Combating Eutrophication: An Ecosystem Scale Analysis of Aluminum Sulfate (Alum) Effectiveness among lakes, with comparison to Alum and Biomanipulation Dual Treatment

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Combating Eutrophication: An Ecosystem Scale Analysis of Aluminum Sulfate (Alum) Effectiveness among lakes, with comparison to Alum and Biomanipulation Dual Treatment

by

Christa Webber

A THESIS

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Combating Eutrophication: An Ecosystem Scale Analysis of Aluminum Sulfate (Alum) Effectiveness among lakes, with comparison to Alum and Biomanipulation Dual Treatment.

Christa Webber M.S

University of Nebraska 2014

Advisor: Amy Burgin

Eutrophic conditions in lakes and reservoirs in agricultural regions often drive summer blooms of toxic cyanobacteria. Aluminum sulfate (alum) applications are commonly used to control cyanobacteria blooms and restore water quality in eutrophic lakes. However, studies of alum treatments often lack true replication, comparison to reference lakes, or comparison to other restoration techniques, such as an alum and biomanipulation combined or “dual” treatments. Without these comparisons, the variation of treatment response between replicate lakes and restoration techniques remains uncertain. Therefore, I sought to assess how water quality is affected by multiple restoration techniques among geographically proximate (1.4 km²) lakes. I hypothesized that: 1) alum restoration would uniformly improve water quality in replicate lakes via nutrient limitation, and 2) dual treatment restoration would out-perform alum treatment alone due to added top-down mechanisms amplifying the alum-only improvements. Regardless of lake or restoration technique, the phytoplankton community was dominated by cyanobacteria pre- and post-treatment in each lake. Treatment success was highly variable among replicate lakes. I found that trends for overall restoration success were not always representative of average lake condition post-treatment, but were often dominated by extreme response in a few lakes. Fully understanding how similar ecosystems are
affected by alum will help determine if lake alum treatments alone can consistently combat algal toxins and other symptoms of eutrophication. Overall, the alum treatment effectively controlled nutrient levels, however, if restoration goals are more biological, adding biomanipulation as a dual treatment may enhance lake restoration success.
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Chapter 1

Comparing Phytoplankton Community Responses to Aluminum Sulfate (Alum) Additions Among Eight Eutrophic Lakes

Abstract

Eutrophic conditions in lakes and reservoirs in agricultural regions often drive summer blooms of toxic cyanobacteria. Aluminum sulfate (alum) applications are an increasingly popular technique to control cyanobacteria blooms in eutrophic lakes. However, studies of alum treatments are often limited to a single lake and lack true replication, leaving the variation of treatment response between lakes uncertain. Therefore, we sought to assess the response of the phytoplankton community structure and cyanobacterial microcystin concentrations to alum treatment between eight replicate, geographically proximate (1.4 km$^2$) lakes. To quantify the effectiveness and variability of alum treatments, we collected bi-monthly plankton community and microcystin toxin samples from lakes pre- and post-alum treatment. Phytoplankton were identified to genus and analyzed using rank-abundance curves and J-evenness to understand changes in density and composition in alum treated lakes. All lakes were dominated by cyanobacteria pre- and post-alum addition. Cyanobacteria density, however, decreased significantly post treatment compared to pre-treatment conditions (p<0.001). Phytoplankton family rank-abundance shifted slightly in three lakes, but did not change in the other five lakes. Fully understanding how similar ecosystems are affected by alum will help determine if lake alum treatments alone can consistently combat algal toxins and other symptoms of eutrophication.
1.1 Introduction

Blooms of cyanobacteria can negatively affect human health and limit recreational use of impacted water bodies (Downing et al. 2001). Species within genera *Anabaena, Planktothrix, Anabaenopsis, Haplosiphon, Nostoc* and *Microcystis* can produce the toxin microcystin (Rantala et al. 2003, Codd et al. 2005). Microcystin is a stable compound that can travel through the aquatic food web, including human consumables (Figueiredo et al. 2004, Codd et al. 2005). Microcystin-producing blooms are becoming more frequent as climate change and cultural eutrophication increase (Johnston and Jacoby 2003, Ekvall et al. 2013, Pitois et al. 2014). Given the increased frequency of toxin-producing blooms, managers often turn to restoration techniques, which aim to alter plankton communities to shift away from toxin-producing taxa (Harris et al. 2014). Therefore, understanding the effect of lake restoration treatments on phytoplankton communities and microcystin toxin production is key for planning successful lake restoration (Figueiredo et al. 2004).

Phosphorus (P) is the main driver of eutrophication symptoms in aquatic systems (Carpenter et al. 1998, Reynolds and Davies 2001). It naturally limits primary production more than nitrogen, as P is mostly in insoluble forms bound to sediment (Schindler 1974, Reynolds and Davies 2001). Therefore, bioavailable P inputs contribute directly to the eutrophic status of a lake, stimulating excess phytoplankton growth (Schindler 1977, Holz and Hoagland 1999, Lewandowski et al. 2003). Phosphorus becomes available through external inputs or internal release from sediments under anoxic conditions (Reynolds and Davies 2001). External inputs from agricultural areas are especially
problematic in the Midwestern US because they contribute non-point source P to aquatic systems (Carpenter et al. 1998, Daniel et al. 1998). Mitigation strategies focused on lowering P are limited to either reducing external loading by eliminating inputs, or reducing internal loading by binding available P through a chemical treatment, often with the addition of aluminum sulfate (Lewandowski et al. 2003).

Aluminum sulfate, commonly called alum, has been used in wastewater treatment for decades and is an increasingly popular management tool for eutrophic lake restoration (Drikas et al. 2001). One of the first documented alum treatments in the U.S. was on Horseshoe Lake, Wisconsin in 1970. Re-evaluation in 1982 showed P concentrations in Horseshoe Lake were significantly reduced (Garrison and Knauer 1984). Following the success of Horseshoe Lake, several alum restorations since have documented decreased phosphorus (Francko and Heath 1981, Steinman et al. 2004) and chlorophyll-a concentrations (Schumaker et al. 1993, Romo and Becares 1994). Alum treatments, however, are not a panacea because some treated lakes show limited water quality improvements or demonstrate only short-term enhancement (i.e. months) (Garrison and Knauer 1984, Lewandowski et al. 2003).

Our understanding of when and where alum treatments will be effective is limited in large part by: 1) a lack of replicated field studies of the treatment and its effects, and 2) a focus on the direct effects of alum on P, without a concurrent effort to understand shifts in the plankton community. Most published studies observed pre- and post-treatment conditions of only a single lake (e.g. Francko and Heath 1981, Cooke et al. 1993, Lewandowski et al. 2003, Reitzel 2005) or at best, replicated areas within a single lake.
(Nogaro et al. 2013). To our knowledge, there are no other a-priori designed studies testing the variability of alum treatment effectiveness at the ecosystem scale. The absence of this information is probably due to the difficulty in achieving ecosystem-level replication, which is often substituted by the use of pseudo-sampling through microcosm experiments (e.g., Reitzel et al. 2003, Egemose et al. 2010, Galvez-Cloutier et al. 2012). Without a solid field-based scientific experiment, it is also difficult to understand how effective alum is on indirect water quality parameters, such as microcystin production, as opposed to direct water quality metrics, such as total phosphorus concentrations. Furthermore, when managing for microcystin toxins, it is impossible to understand the relationship between toxins and alum additions without a full analysis of the phytoplankton community (Jochimsen et al. 2013). Measures of water clarity and chlorophyll-α concentrations are important indicators of water quality, but do not indicate community structure. Microsystins are produced by organisms that experience biological (competition, predation, etc.) and chemical interactions (Sih et al. 1985). Due to complex food-web structures and varied lake morphology, biology may not always react similarly to alum treatment between lakes, even in the same geographic area (Galvez-Cloutier et al. 2012, Nogaro et al. 2013).

