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Contrasting Patterns of Nutrient Limitation in the Littoral and Pelagic Zones of Mesotrophic Maine Lakes

Grace C. Neumiller

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Contrasting Patterns of Nutrient Limitation in the Littoral and Pelagic Zones of Mesotrophic Maine Lakes

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**A thesis submitted to the faculty of the Environmental Studies Program in partial
fulfillment of the graduation requirements for the Degree of Bachelor of Arts with
honors in Environmental Studies**

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ABSTRACT

Intense nutrient loading of nitrogen (N) and phosphorus (P) causes sudden regime shifts in freshwater ecosystems from clearwater to turbid conditions with frequent cyanobacterial blooms. Characterization of nutrient limitation patterns of primary productivity in these ecosystems is necessary for effective management of algal blooms. However, much of this research has focused on pelagic habitats. The influence of lake habitat (i.e. benthic littoral versus pelagic zones) on nutrient limitation of primary production in mesotrophic lakes is largely unknown, particularly in contrast to research on pelagic nutrient limitation in eutrophic systems. Using paired nutrient diffusing substrata and mesocosm experiments, we measured chlorophyll-*a* (chl-*a*) concentrations in response to 4 nutrient treatments (N, P, N + P, Control) to assess nutrient limitation patterns in littoral and pelagic zones of two temperate, mesotrophic lakes in late summer, prior to the fall mixing period (October 2020). While both lakes are mesotrophic, China Lake has approximately 2.18 times higher overall average [chl-*a*] in the water column than Great Pond in this late stratification period. In the pelagic zone, China Lake phytoplankton are colimited by N and P, while Great Pond phytoplankton are P limited. In the benthic zone, China Lake periphyton are serially limited by N then P, and Great Pond periphyton are N limited. These data will improve our understanding of nutrient limitation patterns in mesotrophic systems in danger of eutrophication and allow us to incorporate littoral zone production into our understanding of whole lake ecosystem productivity.

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CHAPTER I: LITERATURE REVIEW

What is nutrient limitation?

Nutrient limitation is a controlling factor of primary productivity in freshwater ecosystems. Liebig's Law of the Minimum states that only one element limits the growth of an organism at any given time (Danger et al. 2008). However, primary productivity, or the biomass produced because of photosynthesis performed by autotrophic organisms such as phytoplankton, algae, and plants, can be limited by many factors. Nitrogen (N), phosphorus (P), various micronutrients, and light have all been shown to limit primary productivity (Dolman and Wiedner 2015). In some cases, primary productivity can be colimited, when cells rapidly shift from N to P limitation and 'bounce' back and forth so rapidly that colimitation appears to be at play. Ecological stoichiometry studies the balance of energy and chemical elements in ecological and living systems (Sterner and Elser 2002). Some communities are likely to adjust their stoichiometry to that of their homeostatic resources, resulting in colimitation over a wide array of supplied nutrient ranges (Danger et al. 2008). Indirect drivers of nutrient limitation modify macronutrient concentrations, stoichiometry, or phytoplankton communities, thereby affecting primary productivity (Lewis et al. 2020).

Nutrient limitation is important to study in freshwater ecosystems because N and P are well-documented drivers of primary productivity lakes (Kolzau et al. 2014, Lewis et al. 2020). Anthropogenic inputs of N and P have altered patterns of primary production in freshwater systems, including the prevalence of cyanobacteria harmful algal blooms (Howarth and Marino 2006, Hamilton et al. 2016, Paerl et al. 2016). Intense nutrient loading of N and P in freshwater ecosystems often leads to sudden regime shifts from clearwater to turbid conditions with a high frequency of toxic cyanobacterial blooms (Smith and Schindler 2009, Urrutia-Cordero et al. 2016). Cyanobacterial presence in lakes, rivers, and reservoirs is a worldwide environmental health issue because some cyanobacterial strains produce toxins that are detrimental to human and ecosystem health (Bláha et al. 2009, Teixeira et al. 2010, Paerl and Otten 2013). Successful management of

lakes depends upon understanding the spatial variability and drivers of nutrient limitation of phytoplankton and periphyton primary productivity (Lewis et al. 2020).

Brief history of nutrient limitation paradigms in lakes

While nutrient limitation is a controlling factor of primary productivity in lakes, there have long been debates about which nutrient (specifically N or P) is the most important limiting nutrient. Broadly, a longtime focus on P limitation and widely accepted evidence that phosphorus and phytoplankton biomass have a linear relationship (Dillon and Rigler 1974) have now shifted towards a modern-day focus to include N limitation and colimitation of primary productivity (Lewis and Wurtsbaugh 2008). An emphasis on P limitation started with the whole-lake experimental work of David Schindler in the 1970s, showing that P limitation overwhelms any potential for N limitation over large spatial-temporal scales (Schindler 1974, 1977). The P limitation focus also became more dominant with the assumption that biological N-fixation can always fulfill the demand for N in primary productivity, causing more systems to be P limited (Abell et al. 2010) since P has no gaseous atmospheric cycle or biological mechanisms to address deficiencies (Schindler 1977).

Not all phytoplankton can fix nitrogen, but some cyanobacterial taxa can. The relative proportion of cyanobacteria present can depend upon the relative ratio of total nitrogen to total phosphorus. For example, through an analysis of 17 lakes throughout the world, a measured TN:TP ratio that exceeded 29:1 by weight resulted in a rarity of cyanobacteria, indicating P as the limiting nutrient of cyanobacterial growth (Smith 1983). Relationships between log-P and log-chlorophyll-*a* can also be sigmoidal (McCauley et al. 1989), suggesting more complicated drivers of lake productivity than simply P limitation. Chlorophyll-*a* concentrations are affected by N concentrations when P concentrations are high, showing that mechanisms affecting phytoplankton biomass change depending on lake conditions (McCauley et al. 1989).

Shifts in the nutrient limitation paradigm from P to N limitation are largely due to atmospheric N deposition that changed the N:P stoichiometric ratio in lakes around the world, indicating altered patterns of phytoplankton nutrient limitation (Howarth and

Marino 2006, Elser et al. 2009). Under low N deposition, phytoplankton growth is generally N limited; however, in high N-deposition lakes, phytoplankton growth is consistently P limited (Elser et al. 2009). The relative magnitude of N loss through denitrification and gain through N₂ fixation is an important barometer for the impact of combined N pollution in aquatic ecosystems (Paerl et al. 2016). Due to widespread anthropogenic emissions of reactive N through the creation of synthetic fertilizer and the combustion of fossil fuels in the 1970s, some estuarine scientists began to concentrate on N as the cause of eutrophication in their studied ecosystems (Galloway et al. 2004). The 1980s saw a continued emphasis on N limitation in temperate estuaries, but research on the N limitation of estuarine eutrophication had little to no influence on the development of water quality management at national scales throughout the 1980s (Howarth and Marino 2006). Throughout the 1990s, there was a growing trend toward a scientific consensus for the need to control N inputs to coastal marine ecosystems due to increasing global inputs of atmospheric N (Howarth & Marino 2006). More recent nutrient enrichment experiments across scales (i.e. bottle bioassays, mesocosms, and whole lakes) show that nitrogen limitation is globally as common as phosphorus limitation (Tank and Dodds 2003, Lewis et al. 2011, 2020).

Despite the growing evidence of N limitation in the late 20th century, implementing N management practices was rare because (1) the control of phosphorus pollution is typically more feasible and cheaper than the control of nitrogen pollution, and (2) P limitation can be induced by aggressive P control, even if algal populations are initially limited by nitrogen deficiency (Lewis and Wurtsbaugh 2008). Additionally, a 37-year whole-ecosystem experiment that fertilized a Canadian lake with N and P (with the share of N decreasing over the years) found that the prevalence of N-fixing bacteria caused chlorophyll-*a* to be proportional to P no matter the amount of nitrogen added to the ecosystem (Schindler et al. 2008). However, especially on short time scales, both N and P have been found to be important drivers of phytoplankton dynamics, and in-lake and catchment characteristics play an important role in determining nutrient limitation status (Tank and Dodds 2003, Lewis et al. 2020). Indeed, characteristics of lakes themselves, such as temperate and tropical climates, mixing regimes, morphology and basin shape, water residence time, etc. all play a role in shaping nutrient limitation

dynamics (Harpole et al. 2011). Arguments against N control based on short-term bottle effects (Schindler et al. 2008) do not hold up under greater scrutiny of results and the consideration of whole-lake experiments, small scale bioassays, and larger-scale incubations (Fee 1979, Paerl et al. 2016).

Another question to consider is the temporal scale of limitation. When nutrient limitation can occur on a scale of minutes, months, seasons, and years, how can we determine which scales of limitation are ecologically relevant, or relevant in terms of management? The nuances of the nutrient limitation debate are important to consider, because although nutrient limitation might seem simple on the surface, the literature shows that even after decades of research, these questions still have not been definitively resolved. It is necessary to be aware of the complexities and contradictions of scale, habitat, watershed, and in-lake dynamics that affect patterns of nutrient limitation (Harpole et al. 2011, Lewis et al. 2020).

Why do lake trophic states matter ecologically and in terms of bloom management?

Classifications of the autotrophic trophic state of lakes are based upon links among nutrient concentrations, phytoplankton biomass, and productivity to describe water quality (Whiles and Dodds 2010). Generally, oligotrophic lakes have low algal biomass, low nutrient content, low algal productivity, high clarity, and deep photic zones. Eutrophic lakes frequently contain cyanobacterial blooms, high total nutrients, large variation in O₂ concentrations, frequent anoxia in the hypolimnion, and frequent fish kills (Whiles and Dodds 2010). Mesotrophic lakes are characterized by moderate water clarity, nutrient concentrations, and phytoplankton biomass. They are sensitive to algal blooms since they have enough nutrients to support a bloom and can be pushed to a higher trophic state (Whiles and Dodds 2010). Conditions such as high nutrient levels, water temperatures, and pH; low light availability, dissolved CO₂, and relative availability of nitrogen and phosphorus; and a stable water column could all put mesotrophic lakes at risk for cyanobacterial harmful algal bloom events (Johnston and Jacoby 2003). Conversely, mesotrophic lakes may be managed to move towards oligotrophic conditions more easily than a eutrophic system. Phytoplankton growth in mesotrophic lakes can be

limited by N, P, both nutrients, or a combination of all 3 scenarios at different points in time (Ding et al. 2019, Bratt et al. 2020, Lewis et al. 2020). Excessive nutrient inputs can lead to harmful algal blooms and an altered stable state in mesotrophic lakes (Whiles and Dodds 2010). Therefore, monitoring chlorophyll-*a* dynamics and nutrient inputs in mesotrophic lakes is important to prevent harmful algal bloom events and a permanent shift to a eutrophic stable state. In terms of management, eutrophic systems have been most heavily studied (Qin et al. 2010, Paerl et al. 2014, 2016), so it is important to expand upon mesotrophic lake research to inform management policies.

Oligotrophic Lakes

In oligotrophic lakes, the demand for nutrients can easily exceed the supply of nutrients, and water conditions are often clear with low turbidity. Phytoplankton biomass accumulation is as likely to be limited by N as P, even in unpolluted oligotrophic lakes (Lewis et al. 2011). In oligotrophic boreal and alpine lakes, TN:TP mass ratios explain less than 30% of the variation in bioassay phytoplankton growth response (Bergström 2010). Thus, TN:TP was a poor predictor for discerning whether phytoplankton was N or P limited in these systems. In oligotrophic lakes, phytoplankton biomass calculated from the concentration of chlorophyll-*a* are overestimates of true phytoplankton biomass, likely due to phytoplankton cell size and species present in oligotrophic lakes, which can affect how much chlorophyll-*a* is found per unit of mass (Kasprzak et al. 2008). This is important in the context of my research because my experimental response variable is chlorophyll-*a* concentration, as a proxy for phytoplankton biomass.

