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The Effect of Cyanobacterium Gloeotrichia echinulata in the Belgrade Lakes, Maine

Harriet T. Rothschild
Colby College

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Harriet T. Rothschild
Environmental Studies Program
Colby College
Waterville, Maine
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Denise Bruesewitz, Adviser	Russell Cole, Reader	William McDowell, Reader

ABSTRACT

Gloeotrichia echinulata is a cyanobacteria species that has been increasingly forming blooms in oligotrophic lakes in the Northeastern United States. The Belgrade Lakes in central Maine have experienced increasing blooms over the past decades. Long Pond and Great Pond in the Belgrade Lakes region are popular locations for summer tourism and year-round residents. Research into *G. echinulata* is important to the Belgrade community because of potential effects to water quality, public health, and recreation. Studying *G. echinulata* bloom density throughout the summer and how it may affect the phosphorous cycle, the nitrogen cycle, and the plankton community will help scientists inform policy makers on water quality initiatives.

G. echinulata can serve as a warning sign for lake eutrophication and blooms can mark tipping points between eutrophication stable states. Further research should focus on luxury phosphorus uptake from the sediment during recruitment because it could mitigate the positive effects alum treatment.

The use of 15-nitrogen (N) stable isotope tracers is a valuable tool for understanding nitrogen cycling in aquatic ecosystems. Traditionally, analytical measurements of ^{14}N:^{15}N ratios involves a time consuming process of incubations to concentrate N onto filters for analysis by isotope-ratio mass spectrometry. The process also requires large sample volumes, which is a challenge for microcosm experiments. Here, we present a technique for measuring ^{14}N:^{15}N in ammonium using ESI-TOF mass spectrometry, to better characterize nitrogen cycling in lake and estuarine systems.

The *G. echinulata* blooms are linked to chlorophyll-*a* concentrations, pheophytin concentrations, and total phosphorus concentrations. *G. echinulata* is possibly a driver of eutrophication and it is an important organism to study, especially in low nutrient lakes.

ACKNOWLEDGMENTS

I would like to thank Professor Denise Bruesewitz for guiding me through this process and convincing me to pursue research. Denise, you are a role model and friend, and I can not thank you enough for your coaching and help along the way.

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CHAPTER I: LITERATURE REVIEW

Importance of Studying Cyanobacteria in Lake Systems

Cyanobacteria are a ubiquitous life form present in a variety of aquatic ecosystems and are commonly referred to as "blue-green algae" because many contain the photosynthetic pigments phycocyanin and chlorophyll-*a* (Speer 1995). Cyanobacteria are among the oldest organisms found in the fossil record, dating back 3,500 Ma (Golubic and Seong-Joo 1999). Cyanobacteria played a role in the evolution of primary production and were the ancestors to the symbiotic origins of plastids in algae and plants (Golubic and Seong-Joo 1999).

Researchers have found evidence of increased cyanobacterial blooms in eutrophic, mesotrophic, and oligotrophic lakes over the past three hundred years (Hallegraeff 1993; Van Dolah 2000; Anderson et al. 2002; Paerl and Huisman 2008, 2009). Many of the blooms occurring in oligotrophic lakes (as well as in higher trophic state lakes) can be attributed to increased nutrient concentrations from anthropogenic sources (Winter et al., 2011). Understanding cyanobacterial bloom dynamics in these very different systems is imperative to maintaining healthy and functioning ecosystems (Carey et al., 2012).

Cyanobacteria Overview

Organisms such as algae, cyanobacteria, and phytoplankton are the basis of food webs in lake ecosystems. Only when their populations expand quickly can they become nuisances. Algal blooms can be detrimental from an economic, health, social and ecological perspective (Istvánovics et al. 1993). *Gloeotrichia echinulata* is the species subject of this research. *G. echinulata* is a nitrogen-fixing cyanobacteria of large filamentous colonies (1-3 mm diameter) that are increasing in abundance in low-nutrient systems in the northeastern United States and Canada (Carey et al. 2008). So far, the abundance of *G. echinulata* in United States lake systems is unknown because phytoplankton monitoring is limited spatially and temporally (Carey et al. 2012).

Impact on Socioeconomics

There is increasing evidence that cyanobacterial blooms could impact water quality and prevent, recreation which, in turn would decrease property values and tourism (Michael et al. 1996). There is a relationship between demand for recreation and water clarity, which could be jeopardized by cyanobacterial blooms (Soderqvist and Scharin

2000). In the present day, Maine lake ecosystems are vital to providing community members with a sense of place combined with economic value through tourism and recreation (Fleming and Love 2012). There are over 5,000 lakes Maine with an area of over one acre and many of these are important culturally (Water Resources Program 1995). Native Americans in Maine used the lakes for transportation, then loggers in the 1800s shuttled lumber great distances across lakes (Water Resources Program 1995). In the early 1900s, the "great lakes" in Maine, including Moosehead Lake and Rangeley Lake, became vacation destinations for the New England elite. As the Great Depression affected the economy and the fish stocks diminished from overfishing, tourism began to decline (Water Resources Program 1995).

Impact on Human and Ecosystem Health

Toxins produced by cyanobacteria pose a threat to water bodies used for recreation and drinking water sources such as lakes and reservoirs. *Gloeotrichia echinulata* is known to cause skin irritation for swimmers, which has a negative effect on recreation (Backer 2002; Serediak and Huynh 2011). Some species of cyanobacteria produce free toxins or toxins bound to the cell that can be dangerous to human health (Pretty et al. 2003). Cyanobacteria can produce four types of cyanotoxins: hepatoxins, neurotoxins, cytotoxic alkaloids, and dermatoxins (Carmichael 1997).

The bioaccumulation of toxins, the build up of toxins in organisms higher in the food chain, and cause a greater toxicity to that organism (Preece et al. 2015). *Gloeotrichia echinulata* produces microcystin-LR toxin, which in turn is an inhibitor of protein phosphatase synthesis and creates oxidative stress in mammalian cells (Corbel et al. 2014). Researchers monitoring an oligotrophic lake in central New Hampshire, USA found hepatotoxin microcystin-LR produced by *G. echinulata* (Carey et al. 2006). The *G. echinulata* contained microcystin-LR at mean concentrations of 97.07 ± 7.78 (1 SE) ng MC-LR g^{-1} dry wt colonies (Carey et al. 2006). Microcystin-LR is released during cell lysis, death or senescence and enters organisms via ingestion (Park et al. 1998; Kinnear 2010). Cyanotoxins are known to bioaccumulate in plants, tadpoles, mussels, rainbow fish, and crayfish (Saker and Eaglesham 1999; Anderson et al. 2003). Bioaccumulation of cyanotoxins is potentially dangerous to human health through the consumption of fish, mussels, and other organisms. This fact suggests that recent outbreaks of *G. echinulata*

in oligotrophic lakes used as water sources throughout New England may pose a health concern to humans (Carey et al. 2006).

Removal and Monitoring of Cyanotoxins

Cyanotoxins are an increasing concern because they are difficult to remove from water sources through common water treatment procedures (Westrick et a., 2010). However, there are several natural processes that can filter out cyanotoxins over an extended period of time. The cyanotoxins are buried in the sediments where biodegrading bacteria (including *Sphingomonas, Paucibacter toxinivorans, microcystinivorans, Sphingosinicella, Burkholderia*) are able to break them down. However, these bacteria are unable to survive in the anoxic hypolimnion common in stratified, temperate mesotrophic and eutrophic lakes, thereby preventing this breakdown (Corbel et al. 2014). Breakdown of cyanotoxins can occur via other mechanisms, such as during brief periods following lake mixing with oxic hypolimnion, warm water temperatures, and an alkaline environment promote cyanotoxin breakdown (D'Anglada et al. 2015). Photodegradation can also aid in the breakdown of cyanotoxins, but this is limited to clear-water lakes and does not occur until after the bloom (Hyenstrand et al. 2003; Corbel et al. 2014). Lastly, benthic soil on the lake bottom with a high concentration of organic carbon and clay can filter toxins (Corbel et al. 2014).

Monitoring, studying and predicting harmful algal blooms are crucial in maintaining clean drinking water and protecting aquatic ecosystems. As harmful algal blooms become more common with climate change (Carey et al. 2012), it is important to understand bloom dynamics to prevent harm to human health. Academic institutions, research institutes, and governmental organizations are focusing on developing equipment that can measure water quality, DNA of microorganisms, concentrations of cyanotoxins. This technology will be important in the future for assessing long term toxin production in many different types of ecosystems (Ryan et al. 2008; Seltenrich 2014).

Cyanobacteria Impact on Plankton Communities

Physical Impact

Lastly, cyanobacterial blooms can have various positive and negative effects on plankton communities. The bloom can be detrimental to the plankton community because they limit sunlight available below the bloom in the water column (Leng 2009). The

limited sunlight will create competition between benthic macroinvertebrates, which decreases the diversity of species (Shaw et al. 2009). Light limitation is a detrimental side effect of cyanobacterial blooms that can lead to decreased biomass and species diversity, especially in eutrophic systems where light limitation is already an issue (Shaw et al. 2009).

Chemical Impact

Cyanobacterial blooms can also affect lake properties by influencing oxygen and nutrient concentrations. An increase in phytoplankton biomass can cause large diel dissolved oxygen shifts (Rangel et al. 2009). These fluctuations in dissolved oxygen disrupt fish, plant, and planktonic growth that can eventually result in oxygen crashes (Leng 2009). Due to light and temperature cues in the early-fall, the cyanobacteria blooms senesce simultaneously creating a period of anoxia as the available oxygen is used in microbial decomposition (Hudnell 2008).

In a nutrient limited ecosystem, the balance of nutrients is critical for phytoplankton growth. Many cyanobacteria possess the ability to sequester luxury phosphorus, stored phosphorus for later use, from the sediment and fix biologically inert nitrogen from the water (Heckey and Kilham 1988). This gives the cyanobacteria a competitive advantage over other phytoplankton and decreases nutrient availability, which can reduce trophic level growth (Heckey and Kilham 1998).

Alternatively, cyanobacteria such as *Gloeotrichia echinulata* have been shown to subsidize plankton grown through nutrient leakage during pelagic growth, senescence, or by injury due to grazing (Foree and McCarty 1970; Agawin et al. 2007). The extra nutrients can facilitate phytoplankton growth in nutrient-limiting environments such as in oligotrophic and mesotrophic lake system (Pitois et al. 1997). Releasing the nutrient limitations on a phytoplankton community can increase the richness and diversity of phytoplankton taxa (Carey et al. 2014b). In eutrophic lakes the effect of extra nutrients from *G. echinulata* is less noticeable and does not cause a significant change in biovolume or species composition (Carey et al. 2014b).

Biological Impact

Cyanotoxins are harmful not only to humans, but to the aquatic ecosystem as well. Common cyanotoxins such as hepatoxins and neurotoxins have different mechanisms of

action and different exposure methods, but both create reduced feeding and survival for zooplankton (Freitas et al. 2014). Importantly, a single type of cyanotoxin is commonly not present alone; different types of cyanotoxins can be present in an ecosystem at the same time. The synergistic effects of multiple cyanotoxins cause more detrimental lasting effects on zooplankton than each individual cyanotoxin alone. Cyanotoxins can make zooplankton less palatable to fish and other predators, which influences every step of the food web (Freitas et al. 2014).

While less understood, there is evidence that cyanobacteria, such as *Gloeotrichia echinulata,* may be providing other subsidies such as protective bioactive secondary metabolites (Gross 2003), and antibacterial or antifungal compounds (Legrand et al. 2003) to the wider plankton community. Secondary metabolites are organic molecules that provide defense against stress and facilitate reproductive process, among other qualities (Mandal and Rath 2015). These protective subsidies in combination with increased nutrients can enhance growth for phytoplankton communities (Gross 2003).

Secondary metabolites are already used as antibacterial and antifungal agents, chemotherapy for cancer, cholesterol-lowering agents, immunosuppressants, anti-parasitic agents, herbicides, diagnostics, and tools for research (Mandal and Rath 2015). Cyanobacteria subsidies are a current area of research in pharmaceutical drug development (Mandal and Rath 2015).

Gloeotrichia echinulata

This research focuses specifically on the cyanobacterium *Gloeotrichia echinulata,* a nitrogen fixing, large (~2 mm), colonial cyanobacteria species that has been increasing globally (Carey et al. 2008). *G. echinulata* is increasing in oligotrophic ecosystems which makes it an interesting study organism due to its potential effect on phytoplankton communities (Carey et al. 2014b), nutrient dynamics (Istánovics et al. 1993), and human health (Carey et al. 2007).

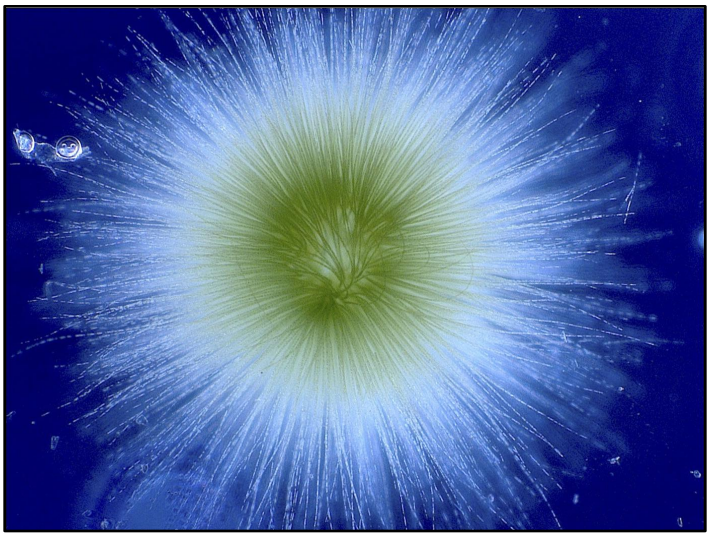

Figure 1. *Gloeotrichia echinulata* colony with individual filaments visible in collections taken from Long Pond, Maine. Image captured by Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, ME.

Gloeotrichia echinulata Range

Over the past decade, reports of *Gloeotrichia echinulata* blooms in oligotrophic and mesotrophic lakes in the northeastern United States have increased (Carey et al. 2012). Scientists hypothesize that the *G. echinulata* spores lay dormant in the sediment for decades or centuries until the climate impacts and anthropogenic effects created more favorable conditions (Karlsson et al. 2003; Carey et al. 2007; Carey and Rengefors 2010). Many lake ecosystems currently experiencing *G. echinulata* blooms have had markedly increased densities compared to the recent past, which has implications for water quality (Carey et al. 2012). This density increase is likely due to a combination of higher global temperatures (Karlsson-Elfgren et al. 2004), increasing sediment P concentrations, and watershed development (Carey et al. 2012).

Gloeotrichia echinulata blooms have been studied in 27 oligotrophic lakes in Maine and New Hampshire between 2002-2006 (Carey et al. 2008). The regional synchronicity of these blooms indicates that light and temperature are possible cues that trigger blooms (Carey et al. 2012). In these studies, Carey et al. (2012) found that the intensity of the blooms varied amoung lakes, but that within lakes the variation was minimal from year to year.

Gloeotrichia echinulata Life Cycle

Gloeotrichia echinulata has a meroplanktonic life history with many stages seasonally (Livingston and Jaworski 1980; Carey et al. 2012). Like many other cyanobacteria species, *G. echinulata* produce dormant cells called akinetes that are embedded in the sediment to protect against adverse environmental conditions (Livingston and Jaworski 1980; Nicholas and Adams 1982; Adams and Duggan 1999; Kaplan-Levi et al. 2010). The akinetes are reproductive spores embedded in a dense mucilage that germinate in the sediment and migrate into the water column (Kaplan-Levi et al. 2010). The *G. echinulata* increase in abundance during the summer by recruitment from the sediment and division that occurs in the water column (Reynolds 2006).

Recruitment occurs due to abiotic factors such as temperature, light conditions, stratification, dissolved oxygen, and nutrients (Forsell and Pettersson 1995; Karlsson 2003; Carey et al. 2008; Wood et al. 2009). Shallow, sheltered coves create microhabitats that have higher recruitment because of wind redistribution, which concentrates the akinetes to certain areas of the lake (Forsell and Pettersson 1995; Karlsson-Elfgren et al. 2003; Wynne et al. 2011; Carey et al. 2014a). The shallow pelagic regions provide the best habitat for recruitment and the highest recruitment occurs at a depth of less than 5 meters (Karlsson 2003; Carey et al. 2008). In the northern hemisphere, maximum recruitment and bloom formation occurs in August, 2-3 weeks after the highest light intensity and warmest water temperature (Barbiero 1993; Forsell and Pettersson 1995). Late season mixing that occurs during August and into September often precedes *G. echinulata* blooms because it introduces nutrients to the water column, promoting bloom formation (Carey et al. 2014a; Yang et al. 2015). *G. echinulata* growth and recruitment is correlated with lake mixing, as years with weaker stratification have shown increased *G. echinulata* abundance (Pierson et al. 1992; Forsell and Pettersson 1995; Karlsson-Elfgren et al. 2005). Sediment mixing by physical disturbance exposes akinetes to light, which can trigger recruitment and adds to bloom formation (Karlsson-Elfgren et al. 2004).

