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NUTRIENT LIMITATION OF ALGAL GROWTH IN FISHERY LAKES

Madeline Ludwig

Biological Engineering Program

Biological and Agricultural Engineering Department

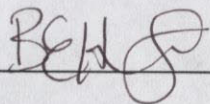
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University of Arkansas

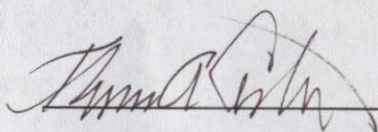
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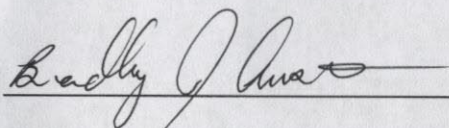
This thesis has been approved by the Biological and Agricultural Engineering Department for submittal to the College of Engineering and Honors College at the University of Arkansas



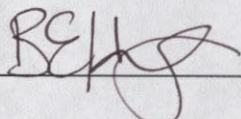
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ABSTRACT

This study investigated the effect of nutrient addition on algal growth in three United States Forest Service lakes for fishery management in Arkansas. In fishery managed lakes, fertilization works by manipulating algae growth, a basal food resource in lakes, to promote the growth of the fish population. For the nutrient addition experiments, water was collected from each lake in cubitainers and spiked with nutrients; the treatments included the control, nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+N +P). When algal growth was visually observed, a water sample was collected from each cubitainer and analyzed for chlorophyll- α . The results showed that lakes were co-limited by nitrogen and phosphorus, and generally continued to be after fertilization. The data on the phytoplankton's nutrient limitations provided the US Forest Service with information that can be used to develop a fertilization plan. Algal cells typically require between 10-20 mole of N per mole of P to promote growth, however, some of the fertilizers used by the USFS fall outside this range. The USFS needs to be consistent and use a balanced fertilizer (10-20:1) on all their lakes. Further research should also be performed to optimize the amount of fertilizer applied to each lake.

INTRODUCTION

Fisheries managers can promote increased fish production (both in number and size) through lake fertilization. Lake fertilization works by manipulating the lower tiers of the food web (e.g., algae) to encourage population growth in the higher trophic levels (Watson and Cichra 2017). In fishery lakes, lakes are fertilized to promote algal growth which in turn increases the population of zooplankton, insects, and bait fish. These organisms are often a food source for sport fish and when their populations increase it can lead to an increase in the sports fish population and size.

However, how you fertilize your lake is important, algal growth is limited by the nutrient level of their food resources (Rothhaupt 1995). The two important nutrients in algal growth are phosphorus (P) and nitrogen (N). These nutrients may limit algal growth individually or in combination (i.e. both as co-limitation) (Dzialowski et al. 2005). Experiments are often set up to determine which nutrients limit algal growth; however, the results are often mixed showing N, P, and co-limitation because of differences in the supply and total N: P ratios in lakes and streams (Dzialowski et al. 2005).

Fertilization is not the only method for increasing sport fish population and size, another common practice is through supplementing lake with bait fish. This, however, can cause strain on the lower level of the food web, which could lead to a collapse of the food web (O'Connor and Donohue 2013). For this reason, it is wise to promote growth at the lowest level of the food chain, which in this case is the algae growing in the water column.

The United States Forest Service (USFS) is charged by congress to managing America's natural resources for the American people and currently manages over 241,000 km of stream and 1,012,000 ha of lakes in national forests and grasslands (Witt 2017). The goal of this project was to determine what nutrient limited algal growth and how fertilizer addition to lakes managed by USFS influenced nutrient limitation. The main objective was to evaluate whether algal growth was limited by N, P, or both N and P in Cove Lake, Lake Wedington, and Spring Lake in Arkansas. I hypothesized that the growth of algae in

the sample lakes will be dependent on both N and P. This research will help the USFS to better manage nutrient fertilization to maximize algal growth and improve fishery management in these lakes.

METHODS

Study Site Description

The lakes, Cove Lake, Spring Lake, and Lake Wedington, managed by the USFS for fisheries, were selected for nutrient limitation testing (Table 1). Cove Lake has a surface area of 65 ha and has a catchment area of approximately 2600 ha, draining mostly forested lands (Wiki Watershed 2018). Cove Lake is in the larger Dardanelle Reservoir Watershed within the Arkansas Valley Level III Ecosystem.