Mesotrophic Lakes

In nutrient limitation literature, there is a general lack of research on nutrient limitation patterns in mesotrophic lakes. The limited data on mesotrophic systems show that they tend to range in limitation status from P limited, N limited, simultaneously colimited, independently colimited, and serially-limited by N and P (Lewis et al. 2020). Simultaneous colimitation occurs when phytoplankton or periphyton respond to the interaction effect between N and P additions, and there are either no main effects from

either nutrient, or main effects from both nutrients (Lewis et al. 2020). Independent colimitation occurs when both nutrients enhance productivity, but there is no interaction between nutrients (Lewis et al. 2020). Serial limitation occurs when there is a main effect on primary productivity from one nutrient, along with a significant interaction between both N and P (Lewis et al. 2020). Due to ecological feedbacks, ecosystems display resistance to state shifts and tend to remain in one state unless perturbations are large enough to shift them into an alternate stable state (Whiles and Dodds 2010). Hysteresis effects during lake disturbance events (i.e. storms) cause changes in the concentration and discharge of nutrients (Hall 1970, Casey and Newton 1973). During hysteresis events, mesotrophic systems are in danger of tipping towards eutrophic or oligotrophic states. To prevent eutrophication of mesotrophic systems, it is important to research nutrient limitation patterns in these understudied systems.

Eutrophic Lakes

Eutrophic lakes have high nutrient content, high turbidity, and are dominated by algae (Whiles and Dodds 2010). Generally, we see that eutrophic systems are P-limited (Vanni and Temte 1990, Hamilton et al. 2016, Paerl et al. 2016). While chl-*a* varies with total nitrogen (TN) and total organic phosphorus (TOP), TN-chl-*a* relationships vary depending on TP. In hypereutrophic lakes above a certain nutrient threshold, TN is not related to chl-*a*, emphasizing the fact that in lakes with extreme nutrient concentrations due to agricultural pollution, chl-*a* might not be a useful predictor of phytoplankton biomass (Filstrup and Downing 2017). In eutrophic lakes, phytoplankton biomass numbers calculated from chl-*a* are underestimates of biomass, likely because cyanobacteria have lower levels of chl-*a* than green algae, which have high concentrations of chl-*a* (Kasprzak et al. 2008). Once an algal bloom begins, different nutrient limitation dynamics can come into play. While many areas of research study initial nutrient limitation patterns of algal blooms, an emerging area of research explores whether primary producers have the N and P they need to sustain the bloom. For instance, labile organic N recycling (in combination with lake morphology and water movement) is critical for the maintenance of non N-fixing cyanobacterial harmful algal

blooms (Gardner et al. 2017). In eutrophic systems with a lot of primary production, where cyanobacteria dominate, there are periods of P limitation that are sustained by N-fixing cyanobacteria (Vitousek et al. 2002). However, this may not be the case year-round, since phytoplankton community composition changes seasonally (Azari et al. 2011).

Algal Bloom Management

Because mesotrophic systems have the propensity to tip towards a eutrophic state, it is important to understand nutrient limitation patterns in these systems to inform management strategies. Algal bloom management strategies either control nutrients at their source in the catchment (preventative management) or mitigate nutrients effects with in-lake strategies (mitigative management) (Whiles and Dodds 2010). Catchment-level methods prevent nutrients, like phosphorus and nitrogen, from entering lakes and triggering cyanobacterial bloom events in the first place—an essential condition to initiate lake restoration and reduce cyanobacterial dominance over the long term (Stroom and Kardinaal 2016). In order to control nonpoint source pollution (i.e. agricultural runoff, urban storm runoff systems, disturbance of watersheds, atmospheric deposition) from entering aquatic systems, policies can be implemented that lower the amount of fertilizer used, time fertilizer applications to reduce flow into aquatic systems, establish erosion-control strategies (i.e. maintaining riparian vegetation and minimizing exposed soil), and restore natural vegetation (Whiles and Dodds 2010). Laws can also be written that specifically regulate point source pollution from specific sources, like a municipal sewage treatment plant or a factory. Removing phosphorus from waste streams can involve chemical treatments with alum or Fe^{3+} to precipitate the phosphate, which is then allowed to settle and the low-phosphorus water is released (Robb et al. 2003, Haghseresht et al. 2009, Whiles and Dodds 2010). Efforts to prevent harmful cyanobacterial blooms must ultimately be directed towards sources of external nutrient loading, whether they are agricultural, related to urban landscape management, or coincident with wastewater treatment (Bullerjahn et al. 2016). The most effective ways to reduce external N and P loading via diffuse non-point sources are vegetative riparian buffers, wetlands for treating

and removing nutrients, and retention ponds for impervious runoff sources (Castelle et al. 1999). These buffer zones aid in the cultivation and stimulation of macrophytes, which can help absorb excess nutrients (Jeppesen et al. 2007, Kuglerová et al. 2020).

Biomanipulation, the deliberate alteration of lake ecosystems by adding or removing species, is a promising tool for improving ecosystem resilience against harmful cyanobacteria (Jeppesen et al. 2007, Urrutia-Cordero et al. 2016). Top-down trophic control of blooms by stocking herbivorous (and specifically cyanobacteria-consuming) fish and shellfish species in lakes can reduce the abundance of cyanobacteria in water bodies (Jeppesen et al. 2007). The success of biomanipulation seems to increase in geographical regions at higher latitudes, because fish biodiversity, omnivory, and density is generally higher in the tropics compared to temperate systems (Urrutia-Cordero et al. 2016). Additionally, planting macrophytes in lakes can remove nutrients like P and N from the freshwater and control cyanobacterial blooms (Wang et al. 2012), although this strategy is critically-dependent on lake morphology. Lower-tech mitigation options, such as cleaning up biofilm scum on lake surfaces or dispersing the blooms, do not stop algae bloom formation, but can reduce health risks associated with the blooms (Stroom and Kardinaal 2016).

Other mitigative management approaches manipulate physical factors in aquatic systems to reduce cyanobacterial bloom proliferation. Some mitigative approaches include precipitating, binding, and immobilizing nutrients in benthic sediments (Robb et al. 2003, Haghseresht et al. 2009). The addition of aluminum sulfate (alum) to lakes has been attempted to control algal blooms, but large doses of alum treatment can damage cyanobacterial *Microcystis* cells, causing the release of harmful cyanotoxins (Han et al. 2013). Altering physical factors that cause cyanobacterial competition versus other eukaryotic phytoplankton can help reduce cyanobacterial bloom effects (Han et al. 2013). Vertical mixing devices, bubblers, and other means of breaking down stratification and decreasing water residence time can control outbreaks and the persistence of harmful cyanobacterial blooms (Paerl and Otten 2013). This is because artificial mixing can lead to a shift in phytoplankton composition from cyanobacterial dominance to green algae and diatoms if the imposed mixing is strong enough to keep cyanobacteria entrained in the turbulent flow (Visser et al. 1996, Mitrovic et al. 2003, Stroom and Kardinaal 2016).

Although often successful, sometimes flushing the water column can alter circulation regimes of receiving water bodies, thus trapping cyanobacterial blooms in the system instead of flushing them out (Huisman et al. 2004). Additionally, in-lake management strategies are expensive and sometimes ineffective, leading to their abandonment and raising questions about their long-term sustainability (Qin et al. 2010). Although biomanipulation has proven efficient in many systems, the outcome is influenced by a lake's trophic status (Hamilton et al. 2016) and geographical region (Paerl et al. 2011a). In-lake manipulation methods have high costs and, in the case of geoengineering, risk being largely ineffective, producing unforeseen outcomes, and causing ecotoxicological effects from the geoengineering material itself (Urrutia-Cordero et al. 2016). Due to their symptom-based approaches, in-lake management actions are unlikely to be propagated in more than a few key lakes (Mackay et al. 2014), undermining their efficacy on large spatial scales.

To improve harmful algal bloom management programs, the general public must be aware of the importance of watershed preservation, value management efforts, and be integrated into management planning and execution (Hamilton et al. 2016). Successful long-term bloom management must consider the human and climatic factors controlling blooms and their impacts on water supply in lakes threatened by accelerating eutrophication, utilizing a combination of preventative and mitigative management tactics (Piehler 2008, Stroming et al. 2020). Understanding nutrient limitation patterns in understudied mesotrophic systems can provide insight into which bloom management strategy would be most effective in a particular system.

How do habitat types (littoral and pelagic zones) impact nutrient limitation patterns?

Most nutrient limitation literature focuses entirely on primary production limitation patterns in lake pelagic zones. However, benthic algae in lake littoral zones can account for a large fraction of primary productivity in lake ecosystems (Qin et al. 2010). Benthic algae may differentially respond to nutrient inputs because they can access nutrients from lake sediments (Vadeboncoeur et al. 2008). Further, do littoral and pelagic zones always show the same limitation patterns? If not, how do we incorporate our

understanding of littoral zones into whole ecosystem management? Understanding nuances in nutrient limitation patterns between the littoral and pelagic zones of lakes is key for the effective management of these systems.

Pelagic Zone

Although most nutrient limitation literature focuses on lake pelagic zones, the definition of pelagic zones can vary across studies. We usually think about lakes as being represented by samples taken at a pelagic site, often at the deepest part of a lake. However, is the pelagic epilimnion representative of an entire lake ecosystem? In terms of productivity and nutrient limitation, phytoplankton are the key players in the pelagic habitat. Sometimes harmful algal blooms can happen in the pelagic zone, although they often blow into shallow bays, affecting the littoral zone.

Pelagic zone primary productivity is impacted by a variety of factors. Lake stratification in the pelagic zone affects phytoplankton distribution and nutrient uptake (Tank and Dodds 2003, Fork et al. 2020). Phytoplankton must stay in the epilimnion for light availability, but nutrients can become depleted as the summer progresses (Salmaso and Tolotti 2020). In the autumn, the epilimnion deepens, allowing sinking phytoplankton taxa to benefit from reaching water layers with higher nutrient availability (Wetzel and Likens 2001). Convective water movements triggered by autumn night cooling increase the thickness of the epilimnetic mixed layer, deepening the metalimnion towards the nutrient-rich hypolimnetic water layers (Yoshiyama 2002). Phytoplankton entrained in the sinking epilimnion can benefit from reaching water layers with higher nutrient availability, maintaining a high growth rate until late autumn (Whiles and Dodds 2010). Some cyanobacteria can even control their position in the water column to evade predators and access nutrients year-round (Salmaso and Tolotti 2020, Aguilo-Ferretjans et al. 2021).

Nutrient limitation patterns vary across pelagic zones in different lake ecosystems. In a suite of microcosm incubation experiments (mirroring my own microcosm experiment methods) across 16 study lakes in the Northeastern U.S., a high prevalence of colimitation, serial limitation, and approximately equal frequency of single nutrient N or P limitation was found (Lewis et al. 2020). Both regional and local lake-

specific drivers explain the observed variation in nutrient limitation types. The prevalence of colimitation and serial limitation can be explained by (1) additional nutrients of one type allowing individual phytoplankton cells to access previously unavailable nutrients and (2) facilitation of colimitation at the community level when various phytoplankton taxa are limited by different nutrients (Lewis et al. 2020). Longitude is the most important factor explaining patterns of nutrient limitation in these study lakes; for instance, the three most western lakes are all N-limited since they have the highest watershed agricultural land among the study lakes (Lewis et al. 2020). These findings are particularly important in the context of my research, as this study researched nutrient limitation patterns in the pelagic zones of 3 mesotrophic Maine lakes, including one of my study lakes (Great Pond). Machine learning analysis of data from 1382 lakes across the U.S. has found that phytoplankton P-limitation is more likely under oligo-mesotrophic or eutrophic conditions and that colimitation of N and P occurs under hypereutrophic chlorophyll-*a* conditions (Liang et al. 2020).

Littoral Zone

The littoral zone is the area near the lake shore where sunlight penetrates to the sediment, allowing aquatic plants (macrophytes) and algal biofilms (periphyton) to grow. Key primary producers in the littoral zone are benthic biofilms, like attached algae and bacteria, that utilize light reaching the lake bottom for primary productivity. Generally, there has been less work on primary production in lake littoral zones, so here I will also draw upon stream literature to provide background on benthic zone nutrient limitation.