Given the unknown interactions between eutrophication, alum additions, and shifts in plankton community structure, we ask: Do phytoplankton density, community structure and associated algal microcystin toxin concentrations respond to alum treatment uniformly among replicate lakes? Our experimental design includes pre- and post-alum community and toxin data collected from eight replicate treatment lakes over two years.
We hypothesized that phosphorus limitation via alum additions will cause a decrease in overall phytoplankton density coupled with a shift from a cyanobacteria dominated community to a more evenly mixed phytoplankton community. Furthermore, we predict that the reduction in phytoplankton density, especially cyanobacteria, will cause a significant decrease in microcystin concentration in all treated lakes.
1.2 Methods

1.2.1 Study Site and Alum Treatment

Fremont Lakes State Recreation Area (FSRA) is located approximately two miles west of Fremont, NE (41°44'16.7"N, 96°35'51.3"W). Annually 800,000 people visit this area, comprised of 20 small sandpit lakes collectively covering 265 ha. These lakes formed when depressions from sand mining filled with groundwater. Shared characteristics among the lakes include small size, groundwater source, shallow depth (<6 m), close geographic proximity (<1.4 km²), and irregular shorelines (Table 1, Figure 2). There are over 800 lakes with similar histories and features located in Nebraska, and likely many others in areas where “borrow pits” were created to aid in road construction. By sampling this subset of Nebraska’s sandpit lakes, we are able to capture the variation of biological response to chemical treatments aimed at altering algal density, community structure and microcystin concentration.

In October 2012, 250 tons of alum were added to lakes 1, 2, 3, 5, 10, 11, 12, and 15 by private contractor (Harmful Algal Blooms Aquatics, Lincoln, NE) at an average dose of 57.5g Al/m² (Table 1). Alum application consisted of a barge spraying alum and a sodium aluminate buffer just under the lake surface. The buffer solution was applied with alum to minimize biologically harmful changes in pH. The crew used GPS to evenly distribute the chemical treatment entirely over each lake.

Prior to the alum addition (summer of 2012), we collected water quality samples every other week from June through October (n=9) in our eight focal lakes (named by number: 1, 2, 3, 5, 10, 11, 12, and 15). At the deepest point in each lake, samples were
collected for total nitrogen (TN), total phosphorus (TP), turbidity, chlorophyll-\(a\), phytoplankton, and microcystin. Post-treatment sampling in 2013 followed the same sampling regime and protocols, collecting samples from June through October (n=9).

1.2.2 Sample Collection and Analysis

We collected TN and TP samples from the epilimnion at 0.5m below the surface of each lake. Samples were taken via a Van Dorn bottle and preserved with 10N sulfuric acid. We refrigerated samples until laboratory analysis by colormetric method (EPA 365.4) with detection limits of 3.57\(\mu\)M for TN and 0.81\(\mu\)M for TP.

Three replicate chlorophyll-\(a\) samples were collected from the epilimnion, filtered through 0.45 micron filters, stored in dark aluminum envelopes, and frozen until analysis. Chlorophyll-\(a\) was extracted using ethanol incubation and florometric reading (Nusch 1980). Replicates were averaged for each timepoint.

We collected phytoplankton samples from the epilimnion at 0.5m below the surface of each lake. Three 100mL replicates were taken via Van Dorn bottle at the deepest point for each sampling period. Ten mL of Lugols solution was added to preserve each sample. Samples were stored in dark cabinets until prep for microscopy analysis.

To quantify phytoplankton density and species richness, we counted individuals and colonies using an inverted microscope at 200x using five mL settling chambers. Settling occurred by allowing 3mL subsamples, taken from vigorously mixed field samples, to sit overnight. After settling, a grid eyepiece aided in accurately identifying and counting at least 300 individuals to the genus level in 10 fields of view, as
recommended by standard protocols (Eaton et al. 1995). To achieve countable phytoplankton densities, dilutions and concentrations were also performed. Totals for each genus were then multiplied by their respective dilution/concentration factors to estimate density of plankton per liter for each lake.

We collected one microcystin sample from the epilimnion at 0.5m below the surface of each lake. Samples were collected via a Van Dorn bottle, stored in amber bottles, and frozen until laboratory analysis. A freeze thaw procedure was used to lyse cells before analysis using a Microcystin ELISA kit (Abraxis, Kansas) with detection limit of 0.075 μg/L.

1.2.3 Statistical Analysis

We analyzed nutrient, phytoplankton count data, and microcystin concentrations from eight alum lakes (1, 2, 3, 5, 10, 11, 12, 15) pre and post-treatment. To understand family dominance we plotted rank-abundance curves for each lake in R (version 2.15.1) using “ggplot2” and calculated phytoplankton community J-evenness using “vegan”. We also used repeated measures analysis of variance (RM-ANOVA) to analyze significant differences between pre and post-treatment microcystin concentrations and phytoplankton densities. To compare trends relating to phytoplankton community evenness and microcystin concentrations, we incorporated water quality metrics into global mixed models. We selected a top model for both J-evenness and microcystin concentrations using backwards selection and AIC values in R using packages “lme4” and “AICcmodavg”.
1.3 Results

Phosphorus was the limiting nutrient for phytoplankton growth compared to nitrogen before treatment (TN:TP = 98.5) given a Redfield N:P ratio of 16:1 (Table 2). Alum treatment created significantly more P limited conditions post-treatment (TN:TP = 129.05) (Table 2) (RM-ANOVA, F=36.5, d.f=1,188, p<0.01). Total phosphorus concentrations in individual lakes were not significantly different post-treatment (Tukey post-hoc test, pvalues range from 1-0.11); however, TP decreased in all lakes post-treatment (Table 2).

Averaged across all lakes, chlorophyll-a concentration significantly decreased 54% overall post treatment (RM-ANOVA, F=18.82, df=1,552, p<0.01)(Figure 2aTotal). Post-hoc Tukey analysis found no significant treatment effect for chlorophyll-a for any individual lake. Mean chlorophyll-a decreased for each lake following treatment except Lake 12 (Table 2, Figure 2a).Across all eight lakes, post-treatment phytoplankton density was not significantly different than pre-treatment conditions (RM-ANOVA, F=0.079, df=1,420, p=0.78). In analyzing phytoplankton density changes in individual lakes, we found that phytoplankton density only significantly decreased in Lake 15 post-treatment (RM-ANOVA, F=7.752, d.f.=1,49 p<0.01) compared to the pre-treatment condition. In Lakes 1, 5, and 12 phytoplankton density increased significantly post-treatment (RM-ANOVA Lake 1 F=22.57, d.f.=1,50 p<0.01; Lake 5 F=20, d.f.=1,50 p<0.01; Lake 12 F=19.24, d.f.=1,48 p<0.01) (Figure 2b).

Overall, microcystin decreased significantly from the pre-treatment condition (RM-ANOVA, F=8.642, d.f.=1,188, p<0.01) (Figure 2c). However, nearby untreated
lakes also experienced an 87% decrease in microcystin concentrations between 2012 and 2013 (data not shown), suggesting that much of this decrease is due to inter-annual variation and not necessarily due to the alum treatments. Microcystin concentrations significantly decreased in Lakes 1, 2, and 5 post-treatment (RM-ANOVA, Lake 1 F=33.52, d.f.=1,20 p<0.001; Lake 2 F=48.42, d.f.=1,20 p<0.001; Lake 5 F=12.55, d.f.=1,20 p<0.01) (Figure 2c).

Phytoplankton communities overlap considerably among the eight alum treated lakes, both before (Figure 3a) and after (Figure 3b) treatment. Overall, there is less variation and more overlap post alum treatment. Both pre- and post-restoration, lakes 10 and 15 overlap more than other lakes due to their connectedness (Figure 1), but are also more distinct from other lakes in the overall group. Lakes 10 and 15 also had the highest average chlorophyll-a across all eight lakes (Figure 2a).

Cyanobacteria consistently dominated the phytoplankton communities in all lakes both pre- and post-treatment (Figure 4). Although cyanobacteria continued to be the most abundant group post-restoration, overall cyanobacterial density significantly decreased 58% following the alum treatment compared to pre-treatment conditions (RM-ANOVA, F=3.964, d.f.=1,420 p<0.05). This decrease in cyanobacteria was coupled with a slight increase in proportional abundance of other families post-treatment (Figure 4).

Changes in the phytoplankton community structure increased community evenness 10%, which was not a statistically significant increase (Figure 4, Table 2). To predict community evenness, our top AIC model used the predictors: lake volume, phytoplankton total density, sampling week, and sampling year with a random effect of
lake by week. Post-treatment community evenness was always greater than pre-treatment evenness across all eight lakes. The lakes differed, however, in seasonal patterns of phytoplankton community evenness with densities increasing (Lakes 2, 11, 12), decreasing (Lakes 5, 10, 15) and increasing pre-treatment but decreasing post-treatment (Lakes 1, 3) (Figure 5).

