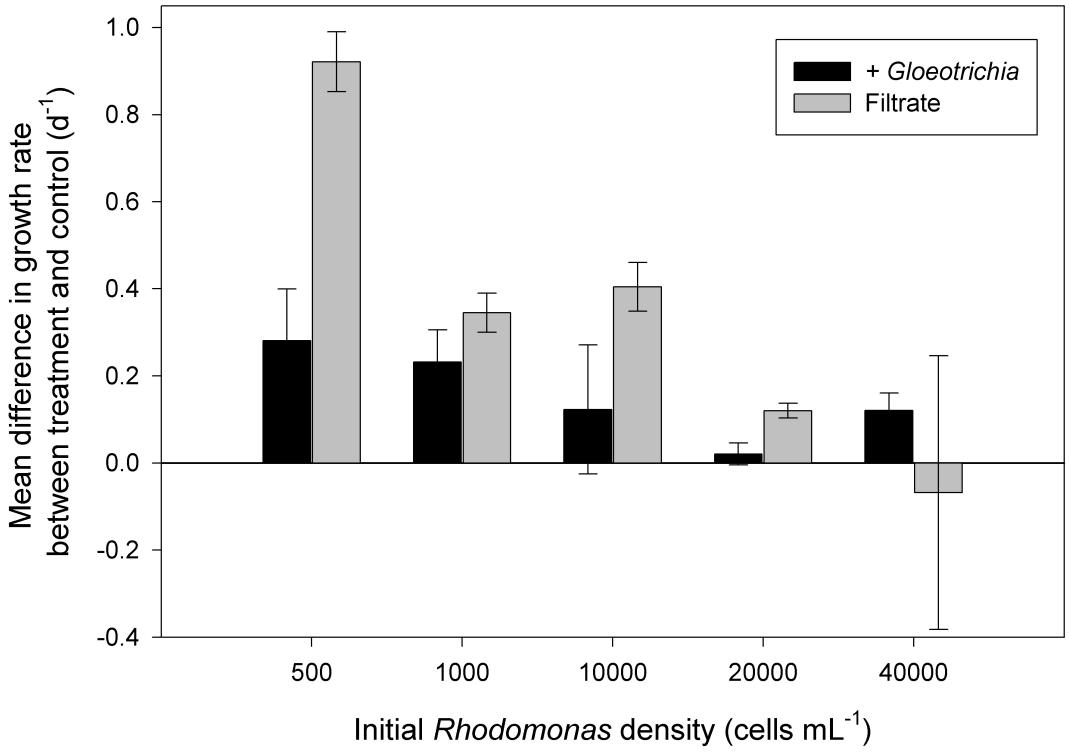

Figure 4.2. The mean difference between the *Rhodomonas lacustris* growth rate in the *Gloeotrichia echinulata* or filtrate treatment and the medium-only control treatment growth rate for each of the five initial densities of *Rhodomonas* (n = 4). Error bars represent 1 S.E.

experiment. Although differences in growth rate between the experiments do exist, the variability within treatments for both experiments is quite low. Thus, even with the variability between experiments, the consistency of the treatment effects indicates that *Gloeotrichia*'s filtrate stimulation is valid.

Third, we tested the effect of different biomasses of live *Gloeotrichia* colonies on an intermediate density of *Rhodomonas* (10,000 cells mL^{-1}). Before the experiment began, we calculated the biomass of *Gloeotrichia* colonies in culture from measurements of colony diameter (assuming *Gloeotrichia*'s density was 1 g cm^{-3}) and grouped the colonies into six biomass classes, with each class representing one treatment. We then chose four live *Gloeotrichia* colonies from each biomass class for each treatment. The live *Gloeotrichia* colonies were added separately to 2 mL wells in 24-well Nunclon microdishes containing 10,000 *Rhodomonas* cells mL^{-1} and MWC medium and incubated for 96 h. The six *Gloeotrichia* treatments consisted of 0 µg biomass (no colony added), 220 ± 0 µg, 610 ± 0 µg, 1020 ± 0 µg, 1890 ± 70 µg, and 3320 ± 170 µg (1 S.E.).

We conducted model selection in the R statistical package (R Development Core Team 2008; http://www.R-project.org) to determine the most appropriate regression model describing the relationship between *Rhodomonas* growth rate and *Gloeotrichia* biomass. We tested four possible models commonly used to describe algal dynamics, two linear and two nonlinear saturating functions: mean (y = a), linear (y = ax + c), and Michaelis-Menten with an intercept term ($y = c + \frac{ax}{s + x}$) and without an intercept term ($y = \frac{ax}{s + x}$) (Briggs and Haldane 1925), to ascertain if

Gloeotrichia linearly or non-linearly affected *Rhodomonas* cell density. We solved for maximum likelihood estimates for each model parameter using a simulated annealing algorithm, a global parameter optimization procedure, with 10,000 iterations, using a normally-distributed error term. We used the Akaike Information Criterion (AIC) to select the most parsimonious model; *i.e*., the best model fit for the fewest parameters (Burnham and Anderson 2002).

Similar to the first two experiments, we observed a stimulatory effect of *Gloeotrichia* colonies on *Rhodomonas* growth rate in comparison with the control treatment; in this case, *Rhodomonas* growth rate increased as a linear function of *Gloeotrichia* biomass (Figure 4.3). We chose the linear regression model (over mean and non-linear models) because it exhibited the lowest AIC value (Table 4.3; Burnham and Anderson 2002).

We found that *Gloeotrichia* exhibited the greatest stimulatory effect at low densities of *Rhodomonas* and at high *Gloeotrichia* biomasses. The most likely explanations for these results, similar to Rengefors and Legrand (Rengefors and Legrand 2007), are that at a low density of a target species, more *Gloeotrichia* exudates are available for each target cell. This finding suggests that *Gloeotrichia*'s stimulatory effect on phytoplankton may be greatest when *Gloeotrichia* biomass in the water column is high (during blooms). Despite that the Michaelis-Menten model's AIC value was similar to the linear model's AIC, we did not observe saturation in *Rhodomonas* growth rate across the wide range of *Gloeotrichia* biomasses tested. Only 35% of the variation in the stimulatory effect on *Rhodomonas* was explained by *Gloeotrichia* biomass, which may be explained by differential production of exudates

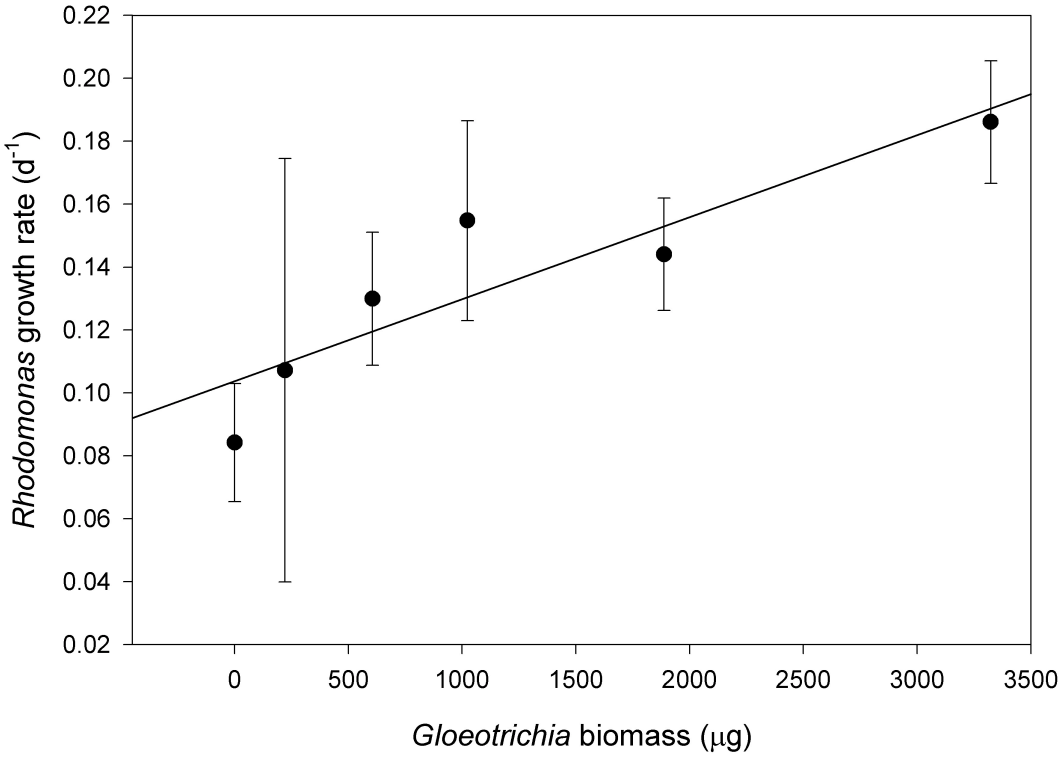

Figure 4.3. Growth rate of *Rhodomonas lacustris* increased linearly with *Gloeotrichia echinulata* biomass. There was non-significant lack of fit for the linear model ($F_{4,16}$ = 0.61, p = 0.66). Error bars represent 1 S.E.

Table 4.3. A list of the regression models tested in Experiment 3 in descending order of best fit, as determined by lowest AIC value.

Model type	Model equation	Parameters with two-unit support intervals	R^2	Corrected AIC
Linear	$ax + c$	a = 0.10 (0.09 – 0.12) c = 0.03 (0.01 – 0.06)	0.35	-72.5
Michaelis-Menten with intercept term	$c + \frac{ax}{s + x}$	a = 0.12 (0.08 – 0.15) c = 0.08 (0.07 – 0.10) s = 0.04 (0.03 – 0.06)	0.40	-72.0
Mean	a	a = 0.14 (0.12 – 0.16)	0	-64.9
Michaelis-Menten without intercept term	$\frac{ax}{s + x}$	a = 0.18 (0.15 – 0.21) s = 0.18 (0.04 – 0.43)	0	-60.4

in the non-clonal *Gloeotrichia* colonies. Differences in allelochemical effect among clones of the same species have also been observed in dinoflagellates (Tillmann et al. 2009).

We cannot determine the exact mechanism responsible for *Gloeotrichia*'s stimulation of other phytoplankton species in this study, but suggest three possibilities. First, *Gloeotrichia* may be releasing nutrients, such as stored P (Nõges et al. 2004, Carey et al. 2008) or fixed N (Stewart 1967). We were unable to measure changes in the medium nutrient concentrations due to the low volume of medium in the microdishes (2 mL), however, due to the short-term nature of our experiments and the very high N and P concentrations in our MWC culture medium (>1 M (14 g L^{-1}) and 0.05 M (1.5 g L^{-1}), respectively), it is unlikely that the target phytoplankton were nutrient-limited. Further, as the *Gloeotrichia* were grown at an irradiance likely close to the compensation level, it is unlikely that the colonies were exuding carbohydrates because of an excess of C over N acquisition (Ana and Massimo 2004). Second, cyanobacteria produce many bioactive secondary metabolites (Gross 2003, Legrand et al. 2003), which phytoplankton may have evolved to recognize and utilize for their own metabolism (Suikkanen et al. 2004). Many phytoplankton species are capable of using dissolved organic compounds (osmotrophy) (Sanders et al. 1990, Tittel and Kamjunke 2004), and thus a positive growth response to algal exudates is not unlikely. Third, *Gloeotrichia*, similar to other cyanobacteria, may produce anti-bacterial or anti-fungal compounds beneficial to other phytoplankton (Legrand et al. 2003).

Our work adds to the growing literature indicating that stimulation, or facilitation, may be an important force structuring communities (Bruno et al. 2003, Halpern et al. 2007). Although Suikkanen et al. (2005) has suggested that stimulation is more likely to occur in natural communities than in laboratory experiments, our results demonstrate strong positive effects of *Gloeotrichia* on other phytoplankton in laboratory experiments. Although we do not know the evolutionary significance of *Gloeotrichia*'s stimulation of other phytoplankton, our findings may indicate co-evolution among phytoplankton taxa. Our results are consistent with observations from Lake Peipsi, Estonia (Nõges et al. 2004), where *Gloeotrichia* blooms stimulated other phytoplankton species to increase in the field. Although this stimulatory mechanism remains to be elucidated, our data suggest that *Gloeotrichia* may be able to enhance eutrophication, particularly in oligotrophic lakes where this cyanobacterium has recently begun to bloom.

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CHAPTER FIVE

THE CYANOBACTERIUM *GLOEOTRICHIA ECHINULATA*: AN ECOSYSTEM FACILITATOR INCREASING RESOURCES AND STIMULATING PHYTOPLANKTON IN NUTRIENT-LIMITED FRESHWATER ECOSYSTEMS

Abstract

Facilitation has gained attention in recent years as an important structuring force in terrestrial and marine communities. Much less is known about the role of facilitators in freshwater ecosystems, especially plankton communities. Freshwater cyanobacteria generally have negative effects on plankton in high nutrient systems because of their thick scums and toxins, yet they may have positive effects on plankton in low nutrient systems because they may be able to increase limiting nutrients (namely, nitrogen and phosphorus). We investigated the effects of *Gloeotrichia echinulata*, a large colonial cyanobacterium that may be increasing in low nutrient lakes in the northeastern USA, on nutrient concentrations and plankton food webs in three experiments. Because it fixes nitrogen and stores large quantities of phosphorus intracellularly, we hypothesized that *G. echinulata* would facilitate

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other phytoplankton in low nutrient systems by increasing available nutrient concentrations in the water column.

High densities of *G. echinulata* had significant positive effects on small-sized (<30 μm) phytoplankton, potentially because the cyanobacterium increased nitrogen and phosphorus concentrations relative to no-*G. echinulata* controls in all experiments. When zooplankton grazers were absent or in low densities, small-sized phytoplankton significantly increased on a gradient of *G. echinulata* density. At high levels of zooplankton biomass, small-sized phytoplankton biomass was higher in *G. echinulata* treatments relative to no-*G. echinulata* controls, but with a surprising twist: phytoplankton stimulation was positively related to zooplankton biomass. *G. echinulata* colonies exposed to high levels of zooplankton biomass exhibited higher levels of damage (potentially through grazing), which may have increased the rate of nutrient leakage from *G. echinulata* to other phytoplankton, thereby intensifying the cyanobacterium's stimulatory effect. Our findings indicate that cyanobacteria may be important facilitators in freshwater systems and may play an important role in structuring food webs by increasing small-sized phytoplankton biomass. In addition, *G. echinulata* blooms in low nutrient lakes may have important consequences for water quality because high densities of *G. echinulata* can increase nitrogen and phosphorus concentrations.

Introduction

Some species have large effects on their surrounding community by substantially affecting food web structure and ecosystem functioning. These species

play critical roles by altering trophic and competitive dynamics, habitat structure, and the flux of nutrients (reviewed by Jones and Lawton 1995). While much ecological research has focused on negative effects via trophic linkages (i.e., predation) and competition (Risch and Boucher 1976, Connell 1983, Keddy 1989, Cherif 1990, Bronstein 1994), there is growing recognition that facilitators, species that have positive effects on other organisms, can also play an important role structuring ecological communities (Bertness and Callaway 1994, Kareiva and Bertness 1997, Stachowicz 2001, Bruno et al. 2003, Brooker and Callaway 2009).

Facilitation, any interaction between organisms in which at least one of the species benefits and neither is harmed (Bruno et al. 2003), ranges from obligate mutualisms to amelioration, in which a benefactor reduces stress (abiotic or biotic) or increases resources for a recipient species (Stachowicz 2001, Bruno et al. 2003, Gomez-Aparicio et al. 2004, Brooker et al. 2008). Amelioration is an important process that substantially affects community structure and nutrient cycling, especially in marine and terrestrial ecosystems (reviewed by Bertness and Leonard 1997, Brooker et al. 2008, Bulleri 2009). For example, nurse plants ameliorate desiccation, heat, and nutrient stress for recipient plants in arid and nutrient-poor terrestrial environments (Franco and Nobel 1989, Valientebanuet and Ezcurra 1991, Callaway 1995, Tewksbury and Lloyd 2001), and salt marsh plants reduce sediment anoxia and salt accumulation, thereby stimulating the establishment of other plants in intertidal systems (Bertness and Hacker 1994, Bertness and Leonard 1997, Hacker and Bertness 1999).

Food web interactions may also mediate the effects and incidence of amelioration. Herbivory, for example, can indirectly increase facilitative interactions among terrestrial plants through shared defenses and associational resistance (Atsatt and O'Dowd 1976, Milchunas and Noy-Meir 2002, Baraza et al. 2006, Smit et al. 2007). Such complexity indicates that amelioration may be dependent on trophic structure, which could potentially reverse, exacerbate, or offset the outcome of the initial species interaction.

Our understanding of positive species interactions in general – and amelioration in particular – is much less extensive for freshwater ecosystems (i.e., lakes, streams, and wetlands; Halpern et al. 2007). Ecological interactions may be more important in aquatic than terrestrial systems (Bruno et al. 2005) because herbivory rates and population densities per species can be greater (Cyr and Pace 1993, Cebrian and Duarte 1994, Cyr et al. 1997), but only a few studies have demonstrated that facilitation actually occurs in freshwater habitats. For example, planting macrophytes in unvegetated reservoirs may facilitate the establishment of other plants through sediment stabilization (Moss 1990, Smart et al. 1998), and some allelochemical assays among freshwater phytoplankton have demonstrated stimulatory effects (Keating 1977, Mohamed 2002, Carey and Rengefors 2010).

Freshwater ecosystems are excellent systems to examine facilitation. Freshwater systems are particularly amenable to controlled replicated experiments (Drenner and Mazumder 1999, Wetzel and Likens 2000, Lampert and Sommer 2007, Spivak et al. 2011), and phytoplankton are extremely tractable organisms for experiments examining species interactions due to their short generation times and,

relative to macrophytes, lack of below-ground competition. As a result, examining how freshwater phytoplankton species affect food webs via positive interactions may contribute new insight to our understanding of how positive interactions can have ecosystem-level consequences.

Here, we tested whether a particular species of phytoplankton, previously shown to have positive effects in laboratory microcosm experiments (Carey and Rengefors 2010), facilitates plankton food webs by increasing nutrient concentrations in low nutrient freshwater systems. We examined these ecosystem-level effects in systems with and without zooplankton grazers to test if trophic interactions mediated any potentially facilitative interactions. In several facilitation studies (e.g., Callaway et al. 2002), the incidence of facilitative interactions decreased with ecosystem productivity; following Grime (1977) that productivity is inversely correlated with stress. Hence, we predicted that we would observe direct amelioration among phytoplankton in freshwater systems with low productivity (i.e., low nutrients). We define a system as being low nutrient if it has a total phosphorus concentration <30 μg/L and total nitrogen concentration <650 μg/L, which includes both oligotrophic and mesotrophic systems (Nürnberg 1996).

Focal Species

We chose a colonial (1-3 mm diameter) cyanobacterium, *Gloeotrichia echinulata*, as our focal phytoplankton species for three reasons. First, *G. echinulata* has been shown to stimulate a diverse array of phytoplankton taxa in laboratory experiments (Carey and Rengefors 2010), indicating that it could potentially stimulate

phytoplankton in natural systems. Second, *G. echinulata* has two important physiological characteristics that may allow it to alleviate nutrient limitation: it is a nitrogen fixer (Stewart 1967, Roelofs and Oglesby 1970, Carr and Whitton 1982) and it takes up and stores large quantities of phosphorus in excess of its immediate metabolic needs (Istvánovics et al. 1993, Pettersson et al. 1993). Both nitrogen (N) and phosphorus (P) may be released to the water column by *G. echinulata* in available forms (Pitois et al. 1997, Nõges et al. 2004), because cyanobacteria leak or release nutrients, especially in oligotrophic systems (Healey 1982, Kankaanpaa et al. 2001, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004). Third, *G. echinulata* may be increasing in oligotrophic and mesotrophic lakes across the northeastern USA and Canada (Carey et al. 2008, 2009, Winter et al. 2011), making it important to determine its effects on aquatic food webs.

We used three experiments to examine the effects of *G. echinulata* on natural plankton communities in low nutrient freshwater systems. In all experiments, we predicted that *G. echinulata* would stimulate other phytoplankton by increasing available N and P. First, we determined if *G. echinulata* facilitated, competed with, or had a neutral interaction with other phytoplankton by adding four different densities of *G. echinulata* to 50 L *in situ* mesocosms in an oligotrophic lake and measuring the phytoplankton response (hereafter referred to as the *in situ* mesocosm experiment). Large zooplankton grazers were absent in this experiment. Second, we conducted a laboratory microcosm experiment to examine if *G. echinulata* colonies increase water column N and P concentrations (hereafter, laboratory experiment). We measured the effects of ten different densities of *G. echinulata* on phytoplankton and nutrients in

oligotrophic lake water in the absence of grazers. Third, we manipulated both the density of *G. echinulata* and zooplankton grazers in 800 L mesotrophic artificial ponds to examine if zooplankton altered the interaction between *G. echinulata* and phytoplankton (hereafter, pond experiment). We expected that any positive effect of *G. echinulata* on phytoplankton would be offset in the presence of zooplankton because of zooplankton grazing on the phytoplankton.

Materials and Methods

In situ mesocosm experiment

We deployed *in situ* mesocosms in a sheltered cove of Lake Sunapee, New Hampshire, USA (43°24'N, 72°20'W) in July 2008 to examine how different densities of *G. echinulata* blooms affected phytoplankton biomass and nutrient concentrations in the absence of large grazing zooplankton. Lake Sunapee is a large (16.55 km^2 surface area; 33 m maximum depth) oligotrophic lake with a 20-year mean total phosphorus (TP) concentration in open water of 4.8 (± 0.01, 1 S.E.) μg/L, a mean Secchi disc transparency of 7.3 (± 0.1) m, and mean chlorophyll *a* concentration of 1.7 (± 0.01) μg/L (Carey et al. 2008).

We suspended 16 clear polyethylene bags (~50 L water volume) from two 4.9 m-long wooden floating frames in the littoral zone. The tops of the enclosures were covered with mesh (1.5 mm) to prevent zooplankton immigration via bird vectors and were situated 0.2 m above the lake surface to prevent water from overtopping the mesocosm rims. We filled the mesocosms with unfiltered water obtained from the upper 0.3 m of the lake on 2 July 2008 (day of year 184) and let them equilibrate for

24 h prior to adding *G. echinulata*. Although we did not specifically exclude large zooplankton, our mesocosms included only small zooplankton (rotifers and nauplii) because we filled them midday with surface lake water 12 d after the summer solstice, when incident light was near maximum levels, and most large zooplankton avoid visual predators during the day by seeking refuge in poorly-lit deep waters (Lampert 1989 and references therein).

We randomly assigned four *G. echinulata* density treatments (with four replicates each) and blocked the treatments by wooden frame so that there were two replicates per treatment per frame. The treatments consisted of four *G. echinulata* densities: 0 (control), 25, 50, and 400 *G. echinulata* colonies/L. As of summer 2008, the highest *G. echinulata* density observed in Lake Sunapee was ~30 colonies/L (K.L.C. et al., unpublished data); however, large *G. echinulata* blooms consisting of ≥5000 colonies/L routinely occur in Lake Erken, Sweden (Eiler et al. 2006), so the densities used in our experiments were well within the range observed in nature.

At 48 h before the experiment began, we collected *G. echinulata* colonies from Lake Sunapee with a zooplankton net (mesh size 100 μm). Colonies were gently rinsed from the net into white plastic 1 L bottles, which were kept in the shade with their caps off until transport to the lab. Once in the lab, colonies were rinsed three times with filtered (Whatman GF/C, 1.2 μm pore size) Lake Sunapee water and individually inspected with a dissecting microscope to manually remove all remaining adhered debris and plankton with micro-scalpels and probes. We chose only the largest, buoyant colonies with all of their trichomes intact for the experiment. We calculated the total number of colonies needed for each mesocosm by multiplying the

bag volume by the treatment density. We cleaned the colonies in aliquots of 100 with a Leica MZ12 dissecting microscope and haphazardly assigned aliquots to treatments. Until the experiment started, we kept the colonies at low density to prevent light limitation from scums in clear 1-L bottles filled with Whatman GF/C-filtered Lake Sunapee water. The bottles were placed in incubators set at 20^{o}C on a 14:10 light:dark cycle that approximated natural conditions in early July and were swirled every 12 h. Less than 1% *G. echinulata* mortality (as indicated by a loss of buoyancy) was observed by the end of this period.

The morning of the experiment, the bottles were brought in insulated coolers to Lake Sunapee and placed in the shade onshore with their caps off while pre-*G. echinulata* addition mesocosm sampling occurred. Immediately after this initial sampling to determine baseline conditions, the *G. echinulata* colonies were added to the mesocosms in one single pulse to create the four treatments.

We sampled each of the mesocosms plus two littoral sites adjacent to the frames immediately before *G. echinulata* addition, 24 h after *G. echinulata* addition, and then every 4-6 d for 20 d. The littoral sites were sampled to provide a reference for mesocosm effects on response variables but were excluded from statistical analyses to maintain a balanced design.

On each sampling day, we measured nutrients and plankton in the mesocosms. We monitored dissolved oxygen and temperature at the water's surface and at 0.5 m depth with a 556 MPS meter (YSI Inc., Yellow Springs, Ohio, USA). With an integrated tube sampler (0.5 m long, 5.1 cm diameter), we collected 1 L from five locations within each mesocosm and pooled the 5 L in a clean, rinsed bucket. We

retained 1 L of this pooled water for chlorophyll *a* analyses, 250 mL for phytoplankton analyses, 500 mL for nutrient analyses, and 250 mL for whole-water microcystin-LR (MC-LR) analyses (on the pre-experiment day and last sampling day only) and returned the unused water to the mesocosm. Water for phytoplankton analyses was collected on each sampling day except the last. We immediately preserved the phytoplankton sample in opaque bottles with Lugol's iodine solution in the field and stored the bottles in darkness until microscopic analysis. On the pre-experiment day and last sampling day only, we collected an additional 1 L each from five locations in the mesocosm (5 L total) with the integrated tube sampler and filtered the water through an 80 μm mesh net for measurements of both *G. echinulata* density and zooplankton. We immediately preserved the collected sample with 70% ethanol and returned the filtrate to the mesocosms. On the interim sampling days, we filtered 3 L of water through an 80 μm mesh net for measurements of *G. echinulata* density, which were immediately preserved with Lugol's iodine solution.

We processed the chlorophyll *a*, nutrient, and plankton samples according to standard protocols immediately upon returning to the laboratory. We measured both total and <30 μm (pre-filtered through a Nitex mesh; Cottingham 1996, Cottingham et al. 2004; hereafter, referred to as 'small-sized') chlorophyll *a* by vacuum-filtering each sample onto Whatman GF/C filters, extracting them in methanol for 24 h, and determining the chlorophyll *a* concentration using a fluorometer (Turner Designs TD 700, Sunnyvale, California, USA) according to Arar and Collins (1997). As *G. echinulata* colonies are 1-3 mm in diameter in northeastern USA lakes (Carey et al. 2008), they were excluded from the small-sized chlorophyll *a* fraction, which

generally represents a size fraction of phytoplankton that zooplankton are able to efficiently graze (Lampert et al. 1986, Cottingham 1996, Cyr 1998).

Of the 500 mL we collected for nutrient analyses, we retained 125 mL for total nutrients (total N and total P; hereafter, TN and TP), and filtered the remaining water through 0.7 μm pore size (Whatman GF/F) filters for ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive P (SRP) analyses. We froze all soluble and total nutrient samples until analysis. Both P fractions (SRP and TP) were analyzed according to Van Veldhoven and Mannaerts (1987) with an acidic persulfate digestion for total samples (method detection limit for SRP and TP = 7.8 μg/L). We analyzed TN samples with spectrophotometric methods after basic persulfate digestion (Crumpton et al. 1992; method detection limit = 74 μg/L). NO_3^- and NH_4^+ samples were analyzed on a Lachat QuikChem 8000 (Lachat Instruments, Loveland, Colorado, USA) according to the QuikChem Phenate method #10-107-106-1-J and QuikChem Cadmium Reduction method #10-107-04-1-A, respectively (method detection limits for NO_3^- and NH_4^+ = 9.7 μg/L).