While nutrient limitation studies on lake littoral zones are few, some studies have shown varied periphyton nutrient limitation responses based on substratum type, water depth, light availability, dissolved organic matter (DOM) content, and nutrient availability in the benthos (Liang et al. 2020). The response of periphyton to water-column N and P additions may depend on whether the water column, the substratum, or the sediment pore water is the primary nutrient source (Vadeboncoeur and Lodge 2000, Rodusky et al. 2001, Fork et al. 2020). During a whole-lake fertilization experiment, periphyton on wood increased primary production, while periphyton on sediment did not

(Sand-Jensen and Borum 1991). Periphyton in the littoral zone can also be primarily limited by the same variables and nutrients limiting phytoplankton in the pelagic zone (Vadeboncoeur and Lodge 2000). When water depths are low enough for sunlight to reach the sediments, the increased light regime in the water column may enhance periphyton growth. Under this scenario, the ability of periphyton to compete with phytoplankton for nutrients may be enhanced, possibly resulting in decreased algal bloom frequency in the ecotone (Rodusky et al. 2001). Additionally, in shallow northern lakes, nutrient enrichment of benthic algae has revealed widespread N limitation with decreasing severity in lakes with higher DOM content (Rodusky et al. 2001). Moderate increases in DOM have the potential to increase benthic primary production, especially for epilithic algae (Fork et al. 2020). Depth ratio and light attenuation strongly determines the maximum possible contribution of benthic algae to lake primary production (Vadeboncoeur et al. 2008), and the benthic proportion of whole-lake primary production has been modeled to decline with increasing nutrients (Fork et al. 2020).

Periphyton responses to nutrient limitation in the benthic zones of streams have been found to vary spatially on smaller spatial and temporal scales. In montane, headwater streams, periphyton responses to nutrient diffusing substrata experiments vary spatially on small and medium scales (Vadeboncoeur et al. 2008). The N + P treatment significantly increased chlorophyll-*a* accrual in three of five study creeks, and the presence/absence of N significantly affected benthic limitation patterns (Irvine and Jackson 2006). N limitation (either N alone or N with P) was the most common response for epilithic algal biofilms in the benthos of 10 North American streams (Irvine and Jackson 2006). In one study, P was never the sole limiting nutrient for epilithic benthic algae (Tank and Dodds 2003).

Nutrient limitation findings in the littoral zone can be impacted by the type of nutrient diffusing substrata (NDS) experiment employed. The three most common NDS methods utilize clay pots, plastic cups, and periphytometers (artificial substrates) as vessels for nutrient diffusion (Tank and Dodds 2003). In an NDS experiment comparing all three methods in a single stream, chlorophyll-*a* biomass is significantly higher for N + P treatments across all NDS methods, except for clay pots when the N:P ratio is 16:1 (Capps et al. 2011). On plastic cups, chlorophyll-*a* biomass is significantly greater in N

treatments than in control treatments, indicating primary N limitation of the stream benthos. Plastic cups are found to have the highest nutrient diffusion rates (in both N and P treatments) (Capps et al. 2011). These findings are important in the context of my study because I utilized the plastic cup NDS method to measure nutrient limitation in the littoral zones of China Lake and Great Pond.

Conclusion

Researching patterns of nutrient limitation in lakes across the trophic spectrum and in different lake habitats is important to understand drivers of primary productivity. Release from nutrient limitation often leads to higher productivity, creating conditions that favor harmful cyanobacterial blooms. The most promising management tool of primary productivity in many situations is the restriction of P supply (Capps et al. 2011) because it is largely assumed that N limitation can be compensated for by N₂-fixing cyanobacteria (Lewis and Wurtsbaugh 2008, Stroom and Kardinaal 2016). However, a review of the literature has shown that different patterns of nutrient limitation are possible across lake habitats and trophic states. Patterns of N limitation in lake and stream benthos are especially prevalent, indicating a need to manage both N and P inputs to mitigate harmful algal blooms. Dual nutrient control has multiple advantages, including controlling nonpoint and background P sources and reducing selective control over species composition and diversity of aquatic communities (Schindler et al. 2008, Stroom and Kardinaal 2016).

This review of nutrient limitation literature highlights a lack of research in mesotrophic systems and in littoral lake habitat zones. Through my research in two mesotrophic Maine lakes, I hope to shed light onto our understanding of nutrient limitation patterns in mesotrophic systems. For instance, do these systems often appear to be ‘tipping’ towards a eutrophic state? If so, how can understanding nutrient limitation in mesotrophic systems help us to prevent eutrophication? In addition, by studying nutrient limitation patterns between the littoral and pelagic zones of China Lake and Great Pond, I hope to help incorporate littoral zones into our understanding of whole lake ecosystem productivity.

CHAPTER II: EXPERIMENTAL RESEARCH

Introduction

Primary productivity in aquatic ecosystems can be limited by many factors. Nitrogen (N), phosphorus (P), micronutrients, and light have all been shown to limit primary productivity (Dolman and Wiedner 2015). Direct and indirect drivers of nutrient limitation (i.e. scale, habitat, watershed, and in-lake dynamics) can modify macronutrient concentrations, stoichiometry, and phytoplankton communities, thereby affecting primary productivity (Lewis et al. 2011). Intense nutrient loading of N and P often leads to sudden regime shifts in freshwater ecosystems, such as a shift from clearwater states to turbid conditions with a high frequency of harmful cyanobacterial blooms (Smith and Schindler 2009, Urrutia-Cordero et al. 2016). Cyanobacterial presence in lakes, rivers, and reservoirs is a global environmental health issue because some cyanobacterial strains produce toxins that are detrimental to human and ecosystem health (Bláha et al. 2009, Teixeira et al. 2010, Paerl and Otten 2013). Locally in Maine, harmful algal blooms have contributed to declining water clarity over decadal timescales (1995-2010), as 79 formerly mesotrophic lakes have become eutrophic and 64 previously oligotrophic lakes have become mesotrophic (Harpole et al. 2011, Lewis et al. 2020). Successful management of harmful algal blooms in lakes depends on understanding spatial variability and direct and indirect drivers of nutrient limitation of phytoplankton and periphyton primary productivity (McCullough et al. 2013).

Mesotrophic lakes are sensitive to algal blooms since they have enough nutrients to support a bloom and can be pushed to a higher trophic state (Whiles and Dodds 2010). Conditions such as high nutrient levels, water temperatures, and pH; low light availability, dissolved CO₂, and relative availability of nitrogen and phosphorus; and a stable water column could all put mesotrophic lakes at risk for cyanobacterial harmful algal bloom events (Johnston and Jacoby 2003). Conversely, mesotrophic lakes may be managed to move towards oligotrophic conditions more easily than a eutrophic system. Phytoplankton growth in mesotrophic lakes can be limited by N, P, both nutrients, or a combination of all 3 scenarios at different points in time (Ding et al. 2019, Bratt et al. 2020, Lewis et al. 2020). Monitoring chlorophyll-*a* dynamics and nutrient inputs to

understand tipping points in mesotrophic lakes, then implementing informed action plans to prevent harmful algal bloom events can prevent permanent shifts to eutrophic states. In terms of management, eutrophic systems have been the most heavily studied (Qin et al. 2010, Paerl et al. 2014, 2016), so it is important to expand upon mesotrophic lake research to inform bloom management policies. For instance, implementing watershed-scale strategies to prevent nutrients from entering mesotrophic systems, as well as investing in in-lake mitigative nutrient control strategies, require insight into which nutrients are limiting in a particular system to maximize the efficacy of management policies.

In addition, most nutrient limitation literature focuses entirely on limitation patterns of primary production in lake pelagic zones. However, benthic algae in lake littoral zones can account for a large fraction of primary productivity in lake ecosystems (Harpole et al. 2011, Lewis et al. 2020). Benthic algae may differentially respond to nutrient inputs because they can access nutrients from lake sediments (Tank and Dodds 2003, Fork et al. 2020). Therefore, understanding nuances between nutrient limitation patterns in lake littoral and pelagic zones is essential for the effective management of these systems. This study will be one of the first to measure nutrient limitation patterns in lake benthic and pelagic zones concurrently.

Objectives and hypotheses

To understand how variation in nutrient limitation patterns is affected by habitat type and trophic status, I conducted simultaneous nutrient enrichment mesocosms and nutrient diffusing substrata bioassays to assess the nutrient limitation of chlorophyll-*a*, an indicator of phytoplankton and periphyton biomass, in two mesotrophic Maine lakes. My questions and hypotheses are the following: (1) How do nutrient limitation patterns compare between higher-mesotrophic China Lake and lower-mesotrophic Great Pond? (2) How do nutrient limitation patterns differ in the benthic versus pelagic zones of China Lake and Great Pond? I hypothesize that I will observe differing patterns of nutrient limitation between China Lake and Great Pond due to the higher-mesotrophic status of China Lake and that I will measure varying patterns of nutrient limitation between the benthic and pelagic zones due to contrasting nutrient availability in different lake regions.

My study took place during a cyanobacterial bloom in China Lake. This paired with slightly higher nutrient availability may lead to different patterns of nutrient limitation.

Methods

Study Sites

Our 2 study sites were in two temperate, mesotrophic Maine lakes: China Lake and Great Pond. China Lake is a higher-mesotrophic temperate freshwater lake located in south-central Maine within the towns of China and Vassalboro in Kennebec County (Table 1). China Lake encompasses 3,937 surface acres and is a relatively deep lake with two main drainage basins (Fork et al. 2020). It has a mean depth of 8.5 meters and a maximum depth of 25.9 meters (Appendix Table 1). China Lake has served as the primary drinking water supply for the Kennebec Water District (KWD) since 1905, supplying water to over 44,000 residents in 6 municipalities. The lake watershed is heavily developed on the north and southeastern lake shores with residential homes accessed by gravel camp roads (China Lake 2001). Based on the presence of elevated phosphorus and elevated chlorophyll data, China Lake has been classified as having a very high risk of algal blooms, with algal blooms occurring near-annually (*Maine Lakes at Risk of Having an Algal Bloom* 2021). My research took place near the western shore of a small inlet in China Lake's west basin, at the inlet for the Kennebec Water District. This site is surrounded by a family farm on one side and a small housing development on the other side (Figure 1). We performed the benthic nutrient diffusing substrata experiments in this area. Water for the mesocosm experiment was taken from the pelagic zone of the western shore in the same inlet (Figure 1), and the experiment underwent a one week incubation period in nearby Messalonskee Stream.

Great Pond is a lower-mesotrophic temperate freshwater lake in south-central Maine within the towns of Belgrade and Rome in Kennebec County (Table 1). Great Pond is part of a larger network of the Belgrade Lakes, in which each lake is connected by a network of streams that descend ultimately into the Kennebec River. Great Pond, the largest of the Belgrade Lakes, has an area of 8,533 surface acres and a perimeter of 46.1 miles. The mean depth is 6.4 meters and the maximum depth is ~ 21 meters (Appendix

Table 1). Based on the volume and in/out flow of water in this lake, it takes approximately 2 years for a complete exchange of water ($0.5 \text{ flushes year}^{-1}$) (*Maine Lakes at Risk of Having an Algal Bloom* 2021). The Belgrade Lakes of Maine are better off than other Maine lakes in terms of their water quality, but algal blooms have become more common in recent years (King and Laliberte 2005). Unlike China Lake, Great Pond has not been classified as at-risk for harmful algal blooms (Kelley 2015). The nutrient diffusing substrata experiment incubated near the shore in Hatch Cove, an eastern basin of Great Pond (Figure 1). This eastern shoreline is heavily forested with periodic housing developments. Water for the mesocosm experiment was taken from the pelagic zone in Hatch Cove, and the experiment incubated in nearby Messalonskee Stream.