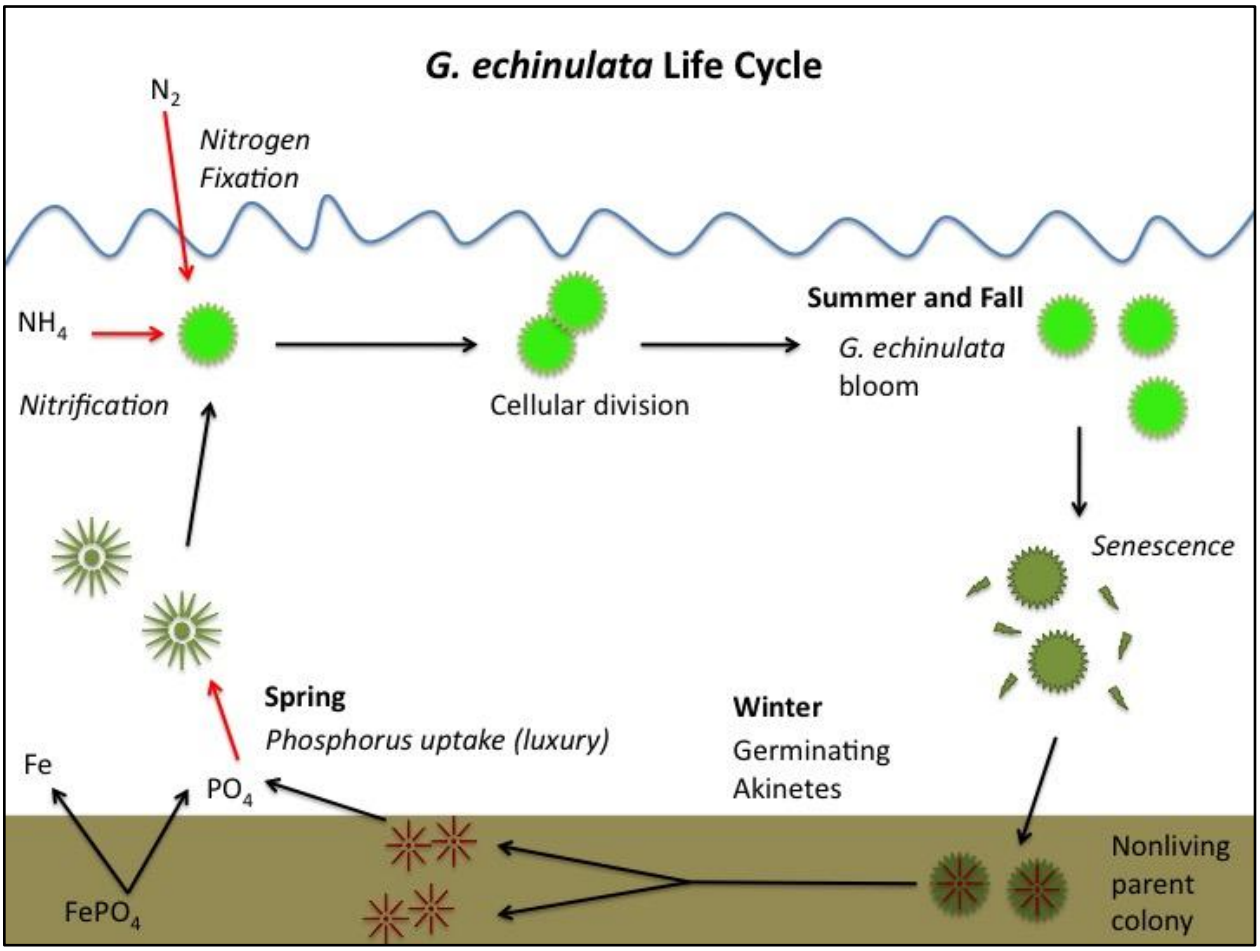

Figure 2. Life stages of *Gloeotrichia echinulata.* Nonliving parent colonies (called akinetes) overwinter in the sediment. Come spring, the akinetes are recruited from the sediment and take up luxury P from the sediment. *G. echinulata* undergoes cellular division during the summer until the blooms senesce in the fall and the cycle starts again.

Gloeotrichia echinulata and Nutrient Cycling

Gloeotrichia echinulata, like many species of cyanobacteria, are unique in their ability to carry out both oxygen-evolving photosynthesis and oxygen-labile N fixation within the same organism (Mitsui et al. 1986). *G. echinulata* is a heterocystous cyanobacteria allowing it to spatially segregate the site of N fixation in the heterocysts from the site of photosynthesis in the vegetative cells (Whitton and Potts 2000).

Gloeotrichia echinulata can also alter the internal P load of a lake. Before undergoing recruitment *G. echinulata* absorb excess P through luxury uptake, a process in which the *G. echinulata* colony takes in more P than is needed (Forsell 1998). Especially in shallow

lakes, luxury uptake of P from cyanobacteria such as *G. echinulata*, can account for 66% of the internal P load (Forsell 1998; Carey et al. 2012; Napiókowska-Krzebietke and Hutorowicz 2015). The increased internal P load due to luxury uptake can contribute to eutrophication and potentially degrade water quality (Carey et al. 2012).

Many cyanobacteria, including *Gloeotrichia echinulata*, can perform the energy intensive process of N fixation, which enables them to access the N from the biologically-inert N_2 gas (Paerl 1988). The nitrogenase enzyme within *G. echinulata* converts N_2 gas into ammonium, which is biologically available. N fixation by *G. echinulata* varies throughout the day, however, it reaches its maximum midday, although it is not light dependent (Finke and Seeley 1978; Stewart et al. 1978). N fixing cyanobacteria often dominate the phytoplankton community when there is excess P and N is limited (Paerl 1988). *G. echinulata* have a competitive advantage over other planktonic organisms because they have access to both P and N (Paerl 1988).

Nutrient Limitation in Lake Ecosystems

There are many elements essential for biological life, but nitrogen (N) and phosphorus (P) are arguably the most important in aquatic systems. Historically, researchers believed that P was the most important limiting nutrient in all lake systems, and was the main driver of eutrophication (Schindler 1974). Recent work by researchers has challenged this idea and has created a new model of co-limitation by both N and P (Abell et al. 2010; Paerl et al. 2014). When both N and P are present in excess in the correct ratio, biomass is not limited by either nutrient. Co-limitation occurs when either nutrient is below the required ratio for growth, so the addition of one nutrient or both can increase aquatic biomass (Harpole et al. 2011).

Nitrogen Cycle

Nitrogen is the most abundant of the five essential elements for life, yet it is the least accessible and can act as a limiting nutrient in aquatic ecosystems (Galloway, 2003). N is both an important nutrient for sustaining life and a factor in eutrophication. A N-limited system with insufficient N will have decreased productivity on all trophic levels. However, an abundance of N in the same ecosystem can cause algal blooms and eutrophication, which have negative consequences (Vitousek 1997). Due to fertilizers, humans have greatly increased the rate of N inputs to terrestrial ecosystems, which results

in N loading in aquatic and marine ecosystems (Vitousek 1997). A well studied and dramatic example of N loading due to fertilizers is the Mississippi River delta which opens into the Gulf of Mexico (Rabalais et al. 2002). The Mississippi River flows through agricultural land that over uses fertilizers and run off carries into the river. This excess N results in large algal blooms in the Gulf of Mexico causing a hypoxic zone that kills all organisms in the area. The size of the hypoxic zone is determined by the amount of N input to the Mississippi from the agricultural land and carried to the Gulf of Mexico (Rabalais et al. 2002). This phenomenon is just one example of how excess nutrients from anthropogenic sources are detrimental to aquatic ecosystems both near and far.

N_2 is a non-reactive gas and is not biologically available unless it is transformed to ammonium (NH_3) via nitrogen fixation. Some bacteria and algae, including many cyanobacteria, are capable of N fixation, but the primary source of ammonium comes from industrial N fixation (Galloway 2003). The Haber-Bosch process was developed by German scientists Fritz Haber and Carl Bosch in the early 1900s to create ammonium for use in fertilizers (Galloway 2003). Reactive N can enter aquatic ecosystems through agricultural/industrial run-off, groundwater, streams, sewer systems, and atmospheric deposition (Holwarth et al. 1996).

Other nitrogen cycle processes include nitrification and denitrification. Nitrification is a two-step process by which chemolithoautotrophic bacteria that live in the sediment or the water column harvest energy from the oxidation of ammonia (Figure 3; Dodds and Whiles 2010). *Nitrosomas* bacteria oxidizes ammonium (NH_4^+) to nitrite (NO_2^-) and then *Nitrobacter* further oxidizes nitrite to nitrate (NO_3^-) (Dodds and Whiles, 2010). Nitrification is often coupled with denitrification (Figure 3). Denitrification is the anoxic process that removes N from the ecosystem in the form of N_2 gas (Dodds and Whiles 2010).

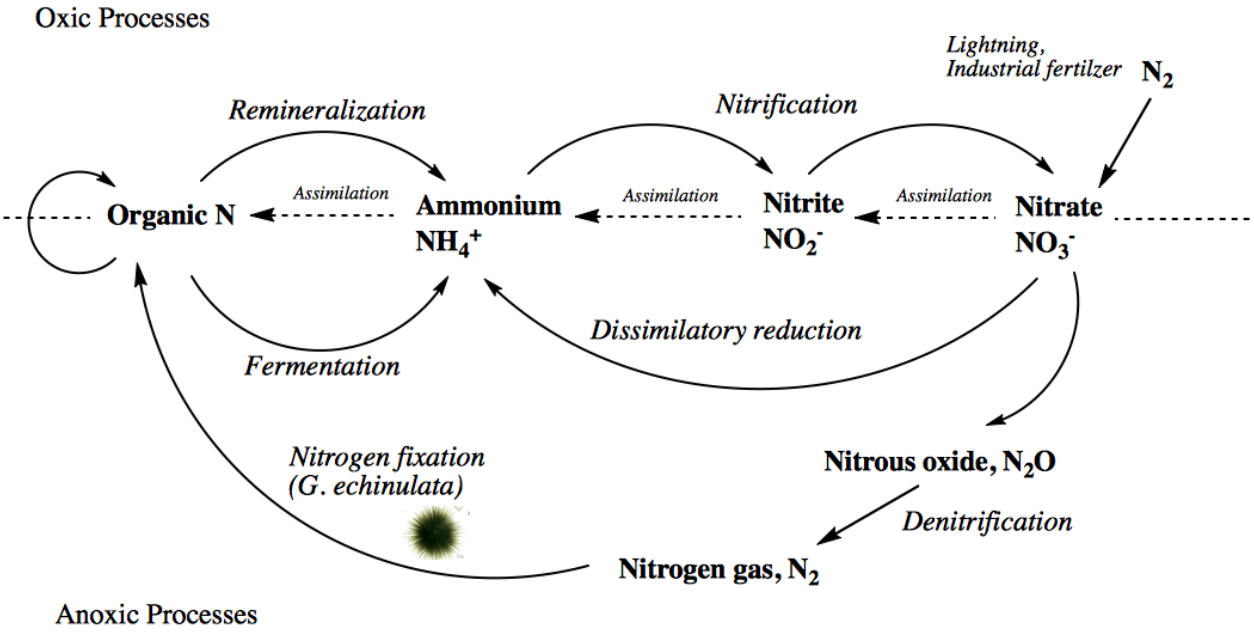

Figure 3. Nitrogen cycle outlined by oxic, the top part of the cycle, and anoxic conditions, the lower half of the cycle. (Bruesewitz, pers. comm.)

Phosphorus cycle

Phosphorus is almost entirely found in the earth's crust and within living organisms, unlike nutrients such as C and N, which are more abundant in living organisms and less in the earth's crust (Smil 2000; Filippelli 2008; Tiessen 2008) (Figure 4). In natural circumstances, P does not have a rapid global cycle because the weathering and erosion of rocks are slow (Smil 2000; Bennett et al. 2001; Bouwman et al. 2009). Anthropogenic activities such as mining P-rich rock contribute two to three times the natural levels of soluble P to aquatic systems (Bouwman et al. 2009). Additionally, anthropogenic P loading comes from the use of P in inorganic fertilizers and animal feeds, urban and industrial waste, and erosion and runoff from agriculture (Tiessen 1996; Smil 2000; Bennett et al. 2001; Bouwman et al. 2009).

Terrestrial P is bound up in rocks in the form of phosphates with calcium and magnesium (Smil 2000; Tiessen,2008). Due to weathering and leaching of calcium by plants, P is released and forms bonds with iron and aluminum in the soil (Smil 2000; Tiessen 2008). The iron and aluminum bound phosphates have a lower solubility than the calcium phosphates, which limits their diffusion by groundwater through the soil or

sediment matrix. The phosphates form tight bonds that prevents them from being absorbed by plants (Smil 2000; Tiessen 2008). Due to soil runoff and other mechanisms, P can be deposited into aquatic systems where it can contribute to eutrophication (Ruttenberg 2003). The increased internal P loading can shift limitation from P to N and add to eutrophication (Nürnberg 1994). Due to accumulation of P in aquatic ecosystems from anthropogenic sources, eutrophication events have escalated worldwide (Soranno et al. 1996).

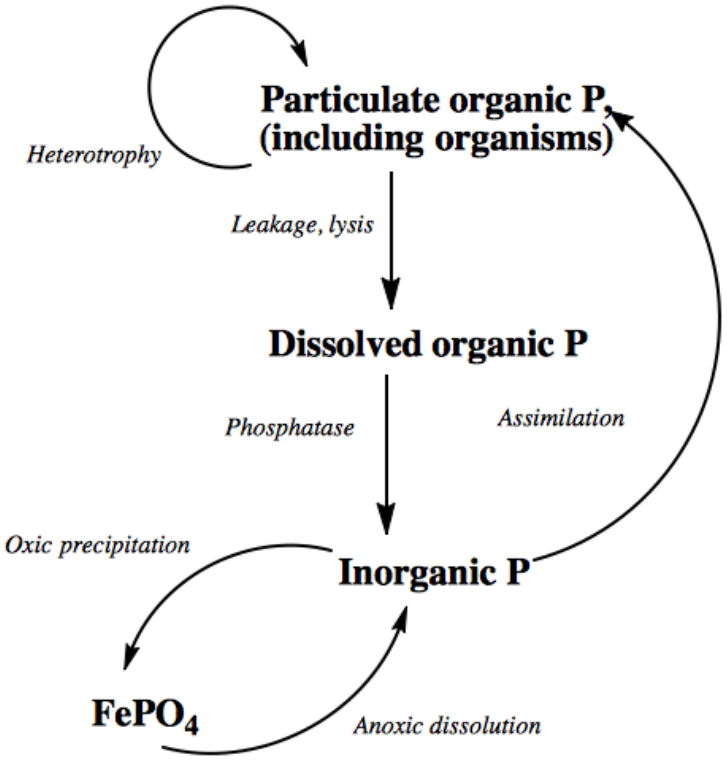

Figure 4. Diagram of the phosphorus cycle in aquatic systems (Bruesewitz, pers. comm.)

Eutrophication

Lake eutrophication, the nutrient enrichment of aquatic ecosystem, happens naturally over time from the gradual accumulation of nutrients and normal lake productivity. Lake eutrophication proceeds through a series of stages: oligotrophic, mesotrophic, eutrophic, and hypereutrophic. At each stage, the level of nutrients, primary production, and plant life increases while the water quality decreases (Addy and Green, 1996). The ecological impacts of eutrophication are due to the increased algal and macrophyte growth, which interferes with recreation, lowers land property values, and degrades drinking water

sources (Carpenter et al. 1998). Cultural eutrophication is the process by which natural eutrophication is accelerated due to anthropogenic effects that cause excess nutrient inputs (Smith et al. 1999). Cultural eutrophication in freshwater lakes is one of the most common and most severe global water quality problems facing humanity (Cloern 2001; Anderson et al. 2002). Anthropogenic activity such as logging, agriculture, cattle husbandry, and urbanization have dramatically increased eutrophication in many aquatic systems worldwide (Vitousek 1997).

Septic systems are onsite wastewater treatment systems that are popular in the Belgrade Lakes and if not functioning properly, can contribute to eutrophication (Robertson et al. 1991). These systems are a known cause of wastewater contamination in groundwater yet their effect can be underestimated because it can be difficult to assess (Yates 1985). Maine has many highly developed lake shorelines that have a high concentration of septic systems due to the rural nature of the area. Septic systems can be a crucial contributor of nutrients to the lakes, leading to an excess of nutrients and summer cyanobacterial blooms. Local governments need to be cognizant of the impacts that septic systems can have on the environment, if not inspected frequently and replaced when necessary.