We settled and concentrated 50 mL of each phytoplankton sample for 3 days and enumerated the cells at 400× magnification according to Utermöhl (1958) on an inverted Nikon MSD microscope. We calculated non-*G. echinulata* phytoplankton biovolume (μm^3/mL) by approximating the cells and colonies to known geometric shapes (Olrik et al. 1998). In addition, we counted *G. echinulata* colonies and zooplankton, which were identified to genus, on an Olympus SZH10 dissecting microscope.

Whole-water MC-LR samples were analyzed with an enzyme-linked immunosorbent assay (ELISA) according to Sasner et al. (2001) at the Center for Freshwater Biology at the University of New Hampshire.

We determined the effects of *G. echinulata* treatments on chlorophyll *a* and nutrient concentrations using one-way repeated measures (RM) ANOVA using both SAS PROC GLM and PROC MIXED (SAS version 9.2, SAS Institute, Cary, North Carolina, USA; Wolfinger and Chang 1999). We report the PROC GLM results here because in almost all cases these analyses, which we corrected with the Greenhouse-Geisser estimate to meet assumptions of compound symmetry and sphericity, were more conservative than the PROC MIXED results. We compared the differences between the highest *G. echinulata* experimental density (400 colonies/L) and the no-*G. echinulata* control treatments with a linear contrast and assessed significance at $\alpha = 0.05$.

We found that more than half of the TP concentrations measured in the mesocosms were below the method detection limit, which prevented the use of RM ANOVA to analyze treatment effects. For TP only, we calculated for each mesocosm the proportion of samples collected after the first *G. echinulata* addition that were above the method detection limit. We then analyzed the effect of *G. echinulata* density on the proportions with one-way ANOVA using JMP version 8.0 (SAS Institute, Cary, North Carolina, USA).

Finally, we analyzed the difference in zooplankton density and MC-LR concentration between the final day and the pre-experiment day of the experiment among treatments with one-way ANOVA (JMP, v. 8.0).

Laboratory experiment

To determine whether *G. echinulata* increased nutrient concentrations in oligotrophic lake water and to examine the effect of a broader range of *G. echinulata* densities on phytoplankton, we tested the effect of a gradient of 10 different *G. echinulata* densities on small-sized phytoplankton biomass (<30 μm chlorophyll *a*), TN, and TP in Erlenmeyer flasks. We collected 20 L of unfiltered water with a 4 L Van Dorn sampler (Wildlife Supply Company, Saginaw, Michigan) from 0.5 m depth in Lake Sunapee and homogenized the water in a large carboy. We brought the carboy back to the laboratory and kept it in the dark at room temperature for ~12 h until the beginning of the experiment.

Simultaneously, because of low *G. echinulata* densities in Lake Sunapee at the time of this study, we collected colonies as described previously but from Lake Morey (43°55'N, 72°8'W, Fairlee, Vermont, USA). Lake Morey is a mesotrophic lake (2.21 km^2 total surface area; 13 m max depth) with a 30-year mean summer TP concentration of 18.4 (± 11.8) μg/L, a mean Secchi disc transparency of 6.7 (± 1.8) m, and mean chlorophyll *a* concentration of 6.8 (± 6.5) μg/L (Vermont Dept. of Environmental Conservation).

We set up the experiment using oligotrophic Lake Sunapee water and Lake Morey *G. echinulata* colonies. At the beginning of the experiment, we collected ~3 L of water from the carboy, filtered it through 30 μm Nitex mesh, and used the filtrate to determine background TN, TP, and chlorophyll *a* in the <30 μm size fraction. We filled thirty 500 mL acid-washed Erlenmeyer flasks with 400 mL of the water and

randomly assigned *G. echinulata* density treatments of 0, 25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 colonies/L with three replicates each. We prepared the *G. echinulata* treatments as described previously, and calculated how many colonies were added to each flask by multiplying the water volume (0.4 L) by the density treatment. After *G. echinulata* addition, the flasks were kept in an incubator at 25^{o}C for 5 d at a 14:10 light:dark cycle and were swirled daily.

At the end of the experiment, we analyzed the effects of *G. echinulata* on chlorophyll *a* and nutrients. We filtered the content of each flask through 30 μm Nitex mesh to remove all *G. echinulata* colonies and used the filtrate for small-sized chlorophyll *a*, TN, and TP analysis. Small-sized chlorophyll a and TN were analyzed as described above, while TP was measured with a higher resolution method, Method 4500-P (American Public Health Association 1980; method detection limit = 1.1 μg/L). We assessed whether *G. echinulata* increased total nutrients in the water column because soluble nutrient concentrations were likely below the method detection limit. Although this method did not measure *G. echinulata* leakage directly, we expected that in such low nutrient water, nutrient uptake would be extremely rapid (Hutchinson and Bowen 1950, Lean 1973, Hudson et al. 2000) and total nutrient concentrations would be dominated by the organic fraction because any leaked nutrients would be quickly incorporated into phytoplankton and microbes.

We $\log_e$-transformed our response variables to equalize variance and used model selection in the R statistical package (R Development Core Team) to determine the most appropriate regression model characterizing the relationship between small-sized chlorophyll *a*, TN, and TP and the *G. echinulata* density treatments. We tested

four possible models (mean, linear, and Michaelis-Menten with and without an intercept term) often used to describe phytoplankton and nutrient interactions to determine if *G. echinulata* density exerted a linear or non-linear effect on the response variables. We used a simulating annealing algorithm, a type of global parameter optimization procedure, with 10,000 iterations and a normally-distributed error term to solve maximum likelihood estimates for each model parameter, and chose the best model fit with the Akaike Information Criterion (AIC; Burnham and Anderson 2002).

We also compared the increased concentrations of TN and TP in the treatments (relative to the non-*G. echinulata* control) with a theoretical upper bound of *G. echinulata* leakage, i.e., the total amount of N and P within the individual colonies added to the flasks. Using data from Pettersson et al. (1993) and Tymowski and Duthie (2000), we estimated that the total amount of P within a colony ranged from 0.02 – 0.08 μg P/colony, and estimated the TN concentration to be 0.14 – 0.58 μg N/colony by multiplying the P concentration by the Redfield ratio (7.2 by atomic weight; Redfield 1934). We multiplied those N and P colony concentrations by the number of colonies added to each flask to determine the potential range of added N and P.

Finally, to examine the *G. echinulata* density threshold at which the effect of *G. echinulata* on small-sized chlorophyll *a*, TN, and TP was significantly different from the non-*G. echinulata* controls, we treated *G. echinulata* density as a categorical variable in a one-way ANOVA with Tukey's HSD tests (JMP v. 8.0). We also tested if there was a significant trend in the three response variables according to the ranks of

the ordered density treatments with linear regression in JMP (v. 8.0) and determined significance if the slope parameter $p \leq 0.05$.

Pond experiment

In summer 2010, we scaled up the size of our mesocosms to test the effect of *G. echinulata* blooms on phytoplankton biomass and nutrient cycling at two densities of herbivorous cladoceran zooplankton. We used a factorial 2 × 2 design, crossing the effects of Zooplankton Biomass (Added Zooplankton vs. No Added Zooplankton) with *G. echinulata* (Added *G. echinulata* vs. No Added *G. echinulata*, i.e., a no-*G. echinulata* control), with four replicates per treatment (n = 16 total). We conducted our experiments in 1136 L (total volume) cattle tank mesocosms (hereafter, ponds; Rubbermaid, Wooster, OH, USA) each filled with 800 L of water, situated in an exposed old field in Etna, New Hampshire, USA (43°41'N, 72°13'W).

We set up the pond experiment following the general methods of Cottingham et al. (2004). Before the experiment began, we acid-washed the inside of each pond with hydrochloric acid (1 N) and immediately covered the ponds with a 1 mm fiberglass mesh to prevent invasion by insects. We stocked each pond in late May 2010 with a mesh bag containing terrestrial leaf litter as a carbon source for the plankton communities before we filled the ponds with water. Each bag contained 200 g of dry leaves (50 g each of sugar maple (*Acer saccharum*), red oak (*Quercus rubra*), white pine (*Pinus strobus*), and American beech (*Fagus granifolia*)) collected from wooded areas near our field site. We removed the leaf bags from the ponds before we added *G. echinulata* in early July.

In mid-June, we filled the ponds with well water and established phytoplankton communities by stocking each pond with 2 L of unfiltered water collected from the top 0.5 m of eight lakes located within a 50 km radius of our field site (16 L total of lake water per pond; see Appendix 1 for pond descriptions). We let the phytoplankton community develop for two weeks before establishing the two randomly-assigned zooplankton treatments with zooplankton collected from four of the eight phytoplankton lakes (Appendix 1). We added all of the zooplankton collected by one 2 m vertical tow with a plankton net (0.5 m diameter, 100 μm mesh) from each lake to each pond. We visually inspected each tow sample and removed *G. echinulata* colonies, large predatory zooplankton, and invertebrates before stocking.

We allowed the zooplankton communities in the tanks to develop for a week and a half and then added *G. echinulata* colonies to the Added *G. echinulata* ponds. Due to dispersal (via overland transport or juvenile zooplankton in the unfiltered phytoplankton water), zooplankton communities also developed in the No Added Zooplankton ponds, but two significantly different levels of zooplankton biomass (hereafter, High vs. Low Zooplankton Biomass) were maintained throughout the experiment (see Results).

We collected *G. echinulata* colonies for the experiment from Lake Sunapee and Lake Morey with the goal of creating Added *G. echinulata* treatments that matched the highest *G. echinulata* density in the *in situ* mesocosm experiment (400 *G. echinulata* colonies/L). We collected colonies at each lake by towing a plankton net (0.5 m diameter, 100 μm mesh) ~25 m alongside a dock with the top of the net at the water's surface. After each 25 m tow, we rinsed the contents into 1-L bottles that

were kept in the shade with their lids off until transport back to the lab. We cleaned the *G. echinulata* colonies from each tow separately using dissection microscopes, as described above, and placed the cleaned colonies into new bottles. We assumed the bottles to have equal colony densities, and divided the bottles by the lake from which the colonies were collected and then assigned them to treatments so that an equal number of bottles from each lake were allocated to every Added *G. echinulata* pond. Colonies from Lake Sunapee and Lake Morey were the same size and appeared identical under a dissecting microscope. Because we were unable to collect enough colonies in one day to reach our target density, we added colonies to the Added *G. echinulata* ponds in four pulses on days of year 189, 192, 202, and 210 (8, 11, 21, and 29 July).

We sampled the ponds 24 h prior to the first *G. echinulata* addition and every 3-4 days thereafter for 37 days, following the general methods of Cottingham et al. (2004). On each sampling day, we recorded the pond water level, measured water temperature and dissolved oxygen with an YSI 556 MPS meter just below the water's surface, and removed arthropod invaders with a dip net. We collected 1 L of water from each of five locations within a pond using a separate 0.5 m long integrated tube sampler for each pond and retained 1.75 L for chlorophyll *a*, nutrient, and phytoplankton analyses as described above (the remaining water was returned to the pond). We sampled zooplankton and *G. echinulata* every 7 d by filtering 7 L of water through 80 μm mesh and preserving the sample with 70% ethanol. We sampled whole-water MC-LR concentrations prior to *G. echinulata* addition and at the end of the experiment (day of year 225). We lost three replicate ponds midway through the

experiment to demonic intrusion (sensu Hurlbert 1984: i.e., the introduction of yellow perch, *Perca flavescens*, despite mesh covering).

We analyzed the chlorophyll *a*, nutrient, and MC-LR samples as described above (using APHA Method 4500-P for P analyses). In addition to examining zooplankton density, we also measured the dimensions of the first 25 animals of each taxa we encountered in each sample to calculate biomass-weighted average total zooplankton mass (hereafter, total zooplankton biomass) and biomass-weighted average cladoceran mass (hereafter, cladoceran biomass; Elser et al. 1987, Elser et al. 1988) from established length-mass regressions (Bottrell et al. 1976, Downing and Rigler 1984). We chose this metric because it weights mean zooplankton and cladoceran biomass by the contribution of each taxon to community biomass rather than community density, thereby preventing taxa that are common but have little biomass (e.g., rotifiers, small cladocerans) from making an overly large contribution to mean zooplankter mass (Elser et al. 1987). Biomass-weighted average total zooplankton and cladoceran mass were calculated using the equation:

$$ZM_B = \frac{\sum_{i=1}^{n}(B_i \times I_i)}{\sum_{i=1}^{n} B_i} \qquad \text{(eqn. 5.1)}$$

where ZM_B is the biomass of either the total zooplankton community or the cladoceran community, weighted by the size of the taxa in the community (μg/animal); n is the total number of zooplankton or cladoceran taxa i; B_i is the total biomass of taxon i on that sampling day (μg/L); and I_i is the mean individual biomass of taxon i on that

sampling day (μg/animal; Elser et al. 1987, Elser et al. 1988). To avoid bias, we estimated log weights individually from each log-transformed length and back-transformed to original units before calculating the mean weight and size of a taxon (Bird and Prairie 1985).

For the dominant cladoceran genus we observed, *Ceriodaphnia*, we also calculated instantaneous birth, death, and growth rates with the egg ratio method (Paloheimo 1974). We estimated the *Ceriodaphnia* egg development time in the ponds using data from Hall et al. (1970), Shuba and Costa (1972), and Anderson and Benke (1994).

We conducted several analyses to examine the effect of *G. echinulata* on phytoplankton and zooplankton communities, as well as on nutrient and MC-LR concentrations. First, to determine if our Low and High Zooplankton Biomass treatments were significantly different, we used two-way RM ANOVA (SAS PROC GLM, as described above) to test the effects of the main factors *G. echinulata* (Added *G. echinulata*/No Added *G. echinulata*) and Zooplankton Biomass (Low Biomass/High Biomass) and their interaction on total zooplankton and cladoceran biomass, as well as to assess if zooplankton biomass responded to the *G. echinulata* treatment. Because the concentration of *G. echinulata* colonies varied in the Added *G. echinulata* treatment throughout the experiment, interactions between *G. echinulata* and time (i.e., *G. echinulata* × time) were used to interpret the main effects of *G. echinulata* addition (following Derry and Arnott 2007, Strecker and Arnott 2010). We repeated this RM ANOVA on the density of aggregate zooplankton groups (cladocerans, copepods, and rotifers). Second, we analyzed the effects of *G.*

echinulata and Zooplankton Biomass on the birth, death, and growth rates of *Ceriodaphnia* with two-way repeated multivariate ANOVA (MANOVA; JMP v. 8.0). Third, we used two-way RM ANOVA to test the effects of *G. echinulata* and Zooplankton Biomass and their interaction on chlorophyll *a* and nutrients. Fourth, we used two-way ANOVA to analyze the effects of *G. echinulata* and Zooplankton Biomass on the change in MC-LR concentrations from the beginning to the end of the experiment.

Finally, we evaluated the effect of zooplankton grazing on damage to *G. echinulata* colonies (i.e., if the colonies exhibited short or missing trichomes and a non-intact central core), following Fey et al. (2010), by constructing separate regression models of the % damaged colonies on each sampling day vs. cladoceran biomass on the previous sampling day. Cladocerans are hypothesized to be potential grazers of *G. echinulata* (Fey et al. 2010), and we expected that the *G. echinulata* damage observed on a sampling day would be more closely related to the cladoceran biomass level it was exposed to at the beginning of the interim period between sampling than at its end.

Results

In situ mesocosm experiment

Prior to *G. echinulata* addition, there were no significant differences in *G. echinulata* density, total and small-sized chlorophyll *a*, non-*G. echinulata* phytoplankton biovolume, TN, or MC-LR among the *in situ G. echinulata* treatments (one-way ANOVA, all $p \geq 0.21$). All TP concentrations prior to *G. echinulata*

addition were below the method detection limit. Throughout the experiment, NO_3^-, NH_4^+, and SRP concentrations were below the method detection limit; more than half (60%) of the TP concentrations were below the method detection limit.

G. echinulata density significantly increased in the mesocosms according to our treatments (25, 50, and 400 colonies/L and a no-*G. echinulata* control; effects of *G. echinulata*, time, and their interaction all had $p < 0.0001$; Figure 5.1; see Table 5.1 for *in situ* mesocosm experiment RM ANOVA statistics). *G. echinulata* addition resulted in scum formation in the 25, 50, and 400 colonies/L treatments, which lasted in the high treatment for ~10 d after addition before subsiding.

We found that *G. echinulata* addition had significant positive effects on nutrients. *G. echinulata* significantly increased TN ($p < 0.0001$; Figure 5.1), with the 400 *G. echinulata* colony/L treatment exhibiting 74% (± 17%, 1 S.E.) higher TN concentrations than the no-*G. echinulata* controls immediately after *G. echinulata* addition (linear contrast $p < 0.0001$). *G. echinulata* addition also significantly increased the proportion of TP samples that were above the method detection limit (one-way ANOVA, $F_{3,12} = 9.63$, $p = 0.002$; Figure 5.1). Mesocosms in the 400 colonies/L treatment exhibited 80% (± 8%) of all TP samples above the method detection limit throughout the experiment, significantly higher than the 25% (± 10%) in the no-*G. echinulata* control (Tukey's HSD test, $p = 0.006$).

Total chlorophyll *a*, which included *G. echinulata* as well as other phytoplankton, also increased significantly in response to added *G. echinulata* ($p <$

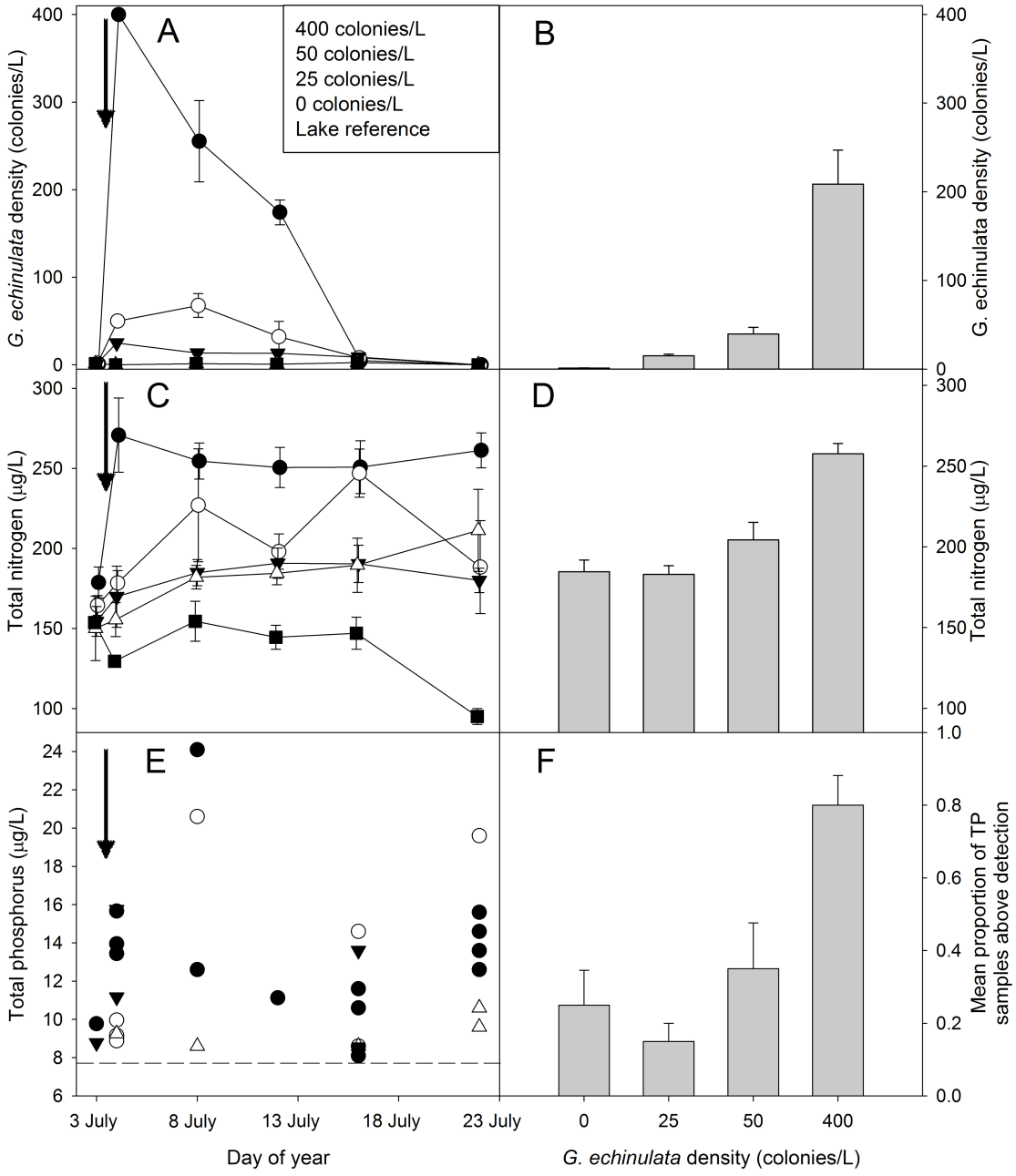


Figure 5.1. (A) *G. echinulata* density (colonies/L ± 1 S.E.) and (C) total nitrogen concentrations, respectively, in the *in situ* mesocosms over time. The 400, 50, 25, and 0 colonies/L treatments were manipulated experimentally within the mesocosms; the lake reference treatment refers to the ambient *G. echinulata* density or total nitrogen

concentration in the lake outside the mesocosms. The arrow refers to the day of *G. echinulata* addition. (B) The mean *G. echinulata* density and (D) total nitrogen concentration observed across all sampling dates after *G. echinulata* addition in the 0, 25, 50, and 400 colonies/L treatments, respectively. (E) Total phosphorus concentrations measured above the method detection limit. (F) The mean (± 1 S.E.) proportion of total phosphorus (TP) samples that were above the method detection limit across all sampling dates after *G. echinulata* addition in each treatment.

Table 5.1. Statistical results from the repeated measures one-way ANOVA analyses testing the effects of four densities of *G. echinulata* (0, 25, 50, and 400 colonies/L) on chlorophyll *a* and nutrient concentrations in the *in situ* mesocosm experiment. The planned *G. echinulata* contrast refers to a pre-determined linear contrast of the 0 colonies/L and 400 colonies/L treatments. DF denotes degrees of freedom, and significant effects ($p \leq 0.05$) are in bold.

Response Variable	**Repeated Measures ANOVA Test**	**DF**	**F-value**	**p-value**
G. echinulata density	***G. echinulata***	3,12	286.64	<0.0001
	Planned *G. echinulata* contrast	1,12	664.84	<0.0001
	Time	4,48	67.50	<0.0001
	***G. echinulata* × Time**	12,48	42.76	<0.0001
Total nitrogen	***G. echinulata***	3,9	28.29	<0.0001
	Planned *G. echinulata* contrast	1,9	61.45	<0.0001
	Time	4,36	1.78	0.19
	G. echinulata × Time	12,36	1.64	0.18
Total chlorophyll *a*	***G. echinulata***	3,12	63.45	<0.0001
	Planned *G. echinulata* contrast	1,12	150.34	<0.0001
	Time	4,48	7.77	0.002
	***G. echinulata* × Time**	12,48	2.91	0.008
Small-sized chlorophyll *a* (<30 μm)	***G. echinulata***	3,11	15.97	0.0003
	Planned *G. echinulata* contrast	1,11	39.57	<0.0001
	Time	4,44	13.03	<0.0001
	G. echinulata × Time	12,44	0.83	0.58
Non-*G. echinulata* phytoplankton biovolume	***G. echinulata***	3,9	21.43	0.0002
	Planned *G. echinulata* contrast	1,9	46.77	<0.0001
	Time	3,27	0.92	0.40
	***G. echinulata* × Time**	9,27	3.80	0.02

0.0001; Figure 5.2), with the *G. echinulata* effect mediated by time. The 400 colonies/L treatment exhibited larger fluctuations in total chlorophyll *a* concentration than the other treatments, resulting in significant *G. echinulata* × time and time effects (both $p \leq 0.008$). Total chlorophyll *a* concentrations in the 400 colonies/L treatment were 255% - 577% higher than in the no-*G. echinulata* control after *G. echinulata* addition, even after the *G. echinulata* density decreased.

Importantly, we observed that *G. echinulata* had strong positive effects on other phytoplankton. Both small-sized chlorophyll *a* (the <30 μm phytoplankton fraction, which excluded *G. echinulata* and other large taxa) and non-*G. echinulata* phytoplankton biovolume increased significantly in response to *G. echinulata* addition (both $p \leq 0.0003$; Figure 5.2), with the non-*G. echinulata* phytoplankton biovolume response mediated by time ($p = 0.02$). The 400 colonies/L treatment experienced a much larger increase in non-*G. echinulata* phytoplankton biovolume than the other treatments, driving the significant *G. echinulata* × time interaction. Small-sized chlorophyll *a* and non-*G. echinulata* phytoplankton biovolume exhibited significantly higher (up to 181% and 2538%, respectively) concentrations in the 400 colonies/L treatment in comparison to the non-*G. echinulata* controls (linear contrast: both $p < 0.0001$). Small-sized chlorophyll *a* decreased slightly during the experiment, resulting in a significant time effect ($p < 0.0001$).

Finally, the 400 *G. echinulata* colonies/L treatment exhibited significantly higher increases in microcystin-LR (MC-LR) concentrations relative to the no-*G. echinulata* controls (Figure 5.3A). MC-LR concentrations increased 198% more in the

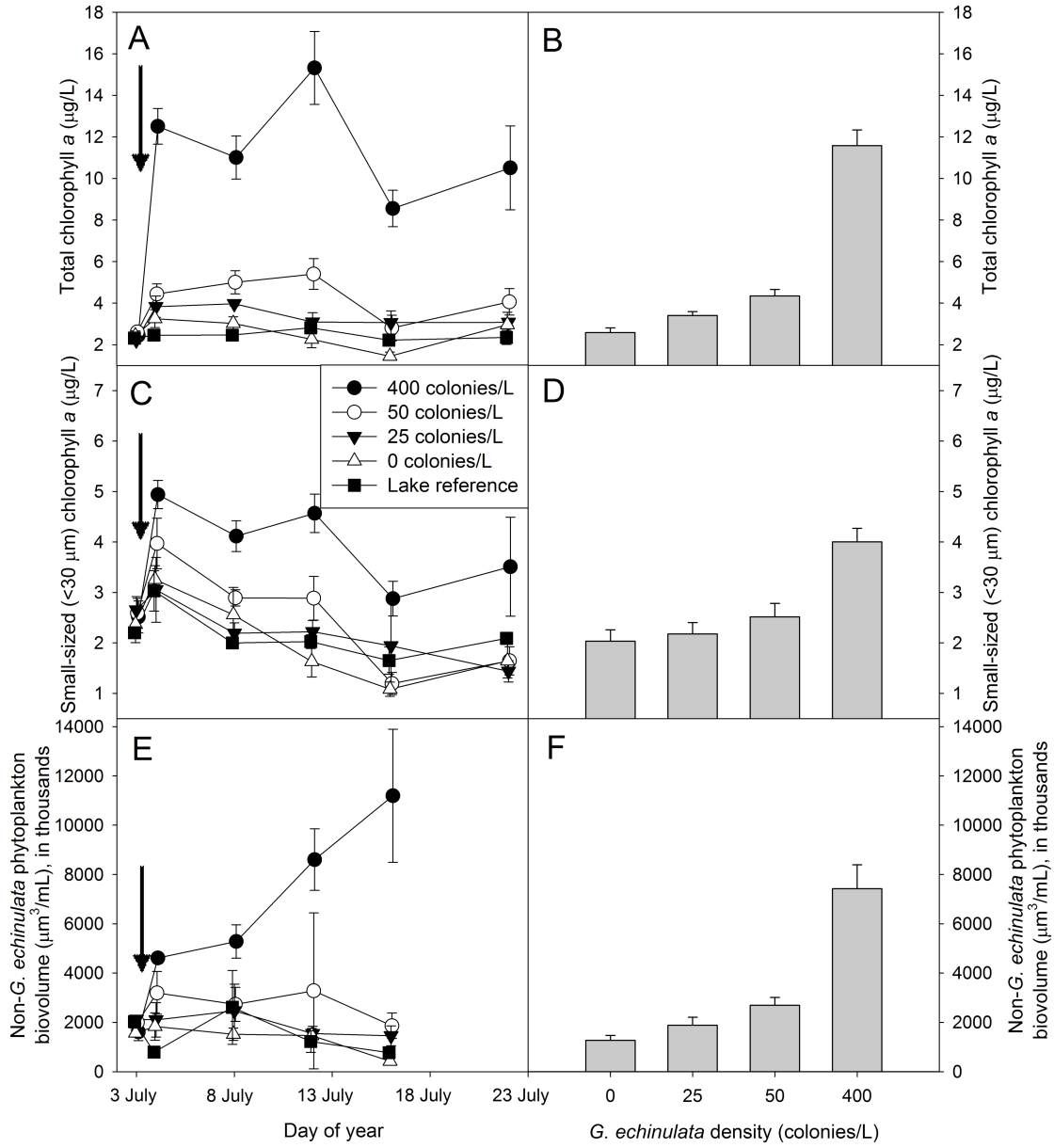


Figure 5.2. A) Total and (C) small-sized chlorophyll *a* and (E) non-*G. echinulata* phytoplankton biovolume across time in the *in situ* mesocosm experiment, respectively. Small-sized chlorophyll *a* refers to the <30 µm fraction and the arrow refers to the day of *G. echinulata* addition. Total and small-sized chlorophyll *a* were determined with fluorometry; the non-*G. echinulata* phytoplankton biovolume was

determined with microscopy. Phytoplankton samples were not collected on day of year 204. (B) The mean total and (D) small-sized chlorophyll *a* and (F) non-*G. echinulata* phytoplankton biovolume observed across all sampling dates after *G. echinulata* addition in the 0, 25, 50, and 400 colonies/L treatments, respectively.