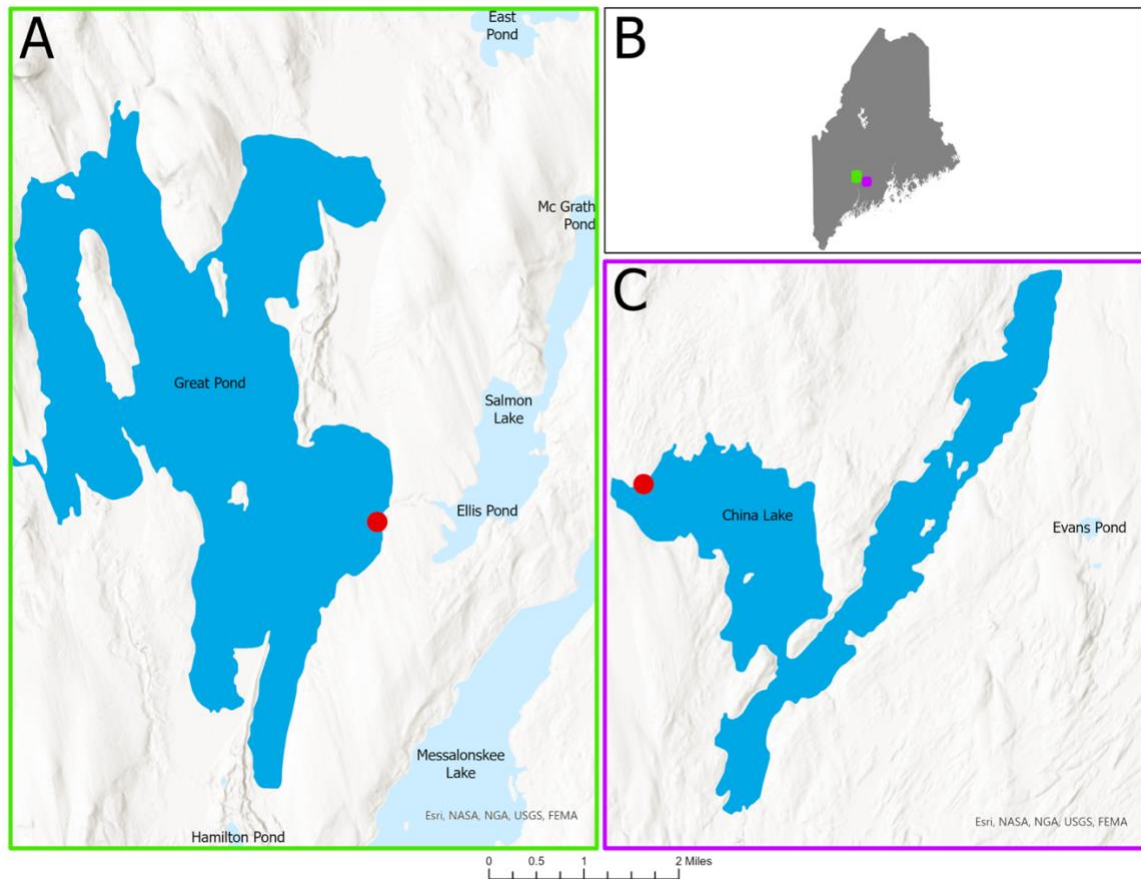


Figure 1. Locations of study lakes within central Maine. A) Great Pond, Belgrade, Rome, Kennebec County, Maine. B) The locations of Great Pond (green) and China Lake (purple) in central Maine. C) China Lake, China, Vassalboro, Maine. Red circles indicate locations of nutrient diffusing substrata experiment incubation and where water was taken for mesocosm experiments.

Table 1. Study sites where mesocosm and nutrient diffusing substrata experiments were conducted within study lakes. Lake and watershed characteristics are also noted. Data sourced from the Maine Lakes Geography and Morphometry dataset from the Lakes of Maine database.

Lake	Great Pond	China Lake
Location	Belgrade, Rome, Kennebec, Maine	China, Vassalboro, Kennebec, Maine
Latitude and Longitude	44.4302452, -69.54731476	44.4302452, -69.54731476
Description	Lower-mesotrophic temperate, freshwater lake	Higher-mesotrophic temperate, freshwater lake
% 500-m buffer in natural land cover	91	70
% 500-m buffer in agricultural land cover	2	14
% 500-m buffer in developed land cover	7	16
% 500-m buffer covered by impervious surface	1.1	3.3
Number of dams on lake	1	1
Lake elevation (m)	75	59
Length of shoreline (m)	65,425	47,040
Shoreline “irregularity index”	3.15	3.37
Mean depth (meters)	6.4	8.5
Max depth (meters)	21	26
NDS Experiment	24	23
Incubation Time (days)		

Mesocosm Experiment

In each study lake, we conducted an *in situ* incubation experiment with factorial N and P amendments to determine the macronutrient limitation of phytoplankton biomass (Figure 2). Experiments were deployed for 1 week beginning 17 October 2020 and

ending 24 October 2020. We prepared 16 ~450 mL microcosms with ~50 mL headspace (6 x 6 inch Bitran bags, Fisher Scientific, USA). We used water collected from 1 m depth and removed larger zooplankton predators with a 125 μm sieve. Each lake had 4 replicates of the following 4 treatments: 0.5 M NH_4Cl (N treatment), 0.5 M KH_2PO_4 (P treatment), both nutrients (N + P treatment), or control (C nutrient amendments). All microcosms were randomly positioned on floating PVC racks and were covered with neutral-density filters to reduce incident light exposure by ~66%, similar to underwater light exposure (Williamson et al. 2010, Lewis et al. 2020) (Figure 2).

Immediately following incubations, we ran a known volume of sampled water from the Bitran bags (ranging from ~300 mL to 400 mL) through a glass fiber filter and stored resulting phytoplankton biofilms on Whatman (Kent, UK) GF/F glass fibre filters (0.7 μm retention) in the freezer. We prepared our samples for the fluorometer approximately 24 hours before analysis. We placed each microfiber glass filter into a flask, added 100 mL of 90% acetone, and shook each solution vigorously before placing the samples into the fridge to thaw gradually. After the samples were in the fridge for ~24 hours, we centrifuged the samples at 300 RPM for 5 minutes to consolidate filter debris at the cuvette bottom out of solution. Using a micropipette, we measured 2.50 mL of sample solution into a glass cuvette, cleansed the cuvette with a Kim wipe, and ran the samples in the 10 AU Turner Designs fluorometer. We diluted the samples so the chlorophyll-*a* concentration values were within range of our fluorometer calibration as needed. After recording the initial chlorophyll-*a* values in $\mu\text{g/L}$, we used a micropipette to add 0.15 mL of 0.1 M HCl acid to the sample, then flicked the sample for 90 seconds to mix the solution. We recorded the chlorophyll-*a* concentration value after the acid addition and repeated the procedure for each sample. We used the equation below to determine the final concentration of chlorophyll-*a* (EPA method 445).

$$[\text{Chl} - a](\mu\text{g L}^{-1}) = \left(\frac{r}{r-1}\right) * (R_b - R_a) * \frac{\text{extraction volume (ml)}}{\text{volume of water filtered (ml)}} * \text{dilution factor},$$

where R_b is the fluorometer reading before acidification of the sample, R_a is the fluorometer reading after acidification, and r is given by R_b/R_a . Acidification of the extracted chlorophyll-*a* converts chlorophyll-*a* to phaeophytin-*a*. The extraction volume

is the volume of the prepared sample that we ran through the fluorometer (10 mL), and the volume of water filtered is the volume (mL) of water that passed through the glass fiber filter apparatus (variable for each sample).

Nutrient Diffusing Substrata Experiment

Nutrient diffusing substrata (NDS) experiment vessels were constructed using 30 mL plastic containers with attached lids filled with a 2% (by weight) agar solution amended with 0.5 M NH₄Cl (N treatment), 0.5 M KH₂PO₄ (P treatment), both (N + P treatment), or not amended as a control (C treatment) (Figure 2). We placed fritted glass disks (r = 1.35 cm) across the tops of the containers to cover the agar completely and serve as inorganic permeable substrata for biofilm colonization (simulating rocks on the lake bottom). The glass frit technique has been commonly used to assess nutrient limitation in the epilithon (Jane and Rose 2018).

Six replicates of each nutrient treatment for inorganic substrata were placed on the stream bottom in plastic racks for 23 days in China Lake and 24 days in Great Pond in October, 2020 (Table 1; Figure 2). Laboratory assays have previously shown that the rate of nutrient diffusion from the 2% agar cups was constant through 17 days and then declined only slightly until day 26 (Tank and Dodds 2003, Tank et al. 2006, Fairchild et al. 2016). At the end of the incubation period, fritted glass disks were removed from the racks and frozen until analysis for chlorophyll-*a* to estimate algal biomass. Chlorophyll-*a* extracts were analyzed fluorometrically following the procedure described above for the mesocosm experiment, but the calculation to determine the final concentration of chlorophyll-*a* on each fritted glass disk was modified to find an area measurement scaled to the glass frit. See chlorophyll-*a* calculation below (Tank and Dodds 2003).

$$[Chl - a](\mu g\ cm^{-2}) = \left(\frac{r}{r-1}\right) * (R_b - R_a) * \frac{\text{extraction volume (ml)}}{SA\ of\ glass\ frit\ (cm)} * \text{dilution factor},$$

where R_b is the fluorometer reading before acidification of the sample, R_a is the fluorometer reading after acidification, and r is given by R_b/R_a. The extraction volume is

the volume of the prepared sample that we ran through the fluorometer (20 mL), and SA is surface area of the fritted glass discs (5.73 cm).

When retrieving NDS racks from Great Pond, one rack was found on its side underneath a dock, missing 5 of its 8 samples and receiving less direct sunlight than the rack with 16 samples. The 3 remaining samples from this rack were subsequently removed from the analysis due to their disproportionately high chlorophyll-*a* concentration values compared to the rest of the samples.

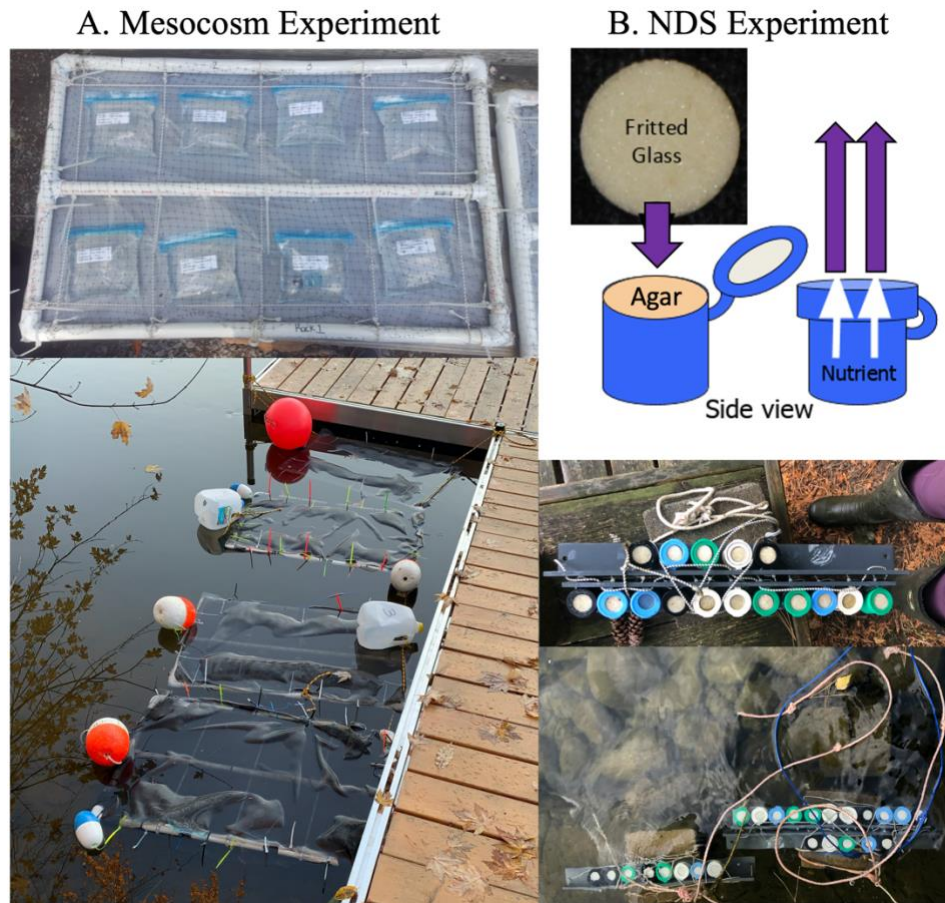


Figure 2. A) Mesocosm experimental set-up to measure pelagic zone nutrient limitation. Each Bitron bag represents a mesocosm with lake water from China Lake and Great Pond and either N, P, N + P, or control treatments (upper left). Mesocosms incubated in nearby Messalonskee Stream for 1 week (lower left). B) Nutrient Diffusing Substrata (NDS) experimental set-up to measure benthic zone nutrient limitation. Side view diagram of cups containing nutrient agar infused with N, P, N + P, or control treatments, and a fritted glass disk placed on top to represent inorganic substrata on the lake bottom. Arrows represent nutrient diffusion from the agar into the surrounding benthic environment (upper right). Racks with NDS cups attached incubated on the lake bottom for ~ 3 weeks in China Lake and Great Pond (lower right).