Many scientists and policy makers focus on efforts to mitigate external P loading to control eutrophication (Søndergaard et al. 2003). There is evidence that reductions in external P can result in improved water quality with re-established submerged macrophyte growth (Cooke et al. 1982; Reitzel et al. 2005). However, internal P loading can be just as important when studying the implications of eutrophication. The release of P from the lake sediment during seasonal changes in oxygen concentrations due to lake turnover can perpetuate symptoms of eutrophication such as algal blooms even after external P loading has been minimized (Reitzel et al. 2003). Internal P loading is the result of particulate iron phosphate in lake sediments becoming aqueous during anoxic conditions (Søndergaard et al. 2003). The increase in biologically available P fuels algal growth and cyanobacterial blooms, making internal P loading a critical component of eutrophication (Sondergaard et al. 2003). Internal P loading is extremely difficult to mitigate, however, solutions such as aluminum application (to bind the mobile P) and hypolimnetic oxygenation (to prevent anoxia) have been used successfully in some lakes.

Internal P loading is important to understand in regard to *Gloeotrichia echinulata* because efforts to quell internal P loading could be undermined by the cyanobacterium life cycle. High-cost strategies such as hypolimnetic oxygenation and aluminum application will only work if the P stays bound and unavailable for a long period of time. A consequence of large-scale recruitment of *G. echinulata* is the translocation of significant amounts of P from the sediment to the water column, possibly negating the efforts of water quality restoration methods such as alum treatment (Driscoll and Schecher 1990). In nitrogen limiting environments, the ability of *G. echinulata* to fix N and sequester luxury P could mitigate the effects of a restoration method.

CHAPTER II: RESEARCH ON THE BELGRADE LAKES

Research Goals

Studying *Gloeotrichia echinulata* in the Belgrade Lakes is important not only to the landowners and stakeholders in the area, but also the wider limnological community. Cyanobacterial blooms are generally studied in eutrophic and mesotrophic lakes which more commonly experience blooms (Downing 2001); much less is known about its dynamics within oligotrophic systems (Barbiero 1993; Jacobsen 1994; Karlsson-Elfgren et al. 2003). The Belgrade Lakes include lakes that are oligotrophic and mesotrophic systems which have experienced *G. echinulata* blooms since the land was first settled, as shown from the sediment record (Downing 2001; Padisák et al. 2003; Lepisto et al. 2005). Ongoing research on *G. echinulata* in the Belgrade Lakes will help scientists understand why the abundance is increasing, what health implications it could have, and how *G. echinulata* affects the N and P cycling in these lakes.

Methods

Study Area

The Belgrade Lakes watershed includes seven large lakes that are at risk for degrading water quality (Diagle 2015). The decreasing water quality in the Belgrades is likely due to mixture of influences, including urban development, aging sewer systems, and ground run off. The Belgrade Lakes have highly developed shorelines with a high concentration of aging septic systems. Septic systems could be a crucial contributor of nutrients to these lakes, leading to an excess of nutrients and summer cyanobacterial blooms.

The Belgrade Lakes are an ideal study site for *Gloeotrichia echinulata* because the bloom intensity has increased in the recent past and it could be a marker of human influence and climate change. Water samples from over the past 40 years indicate increased levels of nutrients to be the leading cause of the decline in water quality (McGuire 2015). The two study lakes are geographically adjacent to each other, but each has a unique trophic state. Great Pond is mesotrophic and Long Pond is oligotrophic; this allows for an interesting comparison of *G. echinulata* population dynamics in lakes subject to the same meteorological forces but different nutrient states.

Local lake associations, stakeholders, and community members are involved in the process of protecting the Belgrade Lakes from eutrophication. Many local residents are enrolled in the LakeSmart Program based in Belgrade Lakes Village that promotes construction and retention of healthy riparian buffers between shoreline development and the lake (Diagle 2015). In 2015, the Belgrade Lakes Association granted 22 homes the LakeSmart certification and 36 homes were awarded a LakeSmart commendation (Diagle 2015). Enrollment in these programs and interest in lake health makes the Belgrade Lakes a socially and environmentally important ecosystem to study.

Study Sites

The sites chosen for sampling *Gloeotrichia echinulata* and water quality comprise a mix of shallow and deep areas in Long Pond and Great Pond (Figure 5). *G. echinulata* abundance differs based on spatial and temporal lake variations. There should be consistency in *G. echinulata* density in deep sites because pelagic conditions are similar within the same lake. Shallow sites are likely susceptible to different weather, land inputs, geological formations, and wind patterns, which will distribute the *G. echinulata* and cause a larger variation in density among sites. The deep sites and shallow sites were chosen to investigate how *G. echinulata* density is affected by these conditions.

The Maine Department of Environmental Protection monitors these lakes at specific sites, typically in the deepest part of each lake, so we adopted those points in our study sites as well. The sites are accessed by public land with the exception of one private property.

Table 1. Descriptions for the 10 sampling sites on Long Pond and Great Pond.

Site ID	Description	Depth (m)
LP1	Belgrade Village: A 2m dock extends into a small artificial bay created to harbor boats. The shoreline consists of commercial buildings, gravel parking lots, a grass lawn, and stone boulders.	2.0 - 3.0
LP2	Resident's Dock: A 2m dock extends into a shallow, secluded cove on the east side of the north basin. Residences in this area are primarily year-round and a busy road runs close to the lake shore. Resident has a LakeSmart certified property.	2.0 - 3.0
LP3	North Basin: Maine Department of Environmental Protection (DEP) survey site. The north basin shoreline has more year-round and seasonal residences than the south basin. Downtown Belgrade also borders the shoreline of the north basin.	40
LP4	South Basin: Maine DEP survey site surrounded by minimal residences.	40
LP5	Public Boat Launch: A 4m dock extends into a shallow bay with a shoreline of natural vegetation to the east and west and a road to the north. A portable toilet is on site.	1.5 - 2.5
GP1	Great Pond Public Boat Launch: A 5m dock extends into a shallow, marsh like habitat. Due to boat traffic in the shallow water, the sediment is continually altered. The shoreline consists of the Great Pond Marina and the parking lot for the Boat Launch. Portable toilets are on site.	1.5 - 2.5
GP2	Goldie Buoy: The Colby owned buoy measures water and light characteristics throughout the water column. The eastern shoreline has seasonal residences and the western shoreline has a mix of seasonal and year-round residences.	62
GP3	North Bay: Sampling occurred in the center of the north bay which had previously restricted access due to a milfoil removal effort by the lake associations and because it is a sensitive nesting area for birds. The shoreline is almost completely natural vegetation with a few residences on the southern shore. Past summers have experienced high *G. echinulata* blooms in this bay.	8.0 - 9.0
GP4	Center of the lake: Maine DEP survey site, minimal residences on the shoreline.	40
GP5	Colby Outing Club Cabin: A 4m dock extends into a shallow, rocky bay on the SE side of Great Pond. Seasonal and year-round houses line the lake shore.	1.0 - 1.5

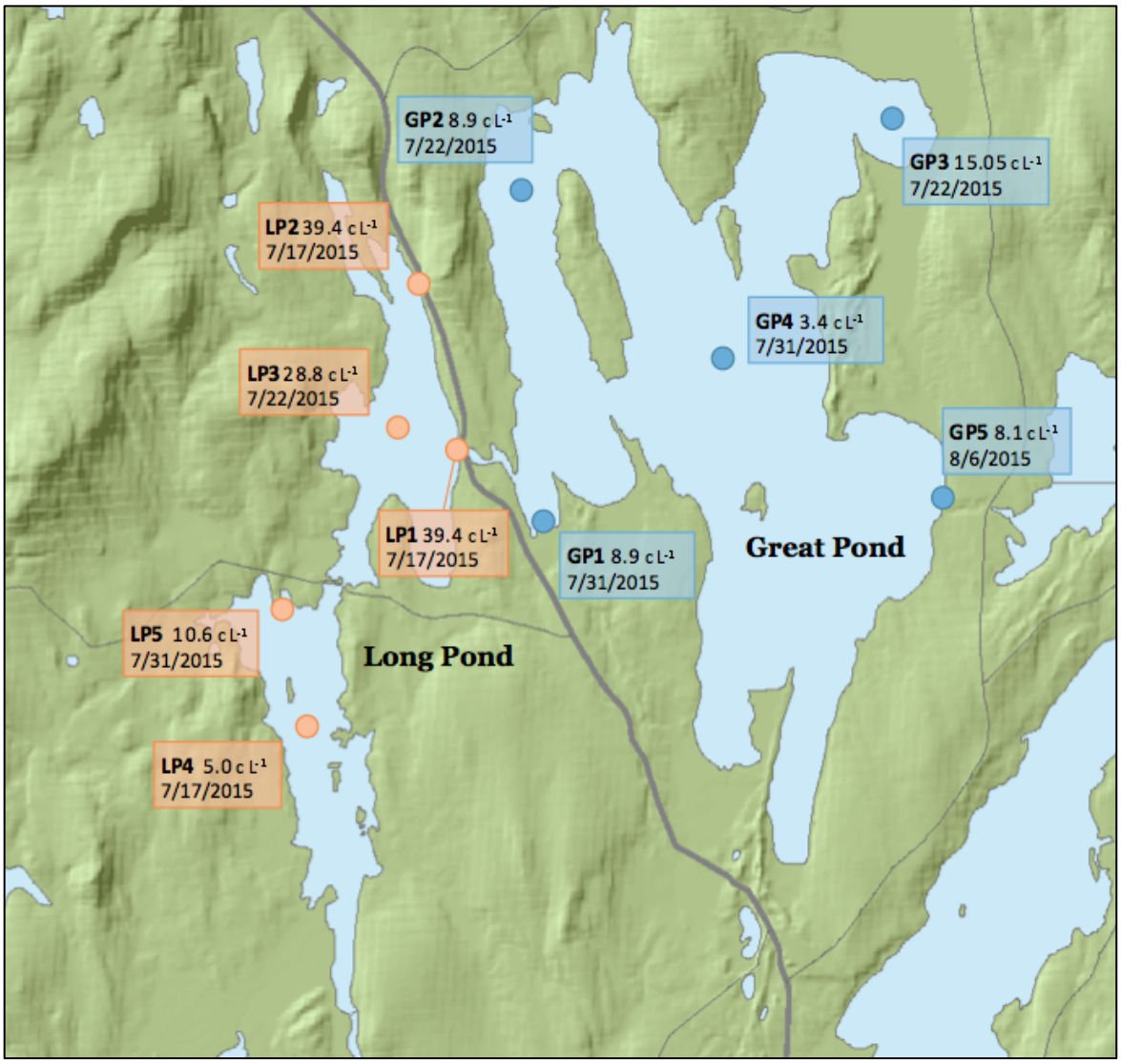

Figure 5. GIS map showing the 10 sampling sites on Long Pond and Great Pond in Belgrade, ME, labeled by site ID. The spatial and temporal distribution of *Gloeotrichia echinulata* peak blooms during summer 2014 measured in colonies L^{-1} is noted along with the date of collection.

Sampling Methods

To sample *Gloeotrichia echinulata* sampling methods of previous studies were used in order to promote consistency between regional datasets (Carey et al. 2008, 2012). Sampling occurred bi-weekly among June and September 2015 to track lake characteristics and *G. echinulata* blooms over the course of the summer. Water data were collected using an In-Situ Inc. SmarTroll Probe to measure temperature, pH, and percent dissolved oxygen (DO) at each site at a depth profile of 0 m, 0.5 m, 1 m, 2 m, 5 m, 10 m.

Corresponding weather data were measured using a weather station at the Maine Lakes Resource Center in Belgrade, Maine. Water collection was taken at 1 m using a Van Dorn 4L water sampler (Wildlife Supply Company, Wildco). *G. echinulata* colonies were collected using a zooplankton net of dimensions 30 cm x 90 cm, and made of 250 micron mesh (Dynamic Aqua-Supply Ltd.). Two tows were taken at 1 m depth at each site and *G. echinulata* colonies were preserved with 2-5 mL of Lugol's Iodine solution in 250mL amber nalgene bottles and stored upright at room temperature.

Gloeotrichia echinulata counting

Hand-counting of *Gloeotrichia echinulata* was conducted by five student research assistants at Colby College using an identical protocol for *G. echinulata* from the summer of 2014 and 2015. Each sample was filtered from storage bottles using a 10 μm mesh screen. Once filtered, the storage bottle was rinsed several times to obtain all possible *G. echinulata* colonies. The sample was then deposited onto a grid-marked square petri dish. Using a dissecting microscope, each sample was analyzed for full *G. echinulata* bodies, "haircut" *G. echinulata* bodies, *G. echinulata* bodies that were less than half, *G. echinulata* bodies that were more than half, and filamentous bundles (Figure 6).

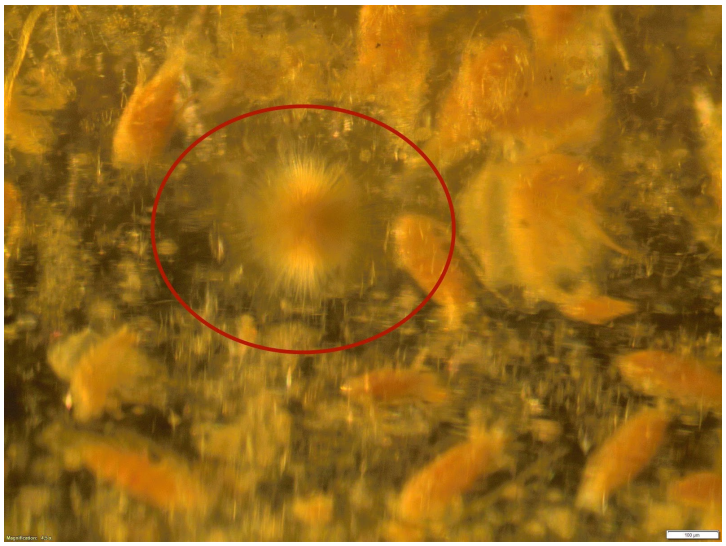

Figure 6. Magnified picture of water sample from Long Pond with full *Gloeotrichia echinulata* body circled.

In order to expedite obtaining accurate counts of *Gloeotrichia echinulata* in samples, we explored the possibility of utilizing a FlowCam, an automated plankton detection instrument, to count the samples. We thought that the FlowCam could provide accurate

counts of *G. echinulata* from lake samples by passing samples through the flow cell and programing the software to identify the *G.echinulata* bodies. We tried many different flow cell sizes and arrangements of tubing, but the FlowCam was never as successful at getting exact counts as the research assistants were. This method was unsuccessful in counting the *G. echinulata* because the flow cell needed to accommodate the size of the *G. echinulata* colonies, which are so large that the accuracy of the FlowCam decreased as it was only able to capture a portion of the flow cell window. When the flow cell size was decreased to improve accuracy the *G. echinulata* colonies formed a blockage in the tubing before entering the flow cell. An accurate and complete count was necessary for this project, so that differences in *G. echinulata* densities between days and sites could be calculated, especially when the densities were low. If a flow cell could be found that was large enough for the *G. echinulata* colonies to pass that still provided high accuracy, there is potential that the FlowCam could be a useful tool in monitoring *G. echinulata* density on a larger scale. We determined after several trials that hand-counting remains the most efficient and accurate way to monitor the density of *G.echinulata.*

Lake Phosphorus Concentrations

To measure total phosphorus (TP) concentrations throughout the summer, water samples were taken weekly at *G.echinulata* sampling sites in Long Pond and Great Pond. Water samples were measured for TP using QuikChem 8500 Series 2 Lachat FIA System after persulfate digestion of the unfiltered water samples using standard methods (10-115-01-1-Q). Samples were prepared by adding 0.05 mL of digestion solution (equal parts ammonium persulfate and sulfuric acid solution) and 9.5 mL of water sample to a test tube, and then digested at high temperatures for 30 minutes in a Market Forge Sterilmatic autoclave. Each site had three replicates for each sample date. Stata 13.1 software was used to determine if there is a significant correlation between *G. echinulata* blooms and nutrient levels.

Phosphorus in Gloeotrichia echinulata Bodies

To measure TP of individual *Gloeotrichia echinulata* bodies, full *G. echinulata* bodies were separated by pipette from lake samples preserved in Lugol's solution. *G. echinulata* bodies were selected from both on Long Pond and Great Pond. For samples containing 10 bodies, *G. echinulata* were taken from site LP5 on the 25th of June 2015. For samples

containing 5 bodies, the *G. echinulata* were taken from GP4 on the 29th of the June 2015. These dates and sites were chosen to get a random sampling of *G. echinulata*. For each trial, varying amounts of *G. echinulata* bodies (n=5, n=10) were placed in test tubes with 0.5 mL of digestion solution (equal parts ammonium persulfate and sulfuric acid solution) and 9.5 mL of deionized water. These samples were digested at high temperature and pressure for 30 minutes in a Market Forge Sterilmatic autoclave. After digestion, samples were analyzed for orthophosphate as described above. The results were analyzed using a linear regression to determine if there is a direct relationship between number of *G. echinulata* bodies and P levels and to estimate an approximate P concentration per colony.