Spring Lake is also in the Arkansas Valley Ecoregion (Woods et al. 2004) but falls into the larger Petit Jean Watershed. Spring Lake has a surface area of 33 ha, and the watershed area is 4400 ha (Wiki Watershed 2018). The land use in the watershed is over 90 percent forested (Wiki Watershed 2018).

Lake Wedington has a surface area of 41 ha, and it is in the Ozark Highland Level III Ecoregion (Wiki Watershed 2018, Woods et al. 2004). This watershed is 1000 ha and is mostly forested (73%) but has greater pasture land use (20%) than the other two lakes (Wiki Watershed 2018).

Table 1: Study Site Lake Characteristics

Lake	Cove Lake	Spring Lake	Lake Wedington
Level III Ecoregion	Arkansas Valley	Arkansas Valley	Ozark Highland
HUC-8 Watershed	Dardanelle Reservoir	Petit Jean	Illinois
HUC-12 Watershed	Upper Short Mountain Creek	Spring Creek-Petit Jean River	Weddington-Illinois
Lake Drainage Area (ha)	2600	4400	1000
Lake Area (ha)	65	33	41
% Urban	3.5	3	4.8
% Forest	89.2	92.8	72.6
% Pasture	4.5	3.6	19.7

Nutrient Limitation Testing

Field data was collected during Summer 2017, and the specific dates of the nutrient limitation experiments were selected based on the USFS schedule for fertilizer addition. For each trial a minimum of 20 one L cubitainers were randomly filled from the lake surface, at the lakes deepest point. The cubitainers, were then separated into four groups: the control, nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+N +P). The cubitainers were spiked to 0.5 mg P L⁻¹ (+P treatment) and 5 mg N L⁻¹ (+N treatment) or both (+N+P treatment) using KH₂PO₄ and NaNO₃. The cubitainers then were incubated in water baths in the greenhouse between four and eight days to allow algal growth. During the incubation period the cubitainers were vented at the same time each day to release any gas.

When algal growth was visually observed, a water sample from the cubitainer was filtered in a dark room, via vacuum filtration, through a Whatman GF/F 25 mm glass microfiber filter using a Buchner Funnel. The filter paper was then collected and placed in plastic test tubes along with 7 mL of 90% acetone. The test tubes were then wrapped in aluminum foil and placed in the freezer to steep at least 24 hours before chlorophyll- α (chl- α) was measured. Fluorescence was used to measure chl- α concentration ($\mu\text{g L}^{-1}$) corrected for pheophytin based on EPA method 445.0 sans tissue grinding (Arar & Collins 1997).

Nutrient limitation was determined from the differences in mean chl- α concentrations between the treatment groups. Single factor analysis of variance (ANOVA) and least significant differences (LSD) were used to evaluate treatments means. Treatment groups were then placed in homogenous groups to aide in determining nutrient dependency.

RESULTS

Cove Lake

The samples for first nutrient limitation experiment at Cove lake were taken on May 15, 2017 and incubated for 8 days. When testing was performed on May 23, 2017, the mean chl- α concentration in the control ($0.62 \mu\text{g L}^{-1}$) was not significantly different from the +N ($1.98 \mu\text{g L}^{-1}$) or +P ($1.37 \mu\text{g L}^{-1}$) treatments (ANOVA LSD, $p > 0.05$) (Figure 1). However, the mean chl- α in the +N+P ($10.65 \mu\text{g L}^{-1}$) was significantly greater than all the other treatments ($p < 0.05$). This suggests that the algal growth was co-limited by N and P at this time.

The samples for the next experiment were taken on June 24, 2017 and incubated for 7 days (Figure 1b). The mean chl- α concentration in the control ($0.78 \mu\text{g L}^{-1}$) was not significantly different from the +N ($2.23 \mu\text{g L}^{-1}$) or the +P ($2.98 \mu\text{g L}^{-1}$) treatments. But, the +N+P treatment ($12.09 \mu\text{g L}^{-1}$) had significantly greater mean chl- α concentrations than all three other treatments on July 1, 2017 ($p < 0.05$). This implied that the lake was co-limited by N and P.

In contrast the last experiment at cove lake showed different results (Figure 1c). The last set of samples were taken on July 30, 2017 and were incubated for 8 days. The mean chl- α concentrations were not significantly different across all four treatments ($p > 0.05$). This shows that neither N and or P were limiting algal growth on July 7, 2017. The control from the last experiment also showed that fertilization was effective. After fertilization, the mean chl- α concentration of the final experiment was greater than the mean concentration before fertilization showing that growth had occurred.