The phytoplankton community shift to a slightly more even assemblage coincided with a 76% decrease in microcystin concentrations (Figure 2c). Microcystin concentrations were best predicted by a linear mixed model using: chlorophyll-a, turbidity, north or south area of the park (Figure 1), Cylindrospermopsis sp. density, and sampling year with a random effect of lake by week. This model consistently shows a decrease in concentration between the two years (Figure b). As with community evenness, microcystin concentrations trends varied by lake, exhibiting increasing trends (Lakes 3, 5, 10, 11, 15) and decreasing trends (Lakes 1, 2, 12) over the season (Figure 6).
1.4 Discussion

1.4.1 Did alum restoration significantly improve water quality?

We predicted that bottom up nutrient limitation via alum would cause a cascade of improved water quality variables of phosphorus, chlorophyll-$a$ and phytoplankton, phytoplankton community $J$-evenness, and microcystin toxins (Figure 7). In our conceptual model (Figure 7) these metrics are ordered one through four with variables more likely to be influenced by alum first, such as phosphorus, working towards more indirect restoration goals, such as microcystin concentrations. We posit that restoration success increases as the number of variables exhibiting significant improvement post-treatment increases. Post-treatment evaluation of each metric results in one of two outcomes: no change or significant improvement (Figure 7). Several possible mechanisms may control each variable. The degree to which alum treatment is successful, as a result of improvements in variables one through four, produces specific restoration implications for each level of treatment success (Figure 7). These implications are listed for the outcome of no change, which implies the end of alum treatment effectiveness.

Overall, the Fremont Lakes alum restoration fits our definition of success because it enhanced water quality in our eight lakes. We observed decreased total phosphorus (Table 2), chlorophyll $a$ (Table 2, Figure 2a), phytoplankton density (Figure 2b), and cyanobacterial density (Figure 4) concurrent with slight increases in community evenness (Table 2, Figure 5), and decreasing microcystin concentrations (Table 2, Figure 3c,
Figure 6) post-treatment. We hypothesized that water quality would improve significantly for all eight lakes; however, in examining individual lakes, we repeatedly observed inconsistent trends for each water quality metric. For example, alum treatment in Lake 15 resulted in significant increase in J-evenness post-treatment (Tukey Post-hoc test p<0.05), but no other significant water quality improvements. One unforeseen consistency across all lakes was that cyanobacteria continued to dominate the phytoplankton communities in all eight lakes, because the increase in evenness was not enough to overcome extreme cyanobacterial dominance. We found that while overall these alum treatments would be considered successful from a management perspective, there remain some limitations to our ability to predict when and where alum will effectively alter phytoplankton communities away from toxin producing groups.

1.4.2 Variation in the success of lake alum additions

Lake restoration treatments can only be successful if they isolate the correct driver of the targeted water quality issue (Hickey and Gibbs 2009, Xu et al. 2010). Thus, quantifying phytoplankton community responses to alum additions is necessary if the ultimate goal is to alter the phytoplankton community away from potentially toxin-producing groups. Restoration treatments are often performed to limit cyanobacterial blooms and associated toxin concentrations to improve water quality for human use (Codd et al. 2005, Jančula and Maršálek 2011). However, many studies only examine P concentrations following alum addition as a means of measuring treatment success (e.g., Francko and Heath 1981, Lewandowski et al. 2003, Steinman et al. 2004). We considered alum lake restoration to be successful if alum’s direct effect on phosphorus continues to
impact chlorophyll-\(a\), phytoplankton density, phytoplankton community structure, and microcystin toxin concentrations (Figure 7). Success at the individual lake level was limited because half of the lakes did not see statistical decrease in chlorophyll-\(a\) or phytoplankton density post treatment.

The variable responses among our eight lakes seem typical of a wider array of the success of alum treatments in lakes across a range of sites and conditions (Table 3). In our search of the literature, we did not find a study with a comparable number of lakes for comparison. The second most replicated study (3 lakes total) was a 12 year evaluation by Garrison and Knauer (1984), who observed phosphorus concentrations in three lakes post alum restoration, one with long-term improvement, one short-term improvement, and one with no change. Conflicting results between studies are also common, for example Smeltzer (1990) observed a decrease in TP and chlorophyll-\(a\) two years post treatment, while Galvez-Cloutier et al. (2012) found an increase in TP and no effect on chlorophyll-\(a\) two months post-treatment. Successful restorations are also plentiful in published results from previous work. A successful restoration in Romo and Becares (1994) observed a one-year decrease in nutrients and cyanobacteria compared to pre-treatment conditions. Another success story in Newman Lake showed alum treatment effectively decreased chlorophyll-\(a\) and cyanobacteria over two years post treatment (Schumaker et al. 1993). However, several other lake alum treatments failed to reduce cyanobacteria (Table 3, Xie et al. 2012, Galvez-Cloutier et al. 2012, Steffen et al. 2014). Similar to our results, Harris et al. 2014 also observed a decrease in cyanobacteria abundance post-alum treatment with no shift in phytoplankton community structure (Table 3).
The treatment’s failure to shift the phytoplankton community away from toxin-producing species (Figure 4) calls in to question the long-term effectiveness of alum alone as a restoration strategy (Welch and Cooke 1999, Steinman and Ogdahl 2008). Processes not influenced by the alum addition may drive persistent cyanobacteria dominance. Our replicate lakes had very similar morphology, but not necessarily the same initial food web structure. Foodweb dynamics are lake specific and dictate energy flow, possibly interfering with chemical alum treatment effects (Moss 1990). Maintaining high grazing pressure on phytoplankton is a key component of managing algal blooms, an issue not addressed by alum (Haney 1987, Mazumder 1994, Gobler et al. 2007). Also, lakes that contain burrowing invertebrates and/or rough fish may be bad candidates for alum treatments as they can quickly resuspend the flocculation layer releasing P back into the water column, re-initiating cyanobacterial algal blooms (Niemisto et al. 2008, Nogaro et al. 2009). Alum may also be ineffective at limiting migration of cyanobacteria from the phosphorus rich sediments into the water column, perpetuating the dominance of cyanobacteria post-treatment (Sonnichson 1997, Head et al. 1999).

Microcystin concentrations in alum treated lakes followed similar trends as nearby untreated lakes (data not shown), calling into doubt whether the observed significant changes due to the alum treatment alone. A similar study comparing an alum treated lake to an untreated lake observed a decrease in TP and chlorophyll-\(\alpha\), but no effect on microcystin (Xie et al 2012). Abiotic factors such as light or pH may explain more of the variation in microcystin between treatments and lakes (Graham et al. 2004, Dziallas and Grossart 2011). Cyanobacterial cell degradation by bacteria in the sediment could also cause a release in microcystin, making alum ineffective for controlling high
microcystin concentrations (Lam et al. 1995, Han et al. 2012). Han et al. (2013) observed microcystin release from cyanobacterial cells up to six days after an alum treatment. Alum’s ineffectiveness to decrease microcystin compared to reference lakes, and the popularity of choosing alum treatments over other restoration techniques that might control this toxin, sets the stage for potential increases in toxic microcystin. This trend is especially problematic in the future as eutrophication becomes more prevalent due to warming temperatures and land use change (Figueiredo et al. 2004, De Senerpont Domis et al. 2007, Paerl et al. 2011).

1.4.3 Implications for managing cyanobacteria blooms and microcystin with alum treatments

If alum restorations have highly variable effectiveness in lakes with similar morphology, extrapolating findings to the management of other lakes based on physical characteristics is unreasonable. It is important to study restoration projects as replicated experiments at the ecosystem level to understand the range of biological response due to complex heterogeneous environments (Schindler 1977, Tilman et al. 1982). However, most alum lake restoration studies only observe one lake, which limits our understanding of the effectiveness of this treatment on water quality (Table 3). Additional replicated, ecosystem-scale studies of morphological, biological, and climate interactions with alum lake restoration treatments may clarify which scenarios are likely to have high success at improving water quality with alum.
Alum additions alone may not be enough to adequately control toxic algae or microcystin production. Lakes that are close in proximity to an external source of phosphorus quickly return to their eutrophic state and continue to experience cyanobacterial blooms post-treatment (Garrison and Knauer 1984, Xie et al. 2012). Therefore, many successful alum treatments include a known P budget and a reduction of external phosphorus sources (Smeltzer 1990, Schumaker et al. 1993) since alum restoration is known to be more effective for managing internal P loading (Søndergaard et al. 2003). Considering a long-term watershed approach or a combination of several treatments (e.g., biomanipulation) may be important to effectively manage eutrophic lakes (Reed-Andersen et al. 2000, Harris et al. 2014, Smith et al. 1999, Webber et al., in preparation).
Table 1. Lake morphometric data of eight alum treated lakes within FSR.