Chlorophyll-a and Pheophytin Concentrations

At each sampling site, 1000 mL of water was collected at a depth of 1m with the Van Dorn water sampler. The 1000 mL were filtered through a GF/F 0.7 μm mesh filter. The filters were stored frozen for 1-3 weeks before being analyzed. Once ready to be analyzed, the filters were defrosted and soaked in 15 mL of 90% acetone for 48 hours in a dark refrigerator. Then the extracted samples were run on a Turner Designs Trilogy Fluorometer and absorbance before and after acidification was measured to determine chlorophyll-*a* and pheophytin, a pigment used by cyanobacteria for photosynthesis.

Isotopic nitrogen experiment analysis

Nitrogen occurs in two stable isotopic forms, ^{14}N and ^{15}N (Lake et al. 2011), and 99.6% of all naturally occurring nitrogen is ^{14}N (Kim et al. 2016). A novel method of measuring isotopic nitrogen was developed by the Colby College Chemistry department in collaboration with the Environmental Studies Program. ^{15}N stable isotope tracer studies are a crucial tool to understand the N cycling mechanisms and rates in aquatic ecosystems (Kim et al. 2016). However, the traditional method is less efficient, less accessible, and requires a far greater volume of water sample than the newly developed method using indophenol (Kim et al. 2016).

The novel method uses an electrospray ionization time-of-flight mass spectrometry to measure nitrogen isotope ratios in the samples. Two phenol molecules trap the nitrogen species, which forms indophenol, a heavier and less reactive compound (Figure 7). Excess reagents are removed from the sample using a C-18 solid phase extraction with

acetonitrile, which concentrates the indophenol four-fold (Kim et al. 2016). From the mass spectrometry spectra, we can measure the isotopic ratios easily and accurately (Figure 8).

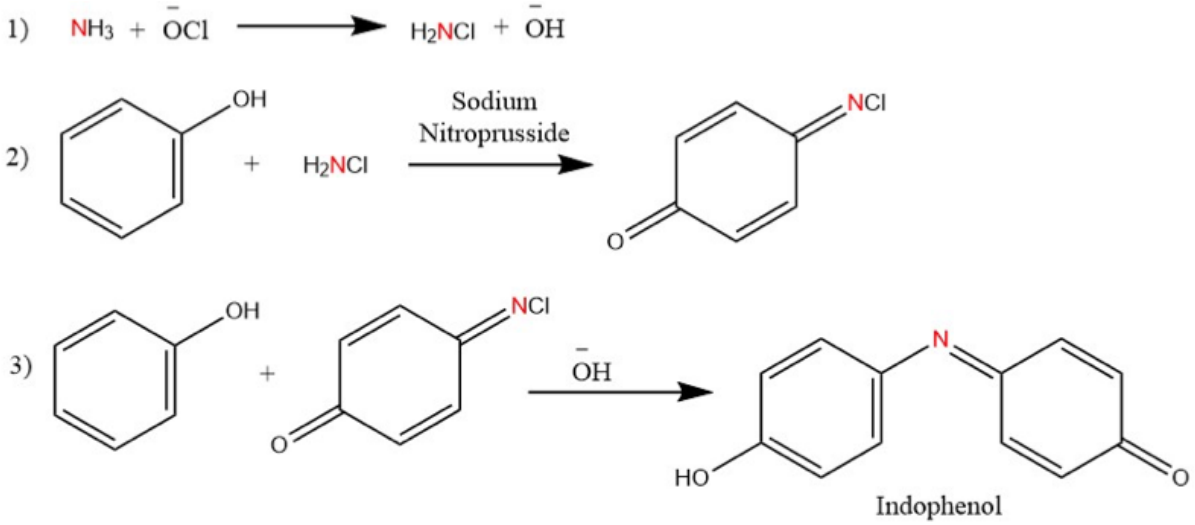

Figure 7. Reaction mechanism for the novel method of measuring isotopic nitrogen ratios in water samples.

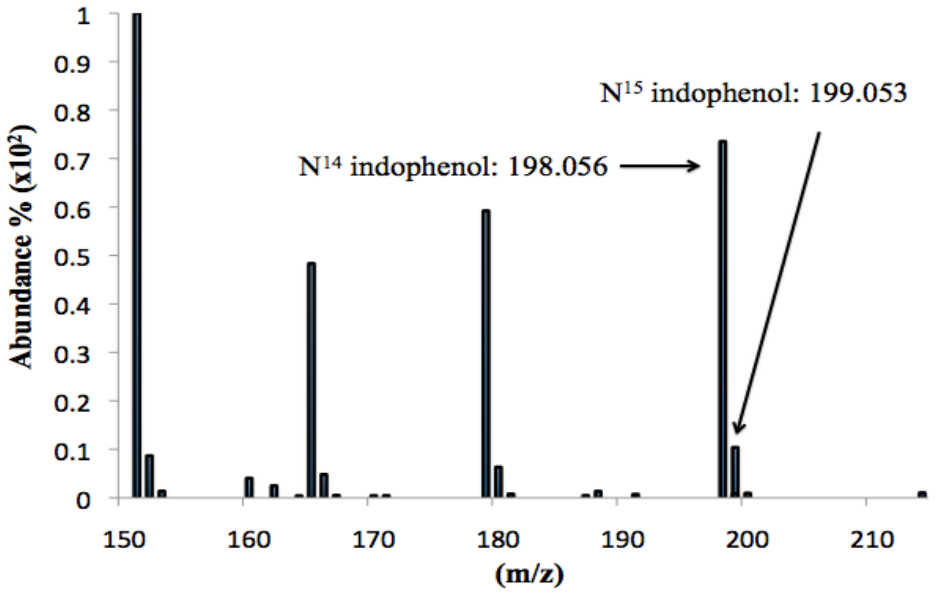

Figure 8. Example of electrospray ionization time-of-flight mass spectrometry for the novel method. The horizontal axis is mass in grams divided by charge. Isotopic ^{14}N indophenol has a peak at 198.056 g and ^{15}N indophenol has a peak at 199.053 g.

There have been setbacks within the time frame of this thesis that have prevented me from analyzing all 1,000+ samples from the isotopic nitrogen experiment. In the future,

these data will provide information on the assimilation and demineralization of organic nitrogen by a phytoplankton community composed primarily of *G. echinulata*.

Isotopic Nitrogen Uptake Experiments

To measure nitrogen uptake of the plankton community throughout the summer, we conducted controlled ammonium (NH_4^+) uptake experiments in the laboratory at Colby College, Waterville, ME. One liter of water was collected using the Van Dorn water sampler at depth of 1 m and stored in opaque white 1 L nalgene bottles on ice. The water was collected at a subset of six sites that were chosen because they experienced high *Gloeotrichia echinulata* abundance in the past and provided information on both shallow and deep sites. The three sites used for Great Pond were the Public Boat Launch (GP1), Goldie Buoy (GP2), and the north cove (GP3). For Long Pond, the three sites were the Belgrade Village dock (LP1), the north basin (LP3), and the south basin (LP4).

Upon returning to the lab from sampling, 180 mL of water was put into six narrow-necked clear glass beakers for each site (Figure 9). 150 µL of 15N was added to the three experiment beakers and the three control beakers were unchanged. 60 mL of water was sampled from each beaker at time 0, 4 hours, and 24 hours to measure uptake of the $^{15}NH_4^+$ over time by plankton at room temperature. The interval samples were stored in vials and kept frozen in a dark space until analysis occurred.

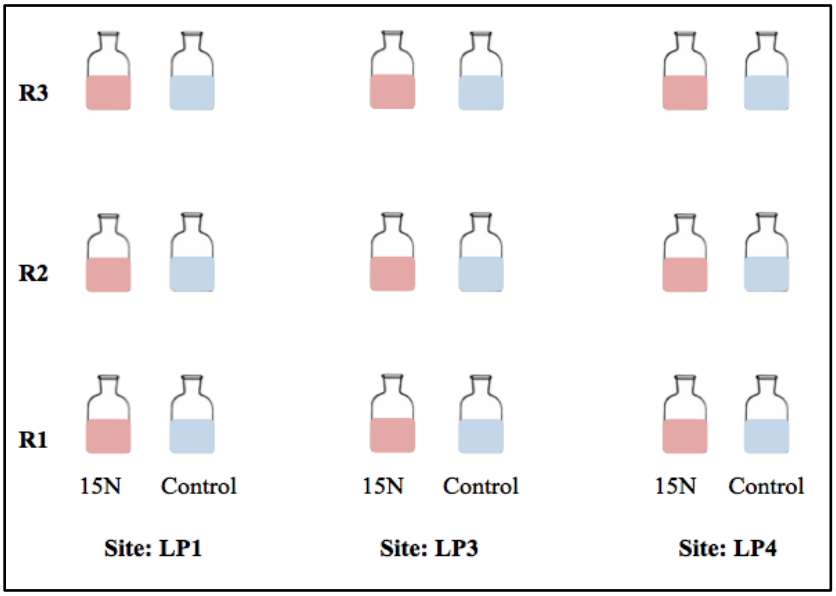

Figure 9**.** Experimental design of beakers for the isotopic nitrogen uptake experiment for Long Pond. The Great Pond experiment was set up identically. Samples were measured at 0, 4, and 24 hours.

CHAPTER III: RESULTS, DISCUSSION, AND CONCLUSION

Lake characteristics

The air temperature at Belgrade Lakes Village, ME increased from the beginning of June to the end of August, with the peak temperature occurring in mid-August (Figure 10). The highest recorded air temperature in Belgrade Lakes Village was 32° C on 18 August 2015. Water temperatures in Great and Long Pond follow the same general seasonal pattern, with peak surface water temperatures of 25.69° C and 26.59° C occurring on 29 July 2015 and 3 August 2015, respectively. The water temperature was measured at different frequencies throughout the water column depending on site depth. Here, I focus on water temperature at 0.5 m depth because *Gloeotrichia echinulata* blooms occur in the top 1 m of the water column (Figure 11). The mean water temperature for Long Pond from June to August 2015 was 22.28 ± 0.33° C. For Great Pond, the mean water temperature from June to August 2015 was 21.88 ± 0.38° C. There was not a significant difference in water temperature between Long Pond and Great Pond (t-test, $p>0.05$; LP n=50, GP n=39).

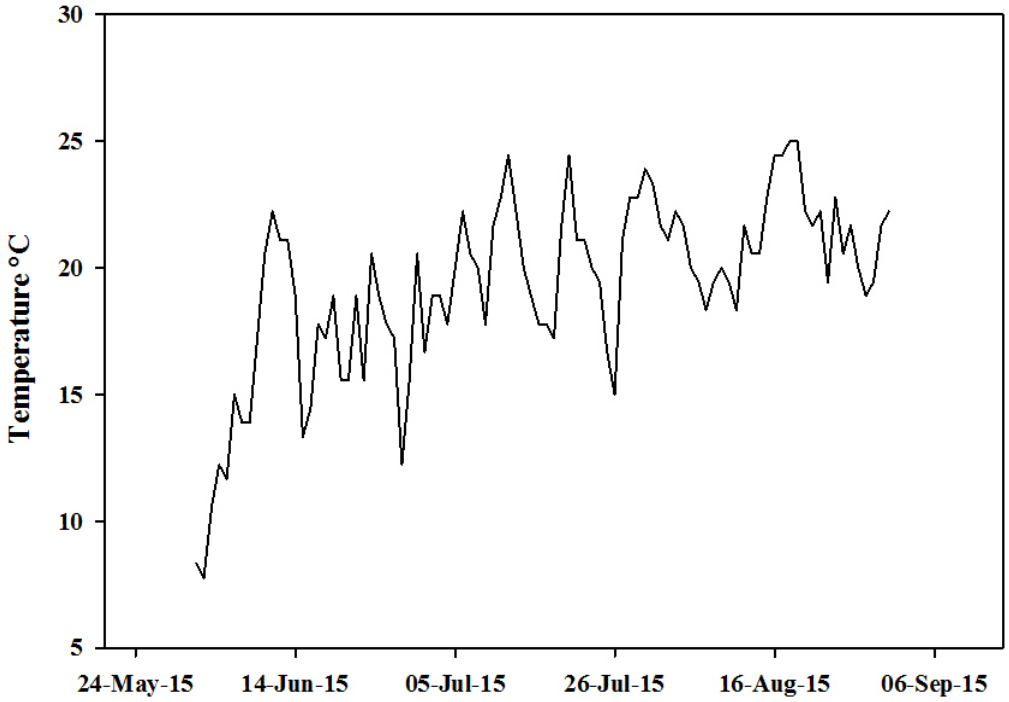

Figure 10. Mean daily air temperatures in Belgrade Lakes Village, Maine, for July and August 2015. Temperatures provided by National Weather Service sampled at 44.53°N 69.86°W.

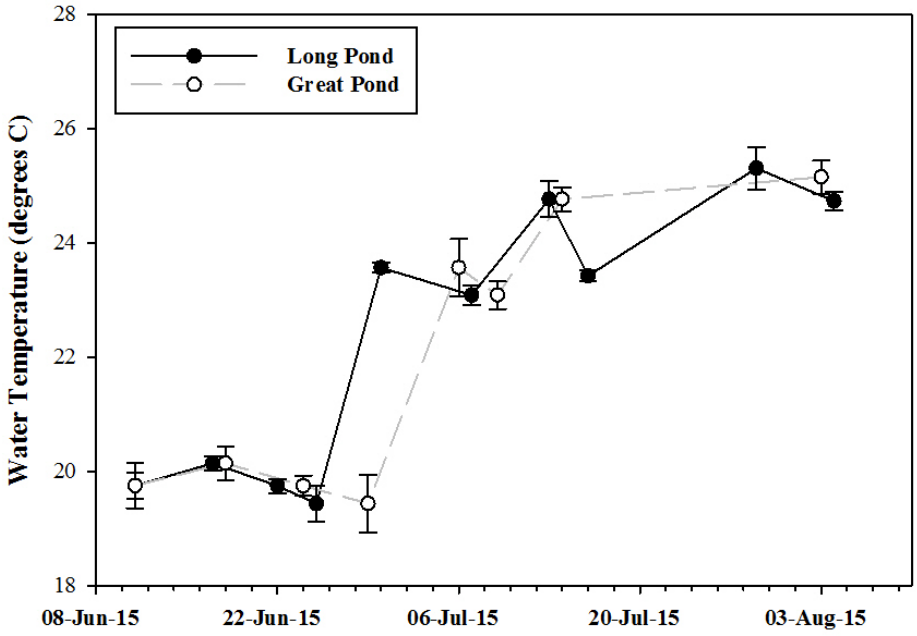

Figure 11. Average water temperature (±SE; °C) in Long Pond and Great Pond at 0.5 m depth from June to August 2015.

We measured DO at many depths, but Figure 12 only displays 0.5 m depth because of its primary relevance for *G. echinulata*. Great Pond had a mean dissolved oxygen of 8.86 ± 0.07 mg L^{-1} from June to August, 2015. Similarly, mean dissolved oxygen in Long Pond from June to August, 2015 was 8.99 ± 0.07 mg L^{-1}. As expected due to their proximity to each other, there was not a significant difference in DO between Long Pond and Great Pond, and both are saturated with oxygen at this depth (t-test, $p>0.05$; GP n=27, LP n=34).

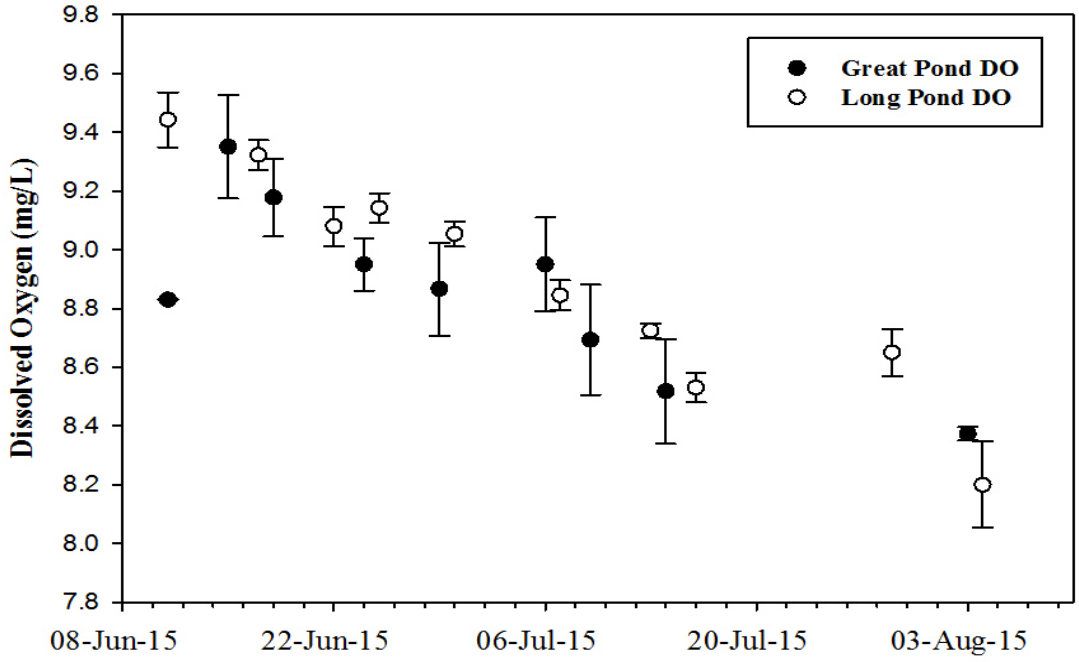

Figure 12. Mean dissolved oxygen (±SE) (mg L^{-1}) in Long Pond (hollow circles) and Great Pond (full circles) from June to August 2015.