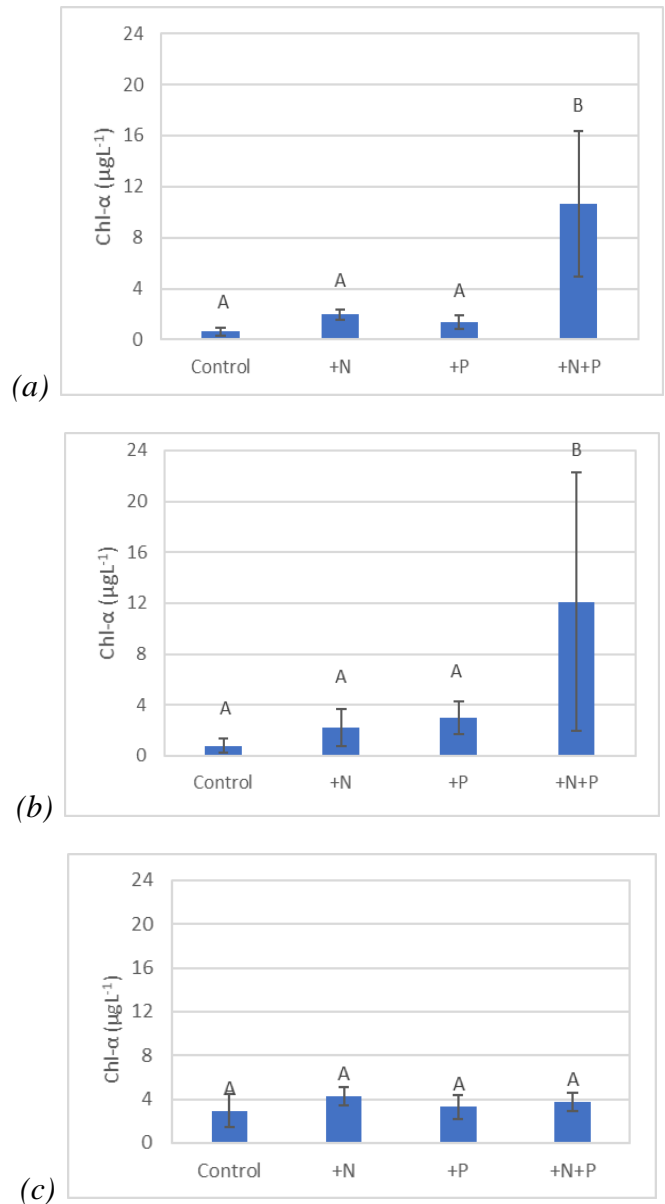


Figure 1: Mean (\pm standard deviation) chlorophyll- α (chl- α) concentration across nutrient amendments at Cove Lake on (a) May 23, 2017 (b) July 1, 2017, and (c) August 7, 2017; treatments include control, nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+N+P) and homogenous groups are identifies by the letters above the bars.

Spring Lake

The first nutrient limitation experiment at Spring lake occurred on May 23, 2017 after samples were incubated for 8 days. The mean chl- α concentration in the control ($0.28 \mu\text{g L}^{-1}$) was not significantly different from the +N ($1.01 \mu\text{g L}^{-1}$) or +P ($1.72 \mu\text{g L}^{-1}$) treatments (ANOVA LSD, $p>0.05$) (Figure 2). However, the mean chl- α in the +N+P ($13.21 \mu\text{g L}^{-1}$) was significantly greater than all the other treatments ($p<0.05$). This implies that the algal growth was co-limited by N and P.

The samples for the second experiment were taken on June 31, 2017 and incubated for 7 days (Figure 2). The mean chl- α concentration in the control ($0.31 \mu\text{g L}^{-1}$) was not significantly different from the +N ($1.12 \mu\text{g L}^{-1}$) or the +P ($0.31 \mu\text{g L}^{-1}$) treatments. But, the +N+P treatment ($7.14 \mu\text{g L}^{-1}$) had significantly greater mean chl- α concentrations than all three other treatments ($p<0.05$). This suggested that the lake was co-limited by N and P on July 1, 2017.

In contrast the last experiment at spring lake showed different results (Figure 2). After the samples were taken on July 14, 2017 and incubated for 7 days, the mean chl- α concentrations were not significantly different between the +N ($14.98 \mu\text{g L}^{-1}$) and +N+P ($13.69 \mu\text{g L}^{-1}$) treatment groups but the +N treatment group was greater than the +P and control. This suggests that N limited algal growth on July 21, 2017.