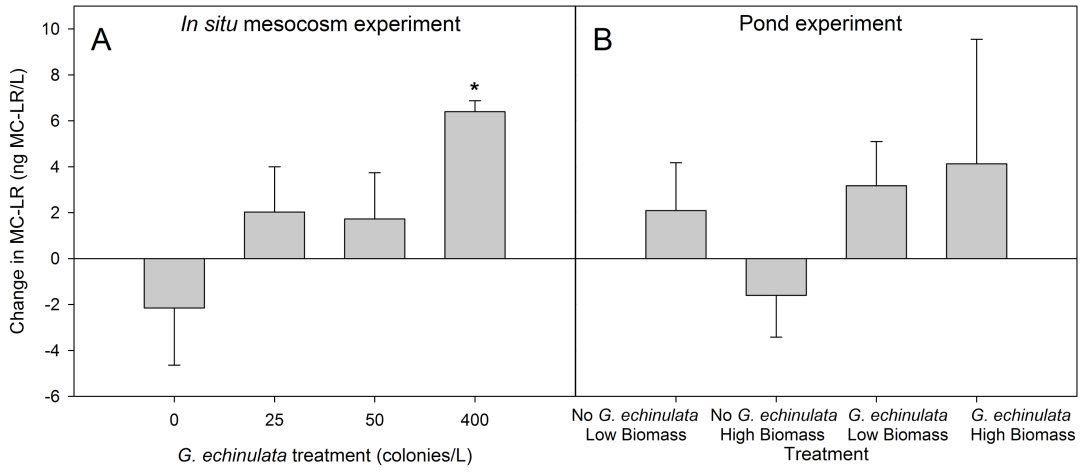


Figure 5.3. The mean difference between the concentration of microcystin-LR (MC-LR; ng MC-LR/L) at the beginning and end of the *in situ* mesocosm experiment and pond experiments. (A) Microcystin-LR change over the *in situ* mesocosm experiment was significantly higher in the 400 *G. echinulata* colonies/L treatment than in the no-*G. echinulata* control. The asterisk denotes the treatment that was significantly different from the control ($p \leq 0.05$). (B) Changes in microcystin-LR concentration during the pond experiment were not statistically different among treatments.

400 *G. echinulata* colonies/L treatment than in the no-*G. echinulata* controls during the experiment (linear contrast: $F_{1,5} = 8.26$, $p = 0.03$; one-way ANOVA model with all density treatments included: $F_{3,11} = 2.70$, $p = 0.097$). We observed no significant differences among treatments in total zooplankton, rotifer, copepod, or cladoceran densities over the course of the experiment (all $p \geq 0.33$).

Laboratory experiment

Small-sized chlorophyll *a*, TN, and TP increased non-linearly as a result of *G. echinulata* (Figure 5.4, Table 5.2, regression equations in table). For all three response variables, a Michaelis-Menten model with an intercept term was the best fitting regression model. Log_e-transformed small-sized chlorophyll *a*, TN, and TP in the flasks at the end of the incubation period, after the *G. echinulata* colonies were removed by filtration, were all strongly correlated (all $r > 0.84$). The increase of TP in the water column was far below the total amount of P added to the flasks within colonies: at the highest *G. echinulata* treatment, 6400 colonies/L, the observed increase of TP in the flasks was 91% (± 0%) lower than the lower bound of the potential total amount of P within the colonies (128 µg TP/L). By comparison, the observed increase of TN in the flasks in the 6400 colonies/L treatment was only 54% (± 4%) lower than the potential total amount of TN added to the flasks within the *G. echinulata* colonies (896 µg TN/L). We did not observe any *G. echinulata* mortality in the flasks during the 5 d experiment.

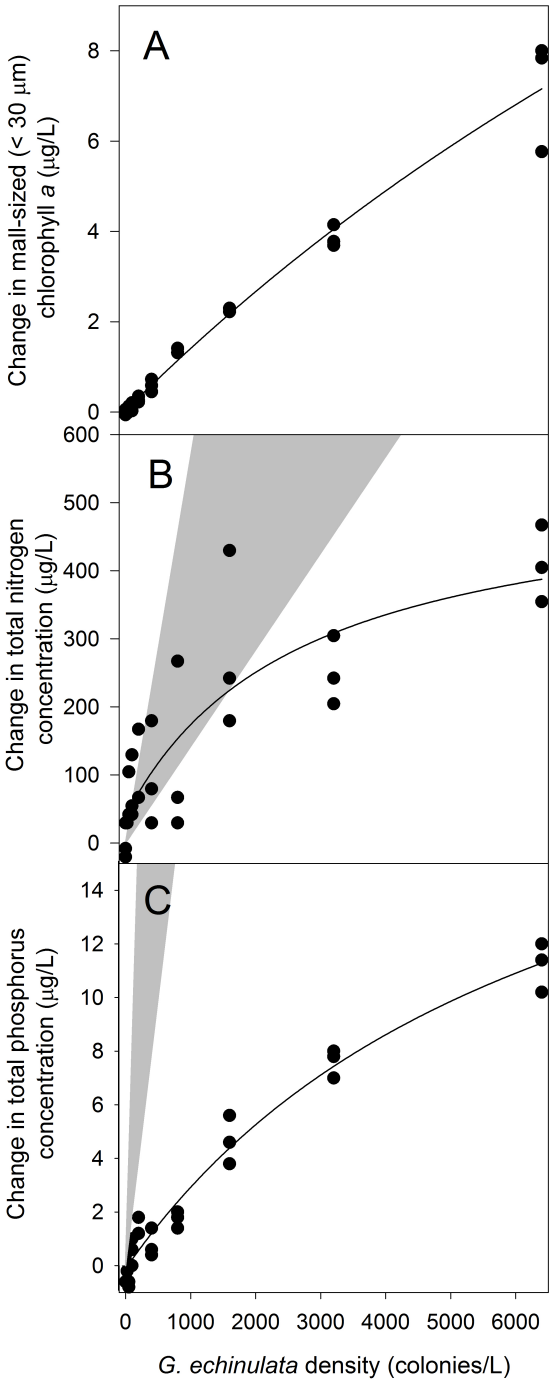


Figure 5.4. The effect of different densities of *G. echinulata* on (A) small-sized chlorophyll *a*, (B) total nitrogen, and (C) total phosphorus concentrations in lake water incubated with *G. echinulata* for 5 d in the laboratory experiment. The concentrations

in the no-*G. echinulata* control have been subtracted from all values for all variables. For total nitrogen and phosphorus, the observed increase in nutrients is compared to the total amount of nitrogen and phosphorus added to the flasks within the colonies (the range of the total amount of N and P in the colonies added to the flasks is represented by the gray shaded area; note that the gray shaded area extends off the scale at high *G. echinulata* densities). For all three response variables, a Michaelis-Menten model with an intercept term was the best fitting regression model (see regression equations in Table 5.2).

Table 5.2. The regression models tested in the laboratory experiment to predict the relationship between *G. echinulata* density and $\log_e$-transformed small-sized chlorophyll *a*, total nitrogen, and total phosphorus in descending order of best fit, as determined by the lowest corrected AIC value. The chosen model for each response variable is in bold.

Response variable	Model type	Model equation	Parameters with two-unit support intervals	R^2	Corrected AIC
Small-sized chlorophyll *a*	**Michaelis-Menten with intercept term**	c + ax/(s+x)	a = 3.01 (2.95 – 3.10) c = -0.50 (-0.54 – -0.45) s = 1419.92 (1320.52 – 1518.50)	0.99	-40.35
	Michaelis-Menten without intercept term	ax/(s+x)	a =19.78 (16.62 – 21.98) s = 46687.21 (41084.75 – 56220.34)	0.84	27.34
	Linear	ax + c	a = 0.00039 (0.00034 – 0.00045) c = -0.097 (-0.24 – 0.04)	0.83	28.44
	Mean	a	a = 0.46 (0.13 – 0.78)	0.00	73.46
Total nitrogen	**Michaelis-Menten with intercept term**	c + ax/(s+x)	a = 1.08 (0.90 – 1.24) c = 5.54 (5.49 – 5.60) s = 1637.49 (1097.12 – 2503.08)	0.72	-3.83
	Linear	ax + c	a = 0.00014 (0.00010 – 0.00017) c = 5.66 (5.60 – 5.71)	0.61	1.73
	Mean	a	a = 5.86 (5.74 – 5.97)	0.00	24.89
	Michaelis-Menten without intercept term	ax/(s+x)	a = 5.93 (5.28 – 6.73) s = 2.07 (0.00 – 22.33)	0.00	115.86
Total phosphorus	**Michaelis-Menten with intercept term**	c + ax/(s+x)	a = 2.19 (2.03 – 2.36) c = 1.03 (0.98 – 1.09) s = 2181.40 (1810.57 – 2572.68)	0.93	-13.57
	Linear	ax + c	a = 0.00026 (0.00022 – 0.0030) c = 1.23 (1.13 – 1.33)	0.80	10.40
	Michaelis-Menten without intercept term	ax/(s+x)	a = 2.29 (2.04 – 2.51) s = 132.64 (74.28 – 234.62)	0.36	42.20
	Mean	a	a = 1.60 (1.38 – 1.82)	0.00	51.87

Finally, the *G. echinulata* density at which the small-sized chlorophyll *a*, TN, and TP responses were significantly different from the non-*G. echinulata* control occurred between 400 and 800 colonies/L for small-sized chlorophyll *a* and TP (Tukey's HSD test) and between 800 and 1600 colonies/L for TN (Tukey's HSD test). Comparisons of treatment means in the 0 colonies/L and the other *G. echinulata* density treatments were non-significant ($p > 0.05$) until the treatment densities were greater than 400 colonies/L for small-sized chlorophyll *a* and TP and greater than 800 colonies/L for TN. All three response variables exhibited a significant increasing trend ordered by treatment density (Linear regression with ordered treatment ranks, all slope parameters $p < 0.0001$).

Pond experiment

Prior to *G. echinulata* addition, there were no significant differences in *G. echinulata* density, total chlorophyll *a*, small-sized chlorophyll *a*, TN, or TP among the *G. echinulata* or Zooplankton Biomass treatments (two-way ANOVA, all main or interaction effects: $p > 0.07$). NO_3^-, NH_4^+, and SRP concentrations throughout the experiment were below the limit of detection and will not be reported.

G. echinulata density increased significantly in the Added *G. echinulata* treatment over the course of the experiment relative to the no-*G. echinulata* control ($p < 0.0001$; Figure 5.5; see Table 5.3 for all pond experiment RM ANOVA statistics). This effect was mediated by significant effects of time and a time × *G. echinulata* interaction ($p = 0.0002$), as the *G. echinulata* densities ranged from 49 (± 7)

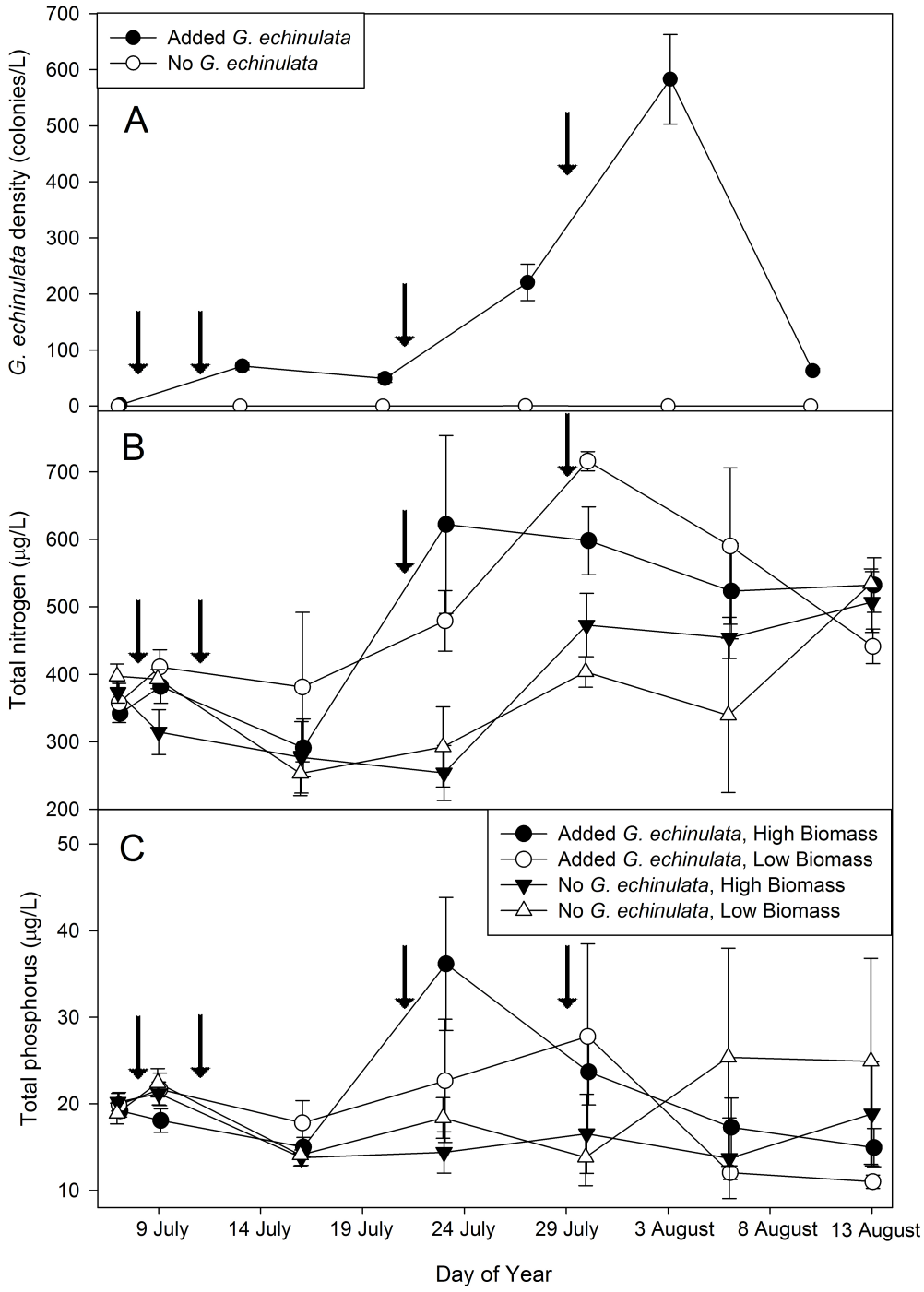


Figure 5.5. (A) *G. echinulata* densities significantly increased in the Added *G. echinulata* treatment in comparison to the No Added *G. echinulata* treatment in the

pond experiment mesocosms. The arrows refer to the days of *G. echinulata* addition. (B) The Added *G. echinulata* treatment exhibited significantly higher total nitrogen concentrations than the No Added *G. echinulata* treatments. (C) Total phosphorus concentrations exhibited a significant interaction between *G. echinulata* and time, as the Added *G. echinulata* treatment exhibited higher total phosphorus concentrations than the No Added *G. echinulata* treatment, but only after the third and fourth *G. echinulata* addition. Zooplankton Biomass did not have a significant effect on either nutrient ($p > 0.05$).

Table 5.3. Statistical results from the repeated measures two-way ANOVA analyses testing the effects and interaction of *G. echinulata* and Zooplankton Biomass treatments on *G. echinulata* density, nutrients, chlorophyll *a*, and zooplankton in the pond experiment. DF denotes degrees of freedom, and significant treatment effects ($p \leq 0.05$) are in bold.

Response Variable	**Repeated Measures ANOVA Test**	**DF**	**F-value**	**p-value**
G. echinulata density	***G. echinulata***	1,9	139.94	<0.0001
	Zooplankton Biomass	1,9	0.51	0.49
	G. echinulata × Zooplankton Biomass	1,9	0.52	0.49
	Time	4,36	53.78	<0.0001
	***G. echinulata* × Time**	4,36	53.68	<0.0001
	Zooplankton Biomass × Time	4,36	2.79	0.12
	G. echinulata × Zooplankton Biomass × Time	4,36	2.75	0.12
Total nitrogen	***G. echinulata***	1,8	12.81	0.007
	Zooplankton Biomass	1,8	0.00	0.97
	G. echinulata × Zooplankton Biomass	1,8	0.04	0.84
	Time	5,40	9.97	0.002
	***G. echinulata* × Time**	5,40	3.79	0.048
	Zooplankton Biomass × Time	5,40	0.58	0.57
	G. echinulata × Zooplankton Biomass × Time	5,40	1.05	0.37
Total phosphorus	*G. echinulata*	1,9	0.15	0.71
	Zooplankton Biomass	1,9	0.00	0.98
	G. echinulata × Zooplankton Biomass	1,9	1.33	0.28
	Time	5,45	1.66	0.12
	***G. echinulata* × Time**	5,45	3.42	0.048
	Zooplankton Biomass × Time	5,45	0.31	0.76
	G. echinulata × Zooplankton Biomass × Time	5,45	1.04	0.38
Total chlorophyll *a*	*G. echinulata*	1,8	1.43	0.27
	Zooplankton Biomass	1,8	0.00	0.95
	***G. echinulata* × Zooplankton Biomass**	1,8	5.12	0.05
	Time	10,80	2.40	0.10
	G. echinulata × Time	10,80	1.31	0.30
	Zooplankton Biomass × Time	10,80	0.18	0.89
	G. echinulata × Zooplankton Biomass × Time	10,80	1.08	0.37
Small-sized	*G. echinulata*	1,9	0.36	0.56

chlorophyll *a*	Zooplankton Biomass	1,9	0.03	0.87
	***G. echinulata* × Zooplankton Biomass**	1,9	10.34	0.01
	Time	10,90	3.83	0.02
	G. echinulata × Time	10,90	0.62	0.60
	Zooplankton Biomass × Time	10,90	0.31	0.81
	G. echinulata × Zooplankton Biomass × Time	10,90	1.06	0.38
Total Zooplankton Biomass	*G. echinulata*	1,9	0.45	0.52
	Zooplankton Biomass	1,9	10.82	0.009
	G. echinulata × Zooplankton Biomass	1,9	0.17	0.69
	Time	4,36	3.89	0.02
	G. echinulata × Time	4,36	1.48	0.24
	Zooplankton Biomass × Time	4,36	0.63	0.61
	G. echinulata × Zooplankton	4,36	1.48	0.24
Cladoceran Biomass	*G. echinulata*	1,9	0.37	0.56
	Zooplankton Biomass	1,9	8.11	0.02
	G. echinulata × Zooplankton Biomass	1,9	0.00	0.99
	Time	4,36	2.73	0.07
	G. echinulata × Time	4,36	0.71	0.55
	Zooplankton Biomass × Time	4,36	1.13	0.35
	G. echinulata × Zooplankton Biomass × Time	4,36	0.9	0.44

colonies/L in the Added *G. echinulata* treatments on day of year 201 (20 July) to 580 (± 80) colonies/L day of year 215 (3 August), while the *G. echinulata* density in the control treatment remained constant. We observed *G. echinulata* scums in the Added *G. echinulata* ponds, which lasted for several days after each addition.

The High Zooplankton Biomass treatment exhibited significantly higher total zooplankton and cladoceran biomass in comparison to the Low Zooplankton Biomass treatment ($p = 0.009$ and $p = 0.02$, respectively), indicating that our biomass treatments were valid (Figure 5.6). However, we did not see any significant main or interaction effect of *G. echinulata* on either zooplankton response variable (both $p \geq 0.52$). Similarly, we did not observe a significant effect of *G. echinulata* on total zooplankton density, cladoceran density, copepod density, rotifer density, or *Ceriodaphnia* density and birth, death, or growth rates (all $p \geq 0.07$; Appendix 2).

The *G. echinulata* treatments, but not the Zooplankton Biomass treatments, significantly increased nutrient concentrations (Figure 5.5). We observed a significant interaction between *G. echinulata* treatment and time for both TN and TP concentrations (both $p = 0.048$), with the Added *G. echinulata* treatments exhibiting up to 113% (mean 31%) higher TN and up to 97% (mean 13%) higher TP concentrations than No Added *G. echinulata* controls after the first *G. echinulata* addition. TN increased in both treatments throughout the experiment, but to a greater degree in the Added *G. echinulata* treatment than in the no-*G. echinulata* control, while TP exhibited higher concentrations in the Added *G. echinulata* treatment than in the control after the third and fourth *G. echinulata* additions, but not consistently

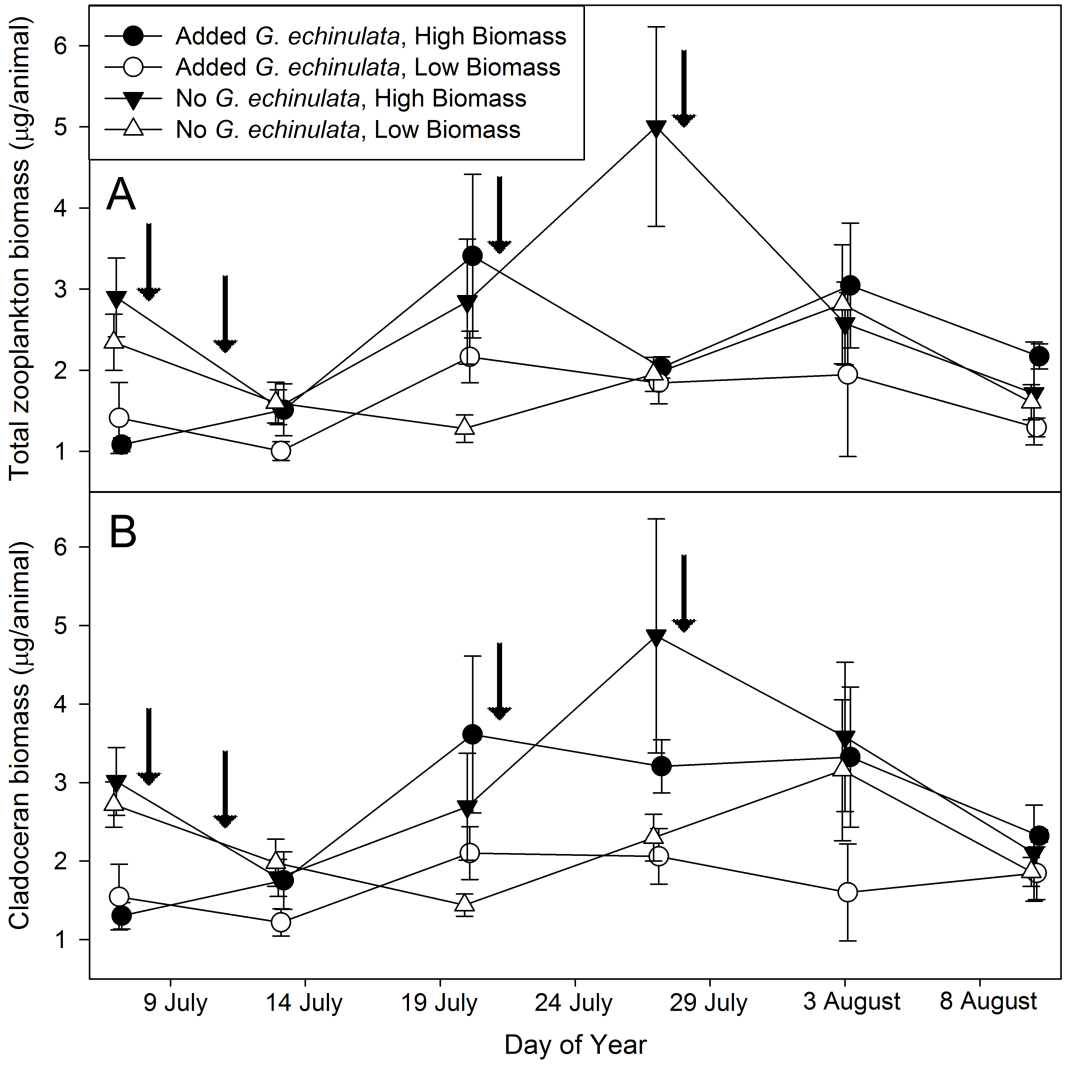


Figure 5.6. In the pond experiment, (A) zooplankton biomass and (B) cladoceran biomass exhibited significantly higher concentrations in the High Zooplankton Biomass treatments than in the Low Zooplankton Biomass treatments; there was no significant effect of *G. echinulata* on either group in the pond experiment ($p > 0.05$).

throughout the experiment. We observed a significant treatment effect of *G. echinulata* on TN ($p = 0.007$), but not TP ($p = 0.71$).

For both total and small-sized chlorophyll *a*, we observed a significant interaction effect of *G. echinulata* and Zooplankton Biomass ($p = 0.05$ and $p = 0.01$, respectively; Figure 5.7). For both fractions of chlorophyll *a*, Added *G. echinulata*/High Zooplankton Biomass treatments exhibited higher concentrations than Added *G. echinulata*/Low Zooplankton Biomass treatments (up to 693% and 404% for total and small-sized chlorophyll *a*, respectively), and No Added *G. echinulata*/Low Zooplankton Biomass treatments exhibited higher concentrations than No Added *G. echinulata*/High Zooplankton Biomass treatments (up to 343% and 239% for total and small-sized chlorophyll *a*, respectively). For both fractions of chlorophyll *a*, the interaction effect was primarily driven by the difference between the two High Zooplankton Biomass treatments (No Added *G. echinulata*/High Zooplankton Biomass and Added *G. echinulata*/High Zooplankton Biomass; see Appendix 3). Additionally, there was a significant time effect on small-sized chlorophyll *a* ($p = 0.02$).

For three out of five zooplankton sampling days after *G. echinulata* addition, we observed a significant ($p \leq 0.05$) or marginally significant ($p < 0.10$) positive effect of cladoceran biomass on the percent grazed *G. echinulata* colonies in the Added *G. echinulata* mesocosms (see Figure 5.8 for regression equations; for all days, the regression $R^2 = 0.32 – 0.75$ and $p = 0.03 – 0.24$). The three significant or moderately significant days (day of year 194, 201, and 208, or 13, 20, and 27 July) occurred in the middle of the experiment.