Long Pond and Great Pond had similar pH values throughout the summer (Figure 13). The mean pH value for Long Pond from June to August 2015 was 7.36 ± 0.08. Great Pond had a mean pH value from June to August 2015 of 7.39 ± 0.04 The mean pH values from June to August 2015 were not statistically significantly different between Long Pond and Great Pond, and both were in a neutral range (t-test, $p>0.05$; GP n=27, LP n=34).

The total rainfall for Belgrade Lakes Village was lower in 2014 than 2015 (Figure 14). Rainfall and strong wind can mix layers within a lake, which results in reduced thermal stability of the water layers within the lakes (Figure 15).

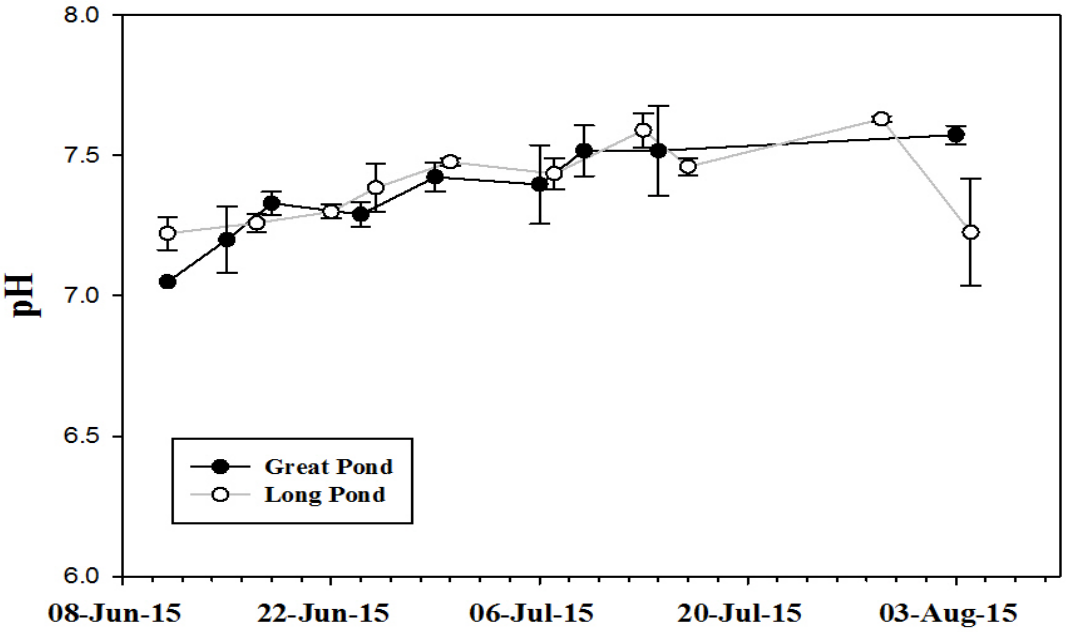

Figure 13. Mean pH values (±SE) for Great Pond (dark circles) and Long Pond (hollow circles) for the summer of 2015.

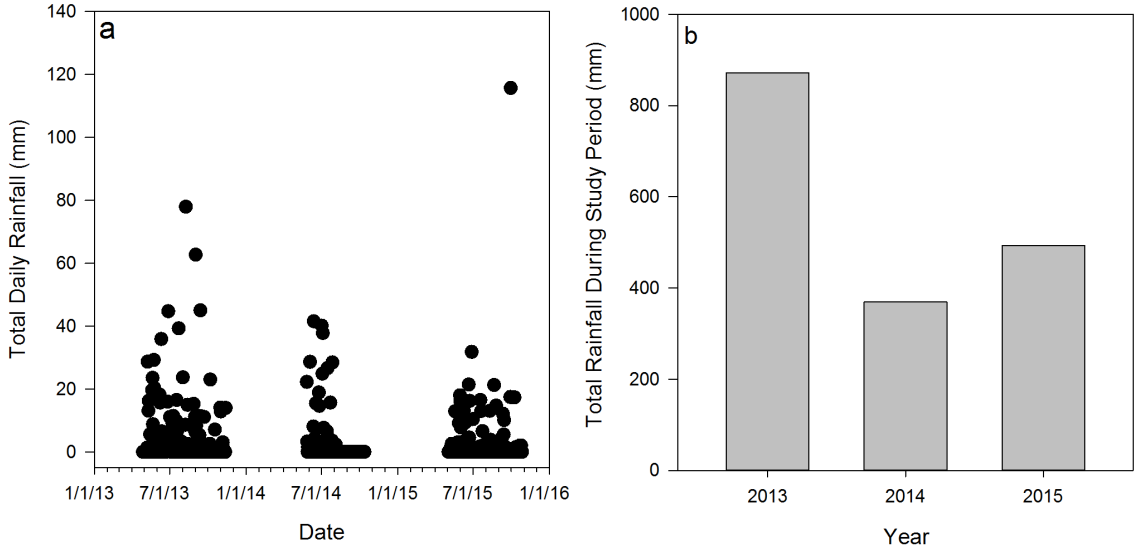

Figure 14. (a) Total daily rainfall in Belgrade Lakes Village for the summers of 2013, 2014, and 2015 measured in mm. (b) Total Rainfall during the study period over the summers of 2013, 2014 and 2015.

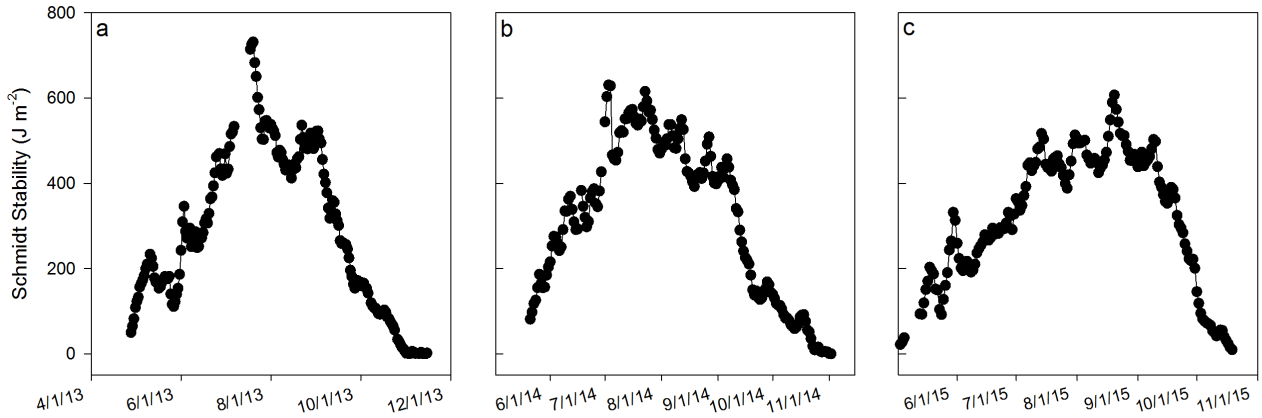

Figure 15. Schmidt stability (thermal stability) (J m^3) for Great Pond for the summers of 2014 (a), 2014 (b), and 2015 (c).

Gloeotrichia echinulata Density

The *Gloeotrichia echinulata* densities throughout the summer of 2015 highlight differences in temporal and spatial distribution across Long Pond and Great Pond. The trend for 2015 blooms mirrored that from 2014 in both lakes with peak blooms of up to 39.4 colonies L^{-1} in Long Pond on July 17, 2015 at LP1 and 15.1 colonies L^{-1} in Great Pond on July 22, 2015 at GP3. For all sites across both lakes the density diminished to 0 colonies L^{-1} by September 1st in both 2014 and 2015.

There is variation in density both between Long Pond and Great Pond and within each lake individually (Figure 16, Figure 17). However when averaged across all sites, Great Pond and Long Pond did not have significantly larger blooms in 2014 (paired t-test, $p>0.05$, $n=10$; data not shown). Likewise, the densities for the lakes as a whole were not significantly different for the summer of 2015 (paired t-test, $p>0.05$, $n=10$). Additionally, the densities of Long Pond in 2014 were not significantly different from the densities in 2015 (paired t-test, $p>0.05$). For Great Pond the same is true, the densities in 2014 and 2015 were not significantly different (paired t-test, $p>0.05$). This is because of the large variability in *Gloeotrichia echinulata* density across the different sites within each lake. However, the means alone show that the magnitude of the densities is lower in 2015 than the summer prior (Table 2).

Table 2. Mean *Gloeotrichia echinulata* densities across both lakes for 2014 and 2015.

Lake	**2014 density**	**2015 density**
Long Pond	4.62±1.48 colonies L^{-1}; n=37	3.97±0.45 colonies L^{-1}; n=78
Great Pond	2.31±0.62 colonies L^{-1}; n=34	1.22±0.23 colonies L^{-1}; n=67

Overall, the peak blooms occur in late July and early August in both lakes, with higher bloom densities in Long Pond than Great Pond. In Great Pond, *Gloeotrichia echinulata* density was not significantly different across the five sites in 2014 (one-way ANOVA ($F(4, 29) = 19.6$, $p > 0.05$)) (Table 3). Likewise, there was not a significant difference among sites for 2014 in Long Pond (one-way ANOVA ($F(4, 32) = 1.15$, $p > 0.05$). However, because the densities vary over time, a repeated measures ANOVA is more appropriate because it partitions our variation due to sampling the same sites through time. When tested with a repeated measures ANOVA, Long Pond was significantly different across sampling sites for 2014 ($F(9, 23) = 4$, $p = 0.0035$) with LP1 site having more *G. echinulata*, whereas Great Pond was not different across sites.

Table 3. F and p value table for Great Pond and Long Pond from one-way ANOVA comparing densities from 2014.

Lake/Site	**1**	**2**	**3**	**4**	**5**	***F* value**	**P value**
Great Pond	n=10	n=5	n=4	n=5	n=10	4,29	>0.05
Long Pond	n=8	n=9	n=5	n=5	n=10	4,32	>0.05

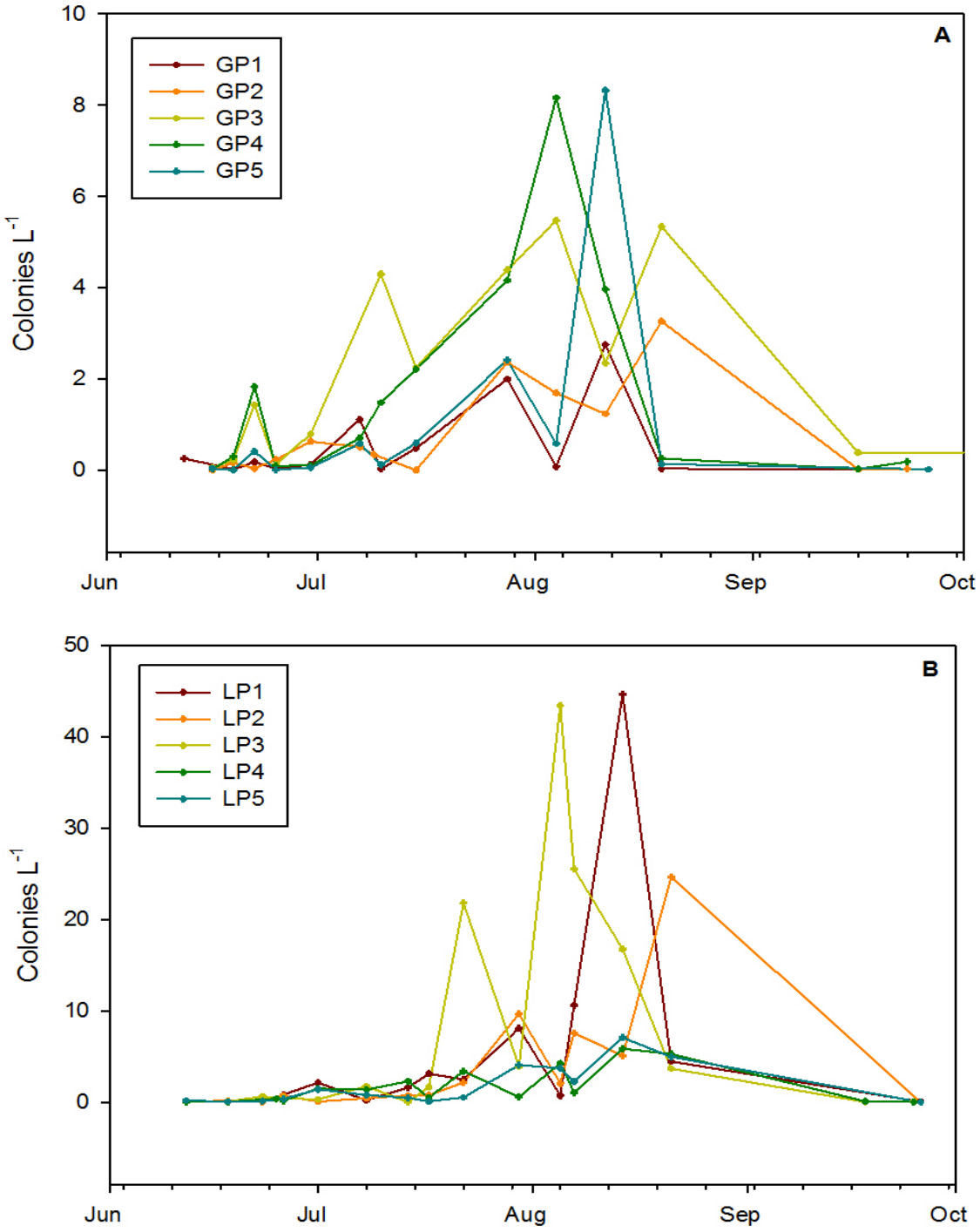

Figure 16. *Gloeotrichia echinulata* density in the surface water of Great Pond (A) and Long Pond (B) from June to October 2015 at five sites on each lake. Density is measured in colonies per liter of water averaged across two plankton net tows at each site.

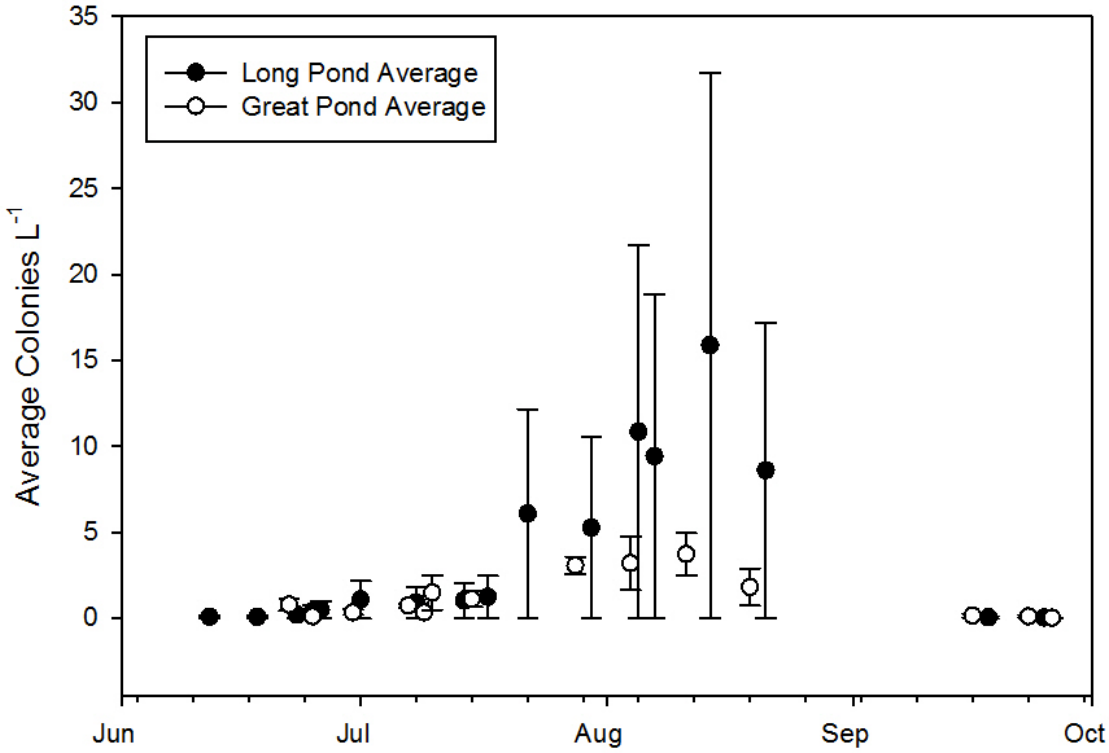

Figure 17. Lakewide means (±SE) of *Gloeotrichia echinulata* density across the five sites for Long Pond (dark circles) and Great Pond (hollow circles) from June to October 2015.