Data Analysis – Determination of Nutrient Limitation

A paired-samples t-test was conducted to determine whether background chlorophyll-*a* concentration in the pelagic zone of each lake was significantly different than chlorophyll-*a* concentration of the control treatment of the mesocosm experiment.

A two-factor analysis of variance (ANOVA) was used to test whether phytoplankton (mesocosm experiment) and algal biofilms (NDS experiment) were significantly affected by N enrichment (presence or absence of NH_4Cl in treatments) or P enrichment (presence or absence of KH_2PO_4 in treatments) (Arar and Collins 1997). Possible outcomes from the ANOVA on the bioassays are summarized in Table 2. Single nutrient limitation was indicated when just one of the additions (N or P) elicited a positive response, but the interaction term in the ANOVA was not significant. If neither N nor P alone significantly increased biomass ($P > 0.05$), but N and P added together (N + P) increased biomass (ie. the interaction term in the ANOVA was significant, $P < 0.05$), we considered the biofilm to be colimited by both N and P. Equivalently, there could also be colimitation by both N and P if they each stimulated biomass relative to controls when added separately, but the positive N and P responses were not different from each other. Secondary limitation was indicated if N or P alone significantly increased biomass, or if both N and P added together caused an even greater increase in biomass, and the interaction term for the ANOVA was significant (Dubé et al. 1997).

Table 2. Interpretation of responses to N and P addition. A diamond in N or P treatment indicates a significant N or P effect in the two-way ANOVA ($P < 0.05$) and a diamond in the N x P treatment indicates a significant interaction between the two treatments. (Modified from (Tank and Dodds 2003) and (Tank and Dodds 2003)).

Interpretation	N effect	P effect	Interaction N x P
N limited	◇		
P limited		◇	
N and P colimited			◇
N and P colimited	◇	◇	
N and P colimited	◇	◇	◇
1° N limited, 2° P limited	◇		◇

1° P limited, 2° N limited	◇	◇
Not limited by N or P		

The limitation status of a particular nutrient is partly due to the statistical power of the experiment (Harpole et al. 2011). Because of this, we also compared the relative magnitude of the response of phytoplankton or algal biomass to nutrient addition between treatment groups. Adapting the approach of Tank & Dodds (2003), the treatment response data were re-expressed by calculating the ratio of the treatment (N, P, or N + P) relative to the control. For example, for the N addition treatment, the N biomass response would be *nitrogen chlorophyll-a response mean/control chlorophyll-a response mean*. Through this method, the response variable (chlorophyll-a) was normalized across lake habitat zones by scaling the mean treatment response (to N, P, or N + P) relative to the control treatment (N : C, P : C, N + P : C). Higher ratios correspond with a greater phytoplankton or periphyton response to nutrient addition. We used ANOVA to compare the biomass responses to nutrient addition in the pelagic zones of each study lake, in the benthic zones of each study lake, between the benthic and pelagic zones within China Lake, and between the benthic and pelagic zones of Great Pond. Tukey HSD *post-hoc* tests followed significant ANOVA ($P < 0.05$) to differentiate between biomass responses. Linear regression was used to test for significant relationships between background annual mean chlorophyll-a data and annual mean TP data in each lake.

Results

Ensuring Chlorophyll-a on GF/F Filters is Representative of In-situ Chlorophyll-a Content

In China Lake and Great Pond, control treatment chlorophyll-a concentration on GF/F filters was not significantly different from ambient chlorophyll-a in the water column (Paired T-test, $P > 0.05$, Table 3). Therefore, the one-week mesocosm experiment incubation time resulted in representative phytoplankton biomass in the water column. Background epilithic biofilm samples from rocks on the lake bottom were not taken, so data are limited to compare chlorophyll-a concentrations to ambient levels in the benthic zone.

Table 3. Results of paired t-test between mean background pelagic chlorophyll-*a* values and mean chlorophyll-*a* values from the control treatment of the mesocosm experiment in each study lake. Background benthic algal biofilms were not sampled, so no t-test was run for these data in the benthic zone.

Study Lake	Paired T-test Results			
	t	df	p-value	Mean of differences
China Lake	1.937	2	0.192	2.051
Great Pond	2.850	2	0.104	0.914

Nutrient Limitation of Phytoplankton in Pelagic Zone

Chlorophyll-*a* concentrations varied between China Lake and Great Pond in the pelagic zone (Figure 3). In China Lake, chlorophyll-*a* ranged from a minimum of 0.13 $\mu\text{g L}^{-1}$ for the control treatment to 12 $\mu\text{g L}^{-1}$ for the N + P treatment (Figure 3). In Great Pond, chlorophyll-*a* ranged from 0.059 $\mu\text{g L}^{-1}$ for the control treatment to 3.1 $\mu\text{g L}^{-1}$ for the P treatment (Figure 3).

To examine nutrient limitation patterns, we tested for a significant phytoplankton response to nutrient addition using two-way ANOVA (Table 4). In China Lake, the pelagic zone was simultaneously colimited by both N and P; in Great Pond, the pelagic zone was P limited (Figure 3; Table 4). All observed nutrient limitation patterns were highly significant ($p\text{-value} < 0.001$; Table 4).

Nutrient Limitation of Periphyton in Benthic Zone

In the benthic zone of China Lake, the lowest concentration of chlorophyll-*a* occurred in the P treatment (790 $\mu\text{g cm}^{-2}$), whereas chlorophyll-*a* concentration was highest in the N + P treatment (4,200 $\mu\text{g cm}^{-2}$) (Figure 3). In Great Pond, chlorophyll-*a* ranged from 430 $\mu\text{g cm}^{-2}$ for the control treatment to 1,100 $\mu\text{g cm}^{-2}$ for the N treatment (Figure 3).

Based on two-way ANOVA test interpretations, benthic nutrient limitation patterns differed from pelagic zone nutrient limitation patterns (Table 4). In China Lake, benthic zone periphyton was serially limited (primarily by N and secondarily by P) ($p\text{-value} < 0.001$; Table 4). In Great Pond, benthic zone periphyton was N limited, although

this limitation pattern was less significant than other observed patterns (p -value = 0.041; Table 4; Figure 3).

Overall Nutrient Limitation Patterns

Overall, between China Lake and Great Pond we see contrasting patterns of nutrient limitation based on habitat zone. The pelagic and benthic zones of China Lake are colimited by N and P (pelagic zone is colimited simultaneously by both nutrients; benthic zone is serially limited by N then P) (Figure 3; Table 4). In contrast, Great Pond's pelagic zone is P limited, and Great Pond's benthic zone is N limited (Figure 3; Table 4).

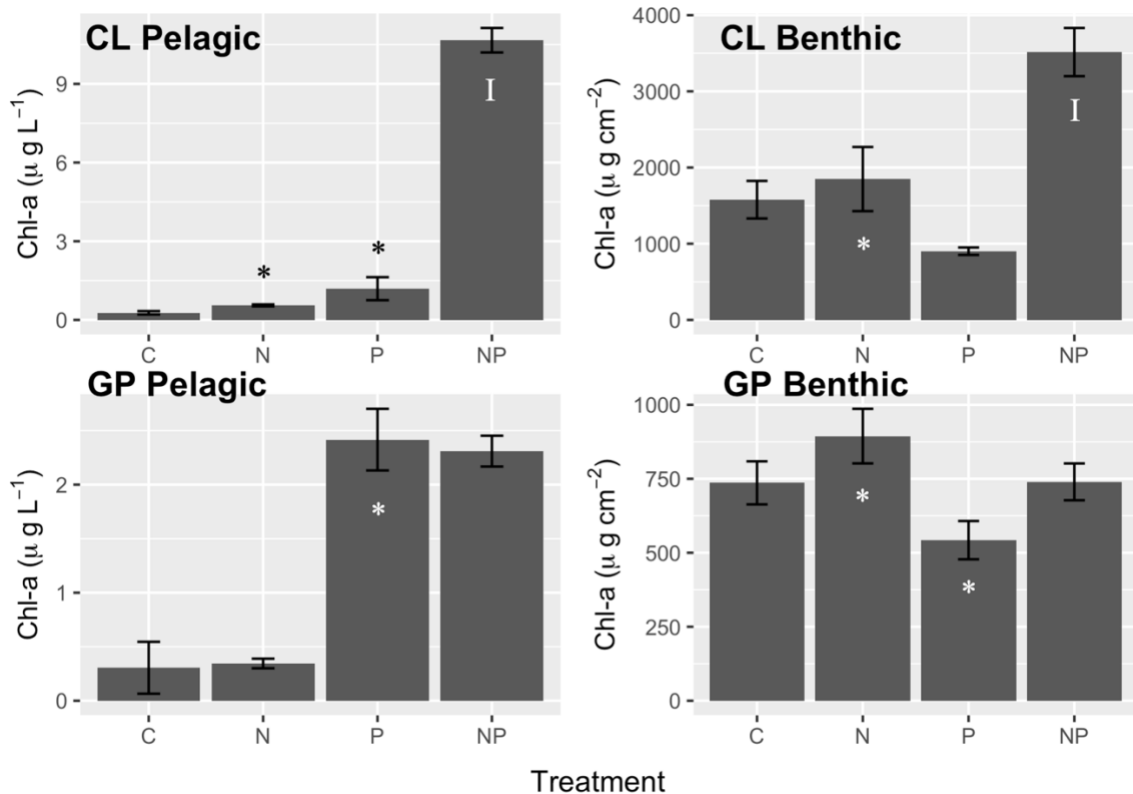


Figure 3. Mean chlorophyll-a on GF/F filters (CL Pelagic and GP Pelagic) and fritted glass disks (CL Benthic and GP Benthic) from the four nutrient treatments; control (C), N alone (N), P alone (P), and N and P added together (NP) for each stream. Means \pm standard errors (SE) are plotted, and each bar represents $n = x$ for each treatment at each site (see Appendix Table 2 for sample replicate numbers by experiment and treatment). Asterisks (*) above bars indicate a significant N effect or P effect ($P < 0.05$), and (I) signifies a significant interaction term as determined by ANOVA ($P < 0.05$).

Table 4. Two-way ANOVA p -values for N and P main effects and interactions for each lake and resulting classification of nutrient limitation. Bold p -values indicate significant effects ($p < 0.05$). S_N indicates serial limitation with N as the primary limiting nutrient. For all analyses, d.f. = 1.