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<th>Elevation (m)</th>
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<th>Volume (m³)</th>
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</table>
Table 2: Chemical data before (2012) and after (2013) alum restoration. Water quality metrics with averages and standard deviations show differences between replicate lakes.

<table>
<thead>
<tr>
<th>Lake #</th>
<th>Total N (µM)</th>
<th>Total P (µM)</th>
<th>Ratio (TN:TP)</th>
<th>Chl a (µg/L)</th>
<th>J-evenness</th>
<th>Microcystin (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Treatment (2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>119.2± 168.5</td>
<td>1.3± 1.0</td>
<td>92.4</td>
<td>22.4 ±23.8</td>
<td>0.70 ± 0.07</td>
<td>11.8 ± 11.3</td>
</tr>
<tr>
<td>2</td>
<td>182.8 ± 245.7</td>
<td>1.6 ± 0.7</td>
<td>113.5</td>
<td>25.9 ± 17.9</td>
<td>0.61 ± 0.04</td>
<td>16.8 ± 16.8</td>
</tr>
<tr>
<td>3</td>
<td>187.1 ± 196.3</td>
<td>1.6± 1.0</td>
<td>116.2</td>
<td>16.4 ± 11.8</td>
<td>0.60± 0.10</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>154.2 ± 154.2</td>
<td>1.6 ±1.3</td>
<td>95.8</td>
<td>24.6 ± 20.0</td>
<td>0.61 ± 0.08</td>
<td>4.9 ± 4.0</td>
</tr>
<tr>
<td>10</td>
<td>274.2 ± 281.3</td>
<td>2.9 ± 1.6</td>
<td>94.2</td>
<td>72.5 ± 54.3</td>
<td>0.48 ± 0.08</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>117.8 ± 159.9</td>
<td>1.3 ± 1.0</td>
<td>91.3</td>
<td>11.8 ± 11.1</td>
<td>0.68 ± 0.04</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>12</td>
<td>114.2 ± 144.2</td>
<td>1.0 ± 1.0</td>
<td>117.8</td>
<td>7.7 ± 5.8</td>
<td>0.65 ± 0.05</td>
<td>3.5 ± 3.9</td>
</tr>
<tr>
<td>15</td>
<td>171.4 ± 193.5</td>
<td>2.6 ± 1.2</td>
<td>66.4</td>
<td>101.4 ± 95.3</td>
<td>0.51 ± 0.09</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>Avg</td>
<td>165.1 ± 53.2</td>
<td>1.7 ± 0.7</td>
<td>98.5 ± 17.1</td>
<td>35.3 ± 33.4</td>
<td>0.64 ± 0.07</td>
<td>4.8 ± 6.25</td>
</tr>
</tbody>
</table>

| After Treatment (2013) | | | | | | |
| 1 | 144.2 ± 137.1 | 0.7 ± 0.3 | 221.9 | 10.8 ± 2.3 | 0.66 ± 0.05 | 2.0 ± 2.2 |
| 2 | 97.1 ± 80.0 | 0.3 ± 0.7 | 303.1 | 11.3 ± 2.1 | 0.71 ± 0.03 | 2.4 ± 2.5 |
| 3 | 79.0 ± 84.00 | 1.0 ± 1.9 | 81.4 | 11.9 ± 7.7 | 0.68 ± 0.03 | 1.8 ± 2.5 |
| 5 | 55.8 ± 55.8 | 0.7 ± 1.0 | 85.8 | 10.7 ± 10.1 | 0.66 ± 0.05 | 0.9 ± 1.0 |
| 10 | 77.1 ±84.3 | 1.0 ± 1.0 | 79.5 | 31.0 ± 18.6 | 0.59 ± 0.07 | 0.1 ± 0.1 |
| 11 | 68.5 ± 87.1 | 0.8± 0.3 | 12.5 | 4.9 ± 2.4 | 0.69 ±0.04 | 0.2 ± 0.1 |
| 12 | 84.3 ± 77.1 | 0.7 ± 0.7 | 129.6 | 7.6 ± 3.7 | 0.67 ± 0.06 | 1.8 ± 1.2 |
| 15 | 77.1 ± 94.2 | 0.7 ± 1.0 | 118.6 | 26.7 ± 11.9 | 0.62 ± 0.06 | 0.1 ± 0.1 |
| Avg | 85.4 ± 26.6 | 0.7 ± 0.9 | 129.1 ± 92.0 | 14.4 ± 9.30 | 0.66 ± 0.04 | 1.2 ± 0.9 |
Table 3. Summary of lake alum application results from the literature. Web of Science search results were narrowed to alum treatments on freshwater lakes. Search terms included “alum + microcystin”, “alum + cyanobacteria”, “alum + phytoplankton”, and “aluminum-sulfate + cyanobacteria”.