Gloeotrichia echinulata Density Across Lake Depths

In both Great Pond and Long Pond, the densities between shallow, littoral and deep, pelagic sites are not significantly different (Table 4, Figure 18) (t-test, $p>0.05$). The difference between the deep sites of Long Pond and Great Pond trends toward being significantly different (t-test, $p= 0.11$). Likewise, there is a trend toward a significant difference between the shallow sites of Long Pond and Great Pond (t-test, $p=0.13$), with Long Pond shallow sites having more *G. echinulata* than Great Pond shallow sites.

Table 4. Mean *Gloeotrichia echinulata* density (± SE) at shallow sites (LP 1,2,5; GP 1,3,5) and deep sites (LP 3,4; GP 2,4) from June-October, 2015.

Lake	Shallow Sites	Deep Sites
Long Pond	3.53 ± 2.04 (n=45)	4.58 ± 3.24 (n=32)
Great Pond	1.354 ± 0.65 (n=33)	1.19 ± 0.98 (n=24)

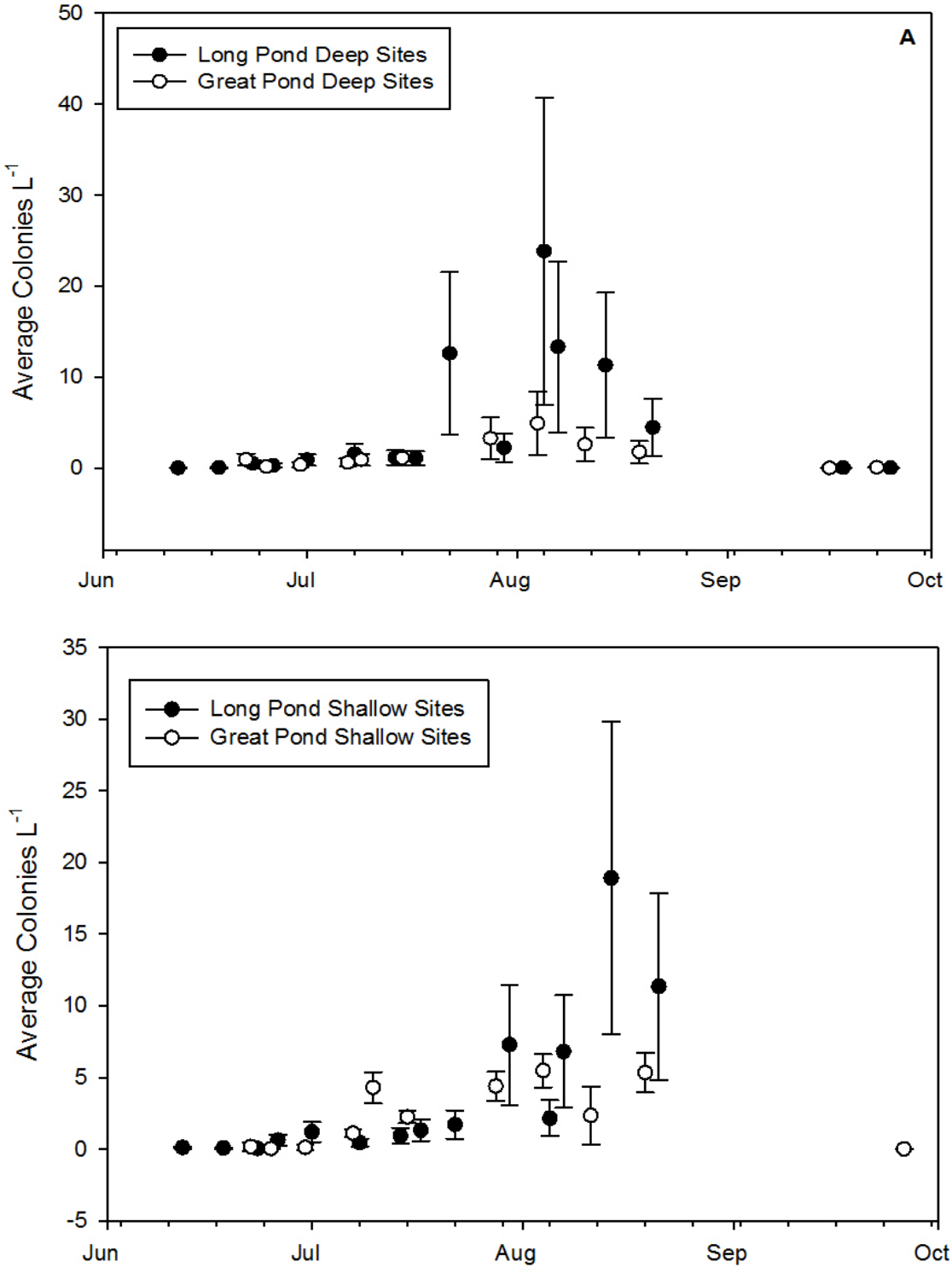

Figure 18. (A) Average (±SE) Gloeotrichia echinulata density at shallow sites (LP: 1, 2, 5; GP: 1, 3 5) for Long Pond (dark circles) and Great Pond (hollow circles) from June to October 2015. (B) Average (±SE) G. echinulata density at deep sites (LP: 3, 4; GP: 2, 4) for Long Pond and Great Pond from June to October 2015.

Total Phosphorus

The total phosphorus (TP) in Great Pond and Long Pond during the summer of 2015 showed large variability. Great Pond had significantly higher TP levels at deep sites than shallow sites (Table 5). Additionally, the five sites on Great Pond had significantly different TP levels over time (MANOVA, $F(4, 11) = 9.38$, $p<0.05$; GP1 (n=33), GP2 (n=39), GP3 (n=42), GP4 (n=42), GP5 (n=39)) (Figure 19). Long Pond, however, did not have a statistically significant difference between the deep sites and shallow sites (Table 5). Also, when compared to each other over time, the sites on Long Pond were not significantly different from each other (MANOVA, $F(4, 12) = 1.88$, $p>0.05$; LP1 (n=1), LP2 (n=35), LP3 (n=35), LP4 (n=37), LP5 (n=36)) (Figure 19). Across both lakes, the TP concentrations averaged across the five sites for each date are not significantly different between lakes (t-test, $p>0.05$, n=12; Figure 20).

We estimate that *Gloeotrichia echinulata* bodies on average contain 0.883 ± 0.079 µg P L^{-1} per *G. echinulata* body (n=50) (Figure 21). See discussion for information on scaling P in *G. echinulata* bodies to lake TP dynamics.

Table 5. Statistical analysis of difference between total phosphorus between deep and shallow sites within each lake.

Lake	**Deep Sites**	**Shallow Sites**	**P-value**
Long Pond	Sites 3, 4 n=72	Sites 1, 2, 5 n=107	T-test, $p>0.05$
Great Pond	Sites 2, 4 n=65	Sites 1, 3, 5 n=95	T-test, $p=0.023$

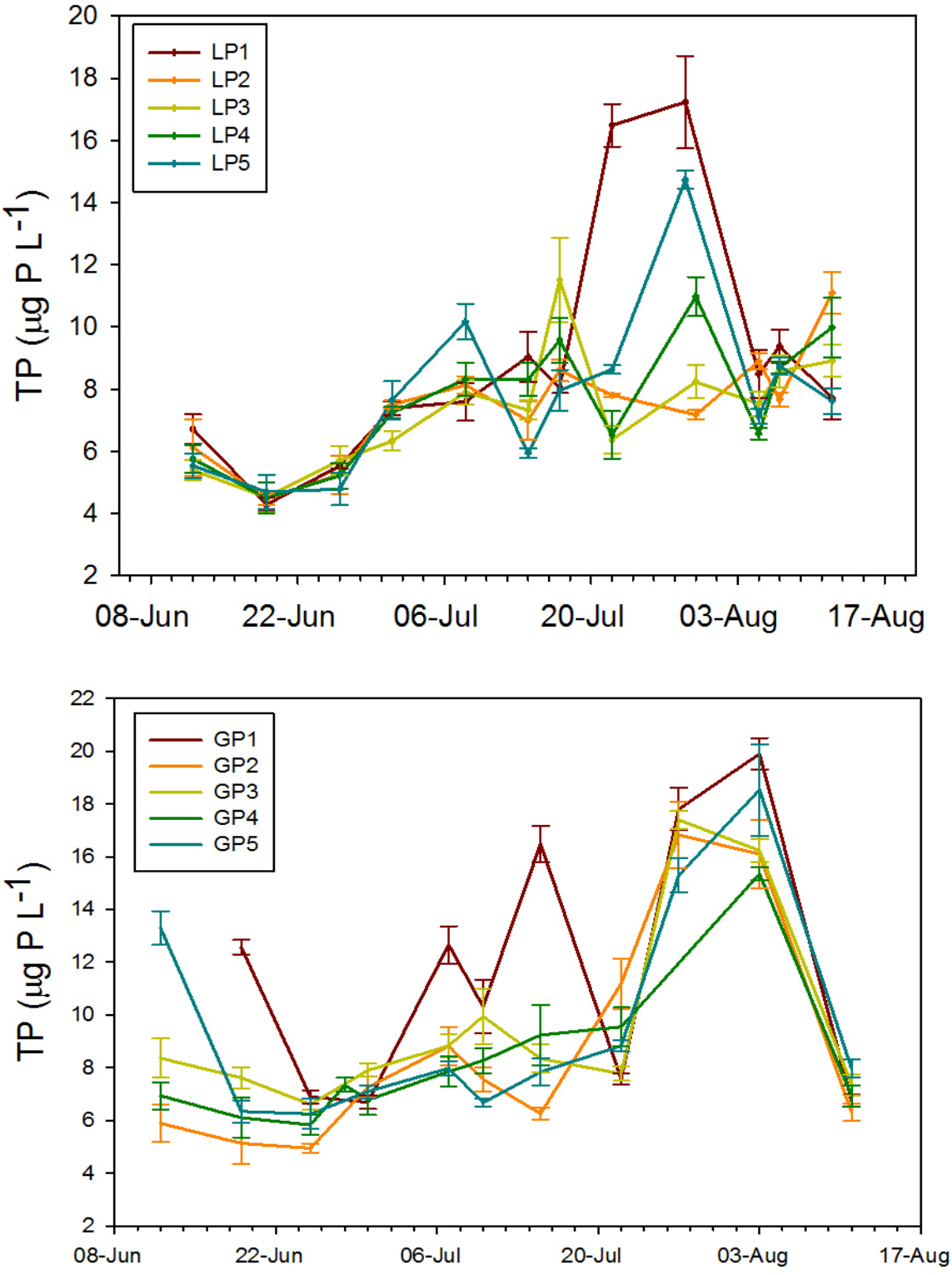

Figure 19. Total phosphorus concentrations (±SE) for Long Pond (top graph) and Great Pond (bottom graph) from June to August 2015.

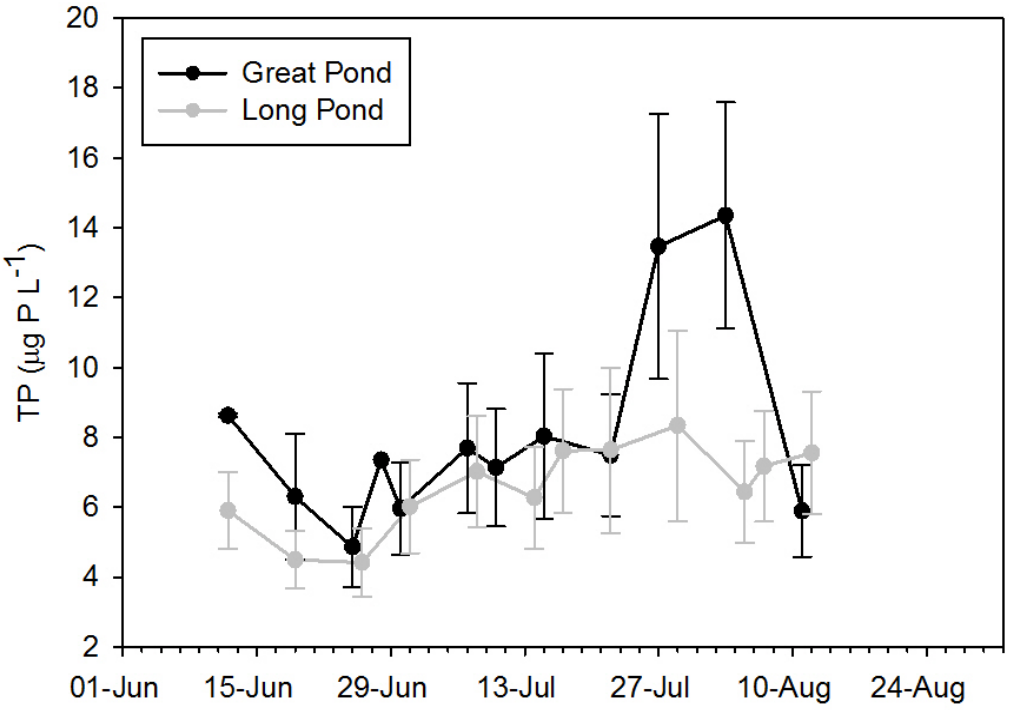

Figure 20. Total phosphorus concentrations averaged (±SE) across the five sites for each lake from June to August, 2015.

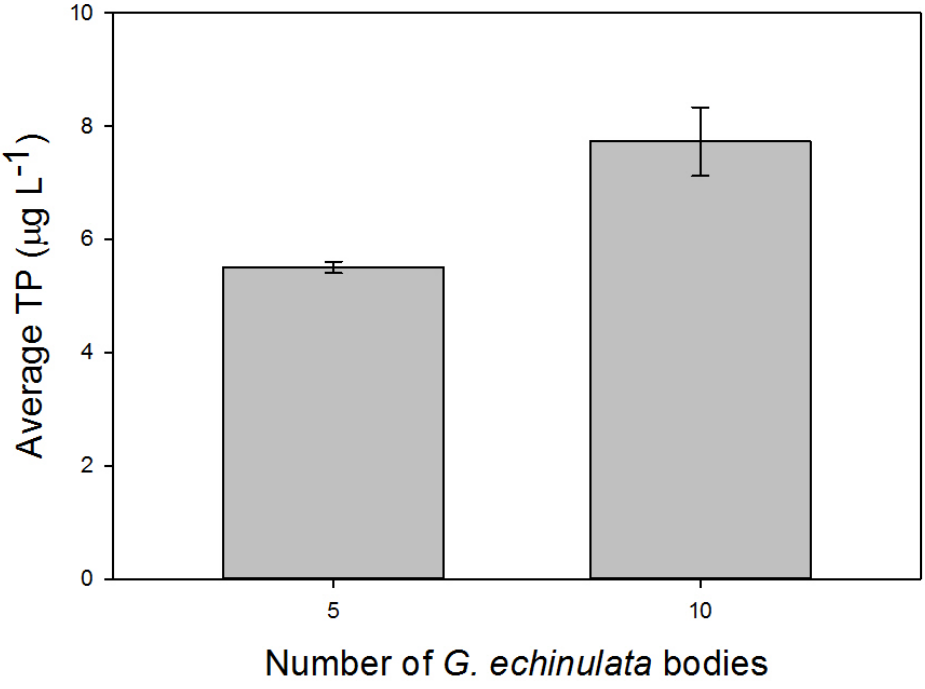

Figure 21. Mean (±SE) total phosphorus concentrations for five and ten *Gloeotrichia echinulata* bodies (n=6).

Chlorophyll-*a* Concentrations

The mean chlorophyll-*a* concentrations for Long Pond and Great Pond (Table 6) for the summer of 2015 reflect the trends of 2014 (data not shown). There is not a significant difference in chlorophyll-*a* concentration among the two lakes when comparing all sites (t-test, $p>0.05$) (Figure 22, Figure 23). Within Long Pond, there is not a significant difference between the sites as determined by a one-way ANOVA (Table 7). Likewise, Great Pond does not have a significant difference between the sites by a one-way ANOVA (Table 7). However, when the sites within a lake are measured across time, both Long Pond and Great Pond show significant differences in chlorophyll-*a* concentrations when using a repeated measures ANOVA (Table 7).