Lake	Lake Habitat	Main effect for N: p -value	Main effect for P: p -value	Interaction effect for N+P: p -value	Interpretation
China Lake	Pelagic Zone	<0.001	<0.001	<0.001	Colimited - simultaneous
	Benthic Zone	<0.001	0.210	<0.001	Serially limited - S_N
Great Pond	Pelagic Zone	0.860	<0.001	0.744	P limited
	Benthic Zone	0.041	0.059	0.803	N limited

Biomass Response to Nutrient Addition in Pelagic Zones of Each Lake

For phytoplankton in the pelagic zone, the biomass responses to nutrient addition in China Lake were significantly higher than biomass responses in Great Pond (ANOVA, $F = 172.2465$, $p < 0.0001$; Figure 4). The China Lake pelagic zone response to N addition was significantly lower than the Great Pond pelagic zone response to N + P addition (Tukey HSD post-hoc, $P = 0.0227$; Figure 4). The biomass response to N + P in the pelagic zone of China Lake was significantly higher than the response to N + P in the pelagic zone of Great Pond (Tukey HSD post-hoc, $P = 0.0153$; Figure 4). Phytoplankton in the pelagic zone of China Lake reacted more strongly to N additions than Great Pond phytoplankton reacted to N + P additions (Tukey HSD post-hoc, $P = 0.0227$; Figure 4). China Lake pelagic zone biomass responses to N + P were significantly higher than the Great Pond pelagic zone response to P (Tukey HSD post-hoc, $P = 0.0131$; Figure 4).

Biomass responses to nutrient treatments varied significantly in the pelagic zone of Great Pond. Within the pelagic zone of Great Pond, P and N + P nutrient additions caused a higher phytoplankton biomass response than N alone (Tukey HSD post-hoc, $P = 0.0083$, $P = 0.0128$, respectively; Figure 4).

Biomass Response to Nutrient Addition in Benthic Zones of Each Lake

Periphyton biomass responses to nutrient addition in the benthic zone of China Lake were significantly higher than responses in the benthic zone of Great Pond (ANOVA, $F = 11.2706$, $P < 0.0001$). Between lakes, the China Lake benthic zone biomass responses to N + P were significantly higher than Great Pond responses to N, P, and N + P (Tukey HSD post hoc, $P = 0.0086$, $P = 0.0003$, $P = 0.0013$, respectively). Within China Lake, benthic zone periphyton responded more strongly to the N + P treatment than separate N and P treatments (Tukey HSD post hoc, $P = 0.0021$, $P < 0.0001$, respectively). These findings re-enforce observed colimitation patterns in the benthic zone of China Lake (Figure 4; Table 4).

Biomass Response to Nutrient Addition in Benthic and Pelagic Zones of China Lake

There was one significantly different biomass response between the benthic and pelagic zones of China Lake (ANOVA, $F = 319.001$, $P < 0.0001$; Figure 4). China Lake's pelagic zone phytoplankton responded significantly higher than China Lake's benthic zone to the P addition (Tukey HSD post hoc, $P = 0.0270$; Figure 4), although both were limited by P.

Biomass Response to Nutrient Addition in Benthic & Pelagic Zones of Great Pond

Biomass responses to nutrient treatments varied significantly between the benthic and pelagic zones of Great Pond (ANOVA, $F = 54.253$, $P < 0.0001$; Figure 4). In the pelagic zone, P and N + P additions caused a stronger biomass response than N, P, and N + P additions in the benthic zone (Tukey HSD post hoc, $P < 0.0001$; Figure 4). Within the pelagic zone of Great Pond, P and N + P nutrient additions caused a higher phytoplankton biomass response than N alone (Tukey HSD post hoc, $P < 0.0001$; Figure 4).

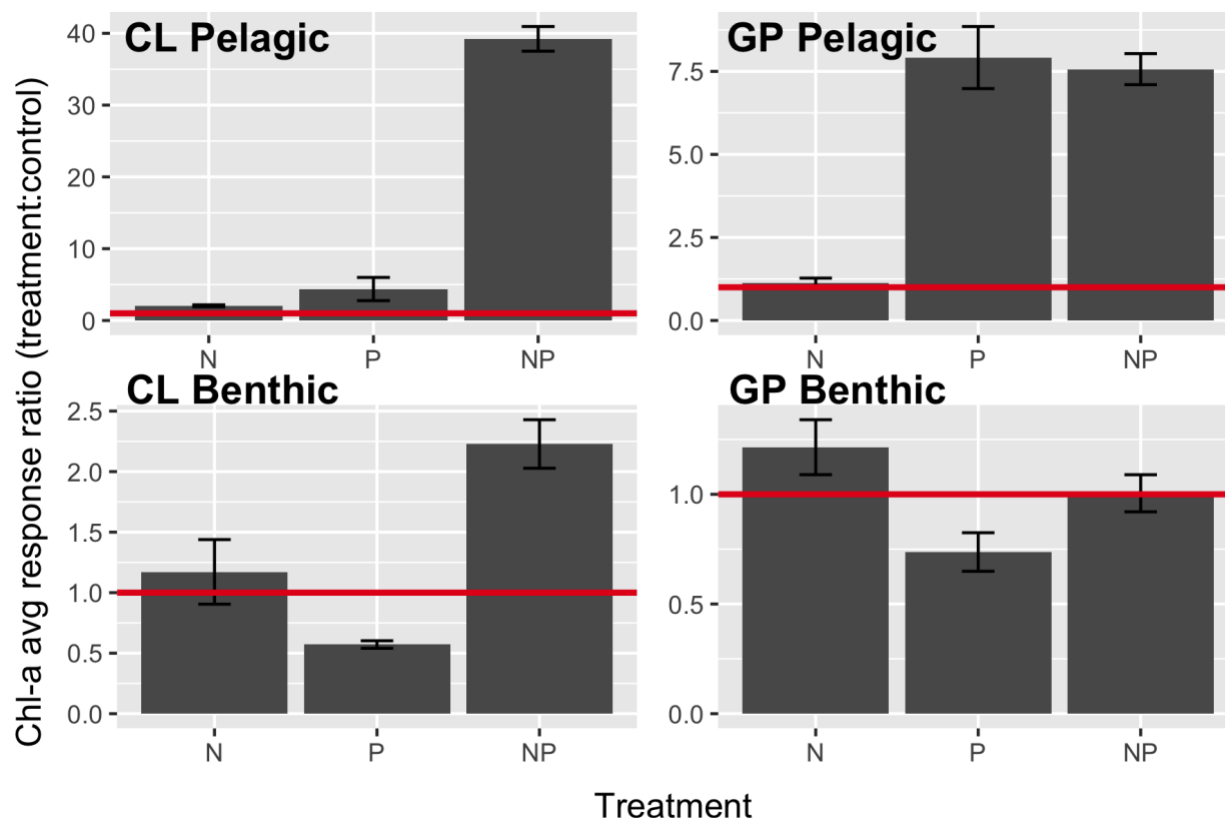


Figure 4. Mean chlorophyll-*a* response to nutrient addition plotted by treatment and habitat type where chlorophyll-*a* response is the ratio of the treatment divided by the unenriched controls [ie. nitrogen = (nitrogen/control)]. Means \pm standard errors (SE) are plotted for chlorophyll-*a* on glass fiber filters (China Lake Pelagic and Great Pond Pelagic) and chlorophyll-*a* on fritted glass disks (China Lake Benthic and Great Pond Benthic). Red line indicates where ratio of treatment : control = 1; values above red line indicate scaled biomass responses to nutrient addition.

Background Lake Nutrient and Chlorophyll-a Content

Total phosphorus (TP) data have been collected in China Lake and Great Pond yearly or every few years since the 1970s. China Lake annual mean TP data ($n = 37$ years) are more abundant than Great Pond annual mean TP data ($n = 24$ years) as China Lake has been more heavily studied due to its higher tendency for algal bloom proliferation. In the epilimnion, overall mean annual TP in China Lake is $18 \mu\text{g L}^{-1}$ (± 3.1 sd) and overall mean annual TP in Great Pond is $8.9 \mu\text{g L}^{-1}$ (± 1.5 sd) (Figures 5 & 6). China Lake mean annual epilimnetic TP is ~2 times higher than in Great Pond (Figures 5 & 6). In the hypolimnion, China Lake annual mean TP is $29 \mu\text{g L}^{-1}$ (± 21 sd) and Great Pond annual mean TP is $20 \mu\text{g L}^{-1}$ (± 20 sd) (Figure 5).

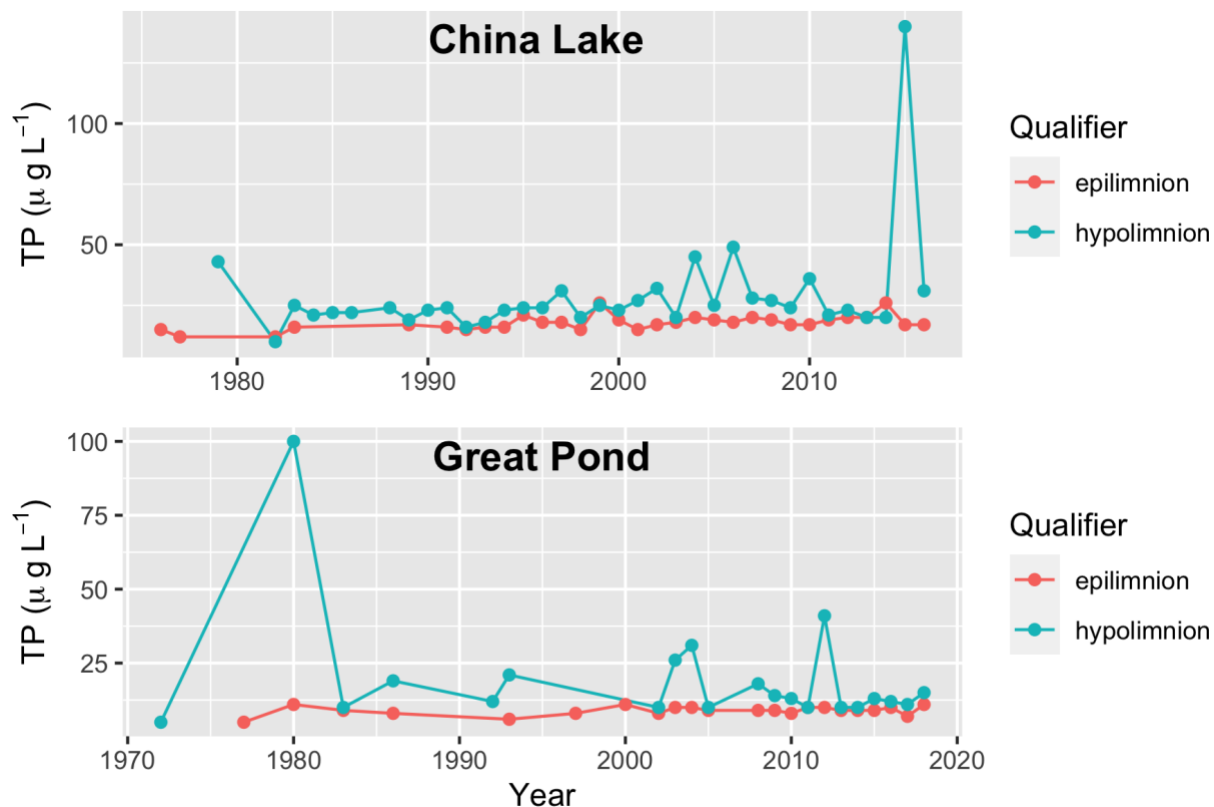


Figure 5. Background annual mean Total Phosphorus (TP) values over time from 1976 to 2016 (China Lake) and from 1972 to 2018 (Great Pond). TP units are micrograms per liter. Data sourced from epilimnetic core samples (epilimnion) and bottom grab samples (hypolimnion) from the Lake Chemistry dataset from the Lakes of Maine database via the Lake Stewards of Maine organization.