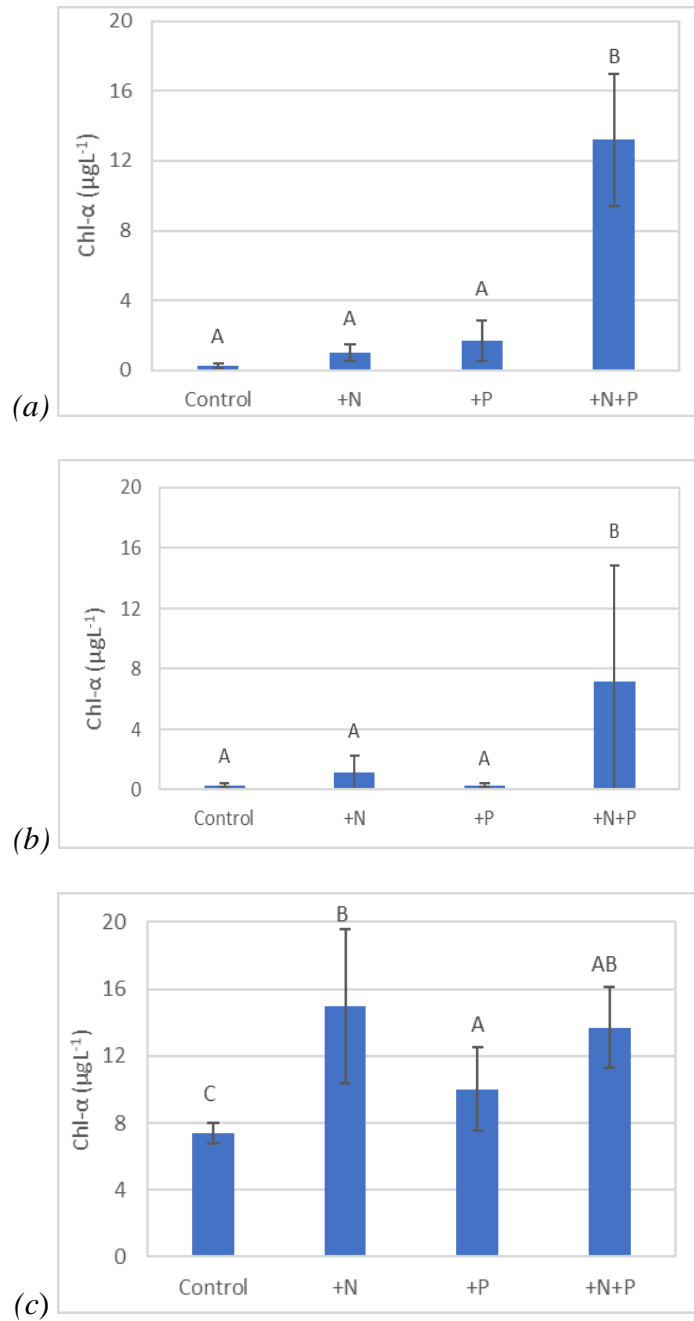


Figure 2: Mean (\pm standard deviation) chlorophyll- α ($\text{chl-}\alpha$) concentration across nutrient amendments at Spring Lake on (a) May 23, 2017, (b) July 1, 2017, and (c) July 21, 2017; treatments include control, nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+N+P) and homogenous groups are identified by the letters above the bars.

Lake Wedington

For first nutrient limitation experiment at Lake Wedington samples were taken from the lake on May 14, 2017 and incubated for eight days. The mean chl- α concentration in the control ($5.95 \mu\text{g L}^{-1}$) was not significantly different from the +N ($8.84 \mu\text{g L}^{-1}$) (ANOVA LSD, $p > 0.05$) (Figure 3a). But, the mean chl- α in the +N+P ($20.90 \mu\text{g L}^{-1}$) and +P ($10.79 \mu\text{g L}^{-1}$) treatments was significantly greater than all the other treatments ($p < 0.05$). This suggest that the algal growth was co-limited by P.

Sampling for the second experiment was done on June 26, 2017 and incubated for 5 days (Figure 3b). The mean chl- α concentration in the control ($0.8 \mu\text{g L}^{-1}$) was not significantly different from the +N ($1.745 \mu\text{g L}^{-1}$) or the +P ($1.62 \mu\text{g L}^{-1}$) treatments. But, the +N+P treatment ($13.045 \mu\text{g L}^{-1}$) had significantly greater mean chl- α concentrations than all three other treatments ($p < 0.05$). This implies that the lake was co-limited by N and P.