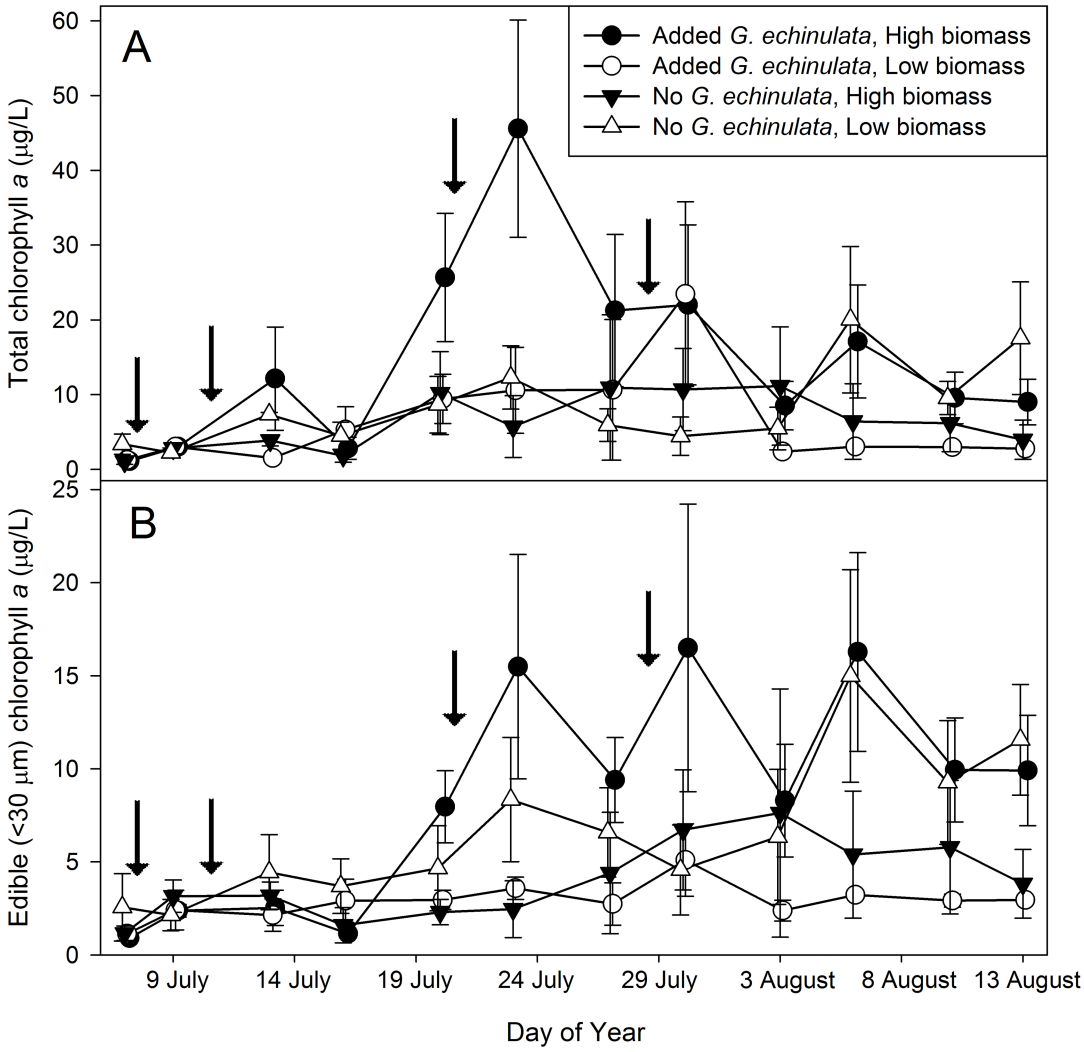


Figure 5.7. (A) Total and (B) small-sized (<30 µm fraction) chlorophyll *a* concentrations in the pond experiment mesocosms. The *G. echinulata* and Zooplankton Biomass treatments significantly interacted to increase both chlorophyll *a* fractions in the Added *G. echinulata*/High Zooplankton Biomass treatment relative to the Added *G. echinulata*/Low Zooplankton Biomass treatment and in the No Added *G. echinulata*/Low Zooplankton Biomass treatment relative to the No Added *G. echinulata*/High Zooplankton Biomass treatment. The arrows refer to the days of *G. echinulata* addition to the mesocosms.

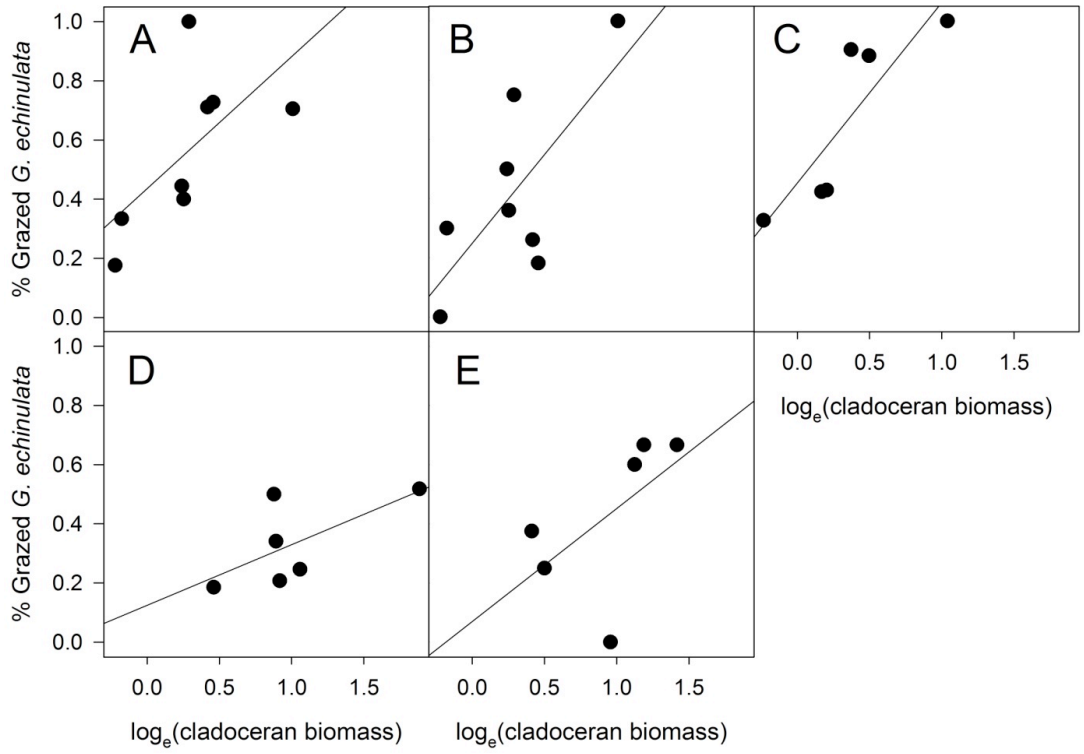


Figure 5.8. The percent damaged *G. echinulata* colonies increased with $\log_e$ cladoceran biomass in the Added *G. echinulata* treatments during the duration of the pond experiment, though the relationship was significant only in the middle of the experiment (day of year 201 and 208) and marginally significant on day 194. (A) % Grazed *G. echinulata* colonies (day of year 194) = 0.44 + 0.45 × $\log_e$(cladoceran biomass); $R^2 = 0.41$, $F_{1,6} = 4.22$, $p = 0.086$. (B) % Grazed *G. echinulata* colonies (day of year 201) = 0.25 + 0.60 × $\log_e$(cladoceran biomass); $R^2 = 0.51$, $F_{1,6} = 6.36$, $p = 0.045$. (C) % Grazed *G. echinulata* colonies (day of year 208) = 0.45 + 0.61 × $\log_e$(cladoceran biomass); $R^2 = 0.75$, $F_{1,4} = 11.71$, $p = 0.03$. (D) % Grazed *G. echinulata* colonies (day of year 215) = 0.12 + 0.20 × $\log_e$(cladoceran biomass); $R^2 = 0.43$, $F_{1,4} = 3.06$, $p = 0.16$. (E) % Grazed *G. echinulata* colonies (day of year 222) =

$0.07 + 0.38 \times \log_e$(cladoceran biomass); $R^2 = 0.32$, $F_{1,4} = 1.89$, $p = 0.24$. Two of the Added *G. echinulata* treatments were lost midway through the experiment due to introduction of yellow perch (*Perca flavescens*), which accounts for the reduced degrees of freedom on days of year 208, 215, and 222.

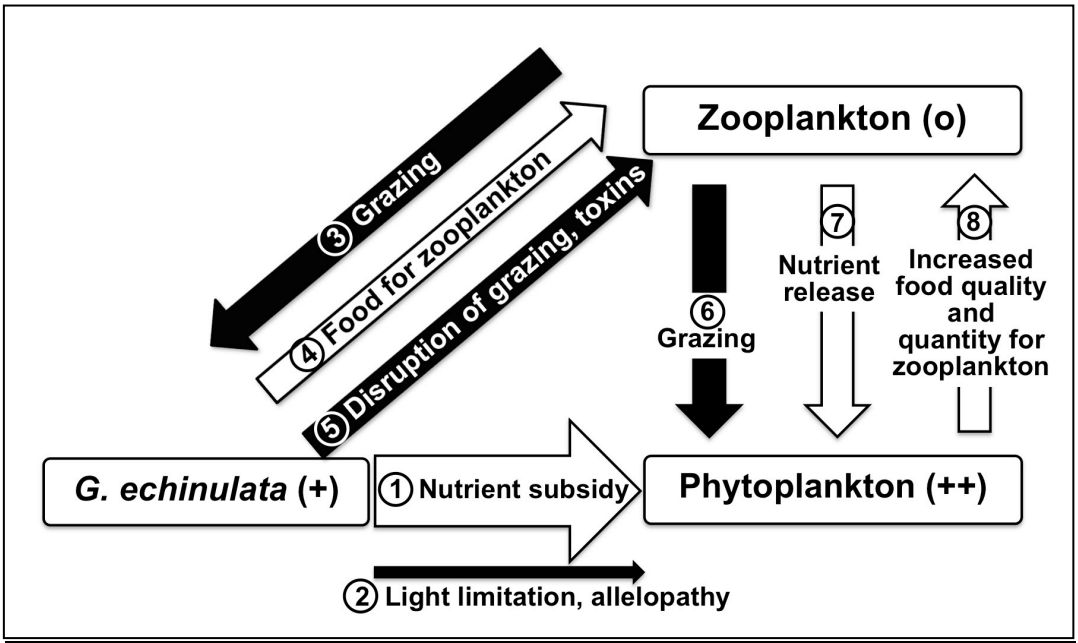
Although the largest increases in mean MC-LR concentration over the course of the experiment occurred in the two Added *G. echinulata* treatments, the differences were non-significant (all two-way ANOVA effects $p > 0.19$; Figure 5.3B).

Discussion

In three different experiments, the cyanobacterium *G. echinulata* played the role of facilitator in freshwater systems by stimulating small-sized phytoplankton. This facilitation likely occurred because *G. echinulata* increased the availability of N and P for other phytoplankton in nutrient-limited systems. Contrary to our predictions, zooplankton intensified the stimulatory effect of *G. echinulata* on small-sized phytoplankton, likely by increasing the cyanobacterium's nutrient release via grazing. Cyanobacteria are typically considered inhibitory, not stimulatory, to other plankton (Paerl 1988, Christoffersen et al. 1990, Bouvy et al. 1999, Huisman et al. 1999, Havens 2008); hence, examining *G. echinulata*'s facilitative effects within the context of its food web may elucidate how a cyanobacterium can facilitate the growth of small-sized phytoplankton (Figure 5.9).

Potential mechanisms and feedbacks: G. echinulata increases nutrients in low nutrient systems, thereby facilitating phytoplankton

In the *in situ* mesocosm and laboratory experiments with low nutrients and no zooplankton grazers, moderate densities of *G. echinulata* (400 colonies/L) resulted in a ~100% increase in small-sized chlorophyll *a* over the no-*G. echinulata* control while



Arrow	Interaction	Evidence supporting interaction
1	*G. echinulata* stimulates phytoplankton by increasing nutrients	Data from *in situ* mesocosm, laboratory, and pond experiments; also observed by Noges et al. (2004) and Pitois et al. (1997).
2	*G. echinulata* may have negative effects on phytoplankton by decreasing available light and releasing allelochemicals	Not observed in this study. Reynolds (1987), Huisman et al. (1999), and Hyenstrand (1999) show that cyanobacteria can limit the growth of other phytoplankton by reducing light availability, and Keating (1977, 1978) demonstrated that cyanobacteria can limit the growth of other phytoplankton by producing allelochemicals. However, these studies occurred in eutrophic, not oligotrophic, systems.
3	Zooplankton damage *G. echinulata* through filament clipping and grazing	Data from pond experiment; also observed by Fey et al. (2010).
4	Zooplankton may graze *G. echinulata*	Data indicate that this interaction *may* be occurring in the pond experiment; Fey et al. (2010) observed *Daphnia pulex* consuming *G. echinulata* colonies and trichomes.
5	Exposure to *G. echinulata* filaments and toxins may disrupt zooplankton grazing	Data indicate that this interaction *may* be occurring in the pond experiment; Fey et al. (2010) observed that *D. pulex* fed *G. echinulata* exhibited lower survivorship and reproduction than *D. pulex* fed other algae; Haney (1987), Lampert (1987), and others (see text) have observed that cyanobacteria can decrease zooplankton grazing because of toxins and filaments that clog filter-feeding appendages.
6	Zooplankton graze phytoplankton	A well-studied trophic linkage (Wetzel 2001, Reynolds 2006); the No Added *G. echinulata*/High Zooplankton Biomass ponds exhibited lower concentrations of small-sized chlorophyll *a* than the No Added *G. echinulata*/Low Zooplankton Biomass

		ponds, indicating that zooplankton grazing of small-sized phytoplankton likely occurred in the pond experiment.
7	Zooplankton recycle and release nutrients that can be taken up by phytoplankton	Data indicate that this interaction *may* be occurring in the pond experiment; Goldman et al. (1979), Lehman (1980) and others (see text) have observed increases in phytoplankton growth and reproduction as a result of nutrient recycling from zooplankton.
8	Small-sized phytoplankton are grazed by zooplankton	A well-studied trophic linkage (Wetzel 2001, Reynolds 2006); data from the pond experiment indicate that zooplankton grazing of phytoplankton most likely occurred in the No Added *G. echinulata* treatments.

Figure 5.9. Conceptual diagram of the effects of *G. echinulata* addition on a simplified aquatic food web. Black arrows indicate adverse effects, white arrows indicate stimulatory effects, and the width of an arrow denotes its hypothesized effect size. After *G. echinulata* addition (represented by the +), small-sized phytoplankton biomass increases (represented by ++) due to nutrient subsidies and zooplankton grazing on *G. echinulata* colonies. Zooplankton do not respond to *G. echinulata* addition, as indicated by the 'o' in parentheses. A description of each numbered food web interaction and the evidence supporting that interaction are detailed in the table below the figure.

high densities (6400 colonies/L) in the laboratory experiment caused a 1180% increase (Figure 5.9, Arrow 1). This facilitation is likely caused by nutrient leakage from *G. echinulata* colonies as a result of diffusion, metabolic processes, grazing on colonies by zooplankton, or senescence, as has been observed for other cyanobacterial taxa, especially in oligotrophic conditions (Healey 1982, Kankaanpaa et al. 2001, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004, Agawin et al. 2007). Facilitation by *G. echinulata* may also be observed in natural systems: nutrient leakage from *G. echinulata* may have triggered other phytoplankton to increase in Antermony Loch, Scotland and Lake Peipsi, Estonia (Pitois et al. 1997, Nõges et al. 2004). When nutrients are limiting, the stimulatory effect of *G. echinulata* on small-sized phytoplankton (Figure 5.9, Arrow 1) may be greater than the inhibitory effects of light limitation and allelopathy (Figure 5.9, Arrow 2).

At moderate densities, *G. echinulata* increased water column TN and TP concentrations in comparison to no-*G. echinulata* controls in all three experiments. The TN increases in the *G. echinulata* treatments were fairly consistent across experiments: we observed 44 – 74% higher TN concentrations in the 400 colonies/L treatment relative to controls in the *in situ* mesocosm and laboratory experiments, and a 44% increase in TN relative to controls in the pond experiment when the *G. echinulata* density was ~400 colonies/L. Effects on TP concentrations were less consistent, but TP was 28 - 63% higher at 400 colonies/L relative to controls in the laboratory and pond experiments.

Although soluble nutrients were below the level of detection, it is likely that the *G. echinulata* colonies were constantly releasing soluble N and P that was immediately taken up by other phytoplankton. This is supported by the increase of small-sized phytoplankton biomass within 24 hours of *G. echinulata* addition in the *in situ* mesocosm experiment. Moreover, after 5 d of incubation in the laboratory experiment, *G. echinulata* significantly increased TN and TP concentrations in the flask filtrate, which was tightly correlated with increases in the concentration of small-sized phytoplankton (as chlorophyll *a*).

G. echinulata colonies increased TN concentrations in the water column more than TP. The mean TN:TP ratio by mass in the 400 colonies/L treatment in the laboratory experiment after 5 d of incubation was 84.6 (± 8.7), substantially higher (87%) than the TN:TP ratio in the flasks at the beginning of the experiment (45.3 ± 16.5). While we were unable to calculate TN:TP ratios in the *in situ* mesocosm experiment because many of the TP measurements were below the method limit of detection, the mean TN:TP ratio in the Added *G. echinulata* ponds on the day of highest *G. echinulata* density was 35.1 (± 16.0), 63% higher than the ratio in the No Added *G. echinulata* ponds (21.6 ± 19.4). The difference in TN:TP ratios between the experiments is mostly likely due to the source of water used in each case: the laboratory microcosms were filled with Lake Sunapee water, whereas the ponds were filled with well water and contained 200 g of dry leaves for several weeks. Nonetheless, both experiments demonstrate that *G. echinulata* enriched the water column with more N relative to P.

The elevated leakage of N relative to P to the water in the laboratory and pond experiments may be due to N fixation. Stewart et al. (1967) found that *G. echinulata* exhibited higher N fixation rates than several other cyanobacterial taxa. Similarly, Vuorio et al. (2006) found that *G. echinulata* had the lowest colonial C:N ratio of 18 algal and cyanobacterial taxa tested and the second lowest $\delta^{15}N$ isotopic signature, indicating N-fixation. In the *in situ*, laboratory, and pond mesocosms, it would be expected that healthy *G. echinulata* colonies would be able to fix ecologically relevant amounts of N, some of which would become available to the water column.

Several lines of evidence support our interpretation that facilitation by *G. echinulata* caused the increase we observed in small-sized phytoplankton biomass (chlorophyll *a*) in the *G. echinulata* treatments. First, the biovolume of the entire non-*G. echinulata* fraction of phytoplankton (not only the <30 μm fraction), significantly increased in response to *G. echinulata* addition in the *in situ* experiment. The total chlorophyll *a* concentration in the 400 colonies/L treatment remained elevated throughout the experiment (Figure 5.2A), despite decreases in the density of *G. echinulata* (Figure 5.1A) and small-sized chlorophyll *a* (Figure 5.2B), indicating that the >30 μm fraction of non-*G. echinulata* phytoplankton must have increased, which we observed (Figure 5.2C). Taxonomic examination of the phytoplankton in this experiment indicates that the increased small-sized chlorophyll *a* concentration represents true stimulation of phytoplankton growth, primarily of diatoms and green algae, not detached *G. echinulata* trichomes (C.C.C. et al., unpublished data). By the end of the experiment, cell densities of diatoms and green algae (chlorophytes) had

increased by more than 2700% and 430%, respectively, in the 400 colonies/L treatment than in the no-*G. echinulata* control.

Second, it is unlikely that the phytoplankton increase was due to cells attached to the added *G. echinulata* colonies because individual colonies were cleaned thoroughly (see Methods). If some phytoplankton were attached to the added *G. echinulata*, chlorophyll *a* should have increased proportionally to *G. echinulata* addition, but it did not. Instead, we observed a saturating effect of *G. echinulata* density on small-sized chlorophyll *a* (Figure 5.4; Table 5.2).

Third, the rates of increase of small-sized phytoplankton correlate with the increases in small-sized chlorophyll *a* observed in the *in situ* mesocosms. *Per capita* phytoplankton growth rates (r) are strongly related to temperature and cell surface-to-volume ratio, with small cells generally having a much higher r than large cells (Reynolds 1989, Reynolds 2006). For phytoplankton smaller than <30 μm, we would expect r values of ~1.0 - 2.0 d^{-1} at 20°C (reviewed by Reynolds 2006). At 22°C, the temperature at 0.5 m depth in the *in situ* mesocosms on the day of *G. echinulata* addition, small-sized phytoplankton with an r of 1 – 2 d^{-1} would be able to increase their biomass by at least three- to seven-fold in a day, indicating that the doubling of the small-sized chlorophyll *a* concentration we observed in the 400 *G. echinulata* colony/L treatment within a day of *G. echinulata* addition was well within the range possible for small-sized phytoplankton growth.

Fluctuations in G. echinulata density represent natural dynamics

The changes in *G. echinulata* density we observed in the *in situ* mesocosms

and ponds are similar to patterns of *G. echinulata* density seen in lakes (Karlsson-Elfgren et al. 2003, Carey et al. 2008). In the *in situ* mesocosm experiment, added *G. echinulata* senesced and sank to the bottom of the mesocosms after 13 d, and in the pond experiment, *G. echinulata* density decreased ~12 d after we stopped adding colonies. This is similar to the estimation by Karlsson-Elfgren et al. (2003) that pelagic *G. echinulata* can remain in the water column for up to 2 weeks, and consistent with patterns in Lake Sunapee (Carey et al. 2008) and other lakes (Roelofs and Oglesby 1970, Barbiero and Welch 1992, Barbiero 1993, Nõges et al. 2004) that natural *G. echinulata* densities increase and decrease quickly. Finally, although our mesocosms were representations of natural ecosystems, we did observe the same result (stimulation of phytoplankton by *G. echinulata* in nutrient-limited aquatic systems) at three different mesocosm scales and experimental set-ups.

Zooplankton damage G. echinulata colonies

In the presence of zooplankton grazers, *G. echinulata* experienced increased damage, specifically, "filament clipping" (Figure 5.8, Figure 5.9, Arrow 3; Schaffner et al. 1994). Similarly, Fey et al. (2010) found that *Bosmina*, *Ceriodaphnia*, and *Daphnia* damaged *G. echinulata* by clipping filaments. All three of those cladoceran taxa were present in the pond experiment, especially *Ceriodaphnia*, and we observed significant or marginally significant relationships between cladoceran biomass and percent damaged *G. echinulata* colonies on three out of five sample days after *G. echinulata* addition. Smaller cladocerans, such as *Bosmina* and *Ceriodaphnia*, can persist or even increase during cyanobacterial blooms (de Bernardi and Guissani 1990,

Ghadouani et al. 2003, Deng and Xie 2008), perhaps because their feeding appendages are less obstructed by cyanobacterial filaments than those of larger species (Lampert 1987, Gliwicz and Lampert 1990, Hambright et al. 2001), or perhaps, as suggested by our data and by Fey et al. (2010), because they may be able to graze large cyanobacterial colonies (Figure 5.9, Arrow 4).

Zooplankton grazing may intensify the stimulatory effect of G. echinulata on phytoplankton

We observed a significant interaction between *G. echinulata* and Zooplankton Biomass in the pond experiment. In the absence of added *G. echinulata*, high densities of zooplankton grazers resulted in a decrease in the biomass of <30 μm phytoplankton. Small-sized phytoplankton are generally considered to be easily consumed by herbivorous zooplankton (Figure 5.9, Arrow 8; Lampert et al. 1986, Sommer et al. 1986, Hambright et al. 2007), so we expected that increasing zooplankton biomass would decrease chlorophyll *a* by grazing (Figure 5.9, Arrow 6; Leibold 1989, Wetzel 2001, Reynolds 2006). However, in ponds with Added *G. echinulata*, high densities of cladoceran zooplankton resulted in an increase of small-sized phytoplankton biomass. In cladoceran-dominated mesocosms, we observed damage to *G. echinulata* colonies through filament clipping (Figure 5.9, Arrow 3) with apparently a net effect of releasing more nutrients and thereby enhancing the growth of small-sized phytoplankton. Zooplankton are well-known for their ability to recycle nutrients by excretion and egestion (reviewed by Sterner and Elser 2002, Vanni 2002), and zooplankton-driven nutrient regeneration has been shown to increase algal growth

and reproductive rates in some aquatic systems (Figure 5.9, Arrow 7; Goldman et al. 1979, Lehman 1980, Sterner 1986, Elser et al. 1988, Sterner et al. 1995).

There are at least four mechanisms by which cladoceran filament clipping may have increased nutrients (Figure 5.9, Arrow 7), thereby intensifying the stimulatory effect of *G. echinulata* (Figure 5.9, Arrow 1). First, if the cladocerans clipped *G. echinulata*'s filaments but did not consume them (Schaffner et al. 1994), the filaments may have decomposed in the water column, providing a nutrient resource. Second, if the filaments were consumed (Figure 5.9, Arrow 4), some fraction would be excreted, which would also increase available nutrients (Sterner and Elser 2002, Vanni 2002). Third, extensive filament clipping can cause *G. echinulata* colonies to senesce (C.C.C., personal observation), further accelerating nutrient leakage. Finally, zooplankton grazing of *G. echinulata* colonies may have decreased consumption of other phytoplankton (Figure 5.9, Arrow 6), allowing them to increase. While our data do not allow us to determine which, if any, of these mechanisms are important in our study, they could have occurred individually or in concert to amplify *G. echinulata*'s stimulatory effect.

Zooplankton did not respond to G. echinulata addition

Despite the fact that *G. echinulata* increased <30 μm phytoplankton biomass, a food source considered edible for zooplankton (Figure 5.9, Arrow 6; Lampert et al. 1986, Reynolds 2006), neither zooplankton biomass or density showed a significant response to *G. echinulata*. Our results are similar to those of Fey et al. (2010), who found that *Daphnia pulex* fed *G. echinulata* exhibited higher survival than *D. pulex*

fed no food, but did not reproduce. Cyanobacteria are considered poor quality food for zooplankton because of their colonial and filamentous morphology (Arnold 1971, Lampert 1982, 1987, Hartmann and Kunkel 1991), toxins (Lampert 1981, 1982, Nizan et al. 1986, Fulton and Paerl 1987), and because they lack essential fatty acids, sterols, and nutrients (Holm and Shapiro 1984, Ahlgren et al. 1990, Gulati and DeMott 1997, Brett et al. 2006, Martin-Creuzberg et al. 2008), all of which decrease survivorship, growth rates, and fecundity in zooplankton (Figure 5.9, Arrow 5).

G. echinulata produces a low level of microcystin-LR (MC-LR; Carey et al. 2007), which can exert adverse effects on zooplankton survival and fecundity (DeMott et al. 1991, Rohrlack et al. 2001, Rohrlack et al. 2005). Treatments of 400 *G. echinulata* colonies/L significantly increased water column MC-LR concentrations relative to no-*G. echinulata* controls in the *in situ* mesocosm experiment. Although there were no large zooplankton grazers in this experiment and the differences in water column MC-LR concentrations between the treatments were small, we expect that direct grazing on *G. echinulata* colonies (Figure 5.9, Arrow 4) in natural systems would likely expose zooplankton to much higher MC-LR concentrations (Figure 5.9, Arrow 5). We did not observe significant differences in water column MC-LR concentrations between the Added *G. echinulata* and control treatments in the pond experiment, potentially because some of the colonies we used were from a different lake than the *in situ* experiment and may have included strains that produce lower concentrations of MC-LR.