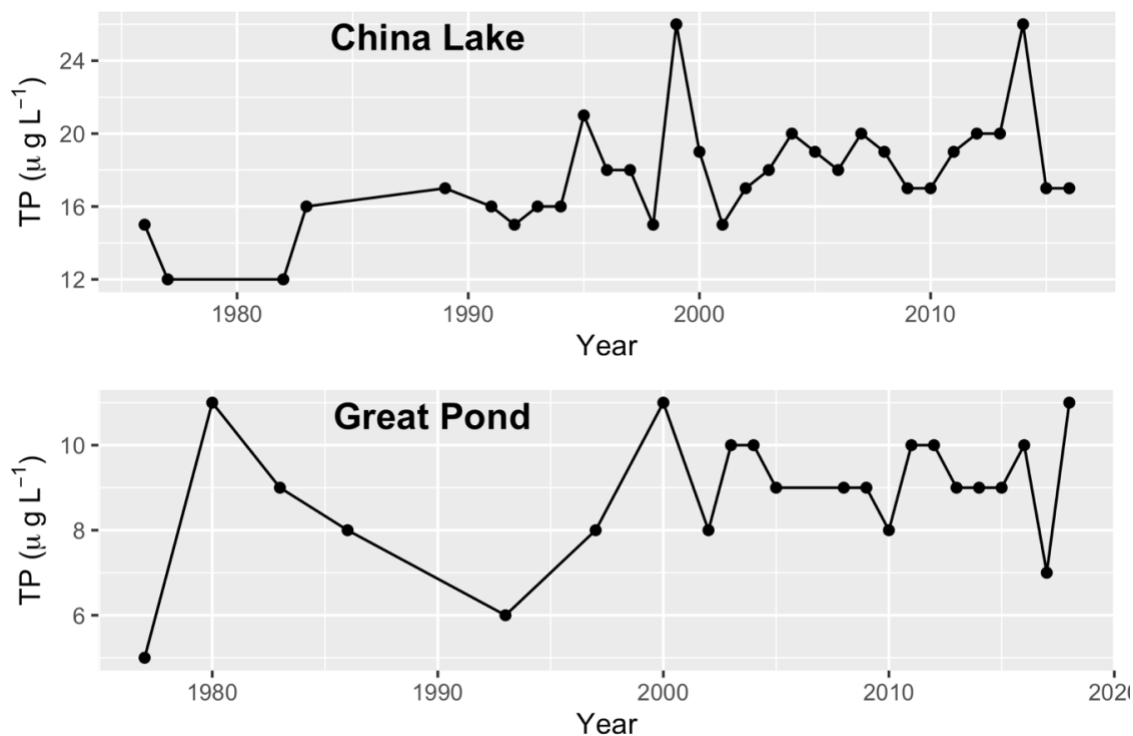


Figure 6. Background annual mean Total Phosphorus (TP) values in the epilimnion over time from 1976 to 2016 (China Lake) and from 1977 to 2018 (Great Pond). TP units are micrograms per liter. Data sourced from epilimnetic core samples from the Lake Chemistry dataset from the Lakes of Maine database via the Lake Stewards of Maine organization.

Chlorophyll-*a* concentrations in lakes are widely used as a proxy for phytoplankton biomass (Francoeur 2001). Overall annual mean chlorophyll-*a* values for China Lake and Great Pond are $12 \mu\text{g L}^{-1}$ (± 5.7 sd) and $4.7 \mu\text{g L}^{-1}$ (± 1.7 sd), respectively (Figure 7). Annual mean chlorophyll-*a* values in China Lake are 2.6 times higher than in Great Pond overall (Figure 7). Therefore, China Lake is higher-mesotrophic (closer to eutrophic), while Great Pond is lower-mesotrophic.

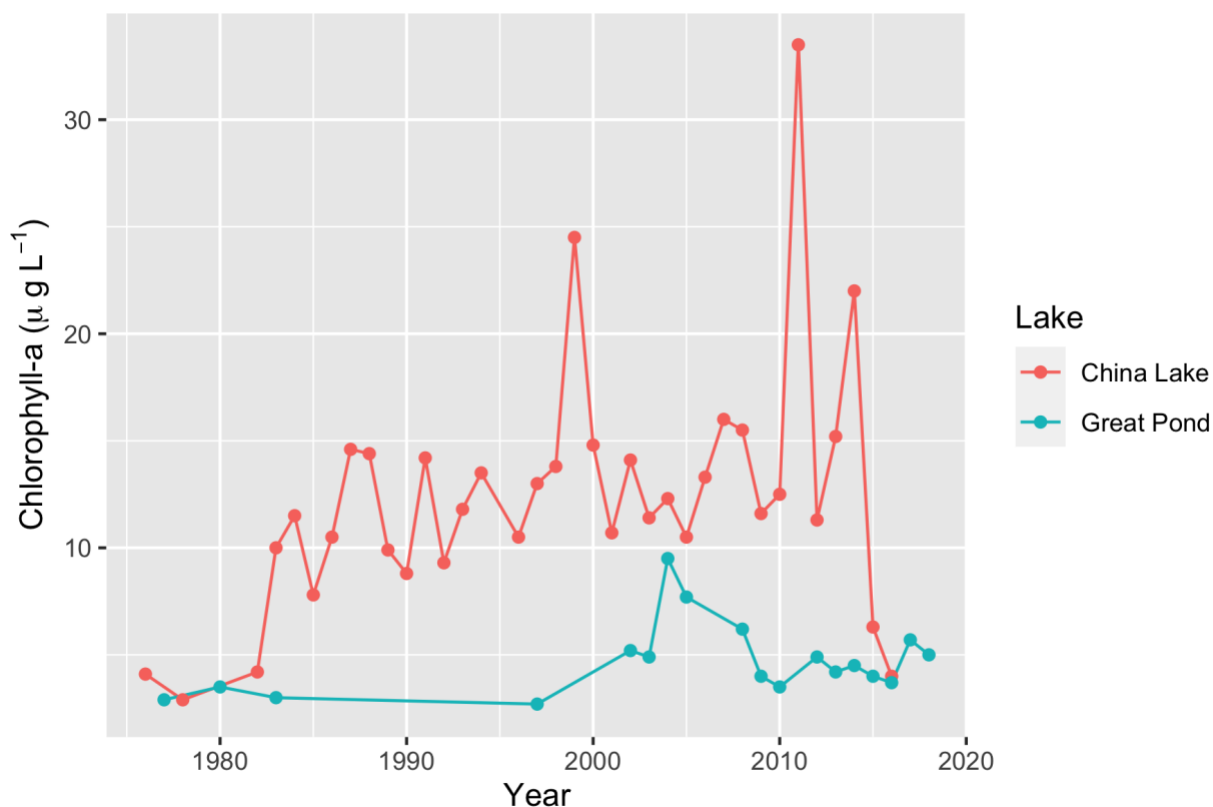


Figure 7. Annual mean chlorophyll-*a* values over time in China Lake and Great Pond from 1976 to 2016 (China Lake) and from 1977 to 2018 (Great Pond). Data sourced from the Lake Chemistry dataset from the Lakes of Maine database via the Lake Stewards of Maine organization.

Annual mean chlorophyll-*a* values (as a proxy for phytoplankton biomass) in the epilimnions of China Lake and Great Pond follow a positive linear relationship when plotted against annual mean TP values (Figure 8). There was a significant positive relationship between annual mean chlorophyll-*a* versus annual mean TP ($r^2 = 0.378$, $P < 0.001$; Figure 8). There was no significant correlation between annual mean chlorophyll-*a* and annual mean TP values in Great Pond.

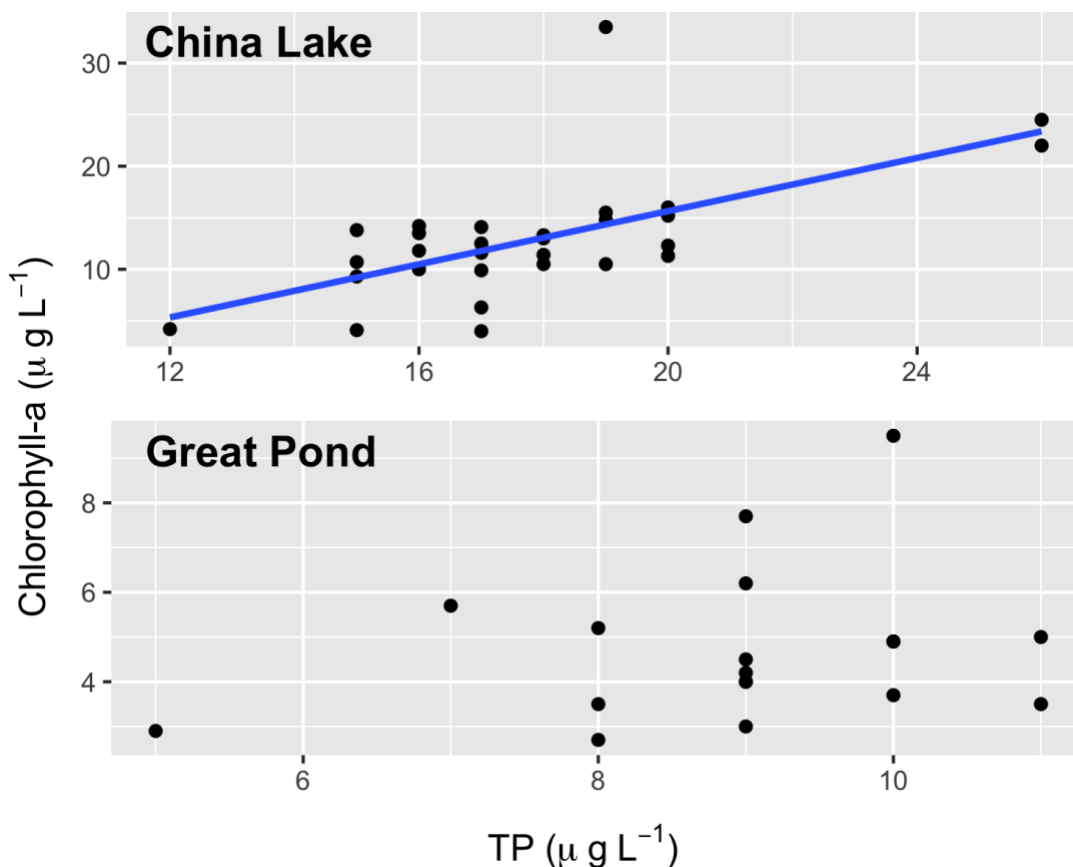


Figure 8. Annual mean chlorophyll-a values plotted against annual mean total phosphorus (TP) values from the epilimnion in China Lake and Great Pond. Points represent annual mean chl-a and TP values from 1976 to 2016 (China Lake) and 1977 to 2018 (Great Pond). In China Lake, there was a significant positive relationship between annual mean chl-a values versus annual mean TP ($r^2 = 0.378$, $P < 0.0001$). Data sourced from the Lake Chemistry dataset from the Lakes of Maine database via the Lake Stewards of Maine organization.

Discussion

Phytoplankton and Periphyton Responses to Nutrient Addition

Autochthonous production is widely known to be affected by nutrients (Wetzel and Likens 2001). In this study, I have used concurrent nutrient enrichment mesocosms and nutrient diffusing substrata bioassays to assess nutrient limitation of chlorophyll-a, an indicator of phytoplankton and periphyton biomass, to evaluate potential nutrient limitation patterns in the pelagic and benthic zones of two mesotrophic Maine lakes. Despite measuring two mesotrophic lakes in close geographic proximity, I found that

patterns of nutrient limitation vary across these two lakes, and even within habitats in the case of Great Pond.

In the pelagic zone mesocosm experiment, I filtered out larger zooplankton grazers before incubating the bags with the nutrient treatments. However, in the NDS experiment, I did not remove grazers from the substrata, as my goal was to assess the potential for nutrient limitation under *in situ* conditions. Grazing pressure by invertebrates has been shown to affect the outcome of NDS bioassays (Tank and Dodds 2003, Lewis and Wurtsbaugh 2008, Vadeboncoeur et al. 2008, Abell et al. 2010, Paerl et al. 2014, Filstrup and Downing 2017, Lewis et al. 2020, Salmaso and Tolotti 2020). Since grazers may consume growth that is stimulated by nutrients in some ecosystems, perhaps grazing affected my observed patterns of nutrient limitation in the benthic zones of each lake. In this case, the periphyton biomass is a net result of both biomass growth and loss, in contrast to the measured phytoplankton biomass that is largely a result of biomass growth.