Table 6. Mean chlorophyll-a concentrations (µg L^{-1}) from Long Pond and Great Pond for the summer of 2015; minimum and maximum chlorophyll-a (µg L^{-1}) with date and site attained. Refer to map for site locations (Figure 5).

Lake	**Mean chlorophyll-*a* (µg L^{-1})**	**Minimum chlorophyll-*a* (µg L^{-1}) and date**			**Maximum chlorophyll-*a* (µg L^{-1}) and date**		
Long Pond	2.273	0.95	6/17/15	LP2	4.91	7/21/15	LP4
Great Pond	2.448	1.34	9/25/15	GP5	7.18	7/21/15	GP4

Table 7. Statistical values for ANOVA and repeated measures tests for Long Pond and Great Pond chlorophyll-*a*.

Lake	**F and P values one way ANOVA**	**F and p values repeated measures**
Long Pond	$F(4, 60) = 0.47$, $p>0.05$ n=13 for all sites	$F(12, 48) = 19.05$, $p<0.001$ n=13 for all sites
Great Pond	$F(4, 60) = 1.23$, $p=>0.05$ n=13 for all sites	$F(12, 48) = 5.9$, $p<0.001$ n=13 for all sites

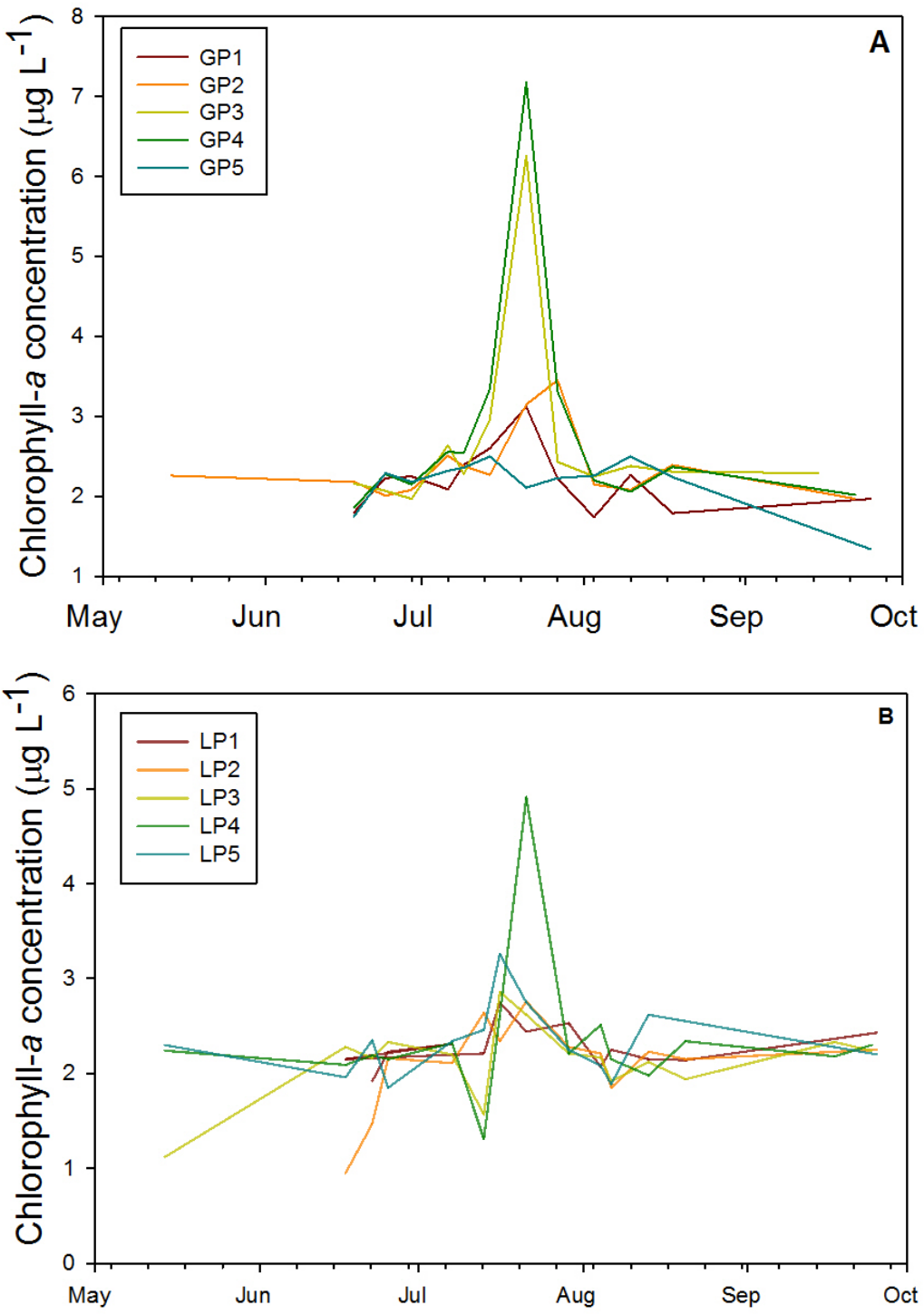

Figure 22. Chlorophyll-*a* concentrations (µg L^{-1}) for Great Pond (A) and Long Pond (B) measured across the five sites at each flake from May to October 2015. Refer to map for site locations (Figure 5).

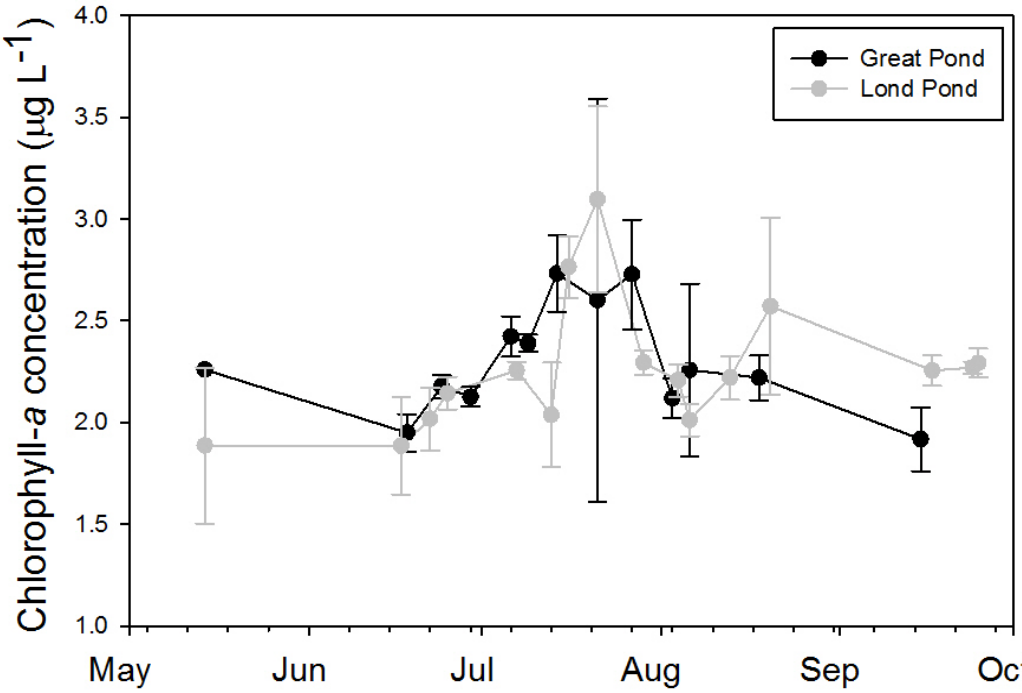

Figure 23. Chlorophyll-*a* concentration (µg L^{-1}) averaged (±SE) for the five sites across Great Pond (dark circles) and Long Pond (grey circles) from May to October 2015. Refer to map for site locations (Figure 5).

Pheophytin Concentrations

The mean pheophytin concentrations for Great Pond and Long Pond (Table 8) for the summer of 2015 are not statistically different (t-test, $p>0.05$) (Figure 24, Figure 25). For both Long Pond and Great Pond, none of the sites within each lake are statistically different from other sites within the same lake (one-way ANOVA; Table 9). Long Pond's northern sites (LP2, LP3, and LP4) had greater *Gloeotrichia echinulata* blooms than the southern site (LP1, LP5; RM-ANOVA; Table 9). Great Pond had greater *G. echinulata* density in the northern cove, GP3, than the other sites (RM-ANOVA; Table 9).

Table 8. Mean pheophytin concentrations (μg L^{-1}) from Long Pond and Great Pond for the summer of 2015; minimum and maximum pheophytin (μg L^{-1}) with date and site attained. Refer to map for site locations (Figure 5)

Lake	**Mean pheophytin** (μg L^{-1})	**Minimum pheophytin** (μg L^{-1})	**Maximum pheophytin** (μg L^{-1})
Long Pond	1.613	0.22 7/7/15 LP2	4.09 6/17/15 LP2
Great Pond	1.656	0.06 9/15/15 GP3	3.32 7/27/15 GP2

Table 9. Statistical values for ANOVA and repeated measures tests for Long Pond and Great Pond pheophytin concentrations.

Lake	**F and P values one way ANOVA**	**F and p values repeated measures**
Long Pond	F(4,62)=0.47, p>0.05 LP1 (n=13); LP2 (n=12); LP3 (n=14); LP4 (n=14); LP5 (n=14)	F(15,47)=4.24, p<0.001 LP1 (n=13); LP2 (n=12); LP3 (n=14); LP4 (n=14); LP5 (n=14)
Great Pond	F(4,60)=0.89, p=>0.05 n=13 for all sites	F(12,48)=5.02, p<0.001 n=13 for all sites

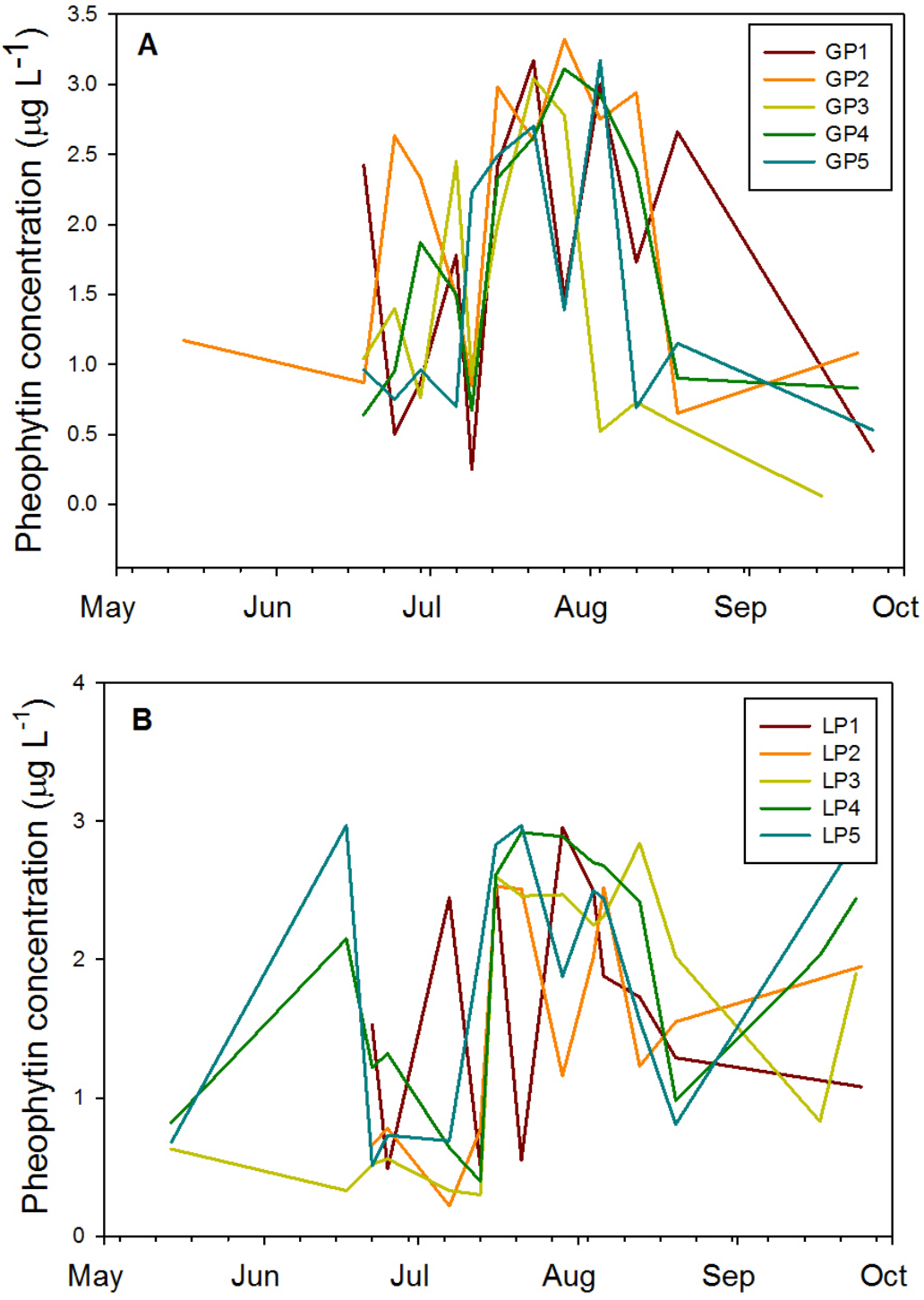

Figure 24. Pheophytin concentrations (µg L^{-1}) for Great Pond (A) and Long Pond (B) for the five sites at each lake from May to October 2015. Refer to map for site locations (Figure 5)

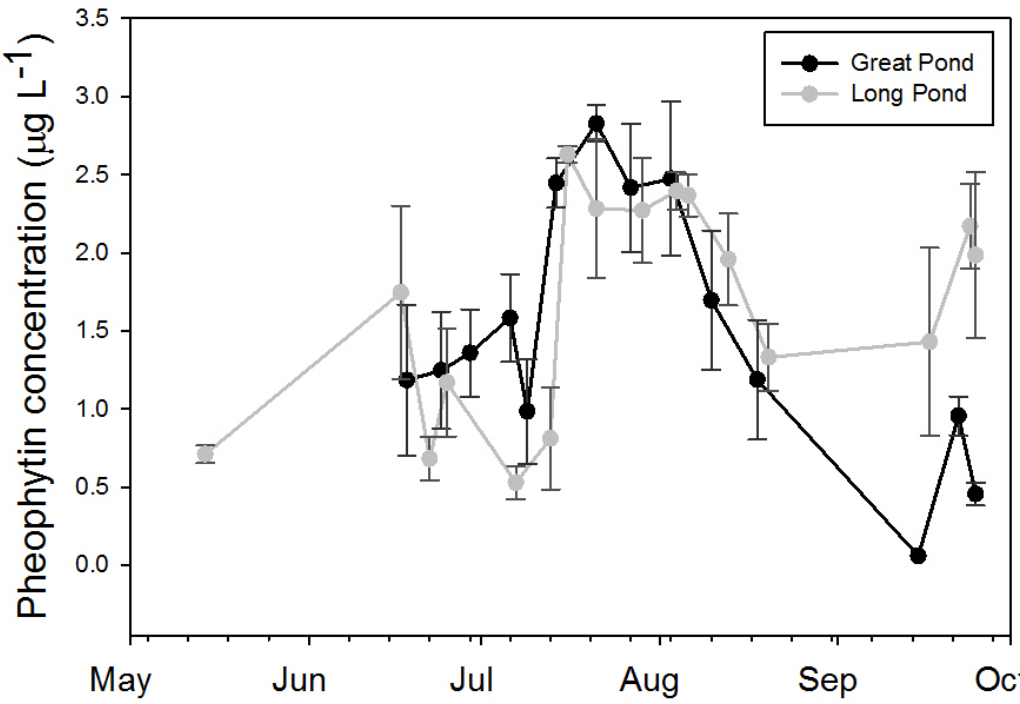

Figure 25. Pheophytin concentrations (µg L^{-1}) averaged (±SE) across the five sites for each lake from May to October 2015. Refer to map for site locations (Figure 5).

Discussion

Lake Characteristics

As the air temperature in Belgrade Lakes Village increased over the summer of 2015, the lake water temperature in Long Pond and Great Pond increased as well. Both Long Pond and Great Pond are at the same latitude, 44.4467° N, 69.8321° W, and elevation, 76 m above sea level. One of the biggest differences between the bodies of water is their topology. Long Pond has a surface area of 10.3 km^2, whereas Great Pond has a much larger surface area of 34.5 km^2 (VLMP, 2016). Additionally, Long Pond is deeper with a mean depth of 10.6 m and a max depth of 32.3 m (VLMP, 2016). Great Pond is shallower with a mean depth of 6.4 m and a max depth of 21.0 m (VLMP, 2016). The lakes experience the same weather and are part of the same watershed with Great Pond flowing into Long Pond, however, the level of lakeshore development differs, with Great Pond being more heavily developed.