The samples for the third experiment were taken on July 7, 2017 and incubated for 4 days (Figure 3c). The mean chl- α concentration in the control ($2.22 \mu\text{g L}^{-1}$) was not significantly different from the +N ($4.74 \mu\text{g L}^{-1}$) or the +P ($2.93 \mu\text{g L}^{-1}$) treatments. But, the +N+P treatment ($9.17 \mu\text{g L}^{-1}$) had significantly greater mean chl- α concentrations than all three other treatments ($p < 0.05$). This shows that the lake was co-limited by N and P. We allowed the third set of samples to incubate for 4 more days and tested them again for chl- α (Figure 3d). The mean chl- α concentration in the control ($3.13 \mu\text{g L}^{-1}$) was not significantly different from the +N ($1.53 \mu\text{g L}^{-1}$) or the +P ($3.01 \mu\text{g L}^{-1}$) treatments. But, the +N+P treatment ($7.87 \mu\text{g L}^{-1}$) had significantly greater mean chl- α concentrations than all three other treatments ($p < 0.05$). This suggested that the lake was continued to be co-limited.

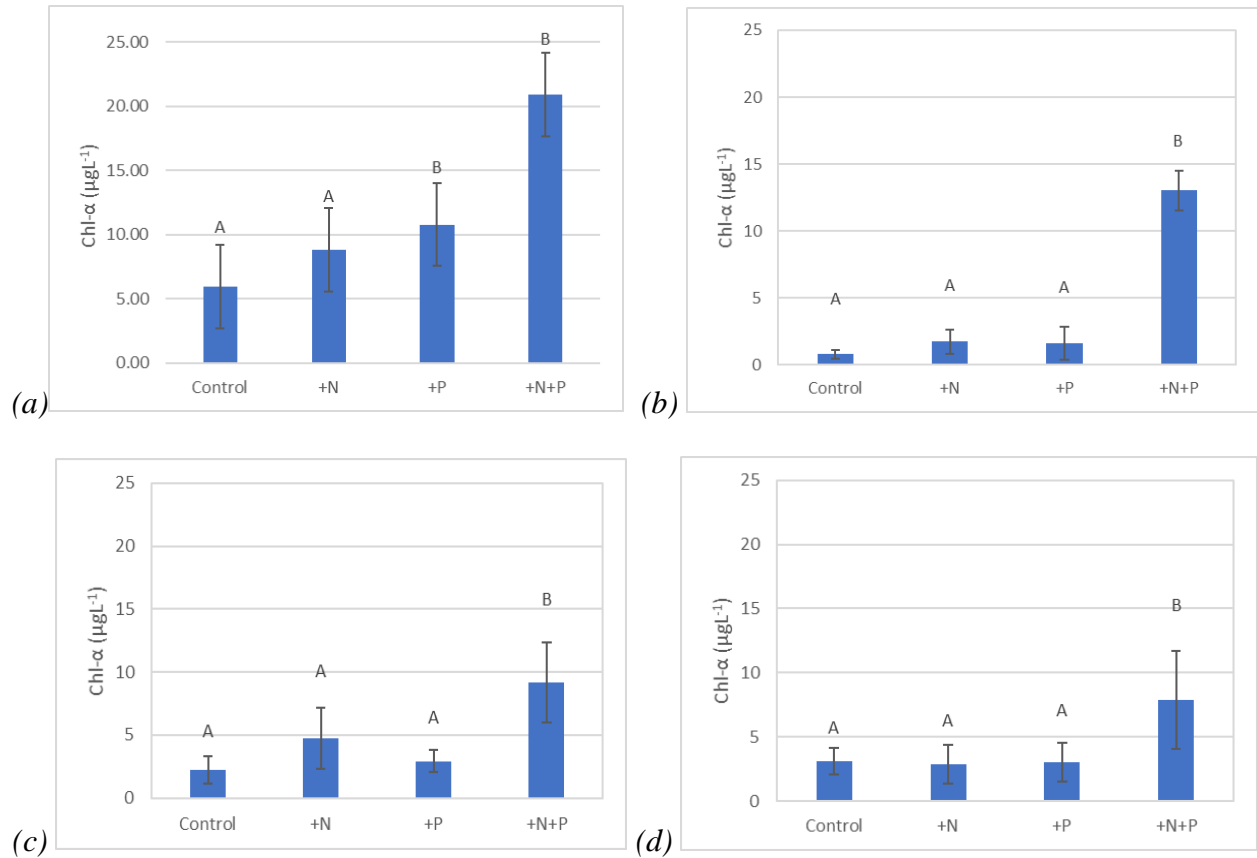


Figure 3: Mean (\pm standard deviation) chlorophyll- α ($\text{chl-}\alpha$) concentration across nutrient amendments at Lake Wedington on (a) May 22, 2017, (b) July 1, 2017, (c) July 11, 2017, and (d) July 13, 2017; treatments include control, nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+N+P) and homogenous groups are identified by the letters above the bars.