Other factors may have prevented us from detecting a significant effect of *G. echinulata* on zooplankton biomass or density. For example, the pond experiment

only lasted for 37 d. However, *Ceriodaphnia* egg development time in the mesocosms was only 1.1 – 3.4 d, so any possible treatment effect, positive or negative, should have been evident. It is also possible that our experimental set-up (~800 L pond mesocosms) may have limited our ability to detect an effect, although we did not observe a decrease in zooplankton biomass or density in the control treatments (Figure 5.6), suggesting that the zooplankton were not too stressed in our mesocosms to respond to *G. echinulata* addition. Mesocosm experiments under similar conditions (same ponds with the identical location, time of year, stocked water, and sampling methods) have been used successfully in other zooplankton manipulations (Cottingham et al. 2004). Taken together, these factors indicate that zooplankton biomass and density, at least under our treatment conditions, do not respond to *G. echinulata* addition or an increase in small-sized phytoplankton due to *G. echinulata*.

G. echinulata may be able to increase eutrophication

Our experiments demonstrate that the principal effects of high densities of *G. echinulata* on low nutrient plankton food webs are to increase nutrients and phytoplankton biomass, important metrics of lake trophic state (i.e., extent of eutrophy). Cyanobacteria are typically thought of as a result, or an indicator, of eutrophication (Hutchinson 1967, Schindler 1974, Wetzel 2001); in contrast, we found that *G. echinulata* may be an *agent* of eutrophication because of its ability to increase nutrients at densities >400 colonies/L, as demonstrated in the laboratory experiment. *G. echinulata* is increasing in low nutrient lakes across the northeastern USA and Canada (Carey et al. 2008, 2009, Winter et al. 2011). If *G. echinulata* densities

exceed 400 colonies/L, we predict that it will increase nutrient availability and consequently phytoplankton abundance in those systems.

G. echinulata as an ecosystem facilitator in freshwater systems

Finally, we provide an example of a species that is a substantial, yet previously-unrecognized, facilitator of other species in freshwater systems. Ecologists typically do not think of cyanobacteria as facilitators of phytoplankton; rather, the opposite: cyanobacterial blooms typically reduce the species richness and biomass of non-blooming phytoplankton (e.g., Paerl 1988, Christoffersen et al. 1990, Bouvy et al. 1999, Huisman et al. 1999, Suikkanen et al. 2004, Havens 2008) and, by being poor quality food, decrease zooplankton grazing (Haney 1987, Lampert 1987, Gilbert and Durand 1990, Gliwicz and Lampert 1990, Gilbert 1996). However, most of these studies were conducted in high nutrient systems in which light, not nutrients, was the main factor limiting phytoplankton growth. Our study adds to the growing body of research demonstrating that cyanobacteria do not always exert inhibitory effects on plankton (e.g., Mohamed 2002, Paterson et al. 2002, Suikkanen et al. 2005, Sarnelle 2007, Wilson and Hay 2007, Neisch et al. In press).

The facilitative interaction between *G. echinulata* and small-sized phytoplankton in low nutrient freshwater systems is both similar to and different from the facilitation observed among terrestrial plants (e.g., Tewksbury and Lloyd 2001, Callaway et al. 2005, Brooker et al. 2008). Like *G. echinulata*, terrestrial plants can facilitate other plants by increasing nutrient availability (Callaway 1995): for example, by increasing N and organic matter in soils, early successional N-fixing

plants can facilitate the establishment and growth of late successional plants (Chapin et al. 1994). However, facilitative interactions dependent on increasing nutrient availability occur on a much shorter time scale between *G. echinulata* and other phytoplankton than among terrestrial plants because *G. echinulata* releases nutrients that quickly diffuse and come into contact with other phytoplankton in a fluid medium. In contrast, it typically takes longer for nutrients leaked or released from terrestrial plants (e.g., from decomposing leaf litter or rhizodeposition) to become available for uptake by other plants because of soil physical properties, soil chemistry, microbial transformations, and other factors (e.g., Melillo et al. 1982, Aerts 1997, Jones et al. 2004, Nguyen 2009). As a result, increasing nutrient availability may be a more common mechanism of facilitation among primary producers in freshwater systems than terrestrial systems because of the immediacy of its effect and because many different taxa of cyanobacteria leak nutrients, especially in oligotrophic conditions (Healey 1982, Kankaanpaa et al. 2001, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004, Agawin et al. 2007). As such, we hypothesize that other cyanobacteria, in addition to *G. echinulata*, could have substantial facilitative effects in low nutrient freshwater ecosystems.

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Appendix 1. The eight lakes from which we collected unfiltered lake water to create phytoplankton communities in the pond experiment. The asterisks (*) denote lakes from which we collected zooplankton to create the Zooplankton Biomass treatments.

Lake Name	Latitude	Longitude	Total phosphorus (μg/L)	Total nitrogen (μg/L)	Nutrient data source
Boston Lot Reservoir	43°40'N	71°17'W	10	251	A.C. Dawson, unpubl.
Broken Tank Pond	43°41'N	72°13'W	437	3007	C.C.C., unpubl.
Deweys Pond*	43°39'N	72°24'W	54	552	A.M. Siepielski, unpubl.
Goose Pond	43°42'N	72°5'W	5	179	A.C. Dawson, unpubl.
Occum Pond*	43°43'N	72°17'W	117	.	C.C.C., unpubl.
Post Pond*	43°50'N	72°09'W	8	215	A.C. Dawson, unpubl.
Lake Sunapee*	43°24'N	72°20'W	5	175	C.C.C., unpubl.
4A Pond	43°29'N	71°58'W	23	145	A.M. Siepielski, unpubl.

Appendix 2. Statistical results from the repeated measures two-way ANOVA analyses testing the effects and interaction of *G. echinulata* and Zooplankton Biomass treatments on zooplankton variables in the pond experiment. DF denotes degrees of freedom, and significant treatment effects ($p \leq 0.05$) are in bold.

Response Variable	**Repeated Measures ANOVA Test**	**DF**	**F-value**	**p-value**
Total zooplankton density	*G. echinulata*	1,9	2.38	0.16
	Zooplankton Biomass	1,9	0.40	0.54
	G. echinulata × Zooplankton Biomass	1,9	0.08	0.78
	Time	4,36	6.45	0.003
	G. echinulata × Time	4,36	1.58	0.22
	Zooplankton Biomass × Time	4,36	1.07	0.38
	G. echinulata × Zooplankton Biomass × Time	4,36	0.72	0.53
Cladoceran density	*G. echinulata*	1,9	0.12	0.73
	Zooplankton Biomass	1,9	1.58	0.24
	G. echinulata × Zooplankton Biomass	1,9	1.09	0.32
	Time	4,36	12.70	<0.0001
	G. echinulata × Time	4,36	0.39	0.82
	Zooplankton Biomass × Time	4,36	0.75	0.56
	G. echinulata × Zooplankton Biomass × Time	4,36	1.00	0.42
Copepod density	*G. echinulata*	1,9	1.23	0.30
	Zooplankton Biomass	1,9	0.42	0.53
	G. echinulata × Zooplankton Biomass	1,9	1.22	0.30
	Time	4,36	8.36	0.01
	G. echinulata × Time	4,36	0.42	0.52
	Zooplankton Biomass × Time	4,36	0.11	0.79
	G. echinulata × Zooplankton Biomass × Time	4,36	0.28	0.65
Rotifer density	*G. echinulata*	1,9	4.16	0.07
	Zooplankton Biomass	1,9	0.08	0.79
	G. echinulata × Zooplankton Biomass	1,9	0.08	0.79
	Time	4,36	1.37	0.28
	G. echinulata × Time	4,36	1.37	0.28
	Zooplankton Biomass × Time	4,36	0.28	0.74
	G. echinulata × Zooplankton Biomass × Time	4,36	0.28	0.74

Ceriodaphnia density	*G. echinulata*	1,9	0.00	1.00
	Zooplankton Biomass	1,9	1.23	0.30
	G. echinulata × Zooplankton Biomass	1,9	1.86	0.21
	Time	4,36	14.25	<0.0001
	G. echinulata × Time	4,36	0.57	0.60
	Zooplankton Biomass × Time	4,36	0.48	0.65
	G. echinulata × Zooplankton Biomass × Time	4,36	0.50	0.64
Ceriodaphnia birth rate	*G. echinulata*	1,9	0.02	0.88
	Zooplankton Biomass	1,9	0.29	0.61
	G. echinulata × Zooplankton Biomass	1,9	0.01	0.93
	Time	4,36	5.87	0.01
	G. echinulata × Time	4,36	1.52	0.24
	Zooplankton Biomass × Time	4,36	2.86	0.08
	G. echinulata × Zooplankton	4,36	0.94	0.41
Ceriodaphnia death rate	*G. echinulata*	1,9	0.99	0.35
	Zooplankton Biomass	1,9	0.04	0.85
	G. echinulata × Zooplankton Biomass	1,9	0.00	1.00
	Time	4,36	9.37	0.0004
	G. echinulata × Time	4,36	0.78	0.50
	Zooplankton Biomass × Time	4,36	0.77	0.51
	G. echinulata × Zooplankton Biomass × Time	4,36	1.49	0.24
Ceriodaphnia growth rate	*G. echinulata*	1,9	1.91	0.20
	Zooplankton Biomass	1,9	0.32	0.58
	G. echinulata × Zooplankton Biomass	1,9	0.00	0.96
	Time	4,36	13.45	<0.0001
	G. echinulata × Time	4,36	0.60	0.62
	Zooplankton Biomass × Time	4,36	1.73	0.18
	G. echinulata × Zooplankton Biomass × Time	4,36	0.62	0.61

Appendix 3.

To determine which *G. echinulata* and Zooplankton Biomass treatments drove the significant interaction effects we observed for in the total and small-sized chlorophyll *a* repeated measures ANOVA analyses, we analyzed all pairwise comparisons of the treatment means with a Bonferroni test, which calculated all pairwise dependent t values, compared them to a critical t with n-1 degrees of freedom, and corrected α for multiple comparisons at:

$$\alpha_{PC} = \frac{2\alpha}{k(k-1)} \qquad \text{(eqn. 1)}$$

where α_{PC} is the corrected significance level for pairwise comparisons; α is the significance level, here, $p \leq 0.05$; and *k* is the number of treatment means (Maxwell 1980). For our four treatments, the $\alpha_{PC} = 0.008$.

Appendix 3, Table 1. Statistical results from Bonferroni tests of all pairwise comparisons of total and small-sized chlorophyll *a* treatment means. G refers to the Added *G. echinulata* treatment, C refers to the No Added *G. echinulata* treatment, L refers to the Low Zooplankton Biomass treatment, and H refers to the High Zooplankton Biomass treatment. No p-values were significant at the α_{PC} (0.008), however, the pairwise comparisons suggest that the difference between the H/G and H/C treatments (in italics) was more important in driving the significant interaction effect for both chlorophyll *a* fractions more than any other treatment mean comparison.

Pairwise comparison of treatment means	Total chlorophyll *a*		Small-sized chlorophyll *a*	
	T-test value	P-value	T-test value	P-value
L/C vs. L/G	$t_8 = 0.39$	0.55	$t_9 = 2.74$	0.13
L/C vs. H/G	$t_8 = 1.01$	0.34	$t_9 = 0.33$	0.58
L/C vs. H/C	$t_8 = 4.25$	0.07	$t_9 = 5.32$	0.047
L/G vs. H/G	$t_8 = 1.77$	0.22	$t_9 = 5.08$	0.05
L/G vs. H/C	$t_8 = 0.58$	0.47	$t_9 = 0.08$	0.78
H/G vs. H/C	*$t_8 = 10.98$*	*0.01*	*$t_9 = 9.70$*	*0.01*

CHAPTER SIX

TROPHIC STATE MEDIATES THE EFFECT OF A LARGE, COLONIAL CYANOBACTERIUM ON PHYTOPLANKTON DYNAMICS

Abstract

Cyanobacteria are typically found in eutrophic lakes, where they commonly exert inhibitory effects on other plankton, however, they are also increasingly reported from oligotrophic and mesotrophic lakes. Here, we explored whether trophic state mediates the effect of cyanobacterial blooms on freshwater ecosystems. We examined the effects of *Gloeotrichia echinulata*, a large, colonial cyanobacterium increasingly prevalent in oligotrophic and mesotrophic lakes in the northeastern United States, on plankton and nutrients. We hypothesized that *G. echinulata*, which has been well-studied in eutrophic lakes, may exert different effects on other phytoplankton in mesotrophic and eutrophic systems. We predicted that (1) *G. echinulata* facilitates other phytoplankton in mesotrophic systems by increasing available nutrients (through fixing nitrogen and transporting phosphorus from the sediments to the water column) and inhibits other phytoplankton in eutrophic systems by producing scums and toxins,

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and (2) that zooplankton intensify negative effects of *G. echinulata* on other phytoplankton by selectively grazing small algae. To test these hypotheses, we manipulated *G. echinulata* presence, nutrient enrichment, and zooplankton biomass in mesocosms. We found that trophic state and zooplankton biomass significantly interacted to affect other phytoplankton. Phytoplankton were stimulated by *G. echinulata* in mesotrophic treatments, potentially because *G. echinulata* significantly increased nitrogen concentrations, whereas in eutrophic treatments, *G. echinulata* presence significantly decreased nitrogen and other phytoplankton. Increasing zooplankton biomass intensified the inhibitory effect of *G. echinulata* on other phytoplankton at eutrophic concentrations; however, increasing zooplankton intensified the facilitative effect of *G. echinulata* on phytoplankton at mesotrophic concentrations. In sum, *G. echinulata* significantly altered plankton food webs and nutrient concentrations in both mesotrophic and eutrophic systems, but trophic state determined if the effect was stimulatory or inhibitory.

Introduction

Aquatic habitats are critically threatened worldwide by eutrophication and its degradation of water quality (MEA 2005, Carpenter et al. 2011, Gleick et al. 2012). One of the most profound and visible symptoms of eutrophication is cyanobacterial blooms, which are typically considered harmful to humans because of their decomposing floating scums, noxious odors, and toxin production (Paerl 1988, Paerl et al. 2001, Huisman et al. 2005, Hudnell 2008). In the past three decades there has been an increase in both their geographic range and frequency (Hallegraeff 1993, Van

Dolah 2000, Anderson et al. 2002, Paerl and Huisman 2008, Sinha et al. 2012). These blooms are predicted to continue increasing under future climate change scenarios (Paerl and Huisman 2009, Carey et al. 2012, Kosten et al. 2012, Paerl and Paul 2012).

Cyanobacterial blooms typically occur in eutrophic systems (e.g., Huisman et al. 2005, Hudnell 2008), where they are considered to be inhibitory to ecosystem functioning (reviewed by Paerl et al. 2001). Modeling and experimental studies have demonstrated that cyanobacterial blooms in high-nutrient systems decrease non-blooming phytoplankton (e.g., Paerl 1988, Christoffersen et al. 1990, Bouvy et al. 1999, Huisman et al. 1999, Hyenstrand 1999, Suikkanen et al. 2004, Havens 2008). This may be due to the numerous physiological adaptations cyanobacteria have that allow them to outcompete other phytoplankton: they produce surface scums that limit light penetration (Mur et al. 1978, Reynolds et al. 1987, Huisman et al. 1999), excrete allelopathic chemicals and toxins (Kearns and Hunter 2001, Legrand et al. 2003, Hu et al. 2005, Leflaive and Ten-Hage 2007), have superior CO_2 uptake kinetics (King 1970, Shapiro 1973, 1984, 1997), store luxury phosphorus (P; Fitzgerald and Nelson 1966, Jensen 1968, Healey 1982), and fix their own nitrogen (N; Fogg and Stewart 1965, Stewart 1967). By decreasing other phytoplankton, cyanobacteria can reduce the flow of energy and nutrients to higher trophic levels, including zooplankton grazers and fish (Havens and East 1997, Nõges 1997, Havens 2008, Rondel et al. 2008). In addition, cyanobacteria can decrease zooplankton growth and reproduction by mechanically interfering with zooplankton feeding (Arnold 1971, Lampert 1982, 1987), producing toxins (Fulton and Paerl 1987, Hairston et al. 2001, Rohrlack et al.

2005), and because they lack certain fatty acids, sterols, and nutrients (Holm and Shapiro 1984, Ahlgren et al. 1990, Gulati and DeMott 1997, Brett et al. 2006).

Although much less studied, cyanobacterial blooms and scums also commonly occur in oligotrophic and mesotrophic lakes (Padisak et al. 2003, Lepisto et al. 2005, Galvao et al. 2008, Ernst et al. 2009, Vareli et al. 2009), where they may also be increasing (e.g., Boyer 2008, Ernst et al. 2009, Winter et al. 2011, Carey et al. In review). The differences between oligotrophic mesotrophic lakes and eutrophic lakes, including nutrient limitation, light availability, and trophic dynamics (Wetzel 2001), may drive fundamental differences in how cyanobacterial blooms impact plankton food webs (Havens 2008). Because of their position at the base of food webs and role in primary production and nutrient recycling, non-blooming phytoplankton are essential to aquatic ecosystem functioning (Graham and Wilcox 2000, Reynolds 2006), and it is important to understand how cyanobacterial blooms affect them.

In oligotrophic and mesotrophic systems, cyanobacteria may have more positive effects on other phytoplankton than in eutrophic systems by stimulating their growth with nutrients. Many cyanobacteria are capable of fixing N and taking up P in excess of their immediate metabolic needs, which can be stored for later periods when P is limiting (Fitzgerald and Nelson 1966, Jensen 1968, Carr and Whitton 1982). This fixed N and stored P may be released into the water column via leakage, cell lysis, or grazing, which could provide a nutrient subsidy to other phytoplankton in N- or P-limited systems (Healey 1982, Kankaanpaa et al. 2001, Ray and Bagchi 2001, Shi et al. 2004). Thus, cyanobacterial blooms in oligotrophic or mesotrophic lakes may have a stimulatory effect on other phytoplankton because they increase nutrient availability.

In contrast, in eutrophic lakes, where nutrients are typically less limiting than light (Wetzel 2001), the additional nutrients provided by cyanobacteria would be unlikely to exert a stimulatory effect on other phytoplankton. If a lake already limited by light began exhibiting cyanobacterial scums, which would exacerbate light and CO_2 limitation for phytoplankton (Scheffer et al. 1997, Passarge et al. 2006, Reynolds 2006), the blooms might create a net inhibitory effect on other phytoplankton. In addition, cyanobacterial allelochemicals and toxins (Kearns and Hunter 2001, Legrand et al. 2003, Hu et al. 2005, Leflaive and Ten-Hage 2007) may contribute to a cumulative inhibitory effect in eutrophic systems.

To the best of our knowledge, no studies have measured the effects of cyanobacterial blooms on other phytoplankton in freshwater systems while also deliberately manipulating trophic state. Most experimental studies testing the effects of freshwater cyanobacteria create blooms in mesocosms by adding nutrients to stimulate cyanobacterial growth or by adding cyanobacteria in culture media, thereby conflating the effect of the cyanobacteria and nutrients (e.g., Ghadouani et al. 2003, Xie et al. 2003, Chen et al. 2005, Rondel et al. 2008). As a result, the negative effects of cyanobacteria on plankton observed in these experiments are confounded by the effect of added nutrients. Here, we conducted a mesocosm experiment in which cyanobacteria were manipulated separately from nutrient treatments, making possible an independent comparison of the effects of cyanobacteria on plankton food webs in mesotrophic versus eutrophic systems.

We analyzed the effects of *Gloeotrichia echinulata*, a large colonial cyanobacterium that is increasing in oligotrophic and mesotrophic lakes in the

northeastern United States (Carey et al. 2008, Carey et al. In review), but also occurs in eutrophic lakes in Europe and the U.S. (Barbiero and Welch 1992, Karlsson-Elfgren et al. 2003). Because *G. echinulata* produces large colonies visible without a microscope (1-3 mm in diameter in northeastern U.S. lakes) and can naturally occur at high densities in oligotrophic and mesotrophic systems (Carey et al. 2008, Carey et al. In review), colony densities can be easily manipulated in mesocosm experiments without contaminating experimental units with added nutrients.

We predicted that *G. echinulata* would exert inhibitory effects on other phytoplankton in eutrophic systems but facilitative effects on phytoplankton in oligotrophic and mesotrophic systems. Following Grime's (1977) paradigm for terrestrial plant communities that productivity is inversely related to abiotic stress, and which Reynolds (1997) applied to freshwater plankton, we developed predictions in accordance with the stress-gradient hypothesis (Bertness and Callaway 1994), which proposes that facilitation is more likely to occur when environmental stress is high (i.e., at low nutrient concentrations). Conversely, competition is predicted to be a more important structuring force than facilitation in environments with lower levels of abiotic or biotic stress (i.e., at high nutrient concentrations; Bertness and Callaway 1994, Callaway and Walker 1997, Stachowicz 2001, Bruno et al. 2003).

The life history characteristics of *G. echinulata* may enable it to facilitate other phytoplankton in oligotrophic or mesotrophic conditions. The cyanobacterium has the ability to fix N (Stewart 1967, Roelofs and Oglesby 1970, Carr and Whitton 1982) and uptake and store P in excess of its immediate metabolic needs (Pettersson et al. 1993), which may be released to the water column through leakage, grazing, or senescence.

In Antermony Loch, Scotland, and Lake Peipsi, Estonia, blooms of *G. echinulata* are believed to have stimulated other phytoplankton by increasing nutrient availability (Pitois et al. 1997, Nõges et al. 2004). Nonetheless, *G. echinulata* can also exert negative effects because it produces gas vesicles that provide buoyancy and enable the formation of large surface scums (Forsell and Pettersson 1995, Eiler et al. 2006, Liess et al. 2006), which may be inhibitory to other phytoplankton when light limits growth. In addition, *G. echinulata* produces microcystin-LR (MC-LR; Carey et al. 2007), a toxin that can inhibit phytoplankton and zooplankton (Kearns and Hunter 2001, Hu et al. 2005, Leflaive and Ten-Hage 2007).

In addition to nutrients, zooplankton may also mediate the effect of *G. echinulata* on other phytoplankton. Zooplankton are a critical component of pelagic food webs and have the capacity to alter phytoplankton interactions through both consumption and nutrient mineralization (Lehman 1980, Lehman and Sandgren 1985, Elser et al. 1988). We predicted that zooplankton would intensify any negative effects of *G. echinulata* on other phytoplankton by grazing down small algae, as was observed by Ventelä et al. (2002) during an *Anabaena* and *Microcystis* cyanobacterial bloom. Zooplankton preferentially graze 'edible,' or small phytoplankton with high food quality (e.g., diatoms, cryptophytes, chlorophytes), over 'inedible' algae, or large or colonial phytoplankton with poor food quality, such as cyanobacteria (Lehman and Sandgren 1985, Lampert et al. 1986, Sommer et al. 1986, Cyr and Pace 1992, Hambright et al. 2007; but see Cyr and Curtis 1999). Consequently, zooplankton grazers may simultaneously moderate the facilitative effects of *G. echinulata* on other phytoplankton by preferentially grazing the stimulated phytoplankton, and intensify

the inhibitory effects of *G. echinulata* by grazing any remaining small phytoplankton. To test these hypotheses, we manipulated *G. echinulata* presence, trophic state, and zooplankton biomass in mesocosms. As increasing eutrophication is causing both increases in cyanobacterial blooms and changes in trophic state in lakes worldwide (e.g., Carpenter et al. 1998, Paerl and Huisman 2008, Dodds et al. 2009, Carpenter et al. 2011), our experiment examined how the effects of a nuisance cyanobacterium may be modified by increasing nutrient concentrations.

Methods

Experimental design and set-up

We conducted a fully factorial 2 × 2 × 2 mesocosm experiment that crossed nutrient levels (Ambient versus Enriched) and zooplankton biomass (Low versus High Zooplankton) with *G. echinulata* (*–G. echinulata* versus +*G. echinulata*). Throughout the experiment, mean total N (TN) and total P (TP) concentrations in the Ambient mesocosms were mesotrophic (350 μg/L ≤ TN ≤ 650 μg/L and 10 μg/L ≤ TP ≤ 30 μg/L), and the Enriched mesocosms were eutrophic (Nürnberg 1996). Each nutrient, zooplankton, and *G. echinulata* treatment combination had four randomly-assigned replicates (n = 32 total). The mesocosms consisted of 1136 L (total volume) cattle tanks (Rubbermaid, Wooster, OH, USA), each filled with 800 L of water and situated away from any tree cover in an old field in Etna, New Hampshire, U.S. (43°41'N, 72°13'W). The experiment ran for 6 weeks from 7 July to 13 August 2010.

In late May 2010, we acid-washed the inside of each mesocosm with hydrochloric acid and immediately covered them with 1 mm fiberglass mesh to

prevent invasion by insects. We added a mesh bag to each mesocosm containing 200 g of dry leaves as a carbon source for the plankton communities before filling the mesocosms with well water in mid-June. Each bag contained 50 g each of sugar maple (*Acer saccharum*), red oak (*Quercus rubra*), white pine (*Pinus strobus*), and American beech (*Fagus granifolia*) leaves collected from a forest near our field site.

We established the nutrient treatments immediately after the mesocosms were filled with water by adding a concentrated solution of KH_2PO_4 and NH_4NO_3 to the Enriched mesocosms, while the Ambient mesocosms received reverse osmosis water controls. N and P were added to the Enriched mesocosms every three days throughout the experiment at daily loading rates of 1 µg P/L and 20 µg N/L. These loading rates were similar to rates measured for eutrophic lakes (Shannon and Brezonik 1972) and approximate the N:P ratio (44:1 molar) observed in nearby oligotrophic and mesotrophic lakes.

We created phytoplankton communities in all of the mesocosms in mid-June by adding 2 L of unfiltered water collected from the top 0.5 m of eight nearby lakes (16 L total of lake water per mesocosm; see Appendix 1 for lake descriptions).

We let the phytoplankton community develop for two weeks before establishing the High Zooplankton treatments using zooplankton collected from four of the eight phytoplankton lakes. At each of these lakes, we collected zooplankton in 2 m vertical hauls with a 100 µm mesh plankton net. We visually inspected each haul sample and manually removed *G. echinulata* colonies, large predatory zooplankton, and invertebrates before adding the contents of one haul from each of the four lakes to every High Zooplankton mesocosm. Zooplankton communities developed in the Low

Zooplankton treatments as a result of dispersal via overland transport and nauplii and zooplankton juveniles in the unfiltered phytoplankton water. The two zooplankton treatments maintained significantly different levels of zooplankton biomass throughout the experiment and were composed of similar zooplankton taxa, predominantly *Ceriodaphnia* (see Results).

We allowed the zooplankton communities to develop for a week and then added *G. echinulata* to the appropriate mesocosms. We collected *G. echinulata* colonies from oligotrophic Lake Sunapee (43°24'N, 72°20'W, Sunapee, New Hampshire) and mesotrophic Lake Morey (43°55'N, 72°8'W, Fairlee, Vermont) with the goal of creating a +*G. echinulata* treatment that matched the highest *G. echinulata* density observed in an oligotrophic or mesotrophic northeastern U.S. lake (250 *G. echinulata* colonies/L; Carey et al. In review).

We collected colonies at each lake by towing a plankton net (0.5 m diameter, 100 μm mesh) for ~25 m just below the water's surface. We rinsed the net contents of each tow into separate 1-L white plastic bottles that were kept in the shade until transport back to the laboratory. We cleaned the *G. echinulata* colonies from each tow separately: we rinsed the colonies three times with GF/C Whatman (1.2 μm pore size) filtered Lake Sunapee water, individually inspected the colonies with a Leica MZ12 dissecting microscope, removed any remaining adhered debris or plankton with micro-scalpels and probes, discarded *G. echinulata* colonies that were missing trichomes or were not buoyant, and placed the cleaned colonies into new bottles. We divided the bottles by the lake from which the colonies had been collected and then assigned them to treatments so that an equal number of bottles from each lake were allocated to every

+*G. echinulata* mesocosm. *G. echinulata* colonies from the two lakes (Sunapee and Morey) appeared identical under a dissecting microscope. We added colonies to the +*G. echinulata* mesocosms in four pulses on days of year 189, 192, 202, and 210 (i.e., 8, 11, 21, and 29 July, or days of experiment 1, 3, 13, and 21) because we were unable to collect enough colonies in one day to reach our target density (250 colonies/L).