As the summer progresses, the air and water temperatures increase, which have effects on lake biology and chemistry. Gases are less soluble in warm water; dissolved oxygen

(DO) levels decrease throughout the summer as the water temperature increases (Aben 2005). In both Long Pond and Great Pond, DO at the surface decreased slightly throughout the summer due to decrease gas solubility in the warmer water, despite gross primary production at the surface providing oxygen. For both lakes at the hypolimnion at 10 m, DO concentration declined throughout the summer due to the decay of phytoplankton (Beutel 2003). An anoxic hypolimnion can cause shifts in nutrients in the sediment along with zones of hypoxia that are dangerous for fish health (Beutel, 2003). Management practices such as oxygen bubbling throughout the summer can be used to prevent anoxia in the hypolimnion (Beutel 2003), but the high cost is prohibitive in many cases.

Throughout the summer of 2015, the pH increased in both Long Pond and Great Pond, similar to trends have been found in other aquatic systems (Okogwu and Ugwumba 2009). Along with reduced dissolved oxygen, an increase pH is associated with cyanobacteria abundance (Okogwu and Ugwumba 2009). Summer cyanobacterial blooms near the Chesapeake Bay have caused an elevated the pH of between 9 and 10 (Gao et al. 2012). An elevated pH promotes desorption of sedimentary inorganic phosphorus and facilitated conversion of ammonium (NH_4^+) to ammonia (NH_3) (Gao et al. 2012). An elevated pH will aid in increasing cyanobacterial abundance by introducing more biologically available nutrients into the water column. Although we do not have measures of pH near the sediment surface where these processes occur, we anticipate that the trends measured in the surface water would follow in the hypolimnion of both Great Pond and Long Pond.

Gloeotrichia echinulata Densities

Lake Sunapee in New Hampshire experiences *Gloeotrichia echinulata* peak blooms in late August (Carey et al. 2008, 2014a), several weeks after peak blooms were observed in the Great Pond and Long Pond. Carey et al. (2014a) suggested lake wide cues such as lake mixing, thermocline depth, Schmidt stability, and minimum air temperatures as important factors in determining bloom dynamics.

Although no significant difference among densities was observed across sites, trends indicate that *Gloeotrichia echinulata* densities were highest at sites in protected coves (GP3) and in shallow areas (GP1, GP5, LP1, LP2, LP5; Figure 17). In Long Pond, the

shallow sites had a lower mean density for the summer than the deep sites. We would expect to see the opposite trend, because Long Pond is a deeper lake with less surface area and two elongated basins. Patterns of wind direction, which is predominantly from South to North, could shift the *G. echinulata* from the open water to accumulate in coves and shallow areas. This movement is most likely occurring in both lakes, but due to Long Pond being oligotrophic, it produces a higher bloom in general, which compensates for the clustering caused by the wind. Great Pond had a higher mean density of *G. echinulata* at the deep sites than the shallow sites. The shape of the lake could help explain these effects; Great Pond has more surface area so the wind does not have as strong an effect on collecting *G. echinulata* into coves as in Long Pond. Higher *G. echinulata* densities in shallow areas were also found Lake Erken, Sweden (Forsell and Pettersson 1995; Karlsson-Elfgren et al. 2003) and Lake Sunapee, NH (Carey et al. 2008, 2014a). This effect was attributed to wind redistribution of *G. echinulata* as well as higher recruitment rates from shallow sediments (Carey et al. 2014a).

There appear to be regional climatic clues such as lake mixing, which trigger similar blooms in Great Pond and Long Pond (Carey et al. 2014). This provides an explanation for the similar peak bloom date across all sites. In addition, variations in timing and bloom magnitude may have been influenced by local in-lake factors such as depth (Karlsson-Elfgren et al. 2004), sediment chemistry and substrate type (Carey et al. 2008, 2009), dissolved O_2 (Barbiero 1993), size of the akinete bank (Forsell 1998), bioturbation (Pierson et al. 1992; Karlsson-Elfgren et al. 2004), and grazing (Rengefors et al. 1998). Future research that includes measurement of *G. echinulata* recruitment from the sediment along with monitoring these variables would further identify which of these factors are drivers in the Belgrade Lakes.

The mean *Gloeotrichia echinulata* densities appear to be lower in 2015 than in 2014, however, this could be a product of the time frame for sampling between years. Monitoring in the summer of 2014 began in mid-July so there were fewer overall samples taken, whereas monitoring in 2015 began in June. Since 2015 included more samples outside of the bloom periods, the overall mean will be lower. We suspect that there was not a significant decrease in *G. echinulata* densities from 2014 to 2015. However, differences in weather patterns between the two years could have altered lake stability or

otherwise altered lake conditions to account for differences between years of sampling. *G. echinulata* grows best in stable, warm, oligotrophic lakes. The summer of 2015 had was more total rainfall and more rainfall in the late summer, which disrupted Schmidt stability and could have limited *G. echinulata* blooms.

Counting Strategies

Routine monitoring is one of the best ways to measure *Gloeotrichia echinulata* blooms in lake systems. Weekly sampling, while labor-intensive, provides a quantitative and continuous data set of *G. echinulata* densities throughout the summer, which can be patchy, with blooms occurring on the scale of a few days and then subsiding. Some lake associations, such as the Belgrade Lakes Association, have established citizen science monitoring systems that engage local residents and stakeholders in water quality issues. These programs have been successful at increasing awareness about lake health and for gathering widespread data across lakes. One of the drawbacks of citizen science monitoring is that it offers a less precise count of *G. echinulata* density, and decreases consistency across sites and lakes with observations from many different people, at different times of the day. In all, weekly sampling combined with citizen science monitoring provide useful tools for establishing long term data sets for measuring lake health.

Once the samples have been collected, the *Gloeotrichia echinulata* are stored in iodine solution to preserve the colonies. Then each sample is carefully counted under a dissecting microscope. This process introduces error because there is variation among the assistants' perception of what constitutes a colony. *G. echinulata* densities are better considered estimations of bloom densities than absolute counts. We minimize this variation by having a small number of trained *G. echinulata* counters in the lab, to decrease variation from individual person counts.

Another strategy considered for counting *Gloeotrichia echinulata* is using a flow cytometry instrument, or FlowCam to take pictures of each colony in a sample of water. The pictures can be identified and then the density can be extrapolated from the count. However, *G. echinulata* colonies are large organisms with varying shapes. This provided a challenge for both taking pictures of the colonies and then using the computer program to identify them. The large colonies clogged the tubing of the FlowCam, but the accuracy

of the count decreased when we used a bigger flow cell because the camera cannot capture the entire depth of the flow cell. The amount of error that this procedure introduced to counting indicated that the FlowCam would need to be modified to achieve accurate estimations.

Total Phosphorus

The TP levels did not significantly differ across Long Pond and Great Pond because of the variability within the sites for each lake. Anthropogenic influences, such as runoff from lawns or roads, and longer water residence time result in significantly higher TP in the shallow sites than in the deep sites for Great Pond. Long Pond also had higher TP in the shallow sites, however, the trend is not significantly different. Higher TP due to anthropogenic effects such as septic system leakage is possible for Belgrade Lakes Village and Great Pond Public Boat Launch. Additionally, runoff from the road and development within Belgrade Lakes Village and sediment suspension by boat motors can contribute to the higher TP at those sites. The lowest TP during the peak bloom (7.17 ± 0.15 μg P L^{-1} occurred at a LakeSmart certified property on Long Pond. The LakeSmart certification entails that the property has a riparian buffer along the shoreline to protect against excess nutrients entering the lake from runoff. LakeSmart is a valuable and successful program in the Belgrade Lakes for protecting lake health (Junker 2016).

Throughout the summer of 2015, the TP levels fluctuated possibly correlating to *Gloeotrichia echinulata* density changes. The TP levels were lowest during June and subsequently increased throughout the summer, peaking in both lakes in early August. Variations in *G. echinulata* distribution could be reflected in the TP measurements as *G. echinulata* typically have the highest recruitment rates at shallow sites (Carey et al. 2008). Along with anthropogenic effects, we suspect that the fluctuations in TP are linked to luxury P uptake by *G. echinulata* during recruitment, P released into the water column during zooplankton grazing, and *G. echinulata* senescence. *G. echinulata* bodies can store a significant amount of P, so we believe variation in *G. echinulata* blooms are likely a driver of TP patterns at sites with high blooms.

Total Phosphorus in Gloeotrichia echinulata Bodies

TP in *Gloeotrichia echinulata* bodies was measured to investigate the role that luxury uptake of P plays in determining TP levels of surface waters. To establish a preliminary

understanding of the potential for *G. echinulata* to transfer P from the benthos to the water column, the approximate mean TP in a *G. echinulata* colony (0.8825 µg P colony^{-1}) was scaled up to reflect the actual *G. echinulata* densities. The mean TP measured per *G. echinulata* body (0.8825 µg P colony^{-1}) and the *G. echinulata* density (0.073 colonies L^{-1}) from the date and site the sample was taken from were used. With this calculation, it was estimated that 0.064 µg P L^{-1} could potentially be transferred from the benthos to the water column by *G. echinulata* recruitment.

If we assume that the TP in *G. echinulata* colonies remains the same throughout their life cycle and is fairly consistent across sites, we can estimate the potential maximum quantity of P that could be transferred from the sediment to the water column by *G. echinulata* recruitment. The largest bloom in Long Pond in 2014 was 39.4 colonies L^{-1} at the resident's dock (LP2) on July 17th. The calculated potential for TP transferred from the benthos to the sediment by *G. echinulata* is 34.7 µg P L^{-1}. For Great Pond, the largest bloom of 15.1 colonies L^{-1} occurred in the north basin on July 22nd; the potential TP transferred was calculated to be 13.3 µg P L^{-1}. This is a significant source of P, especially in oligotrophic lakes which commonly have TP of <5.00 µg P L^{-1}.

These are preliminary calculations and further research is required to fully understand the role of *Gloeotrichia echinulata* introducing P into the water column from luxury uptake. The initial calculations indicate that the potential for *G. echinulata* to increase P loads in a lake is substantial. For more accurate measurements of P load from recruitment, TP of *G. echinulata* would have to be measured throughout lifecycle stages. There are many factors, such as seasonal variation and lake trophic status that would influence the TP level of *G. echinulata.*

Forsell and Pettersson (1995) found that in Green Lake, Seattle, *Gloeotrichia echinuata* accounted for 2/3 of the lake's phosphorus loading into the water column per day. This was equivalent to a flux of 0.4-0.6 mg P m^{-2} day^{-1}. Likewise, at Lake Erken, Sweden, researchers in 1991 found that *G. echinulata* was responsible for 2/3 of total phosphorus loading for the lake, or 2.4 mg P m^{-2} day^{-1} (Forsell and Pettersson 1995). *G. echinulata* increase the total and organic P released into the lake, which could be a source of nutrients for other phytoplankton (Istvanovics et al. 1993). The potential for recruitment to be a source of P for other phytoplankton raises significant concern for the

Belgrade Lakes because *G. echinulata* could essentially resuspend P that was removed from the lake in previous years (King and Laliberte 2005).

It is important to understand luxury uptake of phosphorus by *G echinulata* because it could influence policy-making decisions on whether to implement water quality management strategies. For example, the transfer of P from the benthos during recruitment could undermine the effects of aluminum sulfate (alum) application. Alum is hydrated aluminum sulfate [$Al_2(SO_4)_3$] that works to mitigate internal P loading by binding loosely bound, or mobile P in the water and sediments by forming an aluminum hydroxide [$Al(OH)_3$] floc (Driscoll and Schecher 1990). If *G. echinulata* are able to bind immobile P in the sediment P or from the aluminum hydroxide floc, they could reintroduce P into the water column. This would undermine aluminum sulfate as a mitigation technique. Alum application is being considered by the lake associations in the Belgrade lakes as a potential water quality treatment for the future (Bruesewitz, pers. comm.). Before these important decisions are made, *G. echinulata*'s influence should be taken into account.

Chlorophyll-a and Pheophytin

Peak *Gloeotrichia echinulata* densities in both Great Pond and Long Pond correspond with peak chlorophyll-*a* and pheophytin levels. Increased chlorophyll-*a* concentrations could be an indicator of nutrient subsidies to phytoplankton from *G. echinulata*. Additionally, *G. echinulata* photosynthesis also contributes to chlorophyll-*a* concentration. In some cases, *G. echinulata* have been shown to increase growth rate, Shannon diversity, and taxa richness of phytoplankton (Carey and Rengefors 2010; Carey et al. 2014b). In general, the similar peaks in *G. echinulata* densities, and pheophytin concentrations are expected. Pheophytin is a pigment used by cyanobacteria in photosynthesis and can be used as an indicator of cyanobacteria growth (Hauer and Lamberti 2007).

Broader Context

Gloeotrichia echinulata is both a driver and a consequence of eutrophication, particularly in low nutrient lakes. Its monitoring and management influences many different aspects of lake systems and neighboring communities. This research looks at the impact of *G. echinulata* on larger lake processes including P cycling and primary

production to inform the scientists and stakeholders about the role of *G. echinulata* in eutrophication. On a community level, lake associations can provide useful citizen science monitoring of harmful algal blooms along with increasing awareness about factors affecting water quality.

Gloeotrichia echinulata is found under a wide range of geographic and environmental conditions, from lakes in North America, to Europe, to Asia (Geng et al. 2005; Carey et al. 2009). Reports of *G. echinulata* blooms in low nutrient lakes have been increasing over the past decade, which is a new trend (Carey et al. 2009). The study of *G. echinulata* in low nutrient lakes has been limited mainly to the Northeastern United States and Sweden (Forsell and Pettersson 1995; Carey et al. 2009). The Belgrade Lakes show similar patterns to the low nutrient Lake Sunapee, New Hampshire, another *G. echinulata* study site (Carey et al. 2009). TP throughout the summer of 2015 in Long Pond and Great Pond matched that of Lake Sunapee, which suggests that in some cases, *G. echinulata* creates similar responses. Overall, *G. echinulata* creates both similarities and differences across lakes, trophic states, and geographic regions (King and Laliberti 2005). More research is needed on a broader scale to understand how different lake characteristics influence *G. echinulata* dynamics.

Implications

Modeling P shifts due to *Gloeotrichia echinulata* at the entire lake level would allow researchers to better understand its role in eutrophication. More research is necessary to measure TP uptake through *G. echinulata* life stages to better model P shifts using density data. Furthermore, more research is necessary to understand the interaction between *G. echinulata* and aluminum bound P. Previous studies of alum with *G. echinulata* show that alum treatment has no effect on *G. echinulata* recruitment or bloom formation (Sonnichsen et al. 1997), which could indicate that alum treatment does not inhibit P assimilation by *G. echinulata*. Additionally, it is possible that lakes can shift between stable states, from oligotrophic to mesotrophic to eutrophic, as a result of cyanobacterial blooms (Cottingham et al. 2015). More research on the influence cyanobacteria on the P cycle is needed to understand the potential for *G. echinulata* inducing a shift in stable states in the Belgrade Lakes.

Moreover, microcystin-LR toxin levels need to be investigated at every life stage to prevent dangerous human exposure during blooms. Toxin levels are especially important for other lakes in Maine such as Lake Auburn in the Lewiston-Auburn area that are used as a drinking water source. More information on toxin levels is needed to protect lake users and reservoir watersheds from spikes in toxin levels, especially during summer blooms.

Conclusion

Harmful algal blooms such as *Gloeotrichia echinulata* are becoming increasingly common in freshwater ecosystems globally (Ho and Michalak 2015). *G. echinulata* is an interesting cyanobacteria that has ecological, social, and economic implications. There is more to learn about *G. echinulata* including its role the P introduction, its life cycle, and the toxicity of microcystin-LR. Comprehensive management schemes will need to be implemented to control the progression of *G. echinulata*, especially in oligotrophic lakes (Ho and Michalak 2015). With increasing reports of *G. echinulata* blooms in recent decades in low nutrient lakes in New England, Colby's research efforts are important in monitoring density changes, reducing excess nutrient loads, and measuring toxin levels (Carey et al. 2008, 2012, 2014a).

Possible water quality remediation efforts on Long Pond and Great Pond are not possible without first understanding the nutrient dynamics that *Gloeotrichia echinulata* imposes on these specific ecosystems (King and Laliberti 2005). This research is part of a larger project of measuring nutrient dynamics in the Belgrade Lakes with a focus on *G. echinulata*. The long-term goal is to understand *G. echinulata's* life cycle, nutrient dynamics, and role it plays in water quality remediation. As this research shows, *G. echinulata* in the Belgrade Lakes could be affecting internal P loading, phytoplankton community fluctuations, and water safety concerns for humans. With climate temperatures on the rise and possible increases in external nutrient inputs, cyanobacterial blooms will continue to increase posing an increasing threat to human health, ecosystem functioning, and recreational use on the lake.

CHAPTER IV: LITERATURE CITED

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