Background TP Data in Context with My Findings

In most lake ecosystems, phytoplankton biomass and TP have a linear relationship (Dubé et al. 1997, Lohman et al. 2017). Epilimnetic annual mean chlorophyll-*a* values (as a proxy for phytoplankton biomass) in China Lake and Great Pond follow a positive linear relationship when plotted against annual mean TP values. There is a significant positive correlation between background annual mean TP and chl-*a* in China Lake, but not in Great Pond. However, these relationships do not explain even half of the observed variation in chlorophyll-*a* concentrations, so other factors are important in determining what drives productivity in these systems. While TP is an important controlling factor for primary productivity in lakes, dissolved inorganic nitrogen (DIN) is also potentially limiting for phytoplankton and periphyton biomass (Dillon and Rigler 1974). There is a lack of long-term data on nitrogen concentration in both lakes, so I cannot access similar trends with nitrogen and chlorophyll-*a* over time.

Seasonal Implications

Many nutrient limitation studies focus on primary productivity in the summer when algal blooms are most common (Maberly et al. 2002). However, phytoplankton and periphyton nutrient limitation patterns change seasonally. Colimitation has been shown to become progressively more common as the summer season progresses (Haertel 1976, Carstensen et al. 2007, Chaffin et al. 2014, Paerl et al. 2015). This study helps to fill a gap in the literature about early fall algal bloom nutrient limitation patterns, as all data were collected during October 2020. Additionally, China Lake was in the midst of a cyanobacterial bloom during my experiment. Lake phytoplankton commonly undergo a predictable seasonal succession (Maberly et al. 2002), and my experiments were centered around the time of year when cyanobacteria are most likely to dominate (Tsukada et al. 2006, Carey et al. 2015). Thus, N fixation may be more likely to alleviate N limitation during this late summer period than earlier in the summer when diatoms dominate.

I observed colimitation and serial N limitation in the pelagic and benthic zones of China Lake, respectively, and P limitation in the pelagic zone of Great Pond. In mesotrophic and eutrophic lakes, shortages of N relative to P tend to be compensated for by cyanobacterial N-fixation, one of the mechanisms that leads to P regulation of primary production and eutrophication (Konopka and Brock 1978, Paerl et al. 2014, Sterner 2019). As phytoplankton and periphyton community composition shifts to favor N-fixers in the late summer, growth tends to be limited by P since organisms are fixing their own N for primary production (Schindler 1977). China Lake in particular has a higher background TP content than Great Pond, which could mean that the phytoplankton community has a higher proportion of cyanobacteria since cyanobacteria dominate at higher TP levels (Konopka and Brock 1978, Paerl et al. 2014, Sterner 2019). Thus, N-fixation by cyanobacteria could be contributing to colimitation in the pelagic zone of China Lake and P limitation in the pelagic zone of Great Pond. Conversely, in shallow lake areas, chlorophytes tend to dominate phytoplankton assemblages (Watson et al. 1997, Salmaso and Tolotti 2020), which might explain the serial N limitation in the benthic zone of China Lake, since chlorophytes do not fix nitrogen. However, it is important to note that not all cyanobacteria can fix nitrogen (Salmaso and Tolotti 2020), so these patterns could be explained by other factors.

Lake Habitat Implications

I observed contrasting patterns of nutrient limitation in the benthic and pelagic zones of China Lake and Great Pond. In China Lake, I observed similar nutrient limitation patterns between the pelagic zone (simultaneous colimitation by N and P) and benthic zone (serial limitation by N, then P). In contrast, in Great Pond I observed limitation by different nutrients in the pelagic zone (P limitation) and benthic zone (N limitation).

In China Lake, the similar patterns of colimitation and serial N limitation between the pelagic and benthic zones might be explained by the high overall nutrient content across the lake. Since China Lake is upper-mesotrophic, perhaps the high ambient TP levels and high background primary productivity could overwhelm any zonal differences in nutrient limitation patterns that I might observe. Alternatively, when water depths are low enough for light to reach the sediments, the increased light regime in the water column may stimulate periphyton growth, potentially enhancing the ability of periphyton to compete with phytoplankton for nutrients (Scott and McCarthy 2010). The similar nutrient limitation patterns between habitat zones could suggest competition for nutrients between phytoplankton and periphyton. Additionally, overall benthic contribution to primary production in lakes is linked to light attenuation and depth ratio (DR, ratio of mean depth to maximum depth) and tends to decline with increasing nutrients (Rodusky et al. 2001). In China Lake, the DR is 0.329, and in Great Pond the DR is 0.304, both considered low values on the DR scale. Moderately-deep oligo-mesotrophic lakes have substantial contributions by benthic primary productivity at low DR and when maximum benthic photosynthesis is moderate or high (Vadeboncoeur et al. 2008). Compared to China Lake, Great Pond may have higher benthic contributions to overall primary productivity since it is both lower-mesotrophic and has a lower DR.

In the case of Great Pond, I was surprised to find contrasting nutrient limitation patterns between different habitats of the same lake. A potential factor causing benthic N limitation could be sediment nutrient release that makes an abundance of P available for primary production by periphyton. Although phosphorus release from sediments is known to be dependent on a variety of physical, chemical, and biological factors (Vadeboncoeur et al. 2008), sediment P release in mesotrophic lakes under transient

loading conditions tend to be predominantly generated by a continuous epilimnetic P flux (Psenner et al. 1988). In addition, under oligo-mesotrophic conditions, TP can be more related to chlorophyll-*a* than TN (Soranno & Wagner 2020). Perhaps this stronger relationship between TP and chl-*a* can help explain the observed P limitation in the pelagic zone of Great Pond, because it suggests that phytoplankton growth would have a stronger response to P additions.

Mesotrophic Systems

Understanding nutrient limitation patterns in mesotrophic systems like China Lake and Great Pond is important since enough nutrient inputs could shift the system to a eutrophic state. I observed contrasting nutrient limitation patterns between China Lake and Great Pond, both as a whole and between habitat zones of Great Pond. This means that N and P inputs into these systems from anthropogenic sources will affect primary productivity and algal bloom formation differently in each lake. Therefore, as mesotrophic systems, these lakes could be managed to move towards an oligotrophic state, which would require limiting external loading of both N and P.

A cyanobacterial bloom management priority would be to establish N and P input thresholds below which blooms could be controlled in terms of magnitude, spatial, and temporal coverage (Dittrich et al. 2013). The most effective ways to reduce N and P loading via diffuse non-point sources are vegetative riparian buffers, construction of wetlands for treating and removing nutrients, and building retention ponds for impervious runoff sources (Paerl and Otten 2013). Buffer zones aid in the cultivation and stimulation of macrophytes, which can help absorb excess nutrients (Castelle et al. 1999). The management of point and diffuse sources of nutrients on a landscape scale provides a sustainable approach to management, avoiding ecological tipping points that severely compromise lake ecosystems (Jeppesen et al. 2007, Kuglerová et al. 2020).

Limitations/Sources of Error

There is a historical lack of N data in both China Lake and Great Pond. The concentration of dissolved inorganic nitrogen (DIN) and the molar ratio of DIN to total dissolved phosphorus (TDP) have been shown to be the main environmental factors

controlling the extent of nitrogen or phosphorus limitation at a given site (Bullerjahn et al. 2016, Hamilton et al. 2016). While background TP data is abundant for many Maine lakes in public-access databases, there is little to no N data (of any form) to be found, even through direct contact with Maine state limnologists. Without access to N data, it is difficult to fully understand observed nutrient limitation patterns in China Lake and Great Pond over long temporal scales.

The lack of historical N data and sampling in China Lake and Great Pond could cause algal bloom management strategies to be misinformed. The question of how much N, if any, needs to be managed alongside P is debated (Maberly et al. 2002). While single P management strategies have been proven to be successful in many restoration programs (Schindler et al. 2008, Paerl et al. 2014), it has also been found that excess inputs of both N and P, combined with internal cycling of these nutrients, may overwhelm the ability of a single nutrient to control increasing eutrophication and bloom intensification in large lakes prone to blooms (Smith and Schindler 2009, Stroom and Kardinaal 2016). Therefore, failure to control N inputs may result in continued serious eutrophication problems caused by non-N₂-fixing cyanobacterial blooms (Paerl et al. 2011b, Stroom and Kardinaal 2016).

While my results can be used to understand lake ecosystems, my research may be limited by using mesocosm bags to measure pelagic zone nutrient limitation. Mesocosm bags limit the movement of water, which can lead to ‘enclosure effects’ and limit the herbivory component of the full ecosystems (Paerl et al. 2011b, Stroom and Kardinaal 2016). Microcosms also oversimplify biological communities via exclusion of zooplankton and fish, loss of interaction with the atmosphere and sediments, and omission of natural lake stratification and mixing patterns (Lewis et al. 2020). However, mesocosms are effective because they are easily replicable in factorial treatment designs to test hypotheses of nutrient limitation at broad spatial and fine temporal scales (Dzialowski et al. 2014). Considering this, the mesocosm experiment methods allow for comparison of nutrient limitation patterns of pelagic phytoplankton communities in mesotrophic lakes in the early fall. Additionally, I had a limited ability to include many sample sites in each lake due to time constraints. Including at least 3 different areas in the

littoral and pelagic zones of each lake would have increased the statistical power of my results and offered insight into nutrient limitation patterns on broader spatial scales.

Conclusion

The results of this study are consistent with other lake nutrient limitation studies that find patterns of colimitation and N limitation across smaller spatial and temporal scales. We found contrasting patterns of nutrient limitation between a lower-mesotrophic and higher-mesotrophic lake and between habitat zones within the same lake. My results indicate that both N and P are important drivers of phytoplankton and periphyton dynamics at short time scales, and that lake habitat plays an important role in determining nutrient limitation status. This documented diversity in nutrient limitation patterns in mesotrophic lakes highlights the importance of tailoring management strategies to specific lakes rather than at state or regional scales, especially in response to phytoplankton and periphyton growth over short time scales. As one of the first studies to research patterns of nutrient limitation in both littoral and pelagic zones concurrently, this study provides important evidence for incorporating lake littoral habitats and lake trophic status into our understanding of whole-ecosystem productivity.

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APPENDICES

Appendix Table 1. Characteristics of the two study lakes, China Lake and Great Pond, sourced from the Maine Lakes Geography and Morphometry dataset from the Lakes of Maine database.

Lake Name	China Lake	Great Pond
Area (acres)	3939	8533
Perimeter (miles)	30.4	46.1
Mean Depth (feet)	28	21
Max Depth (feet)	85	69
Volume (acre-feet)	97286.4	195099.3181
Direct Drainage Area (sq miles)	26.10036	31.953636
Total Drainage Area (sq miles)	31.8	82.9
Flushing rate (times/year)	0.65	0.43
Trophic Category	Eutrophic	Mesotrophic
Dam	3	3
Elevation (feet)	196	248
Major Drainage	Kennebec	Kennebec
Sub Drainage	Lower Sebasticook	Messalonskee Stream
Town(s)	China, Vassalboro	Belgrade, Rome
County	Kennebec	Kennebec
Latitude	44.4302452	44.53328716
Longitude	-69.54731476	-69.84094023
Water Quality Statement	Below Average	Above Average
Invasive Plant Infestation	None known	None known
Fishery Management	Coldwater + Warmwater	Coldwater + Warmwater

Appendix Table 2. Number of replicate samples for each experiment treatment within each study lake and lake habitat. Numbers vary due to environmental factors causing sample loss during in situ lake incubation.

Treatment	Control	+ N	+ P	+ N + P	Lake/Habitat
Number of Replicates	4	4	4	4	China Lake Pelagic
	6	6	6	5	China Lake Benthic
	4	3	4	4	Great Pond Pelagic
	4	4	3	4	Great Pond Benthic