We sampled the mesocosms 24 h before the first *G. echinulata* addition and every 3-4 days thereafter, following the general methods of Cottingham et al. (2004). On each sampling day, we measured the mesocosm water level, recorded water temperature and dissolved oxygen just below the water's surface (Yellow Springs Inc. model 556 MPS), and removed insect invaders with a dip net. We lost two replicate mesocosms midway through the experiment in the Ambient Low Zooplankton +*G. echinulata* treatment due to the unexpected arrival of yellow perch, *Perca flavescens*, despite the presence of mesh covering on the mesocosms and the land-locked location of our experiment.

Manipulated variables: Nutrients, Zooplankton, and G. echinulata

We sampled each mesocosm weekly using a separate integrated tube sampler (0.5 m long, 5.1 cm diameter) for chemical and zooplankton analyses. We retained 125 mL for TN and TP analyses, and filtered 500 mL through 0.7 μm Whatman GF/F filters for ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive P (SRP) analyses. We froze all soluble and total nutrient samples until analysis. Both P fractions (SRP and TP) were analyzed using Method 4500-P (American Public Health Association 1980) with an acidic persulfate digestion for total samples. We analyzed TN samples

with spectrophotometric methods after basic persulfate digestion (Crumpton et al. 1992). NO_3^- and NH_4^+ samples were analyzed on a Lachat QuikChem 8000 (Lachat Instruments, Loveland, Colorado, USA) according to the QuikChem Phenate method #10-107-106-1-J and QuikChem Cadmium Reduction method #10-107-04-1-A, respectively.

We sampled zooplankton and *G. echinulata* weekly by filtering 7 L of water through 80 μm mesh and preserving the sample in 70% ethanol, and returned the filtered water to the mesocosms. We counted and identified zooplankton samples to genus on an Olympus SZH10 dissecting microscope and calculated total zooplankton biomass and total *Ceriodaphnia* biomass from established length-mass regressions (Bottrell et al. 1976, Downing and Rigler 1984). Log-transformed weights were calculated individually from each log-transformed length and back-transformed to original units before calculating the mean weight and size of a taxon (Bird and Prairie 1985). We counted *G. echinulata* colonies on an Olympus SZH10 dissecting microscope and determined the effect of zooplankton grazing on damage to *G. echinulata* colonies by calculating the percent of the *G. echinulata* colonies that showed signs of having been grazed (i.e., shortened or missing trichomes, non-intact central core), following Fey et al. (2010).

Response variables: Light Availability, Phytoplankton, and MC-LR

We evaluated the availability of light for phytoplankton photosynthesis in the mesocosms through two indirect methods. First, we examined the size of the phytoplankton scum covering the surface of each mesocosm on each sampling day and

after each *G. echinulata* addition, and ranked the scum on an ordinal scale from 0 (no scum present) to 4 (complete scum cover). The same observer assigned the scum cover rank for every mesocosm throughout the experiment to ensure that the ranks were consistent. Second, because there is a strong positive relationship between phytoplankton in the water column and turbidity (Morel and Maritorena 2001, Effler et al. 2006), we used total chlorophyll *a* as a surrogate measure of light scattering in the water column (Kirk 1994, Effler et al. 2006, Effler et al. 2010).

Two samples were collected for phytoplankton biomass (as chlorophyll *a*) from each mesocosm every 3-4 d. One sample was vacuum-filtered directly onto a Whatman GF/C filter (for total chlorophyll *a*), while the second was pre-filtered through a 30 μm Nitex mesh before being collected on a GF/C filter. This smaller fraction (<30 μm) of phytoplankton excluded *G. echinulata* colonies, which are typically 1-3 mm in diameter (Carey et al. 2008), and represented a size fraction of phytoplankton that zooplankton are generally able to graze (hereafter, small-sized chlorophyll *a*; Cottingham 1996). All chlorophyll *a* samples were frozen for at least 24 h, extracted with methanol, and analyzed with a fluorometer (Turner Designs TD 700, Sunnyvale, California, USA) according to Arar and Collins (1997).

We measured whole-water MC-LR concentrations both prior to *G. echinulata* addition and in the middle of the experiment (day of year 208, 27 July). Whole-water MC-LR samples were analyzed with an enzyme-linked immunosorbent assay (ELISA) according to Sasner et al. (2001) at the Center for Freshwater Biology at the University of New Hampshire.

Statistical Analyses

We conducted several analyses to determine if nutrients and zooplankton mediated the effect of *G. echinulata* on light availability, phytoplankton, and MC-LR concentrations, and assessed significance (α) at $p \leq 0.05$ and marginal significance at $0.05 < p < 0.10$. We first examined if there were significant main effects and interactions of our three treatments (nutrients, zooplankton, and *G. echinulata*) using three-way repeated measures (RM) ANOVA in SAS PROC MIXED (SAS v. 9.2, SAS Institute, Cary, North Carolina, USA) on TN, TP, total zooplankton biomass, total *Ceriodaphnia* biomass, *G. echinulata* density, percent grazed *G. echinulata* colonies, and total chlorophyll *a*. Because total zooplankton biomass and total *Ceriodaphnia* biomass were very highly correlated (on each sampling day, $r = 0.80 - 0.96$), we analyzed the two variables separately in RM ANOVA because MANOVA is not recommended for variables with high collinearity (Quinn and Keough 2002). We analyzed the effects of our three treatments on scum cover rank with non-parametric three-way RM ANOVA in PROC MIXED according to Shah and Madden (2004), and chose a covariance structure for each RM ANOVA analysis using AIC (Kincaid 2005).

More than half of the NH_4^+ and NO_3^- concentrations and approximately half of the SRP concentrations we measured in the mesocosms were below the method limit of detection (9.7 μg/L for NH_4^+ and NO_3^-, 1.2 μg/L for SRP), which prevented use of RM ANOVA to assess treatment effects. For these three nutrients only, we calculated for each mesocosm the proportion of all samples collected after the first *G. echinulata* addition that were above the method detection limit (MDL). We analyzed the effect of

nutrients, zooplankton, and *G. echinulata* on the proportions with three-way ANOVA using JMP (JMP v. 9.0.2, SAS Institute, Cary, North Carolina, USA).

We constructed separate regression models for each sampling day to evaluate the effect of cladoceran grazing potential on the percent damaged *G. echinulata* colonies. We included all cladoceran taxa because Fey et al. (2010) observed that a range of cladoceran genera (including *Bosmina*, *Ceriodaphnia*, *Daphnia*, and *Holopedium*) were able to damage *G. echinulata* trichomes during grazing trials. Because grazing potential is closely related to body size (Knoechl and Holtby 1986), we used a metric developed by Elser et al. (1987, 1988) that weighted the biomass of the total cladoceran community by cladoceran size. Described as biomass-weighted average cladoceran mass by Elser et al. (1987, 1988), here we refer to the metric as cladoceran grazing potential:

$$ZM_B = \frac{\sum_{i=1}^{n}(B_i \times I_i)}{\sum_{i=1}^{n} B_i} \qquad \text{(eqn. 6.1)}$$

where ZM_B is cladoceran grazing potential, or the total biomass of the cladoceran community weighted by the size of the individuals of that community (µg/animal); n is the total number of cladoceran taxa i; B_i is the total biomass of taxon i on that sampling day (µg/L); and I_i is the mean individual biomass of taxon i on that sampling day (µg/L; Elser et al. 1987, 1988). We analyzed the effect of cladoceran grazing from the previous sampling day on the percent grazed *G. echinulata* because we expected that the *G. echinulata* damage observed on a sampling day would be more

closely related to the cladoceran biomass it was exposed to at the beginning of the period between sample dates than at its end (Carey et al. In prep.).

Inherent to our experimental design is the result that small-sized chlorophyll *a* was significantly different among treatments before *G. echinulata* addition because the nutrient and zooplankton treatments had already been established. For example, on day of year 188 (7 July), before *G. echinulata* was added, small-sized chlorophyll *a* was 9.2 (± 2.7, 1 S.E.) µg/L higher in the Enriched mesocosms than the Ambient mesocosms (three-way ANOVA, $F_{1,30} = 18.28$, $p = 0.0003$). As a result, we analyzed the rate of change in small-sized chlorophyll *a* in the mesocosms over time by calculating growth rate, r, on each sampling day:

$$r = \frac{\ln\left(\frac{X_2}{X_1}\right)}{(t_2 - t_1)} \qquad \text{(eqn. 6.2)}$$

where r (d^{-1}) is the rate of change of small-sized chlorophyll *a*, X_2 is the concentration of small-sized chlorophyll *a* on sampling day t_2, and X_1 is the concentration of small-sized chlorophyll *a* on the preceding sampling day t_1.

To determine if the nutrient and zooplankton treatments resulted in positive or negative interactions between *G. echinulata* and other phytoplankton, we subtracted the mean growth rate of small-sized phytoplankton (<30 µm chlorophyll *a*) in a *–G. echinulata* treatment from each of the four replicates in the corresponding *+G. echinulata* treatment on each sampling day. Values greater than 0 indicated positive interactions, or facilitation (i.e., *+G. echinulata* increased the growth rate of small-sized phytoplankton relative to *–G. echinulata*), and values less than 0 indicated negative interactions, or inhibition (i.e., *+G. echinulata* decreased the growth rate of

small-sized phytoplankton relative to –*G. echinulata*). Hereafter, this value is referred to as the Facilitation-Inhibition Index, or FII. This metric is similar to the Relative Interaction Index (RII; Armas et al. 2004) in that it uses basic arithmetic to define inhibition and facilitation on a negative and positive scale balanced around zero, but it is also incorporates the negative values produced by decreasing growth rates.

We analyzed the effect of nutrients and zooplankton on the pattern of FII over time in the four treatments with two-way RM ANOVA in SAS PROC MIXED. In addition, we analyzed all pairwise comparisons of the four RM treatment means with a Bonferroni test, which calculated all pairwise dependent t values, compared them to a critical t with n-1 degrees of freedom, and corrected α for multiple comparisons (Maxwell 1980). Following our hypotheses, we tested if the two Ambient treatments exhibited FII values significantly greater than 0 and the two Enriched treatments exhibited FII values significantly less than 0 after the first *G. echinulata* addition with one-sample t-tests in JMP (v. 9.0.2).

We analyzed the change in MC-LR concentration between the beginning and end of the experiment among treatments with three-way ANOVA, testing the main and interaction effects of nutrients, zooplankton, and *G. echinulata* (JMP, v. 9.0.2). We ln-transformed the MC-LR concentrations to equalize variance.

Results

Manipulated variables: Nutrients, Zooplankton, and G. echinulata

Consistent with our design, TN and TP were higher in the Enriched than the Ambient mesocosms (Figure 6.1; see Table 6.1 for ANOVA statistics). We observed

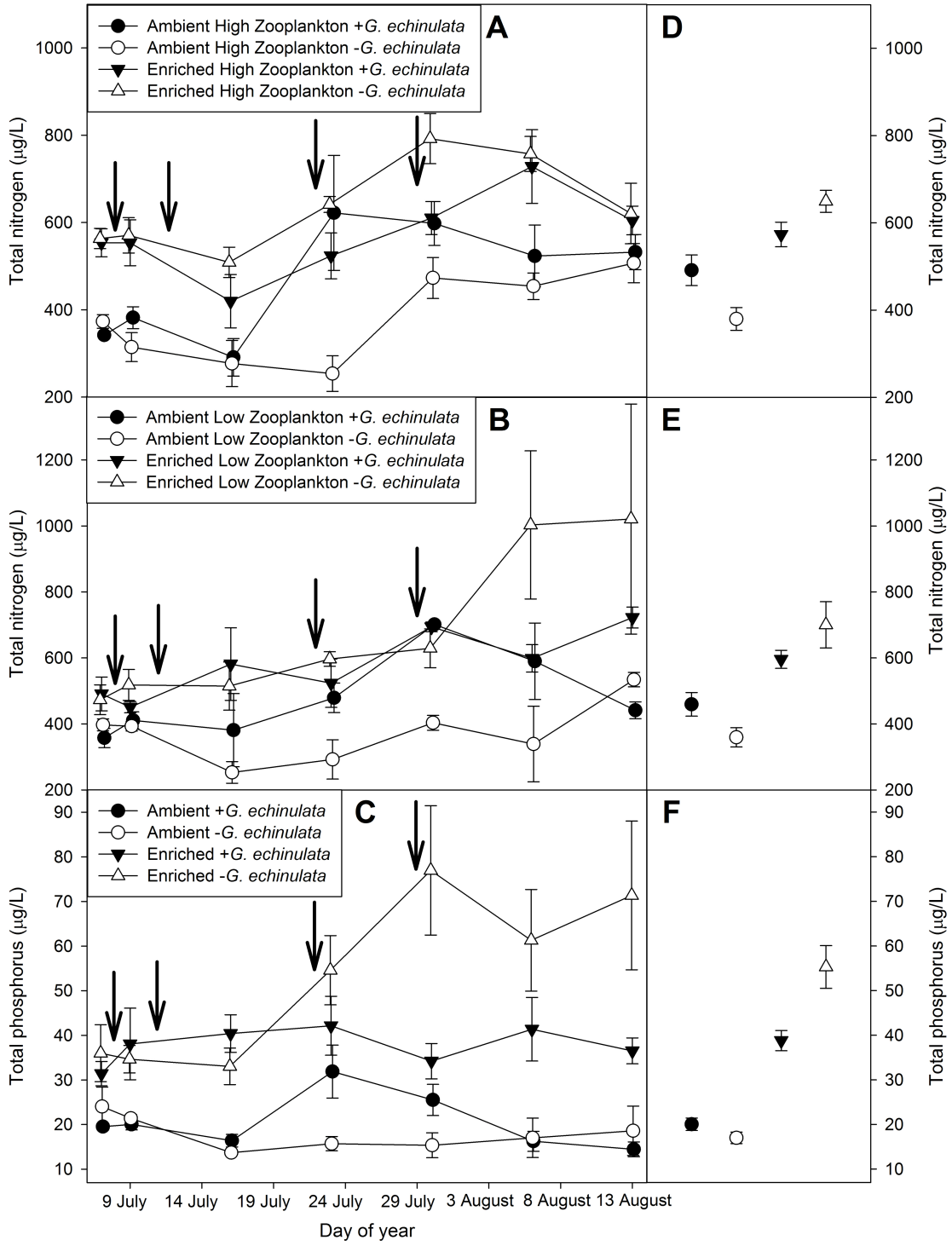

Figure 6.1. The mean (± 1 S.E.) total nitrogen concentrations in the (A) High Zooplankton nutrient and *G. echinulata* treatments and (B) Low Zooplankton nutrient

and *G. echinulata* treatments, and (C) total phosphorus concentrations in the nutrient and *G. echinulata* treatments over time. The arrows refer to the days of *G. echinulata* addition. Nutrient × *G. echinulata* × time and *G. echinulata* × zooplankton × time interactions significantly influenced the total nitrogen concentrations, and nutrients significantly increased the phosphorus concentrations. The mean (± 1 S.E.) total nitrogen concentrations in the (D) High Zooplankton nutrient and *G. echinulata* treatments and (E) Low Zooplankton nutrient and *G. echinulata* treatments, and (F) total phosphorus concentrations in the nutrient and *G. echinulata* treatments observed across all sample days after the first *G. echinulata* addition.

Table 6.1. Statistical results from the three-way repeated measures ANOVA analyses testing the effects and interactions of nutrients, zooplankton biomass, and *G. echinulata* on total nitrogen (µg/L), total phosphorus (µg/L), total zooplankton biomass (µg/L), total *Ceriodaphnia* biomass (µg/L), *G. echinulata* density (colonies/L), and total chlorophyll *a* (µg/L). Non-parametric three-way repeated measures ANOVA was used to analyze the effect of the three treatments on scum cover rank, and two-way repeated measures ANOVA was used to analyze the effects of nutrients and zooplankton biomass on percent grazed *G. echinulata* and the Facilitation-Inhibition Index. DF denotes degrees of freedom, and significant treatment effects ($p \leq 0.05$) are in bold. Marginally significant treatment effects ($0.05 < p < 0.10$) are noted with an asterisk (*).

Manipulated Variables	**RM ANOVA**	**DF**	**F value**	**P value**
Total Nitrogen	**Nutrient**	1,24	63.05	<0.0001
	G. echinulata	1,24	0.28	0.60
	Zooplankton	1,24	0.78	0.38
	Time	5,24	13.69	<0.0001
	Nutrient × *G. echinulata*	1,24	21.34	0.0001
	Nutrient × Zooplankton	1,24	0.52	0.48
	G. echinulata × Zooplankton	1,24	0.00	0.98
	Nutrient × *G. echinulata* × Zooplankton	1,24	0.48	0.50
	Nutrient × Time	5,24	1.34	0.28
	***G. echinulata* × Time**	5,24	3.44	0.02
	Zooplankton × Time	5,24	1.14	0.37
	Nutrient × *G. echinulata* × Time	5,24	2.85	0.04
	Nutrient × Zooplankton × Time	5,24	1.50	0.23
	***G. echinulata* × Zooplankton × Time**	5,24	5.47	0.002
	Nutrient × *G. echinulata* × Zooplankton × Time	5,24	0.94	0.47
Total Phosphorus	**Nutrient**	1,24	26.82	<0.0001
	G. echinulata	1,24	1.81	0.19
	Zooplankton	1,24	0.20	0.66
	Time	5,24	1.92	0.12
	Nutrient × *G. echinulata*	1,24	2.72	0.11
	Nutrient × Zooplankton	1,24	0.28	0.60
	G. echinulata × Zooplankton	1,24	0.16	0.70
	Nutrient × *G. echinulata* × Zooplankton	1,24	0.11	0.75
	Nutrient × Time	5,24	1.06	0.41
	G. echinulata × Time	5,24	1.90	0.13
	Zooplankton × Time	5,24	0.67	0.65
	Nutrient × *G. echinulata* × Time	5,24	1.80	0.15
	Nutrient × Zooplankton × Time	5,24	0.31	0.90
	G. echinulata × Zooplankton × Time	5,24	1.53	0.22
	Nutrient × *G. echinulata* × Zooplankton × Time	5,24	0.52	0.76
	Nutrient	1,24	32.44	<0.0001

Total Zooplankton Biomass	**Nutrient**	1,24	32.44	<0.0001
	G. echinulata	1,24	0.13	0.72
	Zooplankton	1,24	6.02	0.02
	Time	4,24	7.48	0.0005
	Nutrient × *G. echinulata*	1,24	0.02	0.90
	Nutrient × Zooplankton*	1,24	3.31	0.08
	G. echinulata **×** Zooplankton	1,24	1.50	0.23
	Nutrient **×** *G. echinulata* **×** Zooplankton	1,24	0.04	0.85
	Nutrient **×** Time	4,24	1.60	0.21
	G. echinulata × Time	4,24	0.23	0.92
	Zooplankton × Time	4,24	1.10	0.38
	Nutrient × *G. echinulata* × Time	4,24	0.75	0.57
	Nutrient × Zooplankton × Time	4,24	0.92	0.47
	G. echinulata × Zooplankton × Time	4,24	0.74	0.57
	Nutrient × *G. echinulata* × Zooplankton × Time	4,24	0.90	0.48
Ceriodaphnia Biomass	**Nutrient**	1,24	20.90	0.0001
	G. echinulata	1,24	0.88	0.36
	Zooplankton	1,24	8.10	0.009
	Time	4,89	7.81	<0.0001
	Nutrient × *G. echinulata*	1,24	0.86	0.36
	Nutrient × Zooplankton	1,24	4.47	0.04
	G. echinulata **×** Zooplankton	1,24	0.42	0.52
	Nutrient **×** *G. echinulata* **×** Zooplankton	1,24	0.17	0.68
	Nutrient × Time*	4,89	2.37	0.06
	G. echinulata × Time	4,89	0.27	0.90
	Zooplankton **×** Time	4,89	1.41	0.24
	Nutrient × *G. echinulata* × Time	4,89	0.62	0.65
	Nutrient **×** Zooplankton **×** Time	4,89	0.82	0.51
	G. echinulata × Zooplankton × Time	4,89	0.41	0.80
	Nutrient × *G. echinulata* × Zooplankton × Time	4,89	0.64	0.63
G. echinulata Density	Nutrient	1,24	0.34	0.56
	G. echinulata	1,24	828.71	<0.0001
	Zooplankton	1,24	0.30	0.59
	Time	4,24	314.66	<0.0001
	Nutrient × *G. echinulata*	1,24	0.18	0.68
	Nutrient × Zooplankton	1,24	0.02	0.90
	G. echinulata × Zooplankton	1,24	0.19	0.67
	Nutrient × *G. echinulata* × Zooplankton	1,24	0.06	0.82
	Nutrient × Time	4,24	0.50	0.74
	***G. echinulata* × Time**	4,24	323.46	<0.0001
	Zooplankton × Time	4,24	0.35	0.84
	Nutrient × *G. echinulata* × Time	4,24	0.96	0.45
	Nutrient × Zooplankton × Time	4,24	1.56	0.22

	G. echinulata × Zooplankton × Time	4,24	0.19	0.94
	Nutrient × *G. echinulata* × Zooplankton × Time*	4,24	2.40	0.08
Percent Grazed *G. echinulata*	Nutrient*	1,12	3.59	0.08
	Zooplankton	1,12	0.14	0.72
	Time	4,39	1.47	0.23
	Nutrient × Zooplankton	1,12	2.46	0.14
	Nutrient × Time	4,39	1.45	0.24
	Zooplankton × Time	4,39	0.46	0.76
	Nutrient × Zooplankton × Time	4,39	0.27	0.90
Response Variables	**RM ANOVA**	**DF**	**F value**	**P value**
Scum Cover Rank	**Nutrient**	1,24.8	6.57	0.02
	G. echinulata	1,24.8	0.41	0.53
	Zooplankton	1,24.8	0.10	0.75
	Time	14,23.9	22.99	<0.0001
	Nutrient × *G. echinulata*	1,24.8	0.71	0.41
	Nutrient × Zooplankton	1,24.8	0.28	0.60
	G. echinulata × Zooplankton	1,24.8	0.00	0.97
	Nutrient × *G. echinulata* × Zooplankton	1,24.8	0.22	0.65
	Nutrient × Time	14,23.9	4.31	0.0009
	***G. echinulata* × Time**	14,23.9	52.41	<0.0001
	Zooplankton × Time*	14,23.9	1.91	0.08
	Nutrient × *G. echinulata* × Time	14,23.9	1.29	0.29
	Nutrient × Zooplankton × Time	14,23.9	1.52	0.18
	G. echinulata × Zooplankton × Time	14,23.9	1.31	0.27
	Nutrient × *G. echinulata* × Zooplankton × Time	14,23.9	0.83	0.63
Total Chlorophyll *a*	**Nutrient**	1,24	12.57	0.002
	G. echinulata	1,24	0.92	0.35
	Zooplankton	1,24	0.18	0.67
	Time	10,24	3.81	0.004
	Nutrient × *G. echinulata*	1,24	2.58	0.12
	Nutrient × Zooplankton	1,24	0.38	0.55
	G. echinulata × Zooplankton	1,24	0.79	0.38
	Nutrient × *G. echinulata* × Zooplankton	1,24	0.46	0.50
	Nutrient × Time	10,24	0.47	0.89
	G. echinulata × Time	10,24	1.19	0.35
	Zooplankton × Time	10,24	1.64	0.16
	Nutrient × *G. echinulata* × Time	10,24	2.31	0.04
	Nutrient × Zooplankton × Time	10,24	0.96	0.50
	***G. echinulata* × Zooplankton × Time**	10,24	8.35	<0.0001
	Nutrient × *G. echinulata* × Zooplankton × Time	10,24	1.23	0.32
Facilitation-Inhibition	**Nutrient**	1,12	18.59	0.001
	Zooplankton	1,12	1.00	0.34

Index (FII)	Time	10,102	1.35	0.22
	Nutrient × Zooplankton	1,12	12.77	0.004
	Nutrient × Time	10,102	2.65	0.007
	Zooplankton × Time	10,102	2.31	0.02
	Nutrient × Zooplankton × Time	10,102	2.06	0.03

significant nutrient × *G. echinulata* × time, *G. echinulata* × zooplankton × time, nutrient × *G. echinulata*, *G. echinulata* × time, nutrient, and time effects on TN (all $p \leq 0.04$). The +*G. echinulata* treatments generally exhibited higher TN concentrations than the –*G. echinulata* treatments at Ambient levels, while the –*G. echinulata* treatments exhibited higher concentrations than the *G. echinulata* treatments at Enriched levels ($p = 0.04$). The nutrient × *G. echinulata* interaction was more consistent throughout the experiment in the High Zooplankton (Figure 6.1A) than in the Low Zooplankton treatments (Figure 6.1B), driving the significant *G. echinulata* × zooplankton × time interaction ($p = 0.002$). Although the TP concentrations exhibited a similar nutrient × *G. echinulata* pattern as the TN concentrations (Figure 6.1C), the interaction was non-significant ($p = 0.11$), and the only significant effect on TP was nutrients ($p < 0.0001$).