<table>
<thead>
<tr>
<th>Study, Location</th>
<th># of Lakes</th>
<th>Study type</th>
<th>Experimental design</th>
<th>Findings</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Webber et al. 2015, NE</td>
<td>8</td>
<td>Field</td>
<td>pre/post treatment repeated measures n=72</td>
<td>significantly decreased TP, chlorophyll-a, phytoplankton density. Decrease in microcystin suspect.</td>
<td>Limited, phytoplankton community dominated by Cyanobacteria post-treatment</td>
</tr>
<tr>
<td>Garrison and Knauer 1984, WI</td>
<td>3</td>
<td>Field</td>
<td>pre/post long term study</td>
<td>Long-term decrease in P, short term decrease in P, no decrease in P</td>
<td>Limited, magnitude and duration of P decrease was highly variable between lakes</td>
</tr>
<tr>
<td>Xie et al 2012, MI</td>
<td>2</td>
<td>Field</td>
<td>treatment/control n=56</td>
<td>Lower TN, higher SRP, lower TP, higher TN:TP, lower chlorophyll-a, lower turbidity</td>
<td>No, did not control Limnothrix blooms or Cyanobacterial toxins</td>
</tr>
<tr>
<td>Romo and Becares 1994, Spain</td>
<td>2</td>
<td>Field</td>
<td>pre/post treatment 3yr</td>
<td>Long term decrease in nutrients and cyanobacteria, short term improvement for dissolved oxygen, chlorophyll-a, and transparency</td>
<td>Yes, decreased cyanobacteria</td>
</tr>
<tr>
<td>Harris et al 2014, OR</td>
<td>1</td>
<td>Field</td>
<td>treatment/control: in situ mesocosm</td>
<td>Reduced TN, TP, and cyanobacterial biovolume</td>
<td>No, TP decreased but no change in N:P ratio, cyanobacterial relative abundance, or microcystin</td>
</tr>
<tr>
<td>Zhang et al 2008, China</td>
<td>1</td>
<td>Field</td>
<td>post-treatment only, n=7</td>
<td>TN=3.86 mg/L, TP=0.32 mg/L, microcystin=7.68-15.81ug/L</td>
<td>No, nutrient levels and microcystin are above recommended drinking standards</td>
</tr>
<tr>
<td>VanHullebusch et al. 2002, France</td>
<td>1</td>
<td>Field</td>
<td>9 sampling locations</td>
<td>SRP and turbidity decrease for 60 days, no decrease in TP</td>
<td>Limited, no long term change in P concentration, but bioavailability is unknown</td>
</tr>
<tr>
<td>Reitzel et al. 2003, Denmark</td>
<td>1</td>
<td>Field</td>
<td>treatment/controls, mesocosm experiment</td>
<td>Decrease in TP and transparency, but not phytoplankton</td>
<td>Yes, increased transparency</td>
</tr>
<tr>
<td>Schumaker et al. 1993, WA</td>
<td>1</td>
<td>Field</td>
<td>pre/post treatment, 2.5 years</td>
<td>Decreased chlorophyll-a and cyanobacteria</td>
<td>Yes, decreased phytoplankton</td>
</tr>
<tr>
<td>Smeltzer 1990, VT</td>
<td>1</td>
<td>Field</td>
<td>pre/post treatment, 2yr each</td>
<td>Reduced TP and chlorophyll-a, increased transparency</td>
<td>Yes, water quality improved for 4 years</td>
</tr>
<tr>
<td>Franco and Heath 1981, OH</td>
<td>1</td>
<td>Field</td>
<td>pre/post treatment 6 months</td>
<td>Decrease in phosphate</td>
<td>Yes, decrease in phosphate but not other P molecules</td>
</tr>
<tr>
<td>Steffen et al. 2014, OH</td>
<td>1</td>
<td>Field</td>
<td>post-treatment pilot study</td>
<td>Decreased internal P loading 55%</td>
<td>No, this lake maintained hypereutrophic status and cyanobacteria dominated phytoplankton community</td>
</tr>
<tr>
<td>Galvez-Cloutier et al. 2012, Quebec</td>
<td>1</td>
<td>Field &amp; lab</td>
<td>treatment/control: field enclosure N=4, lab microcosm</td>
<td>TP-removal was 72% on day 1, but only 12% on day 69</td>
<td>No, TP still above critical limit of 20 ug/L, no change in chlorophyll-a, phytoplankton, or turbidity</td>
</tr>
<tr>
<td>Nagoro et al 2013, OH</td>
<td>1</td>
<td>Field &amp; lab</td>
<td>treatment/control, lake mesocosms, n=6 each, sediment cores</td>
<td>Decreased TP, TSS, SRP turbidity and chlorophyll-a</td>
<td>No, decreased TP but not consistently between bays, increased dissolved Al and large change in pH</td>
</tr>
<tr>
<td>Steinman 2004, MI</td>
<td>1</td>
<td>Lab</td>
<td>treatment/control, 3 cores each</td>
<td>Decreased P release</td>
<td>Yes, reduced internal loading</td>
</tr>
<tr>
<td>Lewandowski et al. 2003, Germany</td>
<td>1</td>
<td>Lab</td>
<td>treatment/control, 3 cores each</td>
<td>P slightly reduced</td>
<td>No, magnitude and duration of P decrease was less than expected</td>
</tr>
</tbody>
</table>
Figure 1. Map of eight (black) Fremont State Recreation Lakes. Lakes were sampled at the deepest point pre- and post-alum addition.
Figure 2. Water quality metrics of chlorophyll-\(a\) concentration (a; \(n=36\), phytoplankton density (b; \(n=36\), and microcystin concentration (c; \(n=12\) among eight FSR lakes before (white) and after (gray) alum restoration. Eight sub-panels indicate one lake for both years for direct comparison. Sub-panels labeled “Total” average over all eight lakes for both years. The median is represented by black line within each boxplot. Stars indicate significant change between years.
Figure 3. NMDS plot for pre-treatment (panel a) and post-treatment (panel b) for all alum treated lakes. Lake polygons were drawn using phytoplankton community data at the genus level and evaluated using a Bray-Curtis index.
Figure 4. Average phytoplankton rank-abundance between all eight lakes pre and post-treatment condition (n=288). Pre-treatment background conditions in 2012 are represented by a solid line, while post-treatment conditions in 2013 are represented by a dashed line. Symbols represent the mean with error bars of +/- 1 SE.
Figure 5. Top model for phytoplankton community J-evenness by lake and symbolized by year. Solid line indicates pre-treatment data (2012), dashed line indicates post-treatment data (2013).
Figure 6. Top model for microcystin concentration for each FSRA lake and symbolized by year. Solid line indicates pre-treatment samples from 2012. Dashed line indicates post-treatment samples from 2013.
Figure 7. Conceptual model framework connecting water quality metrics, treatment success, and broader implications for alum lake restoration.
References


Chapter 2

Combating eutrophication in freshwater lakes: The benefit of dual alum and biomanipulation treatments

Abstract:

Aluminum sulfate (alum) applications are often used alone as a management tactic to combat lake and reservoir eutrophication symptoms including summer blooms of toxic cyanobacteria. However, studies of alum treatments often lack direct comparison to controls (nearby untreated lakes), other management strategies (e.g., biomanipulation), and are often not replicated at the ecosystem level. Therefore, we sought to compare the response of three untreated (control) lakes, three alum only treated lakes, and three alum + biomanipulation (dual treatment) lakes. We quantified water quality using metrics of dissolved oxygen, total nitrogen, total phosphorus, the nitrogen:phosphorus ratio, chlorophyll-\(a\) concentration, phytoplankton density, cyanobacteria density, microcystin concentration, and zooplankton density. Comparing metrics for these variables between geographically proximate (all in a <1.4 km\(^2\) area) lakes allowed us to accurately quantify treatment success. We collected samples twice a month from lakes for one summer of pre-treatment data and one summer of post-treatment data. Overall, the alum treatment effectively controlled nutrient levels, however improvements in overall water quality consistently occurred in dual-treated lakes. If restoration goals are more biological, adding biomanipulation as a dual treatment may enhance lake restoration success.
2.1. Introduction

A large focus of freshwater management aims to reduce nutrient loading to aquatic environments to improve water quality, especially the reduction of nuisance algae blooms (Cooke et al. 1993, Gulati and Van Donk 2002, Lathrop et al. 2002, Meijer et al. 1999, Søndergaard et al. 2007). Eutrophication, usually driven by increased phosphorus loading, results in a loss of aquatic ecosystem functions including water purification, nutrient cycling, and recreation (Richardson and Jørgensen 1996, Schindler 1974, Smith et al. 1999, Smith and Schindler 2009). Algae blooms are unsightly and can result in anoxic conditions, leading to unpleasant odors and fish kills. Furthermore, blooms are often composed of toxic algae (cyanobacteria) capable of producing microcystin toxins (Rantala et al. 2003). Several genera of cyanobacteria can produce and release hepatotoxic microcystin, which in high concentrations cause unsafe recreational conditions (Codd et al. 2005, Figueiredo et al. 2004, Rastogi et al. 2014). Microcystin also contaminates drinking water supplies, as was the case for the city of Toledo (OH, USA) in Aug. 2014 (Frankel 2014). To combat eutrophication and potential toxin production, various treatments such as chemical additives and fish renovations aim to lower nutrient concentrations, reduce cyanobacteria, and minimize human exposure to microcystin (Annadotter et al. 1999).

Aluminum-sulfate chemical treatments (alum) are a popular management tool for reducing nuisance algae blooms and concomitant microcystin concentrations (Chow et al. 1999, Garrison and Knauer 1984). First used in wastewater treatment plants, alum removes phosphorus and intact cyanobacterial cells from the water column by forming a

Fishery renovation, a form of biomanipulation, is an alternative eutrophication management strategy. A fish community can be restored following rotenone chemical application, which eliminates all fish and allows managers to restock a more desirable community (Reinertsen et al. 1990, Sanni and Wærvågen 1990). This technique reduces sediment resuspension by eliminating benthic feeding fish (Breukelaar et al. 1994, Rowe 2007, Søndergaard et al. 2003, Van Hullebusch et al. 2003) and can change the food-web structure (Van Hullebusch et al. 2003, Wahl et al. 2011). Food-web structures with biomass at specific trophic levels reduce zooplanktivores through the addition of piscavores species through a top-down trophic cascade (Shapiro and Wright 1984, Smith and Schindler 2009). This process can promote high densities of zooplankton, which reduce algae blooms through grazing (Carpenter et al. 1985, Havens 1993, Jeppesen et al. 1999).

Adding other restoration techniques (e.g., biomanipulation) with alum applications might amplify desired water-quality improvements. Combined techniques, called “dual treatments” herein, can strengthen bottom-up nutrient limitation and promote top-down grazing pressure on nuisance algae blooms. Alum additions to eutrophic lakes with burrowing invertebrates or benthic feeding fish, such as common...
carp, may see decreased effectiveness due to sediment resuspension (Lougheed et al. 1998, Nogoro et al. 2009, Parkos III et al. 2003). To circumvent this undesired effect, lake restoration with biomanipulation reduces sediment suspension, allowing the alum “cap” at the benthos to remain intact. An added benefit of dual treatment is a potential increase in zooplankton grazing in conjunction with phosphorus limitation, which may significantly improve water quality over chemical (e.g., alum) treatments alone.