DISCUSSION AND FUTURE OPPORTUNITIES

The results from these nutrient limitation tests showed that all the study lakes were co-limited by N and P in May 2017. This is consistent with many other nutrients limitation studies across central US reservoirs (Dzialowski et al. 2005). However, many reservoirs shift from co-limitation to N limitation in late summer, whereas lakes with forested watersheds were consistently N limited (Hayes et al. 2015). The lakes in this study had mostly forested watersheds, but phytoplankton were generally co-limited by N and P early in the growing season.

Cove Lake was co-limited during the beginning of the growing season. The lake was fertilized on July 27, 2017 using two fertilizers, one that contained a 1.5:1:0 molar ratio of N:P:K and the other that contained only N. The overall molar ratio of N:P of the fertilizer applied was 22:1, and a total of 25 kg P and 233 kg N were applied to the lake. The week following fertilization the phytoplankton's growth was not limited by N or P, where nutrient addition did not increase chl- α . The phytoplankton at Cove Lake might have been limited by something other than N and/or P such as, light, space, or micronutrients (Tilman 1982). To determine if this was the reason algal growth stopped, similar experiments could be performed that would test other limiting factors. The algal growth may also have been maximized because the fertilizer applied was balanced, meeting both N and P needs for algal growth. The fertilization resulted in algal growth at Cove Lake, as the water was noticeably green. The cubitainers could have been filled when the bloom had peaked and the phytoplankton in the cubitainers were dying or being influenced by herbivory (Carpenter 1989). To determine if this is why algal growth was limited, fluorescence test could be performed on water directly after sampling to determine if there was more chl- α before the samples were allowed to incubate.

Spring Lake's phytoplankton population was co-limited by N and P from May through June 2017. The USFS fertilized Spring Lake twice during the growing season, first on July 11, 2017 and then on July 27, 2017. On July 11, 2017 the lake was fertilized with two fertilizers, the first containing a 1.5:1:0 molar

ratio of N:P:K, and the second containing only N. The combined fertilizer used had a 22:1 molar ratio, and a total of 13 kg P and 120 kg N were applied to the lake. After the first fertilization algal growth was limited by N, which might have meant that the phytoplankton needed more N than what was supplied. The ratio used on Spring Lake was close to being balanced and may have met the nutrient needs of the algae while allowing it to remain N limited, like many lakes in forested watersheds (Hayes et al. 2015). The same amount of N and P were applied during second fertilization. This likely caused the algae to remain N limited because the fertilizer that was applied met the nutrient requirements, similarly to the first fertilization.

Phytoplankton at Lake Wedington were co-limited by N and P from May through June 2017. The USFS fertilized the lake on July 5, 2017 using a fertilizer that contained a 1.5:1:0 molar ratio of N:P:K, applying a total of 25 kg P and 18 kg N to the lake. However, the next week the algae were still co-limited by N and P, which continued through the growing season at this lake. This could signify that the nutrient needs of the algae were not met during fertilization, because the ratio was not balanced or in sufficient quantity to meet algal needs.

The results from this study showed that fertilization of these lakes should be balanced with N and P to promote algal growth. Algal cells typically require between 10-20 mol N for every mol P (Klausmeier 2004). The fertilizer that was used on Cove and Spring Lakes had a molar ratio of 22:1, while Lake Wedington applies a 1.5:1 molar ratio of N:P. Since the fertilizer used at Lake Wedington was not balanced (10-20:1 N:P ratio) to meet algal needs, it is likely that some of the nutrients were “wasted”. Further guidance should be given to the USFS to help them adopt a fertilizer that is within the optimal range ratio for all of the lakes they manage.

The USFS should also consider research to find the optimal amount of fertilizer. In several of the studies the lakes returned to colimitation or showed N limitation shortly after fertilization. This could be due to the amount of fertilizer not meeting the needs of the algae, reducing the amount of algal growth

possible with lake fertilization. Leading to the hypothesis that increase of fertilizer could better promote algal growth and the sport fish population.

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