Most of the NH_4^+ and NO_3^- samples (72% and 82%, respectively) and approximately half (45%) of the SRP samples in the mesocosms were below the MDL (Figure 6.2). Nutrients and *G. echinulata* significantly interacted to affect the proportion of NO_3^- samples above the MDL after the first *G. echinulata* addition (three-way ANOVA: $F_{1,30} = 4.78$, $p = 0.04$). At Ambient levels, -*G. echinulata* mesocosms exhibited a higher proportion of NO_3^- samples above the MDL than +*G. echinulata* mesocosms, while at Enriched levels, –*G. echinulata* and +*G. echinulata* mesocosms exhibited similar proportions. Nutrient enrichment significantly or marginally increased proportions of NH_4^+, NO_3^-, and SRP samples that were above the MDL (NH_4^+: $F_{1,30} = 7.04$, $p = 0.01$; NO_3^-: $F_{1,30} = 3.66$, $p = 0.07$; SRP: $F_{1,30} = 76.71$, p

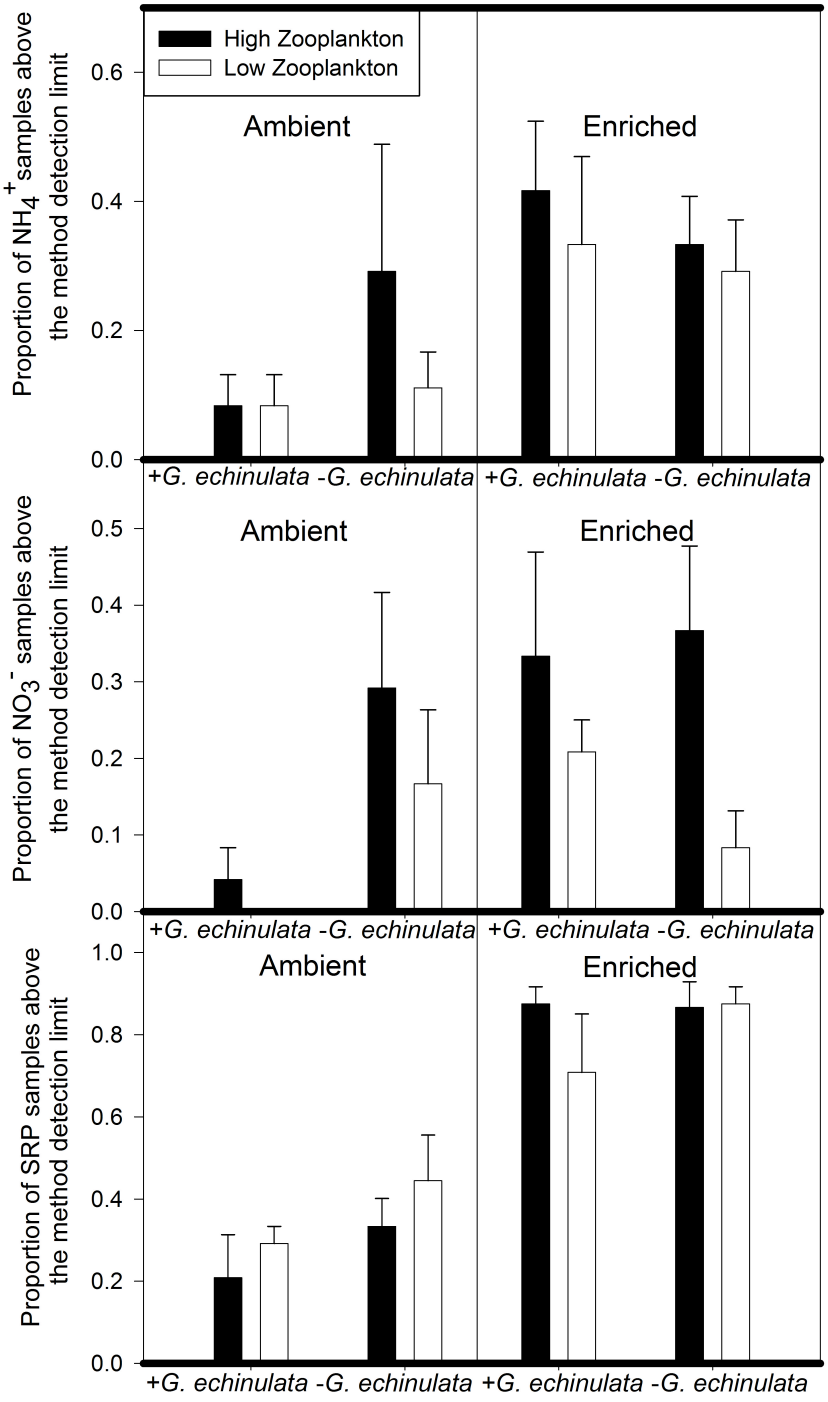

Figure 6.2. The mean (± 1 S.E.) proportion of mesocosms that exhibited (Top) NH_4^+, (Middle) NO_3^-, and (Bottom) SRP samples above the method detection limit throughout the experiment in the nutrient, zooplankton, and *G. echinulata* treatments.

< 0.0001). However, the soluble nutrient concentrations were quite low: when the concentrations below the MDL were omitted, the mean NH_4^+, NO_3^-, and SRP concentrations were only slightly higher (8 µg NH_4^+/L, 5 µg NO_3^-/L, and 1 µg SRP/L, respectively) than the MDL. Zooplankton biomass significantly increased the proportion of NO_3^- samples above the MDL ($F_{1,30} = 5.01$, $p = 0.03$), but there were no significant effects or interactions of zooplankton or *G. echinulata* on NH_4^+ ($p \geq 0.25$) or a main effect of *G. echinulata* on NO_3^- ($p = 0.22$). *G. echinulata* marginally decreased the proportion of SRP samples above the detection limit ($F_{1,30} = 3.48$, $p = 0.07$). There were no significant effects or interactions of zooplankton on SRP ($p \geq 0.14$).

Total zooplankton biomass, which was dominated by the cladoceran *Ceriodaphnia*, was higher in the High Zooplankton than the Low Zooplankton treatments (Figure 6.3). We observed significant or marginally significant effects of nutrient × zooplankton, nutrients, zooplankton, and time on both total zooplankton and *Ceriodaphnia* biomass (total zooplankton biomass: all $p \leq 0.08$; total *Ceriodaphnia* biomass: all $p \leq 0.04$). In addition, we observed a marginally significant nutrient × time interaction on total *Ceriodaphnia* biomass ($p = 0.06$). The difference in total zooplankton and *Ceriodaphnia* biomass concentrations between the Low and High Zooplankton treatments was greater at Enriched concentrations, causing the nutrient × zooplankton interactions (both $p \leq 0.08$). On average, the High Zooplankton treatment exhibited 14.3 (± 43.8) µg/L higher total zooplankton biomass and 24.7 (± 22.5) µg/L higher *Ceriodaphnia* biomass than the Low Zooplankton treatment at Ambient concentrations, while the High Zooplankton treatment exhibited 257.3 (± 87.6) µg/L

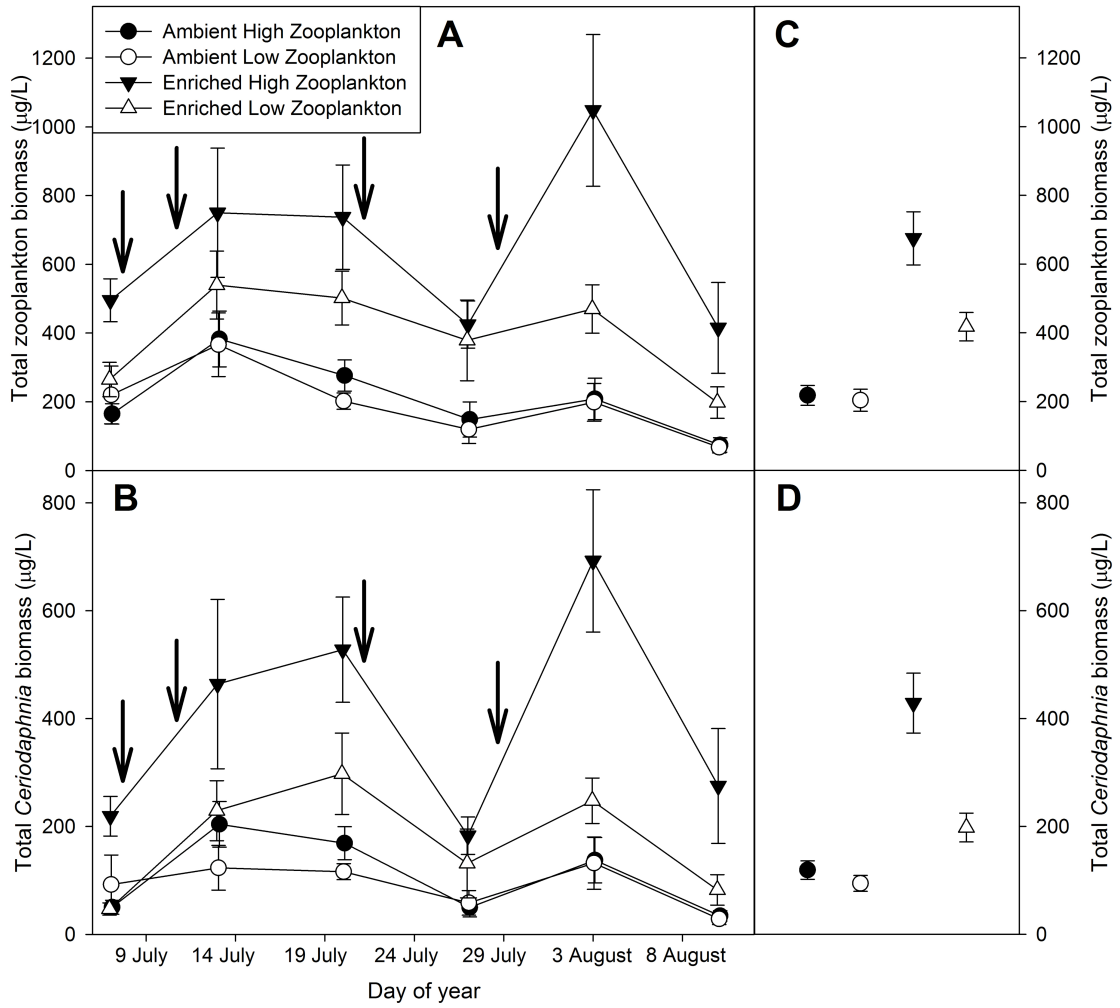

Figure 6.3. (A) The mean (± 1 S.E.) total zooplankton biomass and (B) total *Ceriodaphnia* biomass concentrations in the nutrient and zooplankton treatments over time. The arrows refer to the days of *G. echinulata* addition. Nutrients significantly interacted with zooplankton to increase the difference between the High and Low Zooplankton treatments at Enriched nutrient levels. (C) The mean (± 1 S.E.) total zooplankton biomass and (D) total *Ceriodaphnia* biomass concentrations observed across all sample days after the first *G. echinulata* addition within the nutrient and zooplankton treatments.

higher total zooplankton biomass and 230.7 (± 61.6) µg/L higher *Ceriodaphnia* biomass than the Low Zooplankton treatment at Enriched concentrations. There was no significant effect or interaction of *G. echinulata* on either total zooplankton or *Ceriodaphnia* biomass (all $p \geq 0.23$).

G. echinulata densities were significantly higher in the +*G. echinulata* mesocosms than in the –*G. echinulata* mesocosms ($p < 0.0001$; Figure 6.4A). The *G. echinulata* density in the +*G. echinulata* treatment peaked at 521 colonies/L on day of year 215 (3 August) after four additions of *G. echinulata* colonies, resulting in significant effects of time and a *G. echinulata* × time interaction (both $p \leq 0.0001$). We did not observe significant main effects of nutrients or zooplankton biomass on *G. echinulata* density (both $p \geq 0.56$).

Nutrients marginally decreased the percent of *G. echinulata* colonies that were grazed by zooplankton: we observed a lower percentage of grazed *G. echinulata* colonies in the Enriched treatments than in the Ambient treatments ($p = 0.08$). On average, over the course of the experiment, 31 – 59% of colonies in the Enriched treatment exhibited damage from grazing, in comparison to 45 – 76% of *G. echinulata* colonies in the Ambient treatment. We did not observe a significant effect or interaction of zooplankton biomass on percent grazed *G. echinulata* ($p \geq 0.14$), however, the percentage of grazed *G. echinulata* colonies significantly or marginally increased as a function of cladoceran grazing potential in the Ambient mesocosms on 3 out of 5 sampling days (for all days, slope = 0.21 – 0.61, regression $R^2 = 0.32 – 0.75$, and $p = 0.03 – 0.24$; Table 6.2). In the Enriched mesocosms, increasing cladoceran grazing potential resulted in a significantly lower percent of grazed *G. echinulata*

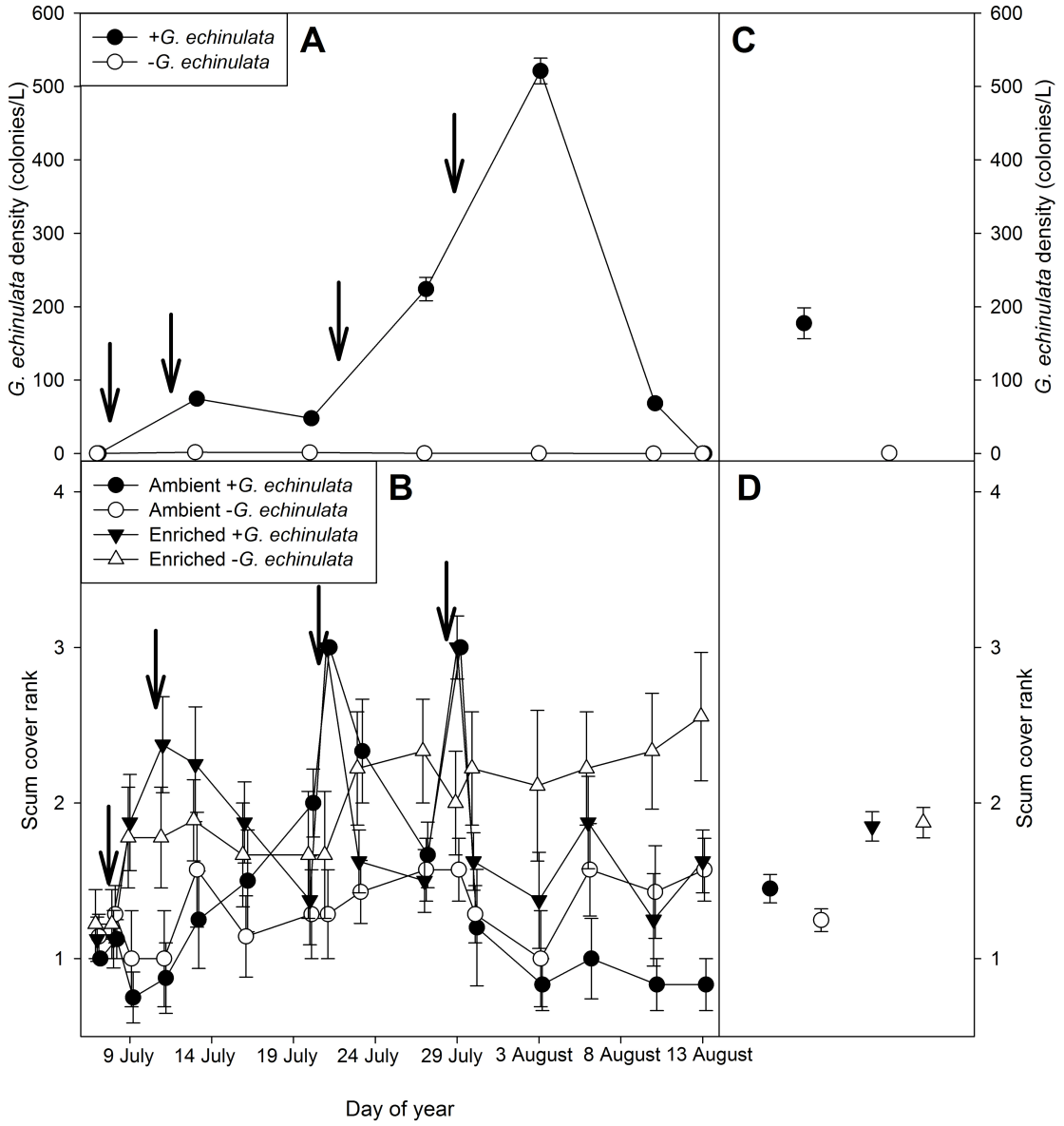

Figure 6.4. (A) The mean (± 1 S.E.) density of *G. echinulata* colonies in the *G. echinulata* treatments and (B) scum cover rank in the nutrient and *G. echinulata* treatments over time. The arrows refer to the days of *G. echinulata* addition. The scum cover rank ranged from 0 (no scum present) to 4 (complete scum cover). Both *G. echinulata* density and scum cover rank were significantly affected by *G. echinulata* × time, *G. echinulata*, and time. In addition, nutrients significantly

increased scum cover rank. (C) The mean (± 1 S.E.) density of *G. echinulata* colonies in the *G. echinulata* treatments and (D) scum cover rank in the nutrient and *G. echinulata* treatments observed across all sample days after the first *G. echinulata* addition.

Table 6.2. Summary statistics of the linear relationship between cladoceran grazing potential and percent grazed *G. echinulata* colonies (referred to as % Grazed) every week after the first *G. echinulata* addition. The relationship between cladoceran grazing potential and % Grazed in the Ambient and Enriched mesocosms are listed separately. DF denotes degrees of freedom. Days when significant treatment effects ($p \leq 0.05$) were observed are in bold and days when marginally significant treatment effects ($0.05 < p < 0.10$) were observed are noted with an asterisk (*).

Sample day (day of year)	Nutrient Level	R^2	DF	F value	P value	Regression equation
194*	Ambient	0.41	1,6	4.22	0.08	% Grazed = 0.44 + 0.45 × ln(grazing potential)
201	Ambient	0.51	1,6	6,36	0.045	% Grazed = 0.25 + 0.60 × ln(grazing potential)
208	Ambient	0.75	1,4	11.71	0.03	% Grazed = 0.45 + 0.61 × ln(grazing potential)
215	Ambient	0.49	1,3	2.93	0.18	% Grazed = 0.14 + 0.21 × ln(grazing potential)
222	Ambient	0.32	1,4	1.89	0.24	% Grazed = 0.07 + 0.38 × ln(grazing potential)
194	Enriched	0.01	1,6	0.07	0.80	% Grazed = 0.60 – 0.09 × ln(grazing potential)
201	Enriched	0.60	1,5	7.42	0.04	% Grazed = 0.44 – 0.54 × ln(grazing potential)
208*	Enriched	0.44	1,6	4.72	0.07	% Grazed = 0.29 + 0.43 × ln(grazing potential)
215	Enriched	0.54	1,6	6.92	0.04	% Grazed = 0.57 – 0.34 × ln(grazing potential)
222	Enriched	0.20	1,6	1.53	0.26	% Grazed = 0.24 + 0.46 × ln(grazing potential)

colonies on two sampling days, a positive marginally significant effect on one sampling day, and non-significant relationships on the other two sampling days (for all days, slope = -0.54 – 0.46, regression R^2 = 0.01 – 0.60, and p = 0.04 – 0.80; see Appendix 2).

Response Variables: Light Availability, Phytoplankton, and MC-LR

We observed significant nutrient × *G. echinulata* × time, *G. echinulata* × zooplankton × time, nutrient, and time effects on total chlorophyll *a* (all $p \leq 0.04$; Table 6.1), which complemented our observations of scum cover rank in the mesocosms (Figure 6.4B). Before the first *G. echinulata* addition, total chlorophyll *a* concentrations were significantly higher in the Enriched mesocosms (13.4 ± 2.7 µg/L) than Ambient mesocosms (1.6 ± 0.4 µg/L; $F_{1,30} = 18.89$, p = 0.0002); there were no significant treatment effects or interactions for scum rank (all $p \geq 0.48$).

G. echinulata additions and nutrient enrichment significantly increased the scum rank, which resulted in significant *G. echinulata* × time, *G. echinulata*, nutrient, and time effects (all $p \leq 0.02$; Figure 6.5). In the Ambient mesocosms, there was little or no scum cover until the third and fourth *G. echinulata* additions, which caused large surface scums composed of buoyant *G. echinulata* aggregated with other phytoplankton in the +*G. echinulata* mesocosms (Figure 6.4B). The scums quickly dissipated in the +*G. echinulata* mesocosms while scum cover in the –*G. echinulata* mesocosms remained low, resulting in lower overall mean scum rank in the Ambient – *G. echinulata* mesocosms (1.33 ± 0.07; Figure 6.5C) than the Ambient +*G. echinulata* mesocosms throughout the experiment (1.45 ± 0.09; Figure 6.5D).

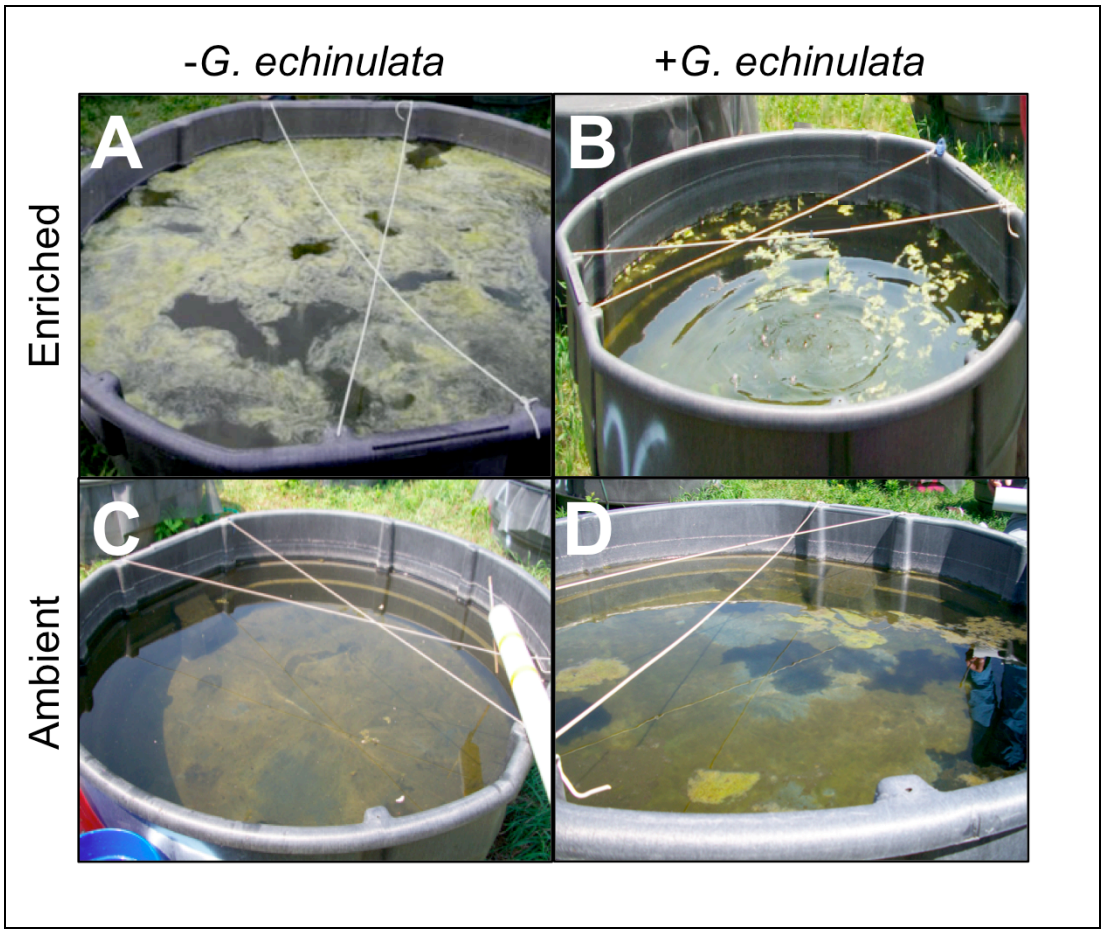

Figure 6.5. Photographs of (top row) Enriched and (bottom row) Ambient mesocosms that summarize the differences in scum cover between the (left column) –*G. echinulata* and (right column) +*G. echinulata* treatments six days after the third *G. echinulata* addition. (A) The Enriched –*G. echinulata* mesocosms exhibited large scums which lasted throughout the experiment, whereas the scums in the (B) Enriched +*G. echinulata* mesocosms decreased after *G. echinulata* additions. The scum rank for (A) was 3, indicating a large-sized scum, and the scum rank for (B) was 1, indicating a small-sized scum. By comparison, the (C) Ambient –*G. echinulata* mesocosms exhibited low or no scum cover throughout the experiment, while the scums in the (D) Ambient +*G. echinulata* mesocosms had decreased to low levels by

six days after the third *G. echinulata* addition. The scum rank for (C) was 0, or no scum cover present, and the scum rank for (D) was 1, indicating a small-sized scum.

In the Enriched treatment, the –*G. echinulata* and +*G. echinulata* mesocosms exhibited medium-sized surface scums until the third and fourth *G. echinulata* additions, which created larger scums in the +*G. echinulata* mesocosms than the –*G. echinulata* mesocosms. The scums in the +*G. echinulata* mesocosms quickly decreased and remained at lower levels than were observed before the additions, while the –*G. echinulata* mesocosms continued to exhibit medium-sized to large scums. As a result, the Enriched –*G. echinulata* mesocosms exhibited a higher overall mean scum rank throughout the experiment (1.98 ± 0.09; Figure 6.5A) than in the Enriched +*G. echinulata* mesocosms (1.85 ± 0.09; Figure 6.5B). Total chlorophyll *a* followed a similar pattern: at Ambient levels, the +*G. echinulata* treatments generally exhibited higher mean total chlorophyll *a* concentrations than the –*G. echinulata* treatments, while the –*G. echinulata* treatments exhibited higher mean concentrations than the +*G. echinulata* treatments at Enriched levels, especially at the end of the experiment ($p = 0.04$; see Appendix 3). Low Zooplankton –*G. echinulata* treatments exhibited higher mean total chlorophyll *a* than High Zooplankton –*G. echinulata* treatments, while the High Zooplankton +*G. echinulata* treatments exhibited higher total chlorophyll *a* than Low Zooplankton +*G. echinulata* treatments ($p < 0.0001$), and nutrients significantly increased total chlorophyll *a* ($p = 0.002$).

Nutrients, zooplankton, and time significantly interacted to affect the incidence of *G. echinulata* facilitation of small-sized chlorophyll *a* growth rate, as determined by the Facilitation-Inhibition Index (FII; Figure 6.6). Overall, with no significant main effect of zooplankton ($p = 0.34$), *G. echinulata* facilitation was significantly more likely to occur in Ambient mesocosms than Enriched mesocosms ($p = 0.001$). The

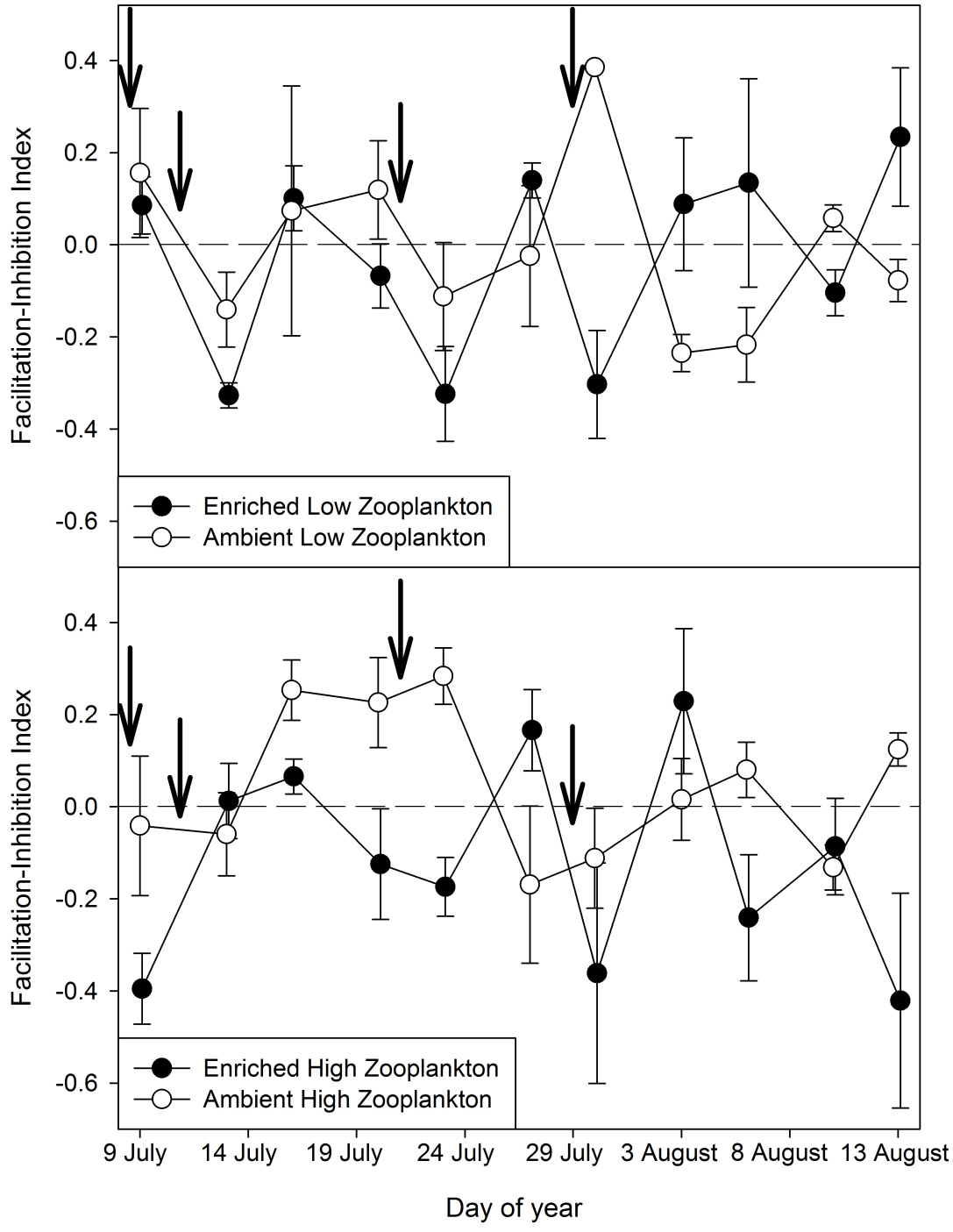

Figure 6.6. The Facilitation-Inhibition Index (± 1 S.E.), or the difference in small-sized chlorophyll *a* growth rate between the +*G. echinulata* and –*G. echinulata*

treatments for the two Low Zooplankton treatments (top) and two High Zooplankton treatments (bottom) over time. The arrows refer to the days of *G. echinulata* addition. The Ambient High Zooplankton treatment was significantly greater than zero, the Enriched High Zooplankton treatment was significantly less than zero, while the two Low Zooplankton biomass treatments were not significantly different from zero.

largest increases in facilitation in the Ambient mesocosms and the largest increases in competition in the Enriched mesocosms occurred immediately after *G. echinulata* additions (nutrient × time, $p = 0.007$).