Combining chemical and biological treatments should consistently result in improved water quality compared to reference conditions (Kaihong et al. 2012, Reitzel et al. 2003, Van Hullebusch et al. 2002). However, a replicated experimental design directly comparing these restoration options is necessary to fully evaluate the added impact of using a dual alum-and-biomanipulation treatment. Fish are known to negatively impact alum restoration (Holz and Hoagland 1999, Schauser et al. 2003), however, it is unknown how the fish community affects alum treatment success at the ecosystem level. To our knowledge, there are no studies that compare these treatments at the ecosystem level with reference to untreated, control lakes to evaluate direct impacts on nutrients and indirect effects on plankton community structure and microcystin toxins.

To evaluate lake management techniques we asked: How does water quality improve when comparing control, alum only, and alum-and-biomanipulation (dual treatment) lake restoration? We quantified pre- and post-treatment effects in nine lakes (n = 3 per treatment group) to discover which option is most effective for improving dissolved oxygen (DO), TN, total phosphorus (TP), chlorophyll-a, phytoplankton density, cyanobacteria density, zooplankton density, and microcystin concentrations. We
hypothesized that the dual treatment would be most effective for increasing water quality through enhanced bottom-up nutrient limitation and top-down grazing pressure.
2.2 Materials and Methods

2.2.1 Study Site and Restoration Description

Fremont Lakes State Recreation Area (FSA) is located about 3.22 km west of the city of Fremont, NE (41.44167, -96.55833, 41°26'30"N, 96°33'30"W). FSA includes 20 small sandpit lakes that cover a total of 265 ha. The lakes were formed when groundwater filled depressions created by sand mining. These lakes share common characteristics of being small, shallow (<5 m), groundwater fed, irregularly shaped, and have similar elevation (365.6m ± 1.9m) (Table 1, Figure 1). There are over 800 lakes with similar histories and features in Nebraska (NDEQ 2009), and likely hundreds more in areas of major highway construction (e.g., interstate highways). We sampled a subset of nine lakes, grouped by treatment: control (lakes named by number: 4, 18, 19), alum treated (2, 10, 11) and dual treated (8, 9, 13) (Figure 1, Table 1).

Fish renovation via rotenone application to lakes 8, 9, and 13 began in August 2012. Fall is typically the best time to apply rotenone as water temperatures are high and water levels are low. The Nebraska Game and Parks Commission (NGPC) oversaw the application according to standard procedures of three mL/m$^3$. All three lakes were restocked with juvenile largemouth bass and bluegill in September 2012 for Lake 8 and 9 and in May 2013 for Lake 13.

In October 2012, 258 tons of alum were added to lakes 2, 8, 9, 10, 11, and 13 (Figure 1, Table 1) by private contractor (Harmful Algal Blooms Aquatics, Lincoln, NE) at an average dose of 57.5g Al/m$^2$. Alum was applied by a barge, which sprayed a mixture of alum and a sodium aluminate buffer just under the lake surface. The buffer
solution was applied with alum to minimize biologically harmful changes in pH. The crew used GPS to evenly distribute the chemical treatment within a lake.

2.2.2 Water Quality Sample Collection and Laboratory Analysis

Field collection of water quality samples occurred twice a month and began the first week of May and ended the last week of October of 2012 for pre-treatment data. Sampling resumed in May 2013 for post-treatment year sampling, and proceeded through the end of October. This sampling regime resulted in twelve sampling periods throughout the summer for each lake for each of two years. All samples were taken at the deepest point of each lake. All water chemistry and phytoplankton samples were collected via Van Dorn bottle. We collected total nitrogen (TN) and total phosphorus (TP) samples at 0.5m below the surface (epilimnion). These nutrient samples were preserved with 10N sulfuric acid and refrigerated until analysis. We ran laboratory analysis by colorimetric method (EPA 365.4) with detection limits of 3.57µM for TN and 0.81µM for TP. Three replicate chlorophyll-\(a\) samples were collected from the epilimnion, filtered through 0.45 micron filters, stored in dark aluminum envelopes, and frozen until analysis. Chlorophyll-\(a\) was extracted using ethanol incubation and florometric reading (Nusch 1980). Replicates were averaged for each timepoint. One microcystin sample from the epilimnion was collected in a dark bottle and frozen until analysis. A freeze thaw procedure was used to lyse cells before analysis using a Microcystin ELISA kit (Abraxis, Kansas) with detection limits of 0.075 μg/L.

Three 100mL replicate phytoplankton samples from the epilimnion were preserved with 10mL of Lugols solution and stored in the dark. To quantify phytoplankton density and species richness, we counted individuals and colonies using
five mL settling chambers viewed through an inverted microscope at 200x. Settling occurred by allowing 3mL subsamples taken from vigorously mixed field samples to sit overnight. After settling, a grid eyepiece aided in accurately identifying and counting at least 300 individuals to the genus level in 10 fields of view as recommended by standard protocols (Eaton et al. 1995). To achieve countable phytoplankton densities, dilutions and concentrations were also performed. Totals for each genus were then multiplied by their respective dilution or concentration factors to estimate density of plankton per liter for each lake.

Zooplankton samples were collected via three replicate vertical tows using a Wisconsin net with mouth gauge of 0.03m$^2$ and 35µm mesh. Concentrated samples were preserved at a 1:1 ratio with 10% sugar formalin and stored in the dark. Zooplankton counts were performed using 1mL Sedgwick-Rafter cells viewed through a compound microscope at 10x. To count a sample, a Hensen-Stempel pipette was swirled through the jar in a figure eight pattern to suspend zooplankton that settled to the bottom. A 1mL sample was then taken with the pipette and transferred to the Sedgwick-Rafter cell. At least 300 plankton were identified. Counts were then used to calculate density of each genus for each time point.

2.2.3 Statistical Analysis

To differentiate treatment effects from year-to-year variation, we performed a before-after-control-impact (BACI) analysis. This was accomplished by evaluating the treatment by year (treatment*year) interaction, using two-way analysis of variance (ANOVA), for linear mixed-effects (LME) models in R package “nlme” with random effects of “lake” and “week”. We selected this model by comparing the residuals
of random effect combinations of no effect, “lake”, and “week”. We also evaluated these same model combinations with a smoothing spline of “week”, however, the addition of a spline did not improve residual patterns, and therefore was not used in this analysis. A significant BACI effect indicates a treatment effect while standardizing for temporal variation between years (Schwarz 2014). When we did not detect a significant BACI effect, we did a power analysis to quantity how many lakes we need to sample to detect a significant effect (0.05 level). We performed the post-hoc power analysis in the R “pwr” package.

To further explore water quality inter-annual variation, we evaluated the week by BACI effect interaction with a random effect of lake of linear mixed-effects models (LMER) in R package “lme4” and “effects”. Standard two-way ANOVA was used to evaluate significant changes over time of LMER models for each water quality variable. The focus of our ANOVA results is on the week by year by treatment interaction term, which indicates different rates of change between treatment groups over the spring-fall growing season. We compared rank-abundance curves to analyze plankton community dynamics for each treatment. Proportional abundance was calculated by taking each phytoplankton or zooplankton family density divided by the overall total density. We conducted this analysis in R using “reshape2” and the base package in R version 2.15.1.
2.3 Results

Overall, chemical water quality parameters, including TP, TN and TN:TP, improved in 2013 compared to pre-treatment conditions in 2012, except for DO (Figure 2, Table 2a). DO was significantly lower in 2013 compared to 2012 (p<0.05, Figure 2a, Table 2a), however, we cannot contribute this difference to a treatment effect (p=0.83, Figure 2a, Table 2a) or BACI effect (p=0.52, Figure 2a, Table 2a). Power analysis indicated 20 lakes would be required to detect a significant DO BACI effect. TN was not significantly different between years (p=0.65, Figure 2b, Table 2a), treatment groups (0.76, Figure 2b, Table 2a), and did not have a BACI effect (p=0.77, Figure 2b, Table 2a). Power analysis indicated 12 lakes would be required to detect a significant TN BACI effect. TP was significantly lower in 2013 compared to 2012 (p<0.01, Figure 2c, Table 2a) with a BACI effect, indicating the treatment was effective at reducing TP between years (p=0.01, Figure 2c, Table 2a). In comparing the BACI effect between treatments, the dual treatment lakes had significantly lower TP compared control lakes (p<0.01, Figure 2c, Table 2a) and alum treated lakes (p<0.05, Figure 2c, Table 2a), but we did not detect a difference between control and alum treated lakes (p=0.65, Figure 2c, Table 2a). The TN:TP ratio was significantly higher in 2013 compared to 2012 (p=0.01, Figure 2d, Table 2a), but differences were not due to treatments (p=0.63, Figure 2d, Table 2a), or BACI effect (p=0.88, Figure 2d, Table 2a). We could not conduct a Power analysis for TN:TP ratio BACI effect because the model did not converge.