The significant effect of nutrients on *G. echinulata* facilitation was mediated by interactions with zooplankton, and zooplankton × time (both $p \leq 0.03$; Figures 6.6 and 6.7). High Zooplankton biomass significantly amplified the stimulatory or inhibitory effect of *G. echinulata* on other phytoplankton in Ambient or Enriched mesocosms, respectively. In the Ambient mesocosms, the High Zooplankton treatment exhibited a positive FII (Facilitation-Inhibition Index; i.e., facilitation) significantly greater than zero (one-sample t-test, $t_{42} = 1.87$, $p = 0.03$), whereas the FII of the Low Zooplankton treatment was not significantly greater than zero ($p = 0.53$). In the Enriched mesocosms, the High Zooplankton treatment exhibited a negative FII (i.e., competition) significantly less than zero ($t_{43} = -2.49$, $p = 0.008$), whereas the FII of the Low Zooplankton treatment was not significantly less than zero ($p = 0.18$). The FII of the two Enriched treatments were significantly different from each other ($p \leq 0.006$; Figure 6.7), as were the FII of the two High Zooplankton treatments ($p < 0.0001$), while the FII of the two Ambient treatments were marginally different from each other ($p = 0.08$), and the FII of the two Low Zooplankton treatments were not significantly different from each other ($p = 0.93$). Finally, we also observed significant zooplankton × time and nutrient × zooplankton × time effects on the FII (both $p \leq 0.03$), as the difference between the High Zooplankton and Low Zooplankton treatments fluctuated during the experiment.

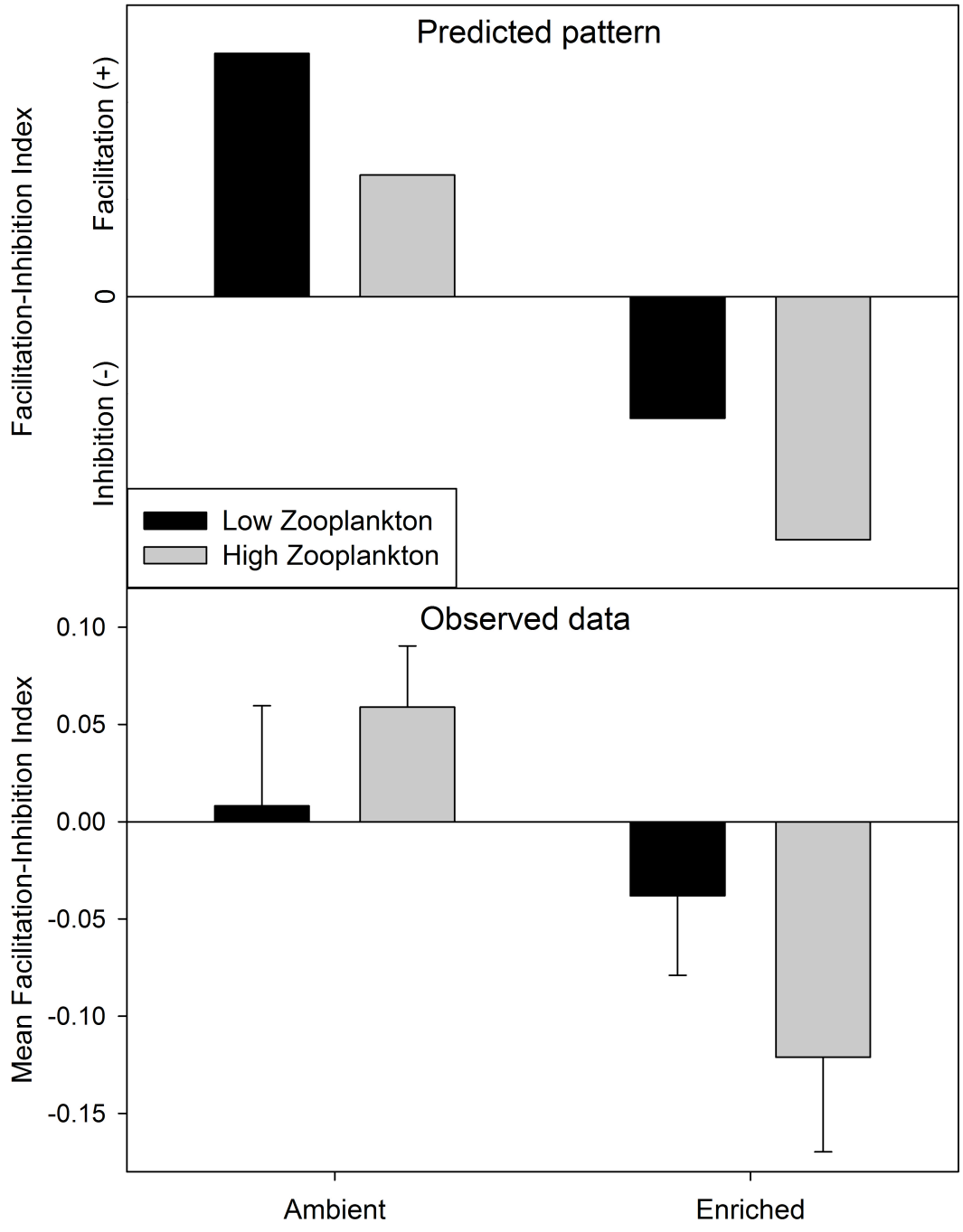

Figure 6.7. (Top) The predicted pattern of the Facilitation-Inhibition Index (FII) for the four nutrient and zooplankton treatments. We hypothesized that *G. echinulata* would exhibit facilitative effects on small-sized phytoplankton in Ambient nutrient

treatments (FII > 0) and inhibitory effects in Enriched treatments (FII < 0), and more inhibitory interactions would occur with High Zooplankton than Low Zooplankton. (Bottom) The observed pattern of the mean FII for the four nutrient and zooplankton treatments. Unique letters refer to treatments that are significantly or marginally different from each other. Following our predictions, *G. echinulata* exhibited significantly higher facilitation in Ambient treatments than Enriched treatments. However, contrary to our expectations, High Zooplankton intensified the nutrient effect, increasing facilitation between *G. echinulata* and other phytoplankton at Ambient levels and increasing competition at Enriched levels.

We observed no significant effect or interactions of nutrients, zooplankton biomass, or *G. echinulata* on MC-LR concentrations (overall three-way ANOVA model, $p = 0.90$).

Discussion

Although many studies have focused on the inhibitory effects of cyanobacteria, recent research has indicated that the effects of cyanobacterial blooms are more complex and context-dependent than previously realized (Havens 2008, Ibelings et al. 2008). A growing number of studies (all on taxa other than *G. echinulata*) indicate that cyanobacteria can stimulate the growth and division of other phytoplankton in both the laboratory and field (Keating 1977, Mohamed 2002, Suikkanen et al. 2005, Karjalainen et al. 2007, Neisch et al. In press). Why do some systems exhibit inhibitory and other systems exhibit stimulatory effects of cyanobacterial blooms? The majority of the studies of inhibitory blooms have been conducted in eutrophic and hypertrophic systems (reviewed by Huisman et al. 2005, Hudnell 2008), whereas the studies of stimulatory blooms have been primarily performed in less nutrient-rich systems, suggesting that nutrient concentration may be an important determinant of how cyanobacterial blooms affect small-sized phytoplankton.

Our experimental data indicate that trophic state can play a substantial role in mediating the effect of *G. echinulata* on the small-sized phytoplankton community. Although there was variability in the FII time series, *G. echinulata* generally had a facilitative effect on small-sized phytoplankton at Ambient nutrient concentrations, whereas we observed an inhibitory effect at Enriched concentrations. These findings

agreed with predictions from the stress-gradient hypothesis (SGH) that increased nutrients would result in a greater incidence of competition, and lower nutrients would result in a greater incidence of facilitation (Bertness and Callaway 1994). Species interactions on a stress gradient have been successfully predicted by the SGH in several different terrestrial and marine systems (e.g., Bertness et al. 1999, Choler et al. 2001, Callaway et al. 2002, but see Maestre et al. 2005), but there have been fewer tests of the SGH conducted in freshwater systems (Halpern et al. 2007). It is also important to note that the effect on small-sized chlorophyll *a* was a community-level response of the <30 μm phytoplankton. Small-sized chlorophyll *a* is nevertheless a useful metric because it captures the net effect on the community of phytoplankton considered most vulnerable to grazing (e.g., Lehman and Sandgren 1985, Lampert et al. 1986, Cyr and Pace 1992).

We hypothesize that the incidence of facilitation was higher in the Ambient mesocosms than Enriched mesocosms because *G. echinulata* significantly increased nutrients for other phytoplankton. We observed that the Ambient +*G. echinulata* mesocosms exhibited significantly higher TN concentrations than Ambient –*G. echinulata* mesocosms, as has been documented in other experiments (Carey et al. In prep., Carey et al. In review). *G. echinulata* may release some of its nutrients into the water column, which could potentially stimulate phytoplankton growth (Pitois et al. 1997, Nõges et al. 2004), similar to other cyanobacteria in low-nutrient systems (Healey 1982, Kankaanpaa et al. 2001, Ray and Bagchi 2001, Wetzel 2001, Shi et al. 2004, Agawin et al. 2007). While *G. echinulata* did not significantly increase TP in the Ambient mesocosms, we did observe higher TP concentrations in the Ambient +*G.*

echinulata mesocosms relative to Ambient –*G. echinulata* mesocosms when *G. echinulata* densities were at their highest, after the third and fourth additions. Previous experiments have demonstrated that *G. echinulata* releases more N relative to P to the water column, which is most likely due to the cyanobacterium's N fixation (Carey et al. In prep.).

The increase in TN concentrations in the Ambient +*G. echinulata* treatment may be due to N added within the colonies to the mesocosms. Estimates for the total amount of P in a *G. echinulata* colony range from 0.02 – 0.08 µg P/colony (Pettersson et al. 1993, Tymowski and Duthie 2000), and by multiplying these estimates by the Redfield N:P ratio (7.2:1 by atomic mass; Redfield 1934), an approximate amount of N in a colony ranges from 0.14 – 0.58 µg N/colony. On day of year 215 (3 August), when the +*G. echinulata* mesocosms exhibited their highest colony density (521 ± 17 colonies/L), the TN concentrations in the Ambient +*G. echinulata* mesocosms were 194 ± 50 µg N/L higher than in the Ambient –*G. echinulata* mesocosms. By multiplying the *G. echinulata* density on day of year 215 by the colony N concentration, we calculate that the amount of N that was added to the Ambient +*G. echinulata* mesocosms within *G. echinulata* colonies was 73 – 302 µg N/L, which is within the same range as the observed increase in TN concentrations. There were lower proportions of NO_3^- and SRP samples above the MDL in the Ambient +*G. echinulata* treatment than in the Ambient –*G. echinulata* treatment, which may be because the increased phytoplankton growth rate and *G. echinulata* in the Ambient +*G. echinulata* mesocosms resulted in higher demand for available forms of N and P.

We hypothesize that *G. echinulata* may have exhibited more inhibitory effects in the Enriched mesocosms than in the Ambient mesocosms because it decreased light availability for other phytoplankton. In eutrophic systems, light is often a more important limiting factor for phytoplankton growth than nutrients (Wetzel 2001, Reynolds 2006, Lampert and Sommer 2007). Mesocosm experiments have demonstrated that nutrient additions decrease light availability in the water column by increasing phytoplankton, which results in higher light attenuation with depth (Kumagai et al. 2000, Jones et al. 2005). Consequently, because the Enriched mesocosms had higher nutrient and total chlorophyll *a* concentrations than the Ambient mesocosms at the beginning of the experiment, it is highly probable that the Enriched mesocosms had lower light availability in their water columns before the first *G. echinulata* addition.

Because of greater light limitation in the Enriched mesocosms, the *G. echinulata* additions were likely more inhibitory to other phytoplankton in the Enriched mesocosms than in the Ambient mesocosms. Buoyant *G. echinulata* significantly increased scums in both Ambient and Enriched +*G. echinulata* mesocosms, but only the Enriched mesocosms consistently exhibited decreases in phytoplankton (as FII and total chlorophyll *a*) after *G. echinulata* additions. In eutrophic Lake Erken, Sweden, *G. echinulata* forms large scums that decrease light availability substantially, resulting in lower littoral periphyton growth (Liess et al. 2006). Cyanobacteria can outcompete other phytoplankton under conditions of low light and can also create a higher turbidity per unit of P than any algal group (Scheffer

et al. 1997), so it is likely that the scums produced by *G. echinulata* decreased other phytoplankton, especially in the Enriched mesocosms.

Our data indicate that nutrients were likely less limiting for phytoplankton in the Enriched treatments than in the Ambient treatments. TN and TP concentrations were significantly higher in the Enriched mesocosms than in the Ambient mesocosms throughout the experiment, and the Enriched mesocosms exhibited significantly or marginally higher proportions of NH_4^+, NO_3^-, and SRP samples higher than the MDL than the Ambient mesocosms, although the soluble concentrations were very low overall. The maximum amount of N and P contributed by *G. echinulata* colonies on a sampling day (using the most conservative estimate: 73 ± 2 µg N/L; 10.4 ± 0.3 µg P/L), represents a much lower proportion of the mean TN and TP concentrations observed throughout the experiment in the Enriched –*G. echinulata* mesocosms (672 ± 34 µg TN/L; 55.3 ± 4.8 µg TP/L) than in the Ambient –*G. echinulata* mesocosms (371 ± 19 µg TN/L; 17.0 ± 1.3 µg TP/L). As a result, even though *G. echinulata* likely released or 'leaked' nutrients in both the Ambient and Enriched mesocosms, the net effect of *G. echinulata* on other phytoplankton in the Enriched mesocosms was inhibitory because *G. echinulata* scums reduced light, which was likely already limiting growth, and the *G. echinulata* additions represented a minimal increase of nutrients proportional to the baseline concentrations. In the Ambient mesocosms, where the scum cover and total chlorophyll *a* concentrations were significantly lower and nutrients were likely a more important limiting factor of growth than light, *G. echinulata* had a stimulatory effect on other phytoplankton because it substantially increased nutrients relative to baseline concentrations. Although the *G. echinulata*

scums may have decreased light in the Ambient +*G. echinulata* mesocosms, it is unlikely that they caused as inhibitory an effect on other phytoplankton as in the Enriched +*G. echinulata* mesocosms because light was not as limiting in the Ambient mesocosms before *G. echinulata* addition, and the scums never completely covered the entire surface of the mesocosms (i.e., scum rank throughout the experiment was <4; Figure 6.4B), so some light was always able to penetrate the water surface.

Although the Enriched treatments had significantly higher overall TN and TP concentrations than the Ambient treatments, Enriched +*G. echinulata* mesocosms exhibited lower TN and TP concentrations relative to the Enriched –*G. echinulata* mesocosms. It is possible that the loss of TN and TP from the water column represented senesced phytoplankton that settled to the bottom of the mesocosms. Alternatively, it is possible that the TN and TP concentrations decreased because those nutrients went into zooplankton production; however, there were no significant main or interaction effects of *G. echinulata* on total zooplankton or *Ceriodaphnia* biomass.

There may be other explanations for why *G. echinulata* had an inhibitory effect on other phytoplankton in the Enriched mesocosms. Many cyanobacteria produce allelopathic compounds (reviewed by Gross 2003, Legrand et al. 2003, Leflaive and Ten-Hage 2007), and it is possible that *G. echinulata* excreted more allelopathic compounds at Enriched concentrations. However, most allelopathic screenings have indicated that phytoplankton produce more allelochemicals and are more sensitive to them when nutrients are limiting (von Elert and Juttner 1997, Ray and Bagchi 2001, Rengefors and Legrand 2001), opposite to what we observed here. Alternatively, microcystin-LR, a toxin that *G. echinulata* produces (Carey et al. 2007), has been

shown to have inhibitory effects on phytoplankton in laboratory experiments (Kearns and Hunter 2001, Hu et al. 2005). However, microcystin-LR concentrations were not significantly different among our treatments, making this mechanism unlikely to have been responsible for the inhibitory effects we observed. It is also possible that the *G. echinulata* scums in the Enriched mesocosms decreased CO_2 concentrations for other phytoplankton, as has been observed for other cyanobacteria (Shapiro 1973, 1984, 1997), resulting in CO_2 limitation. CO_2 may have already been limiting in the Enriched mesocosms before the *G. echinulata* additions as a result of the high total chlorophyll *a* concentrations, and the addition of *G. echinulata* scums may have exacerbated CO_2 limitation for other phytoplankton.

Importantly, nutrients also mediated the effect of zooplankton on the *G. echinulata* – phytoplankton interaction. In the Ambient mesocosms, contrary to our predictions, High Zooplankton biomass increased the facilitative effect of *G. echinulata* on the growth rate of small-sized phytoplankton in comparison to treatments with Low Zooplankton biomass. Conversely, High Zooplankton in the Enriched mesocosms significantly increased the inhibitory effect of *G. echinulata* on small-sized chlorophyll *a* growth rate. Hence, for the Ambient mesocosms, the effect of the two stressors (Ambient nutrients and High Zooplankton biomass) on the incidence of facilitation was additive: high abiotic stress (Ambient nutrients) and high biotic stress (High Zooplankton) resulted in a higher incidence of facilitation. In abiotically stressful environments, biotic stress may further increase the incidence of positive interactions. This synergistic interaction of abiotic and biotic stress, demonstrated here for a freshwater pelagic ecosystem, has also been observed in

terrestrial ecosystems (Graff and Aguiar 2011; but see Eskelinen 2008). For example, on the arid Patagonian steppe, Graff et al. (2007) found that sheep grazing increased facilitative interactions between unpalatable and palatable grasses by associational defenses. In the less abiotically stressful Enriched mesocosms, however, increased biotic stress compounded the incidence of negative interactions. Within our experiment, the direction of the species interaction (e.g., stimulatory or inhibitory) between *G. echinulata* and other phytoplankton was driven primarily by the abiotic nutrient stress gradient, but the magnitude of that interaction was determined by the biotic stress gradient.

Zooplankton biomass may have amplified the facilitative effect of *G. echinulata* on other phytoplankton in Ambient mesocosms because elevated grazing pressure increased damage to *G. echinulata* colonies (Fey et al. 2010, Carey et al. In prep.). In the Ambient mesocosms, increased cladoceran grazing potential increased the percentage of grazed *G. echinulata* colonies. Damaged *G. echinulata* colonies may have released more nutrients than non-damaged colonies, which small-sized phytoplankton could access. In addition, the zooplankton may have excreted and defecated nutrients obtained from consuming *G. echinulata* trichomes (Fey et al. 2010), although we did not detect significant differences among zooplankton treatments in the proportion of NH_4^+ or SRP (the form of N and P that cladocerans generally excrete; Vanderploeg et al. 1986, Frost et al. 2004) samples above the MDL.

In the Enriched mesocosms, High Zooplankton biomass decreased other phytoplankton, amplifying the inhibitory effect of *G. echinulata*. We observed lower percentages of grazed *G. echinulata* in the Enriched mesocosms relative to the

Ambient mesocosms, as well as inconsistent relationships between cladoceran grazing potential and percent grazed colonies on sampling days. These findings suggest that *G. echinulata* colonies were a less-preferred food source when nutrients were high and other phytoplankton food was abundant. Hence, High Zooplankton may have intensified the inhibitory effect of *G. echinulata* on other phytoplankton because the zooplankton preferentially grazed small-sized phytoplankton. Cyanobacteria are typically considered poor food quality for zooplankton (Fulton and Paerl 1987, Lampert 1987, Gulati and DeMott 1997, Rohrlack et al. 2005), so it is not surprising that zooplankton grazed *G. echinulata* less when other phytoplankton were readily available in the Enriched mesocosms. By comparison, in the Ambient mesocosms, which exhibited significantly lower growth rates of small-sized phytoplankton than Enriched mesocosms, *G. echinulata* may have represented an important food source for food-limited zooplankton. In addition, it is possible that the lower grazing rate of *G. echinulata* in the Enriched mesocosms may be due to faster regrowth of trichomes in higher nutrient conditions.

Despite decreasing other phytoplankton competitors in the Enriched mesocosms and increasing phytoplankton competitors in the Ambient mesocosms, *G. echinulata* densities were not significantly higher in the Enriched mesocosms. The lack of a difference in *G. echinulata* density may be because the colonies in both treatments experienced the same pre-addition collection and cleaning, which may have stressed the colonies and prevented them from dividing in the water column. Alternatively, even though grazing rates were lower in the Enriched mesocosms, the

incidence of grazing was still substantial (31 – 59%), which may have resulted in the colonies not being able to increase despite fewer phytoplankton competitors.

In lieu of a significant *G. echinulata* effect, total zooplankton and *Ceriodaphnia* biomass concentrations were primarily driven by the nutrient and zooplankton treatments. It is possible that zooplankton biomass did not respond to *G. echinulata* addition because grazing of *G. echinulata*, as has been observed for other cyanobacteria, decreased zooplankton feeding rates (Arnold 1971, Lampert 1982, 1987). However, despite the many negative effects that cyanobacteria are known to exert on zooplankton survival and fecundity, it is notable that zooplankton biomass did not decrease in response to *G. echinulata*. Nutrient enrichment, which was closely tied to chlorophyll *a*, was a far more important predictor of zooplankton biomass than *G. echinulata* addition.

Nutrient pollution is increasing in many lakes globally (e.g., Carpenter et al. 1998, Dodds et al. 2009, Carpenter et al. 2011). Simultaneously, cyanobacterial blooms are increasing in oligotrophic, mesotrophic, and eutrophic systems (Hallegraeff 1993, Van Dolah 2000, Anderson et al. 2002, Boyer 2008, Paerl and Huisman 2008, Ernst et al. 2009, Winter et al. 2011, Sinha et al. 2012, Carey et al. In review). Consequently, it is important to know how nutrient concentrations mediate the effects of cyanobacteria on plankton food webs. Our data indicate that increasing nutrient loads to lakes will have nonlinear effects on the role of *G. echinulata* in pelagic ecosystems as they transition from a stimulatory role in low-nutrient lakes to an inhibitory role in high-nutrient lakes.

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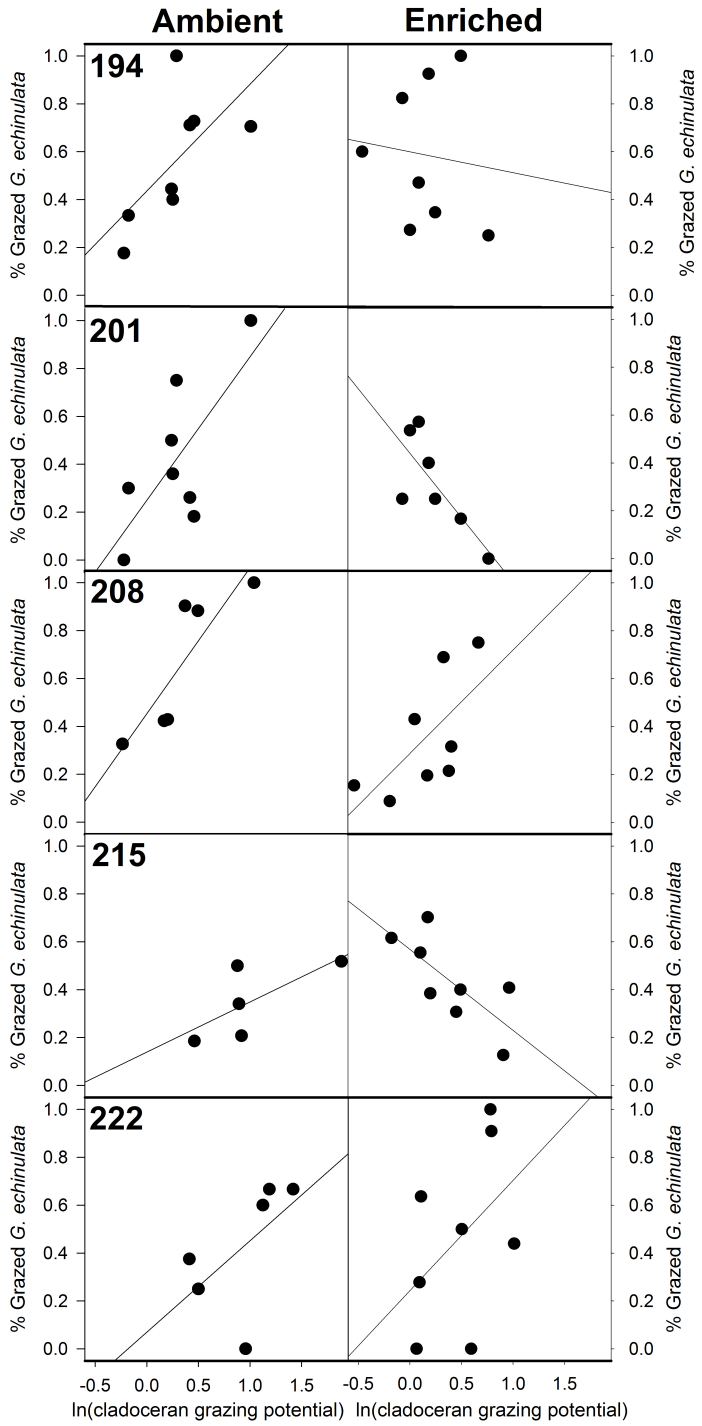
Appendix 1. The eight lakes from which we collected unfiltered lake water to create phytoplankton communities in the pond experiment. The asterisks (*) denote lakes from which we collected zooplankton to create the Zooplankton Biomass treatments.

Lake Name	Latitude	Longitude	Total phosphorus (μg/L)	Total nitrogen (μg/L)	Nutrient data source
Lake Sunapee*	43°24'N	72°20'W	5	175	C.C.C., unpubl.
Goose Pond	43°42'N	72°5'W	5	179	A.C. Dawson, unpubl.
Post Pond*	43°50'N	72°09'W	8	215	A.C. Dawson, unpubl.
Boston Lot Reservoir	43°40'N	71°17'W	10	251	A.C. Dawson, unpubl.
4A Pond	43°29'N	71°58'W	23	145	A.M. Siepielski, unpubl.
Deweys Pond*	43°39'N	72°24'W	54	552	A.M. Siepielski, unpubl.
Occum Pond*	43°43'N	72°17'W	117	.	C.C.C., unpubl.
Broken Tank Pond	43°41'N	72°13'W	437	3007	C.C.C., unpubl.

Appendix 2. The relationship between percent grazed *G. echinulata* colonies and ln-transformed cladoceran grazing potential in the (left) Ambient and (right) Enriched mesocosms on each sampling day (day of year 194, 201, 208, 215, and 222).



Appendix 3. (Top) The mean total chlorophyll *a* concentration (± 1 S.E.) in the (Top) Low Zooplankton and (Bottom) High Zooplankton treatments over time. Nutrients × *G. echinulata* × time and *G. echinulata* × zooplankton × time significantly influenced total chlorophyll *a*. The arrows refer to the days of *G. echinulata* addition.