Chlorophyll a, phytoplankton density and cyanobacteria density in the control lakes remained consistent between years, but were more variable in alum and dual treated
lakes (Figure 3a panel a-c). Chlorophyll-α significantly decreased in 2013 compared to 2012 (p<0.01, Figure 3a, Table 2b), however, we did not detect a significant treatment effect overall (p=0.31, Figure 3a, Table 2b) despite more substantial decreases in the alum and dual treatments compared to the relatively stable control lakes. Chlorophyll-α had a significant BACI effect (p<0.01, Figure 3a, Table 2b); chlorophyll a decreased in dual treated lakes compared to alum and control lakes (p<0.01, Figure 3a, Table 2b). Also, chlorophyll a in alum treated lakes decreased compared to the control group BACI effect (p<0.01, Figure 3a, Table 2b). Total phytoplankton density and cyanobacteria density was not significantly different between years (p=0.74, 0.25, Figure 3b-c, Table 2b) or by treatment (p=0.18, 0.12, Figure 3b-c, Table 2b). However, we did detect a significant BACI effect (p<0.01, Figure 3b-c, Table 2b), corresponding to lower density for phytoplankton and cyanobacteria in dual treated lakes compared to alum and control lakes (p<0.01, Figure 3b-c, Table 2b). Also, alum decreased for phytoplankton and cyanobacteria compared to the control group for the phytoplankton and cyanobacteria BACI effect (p<0.01, Figure 3b-c, Table 2b). Microcystin concentrations decreased sharply in 2013 compared to 2012 (p<0.01, Figure 3d, Table 2b), however, we cannot attribute this difference to a treatment effect (p=0.66, Figure 3d, Table 2b) or BACI effect (p=0.32, Figure 3d, Table 2b). Power analysis indicated 255 lakes would be required to detect a significant microcystin BACI effect. Zooplankton density changed between years (p<0.05, Figure 3e, Table 2b), however, we cannot attribute this difference to a treatment effect (p=0.52, Figure 3e, Table 2b). For zooplankton density we could not evaluate interactions for the control group due to a lack of data (not collected for 2012). Zooplankton had a BACI effect overall (p<0.01, Figure 3e, Table 2b) and we detected a
significant decrease in zooplankton density in dual treated lakes compared to alum treated lakes (p<0.01, Figure 3e, Table 2b).

To evaluate intra-annual treatment effects, we analyzed the interaction between sampling week by BACI effect for each chemical and biological water quality response variables (Figure 4-5, Table 3-4). Throughout the growing season, DO decreased over time (Figure 4a&b) in all treatments over both years, whereas TN, TP, and the TN:TP ratio generally increased (Figure c-h). The increases in TN and TP, however, were more substantial in 2012 compared to 2013 (Figure c-f). Despite these general patterns, we did not detect significant three way interactions (week*treatment*year) for any chemical water quality metrics (Figure 4a-l, Table 3a), indicating treated lakes behaved similarly to untreated lakes throughout the season. Chlorophyll-α concentrations generally decreased over time (Figure 5 g&h, Table 3b) in all treatments over both years, but we did not detect a significant three-way interaction. Phytoplankton density generally increased over the field season. Alum treated lakes increased faster than controls (p<0.05, Figure 5a&b, Table 3b) and dual treatment (p=0.01, Figure 5a&c, Table 3b). Control lakes increased faster than dual treated lakes (p=0.01, Figure 5b&c, Table 3b) for chlorophyll $a$ throughout the 2013 season. The increase of cyanobacteria density over time had similar trends as total phytoplankton between years. Cyanobacteria density in alum treated lakes increased faster than in control lakes (p<0.01, Figure 5c&d, Table 3b) or dual treated lakes (p<0.01, Figure 5c&d, Table 3b). We did not detect any difference between alum and dual treatments for phytoplankton density (p=0.10, Figure 5c&d, Table 3b). Microcystin concentrations were generally higher and more variable in 2012 than 2013.
Control lake microcystin concentrations remained constant compared to an increasing concentration in alum treated lakes over time (p<0.01, Figure 5i&j, Table 3b). Microcystin in alum treated lakes also increased throughout the 2013 season compared to dual treated lakes (p<0.01, Figure 5i&j, Table 5b), but there were no differences in microcystin between control and dual treated lakes (p=0.17, Figure 5i&j, Table 3b).

Zooplankton density was generally higher in 2012 compared to 2013; in 2012 density decreased over the field season, but density increased across all three treatments over the 2013 season (Figure 5e&f). For zooplankton density, we could not evaluate interactions for the control group due to a lack of data (zooplankton were not collected in control lakes for 2012). The change in zooplankton density throughout the season between years was similar between alum and dual treated lakes (p=0.97, Figure 5e&f, Table 3b).

Regardless of treatment or year, cyanobacteria dominated the phytoplankton community and rotifers dominated the zooplankton community (Figure 6a-f). We saw very few shifts in the plankton communities at the family level. The only shift in phytoplankton relative abundance ranking was in the dual treatment group between Euglenophyceae and Chlorophyceae (Figure 6a). One shift in zooplankton relative abundance ranking occurred in alum lakes when Copepods gained rank and Cladocerans lost rank post-treatment (Figure 6f).
2.4 Discussion

We predicted that, compared to control (untreated) lakes, water quality would improve in alum treated lakes, but that improvements would be greatest in the dual treated lakes due to multiple synergistic mechanisms (Figure 7). No mechanism to improve eutrophic conditions exists in the control group, and responses measured between years result largely from year-to-year weather variation (Figure 7; e.g., 2012 was a hot drought year whereas 2013 was colder and wetter). Alum treatment directly lowers phosphorus concentrations in the water column through chemical flocculation, resulting in chemical improvement of water quality (Figure 2b-d, Figure 4d&f). Limited biological improvements occurred with the alum only treatments, including decreased chlorophyll-\(a\) concentrations and increased zooplankton density (Figure 3a&e).

Biological water quality improvements, however, were greatest in the dual treated lakes due to a greater number of mechanisms at work (Figure 2b-d, Figure 3a-d, Figure 4d,f&h, Figure 5b,d,h&j, Figure 7). The dual treatment increases water quality by chemically flocculating phosphorus and by limiting nutrient release from the sediment by benthic feeding fish. This treatment also employs top-down control by reducing planktivorous fish and potentially increasing zooplankton grazing on the phytoplankton. Therefore, results from the dual treatment group include weather patterns, chemical and biological control of water quality (Figure 7). Below, we discuss the additive effects of the alum and biomanipulation restoration techniques, the effects of lake restoration on phytoplankton communities, the role of experimental design in parsing out treatment effects from year-to-year variation and the implications of our findings for managing eutrophication.
4.1 Is alum addition coupled with biomanipulation more effective at improving water quality than alum additions alone?

Dual treatment often had the greatest improvement in water quality compared to other treatment groups (Figure 2c, 3a-c). The dual treatment was not only more effective at lowering phosphorus (Figure 2c), but also decreased indirect water quality metrics, such as chlorophyll-α, phytoplankton density, and cyanobacteria density (Figure 3a-c). This treatment was more effective compared to the alum treatment, which only significantly decreased direct treatment effects of TP (Figure 2c) and chlorophyll-α (Figure 3a). A review of the success of Danish lake restoration concluded that combining treatment options with biomanipulation is generally more successful than restoration using a single technique (Gulati and Donk 2002). As we predicted, biomanipulation and alum together enhanced this restoration in the short term, and may see even greater long-term benefits if alum remains at the benthos instead of being resuspended (Egemose et al. 2010). We observed this trend where degradation of water quality is slower in the dual treatment group compared to controls and alum treatment (Figure 5b,d,&h).

Regardless of treatment, water quality decreased over the summer growing season from June-October (Figures 4&5). This is likely due to the typical succession of changes that occur in temperate lakes over the growing season. However, it is possible that treatment effects were decreasing through time (Lewandowski et al. 2003). A laboratory study on the longevity of alum showed a 50% decrease in phosphate sorption six months following treatment (Berkowitz et al. 2006). Our control lakes, however, indicate that observed patterns can be explained by normal, seasonal fluctuation (Figure 4&5). This is
a typical pattern as algae blooms often climax in the late summer or early fall (Grover and Chrzanowski 2006, Sommer et al. 1986).

The alum treated lakes deteriorated to a greater extent compared to control and dual treatment lakes (Figure 5b, d&j). This pattern was largely influenced by high total phytoplankton and cyanobacteria density in Lake 2 post-alum treatment. This lake has a history of high microcystin concentrations compared to other lakes at Fremont. It also has many shallow areas used extensively by Canada Geese. More research is needed to identify the driver of high phytoplankton, cyanobacteria, and microcystin in Lake 2.

4.2 How did lake restoration treatments impact plankton communities?

Our data does not support the common management philosophy that limiting TP eliminates cyanobacteria’s competitive edge over phytoplankton groups, resulting in the dominance of more desirable species (Figure 6). Total phosphorus is often cited as the main nutrient driver of freshwater phytoplankton growth (Schindler 1974, Wang et al. 2008, Watson et al. 1997) and we did observe decreased chlorophyll-\(a\) concentrations post alum additions (Figure 3). Analysis of phytoplankton biomass of Lake Constance indicated that all taxa, except dinophytes, were significantly correlated with TP availability (Jochimsen et al. 2013). However, there has been limited success at significantly reducing indirect, foodweb metrics such as cyanobacteria and microcystin concentrations with alum alone (Han et al. 2013, Van Hullebusch et al. 2002, this study, Webber et al. in prep ch.1). Two lakes in Michigan also failed to adequately control cyanobacteria and microcystin toxins following an alum addition (Xie et al. 2012). Similarly, in Harris et al. (2014) alum slightly reduced cyanobacterial biovolume, but failed to lower cyanobacterial relative abundance.
Adding biomanipulation with alum (dual treatment) further decreased cyanobacteria (Figure 3), but not enough to significantly alter the community structure (Figure 6). A similar study compared Phoslock (phosphorus binding clay) additions to dredging and found slight decreases in cyanobacteria biomass, but no change in microcystin for each technique (Lürling and Faassen 2012). Even after water quality increased following Phoslock additions and dredging combined, Lürling and Faassen still recommend adding biomanipulation to the management of the lake (Lürling and Faassen 2012). A similar study on lake resilience to restoration from Ibelings et al. (2007) found that a drastic increase in water quality only occurred when nutrient limitation was coupled with fish removal. For any restoration treatment option, sampling time-scale may influence results, since long term monitoring post-treatment is not frequently incorporated into studies (Jochimsen et al. 2013). Increasing the restoration monitoring timeline may be especially helpful with regards to our dual treatment lakes, as hatchery piscivorous fish take time to mature and change trophic status.

Zooplankton community response was different between treatment groups as alum treatment resulted in increased density and dual treatment resulted in decreased density between years. However, there was only one shift in rank-abundance, allowing rotifers to continue to dominate these lakes (Figure 6f). Thus, our data doesn’t support our hypothesis that dual treatment would enhance zooplankton density and promote grazing, as we did not observe any change in zooplankton density compared to control groups post-treatment. Dual treated Lake 13 had very low zooplankton density, possibly due to detection problems caused by dense macrophyte growth (Cazzanelli et al. 2008). A similar three-year study in Medical Lake, WA observed zooplankton dynamics in
response to alum addition and trout fishery establishment (Mires et al. 1981). In contrast to the Fremont Lakes, Mires et al. (1981) found a decrease in rotifer dominance and an increase in community evenness following an alum addition. Other studies on lake biomanipulation also confirm similar zooplankton abundance post-treatment (Brett and Goldman 1996, Mires et al. 1981). A sediment capping experiment using modified zeolite saw similar results of no significant change in zooplankton post-treatment in Lake Okaro, New Zealand (Ozkundakci et al. 2011). Although changes in zooplankton are not significant in our study post-treatment, the reduction of zooplankton was significant in the dual treatment between years. This could be a short-term effect due to the addition of juvenile hatchery fish feeding heavily on zooplankton or inter-annual variation.

**4.3 Detecting restoration treatment effects requires adequate experimental design**

Currently, it is difficult to generalize restoration recommendations for environmental managers since most studies observe a single lake (Holz and Hoagland 1999, Reitzel et al. 2003, Schumaker et al. 1993, Steinman et al. 2004) and often present conflicting results. While synthesis papers include results from several lakes, these compilations are indirect comparisons as they include varying treatment applications, timelines, or sampling regimes (Gulati and Donk 2002, Meijer et al. 1999, Jeppesen et al. 1997, Søndergaard et al. 2008). Our replicated, ecosystem-level study shows alum is an effective tool to lower TP and chlorophyll $a$ concentrations, as has been shown in many other studies (e.g., Nogoro et al. 2013, Smeltzer 1990, Xie et al. 2012). However, if management goals include more indirect metrics such as decreased phytoplankton or
cyanobacteria density, our study indicates a dual treatment could likely yield better results.

The BACI experimental design of this study captured inter-annual variation via control lakes, intra-annual variation from spring to fall, and variation between replicate lakes within treatment groups. This design was essential in our study to understand if changes in water quality were due to year effects alone or included a BACI effect between treatments, as year effects were more frequently significant than were treatment effects. The interpretation of our results relies heavily on comparing natural variation in controls to variation in treatments. Evaluating post-treatment data alone yields different conclusions for treatment effectiveness, especially for microcystin concentrations (Figure 3d). This study design captures much more variation than a post-treatment field study of one lake (e.g. Zhang et al. 2008) or laboratory microcosm experiment (e.g. Lewandowski et al. 2003). Long-term monitoring of treated lakes compared to controls may be necessary to further differentiate true treatment effects from background conditions. We also included replicate lakes, possibly assessing a variety of foodweb structures, which would not be the case in making comparisons to mesocosm experiments (Mazumder 1994). We calculated it could take up to 255 lakes to detect a significant BACI effect for microcystin with our current sampling regime. High variation in our results may be a product of our careful inclusion of variation through space and time, which is not accounted for in other studies. This variation is important to recognize and quantify in order to understand the limitations of lake restoration studies for generalization and extrapolation (Englund and Cooper 2003).
The exact mechanisms by which biomanipulation further decreased TP concentrations compared to the alum treatment group remain unclear. Consistent with our results, total phosphorus is often significantly reduced by alum restoration treatments via direct chemical reaction (Steinman et al. 2004). However, biomanipulation could reduce phosphorus via biomass removal (Vanni and Layne 1997), trophic cascade (Mazumder 1994), or reduced bioturbation (Søndergaard et al. 2003, Breukelaar et al. 1994) (Figure 7). Further study is needed to isolate which mechanism is driving lower TP, as these processes are confounded in our experimental design (Meijer et al. 1999).

Our study only focuses on in-lake management techniques for combating eutrophication, and thus does not address issues related to external nutrient loading (e.g., from the watershed). A constant influx of nutrients from the watershed could continue to drive eutrophication symptoms in our nine sample lakes, as was described by Brookes and Carey 2011. A watershed management plan to limit external loading to Lake Newman preceded the successful alum restoration in Spokane, WA (Schumaker et al. 1993). The combination of in-lake restoration techniques, as those studied here, together with watershed management techniques likely results in the greatest success for managing water quality issues.
Table 1. Fremont Lakes State Recreation Area morphological characteristics.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Treatment Group</th>
<th>Area (m²)</th>
<th>Volume (m³)</th>
<th>Max depth (m)</th>
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<td>4</td>
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<td>78,202</td>
<td>4.25</td>
</tr>
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<td>18</td>
<td>control</td>
<td>32,375</td>
<td>118,414</td>
<td>5.5</td>
</tr>
<tr>
<td>19</td>
<td>control</td>
<td>19,830</td>
<td>42,308</td>
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</tr>
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<td>213,979</td>
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<td>10</td>
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<td>82,002</td>
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</tr>
<tr>
<td>8</td>
<td>dual</td>
<td>45,629</td>
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<td>dual</td>
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### Table 2. ANOVA table for chemical (panel a) and biological (panel b) water quality variables. Each response variable was modeled using linear mixed-effects (LME) models with a random variable of (week|lake).

#### a.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>DO</th>
<th>log(TN)</th>
<th>TP</th>
<th>log(Ratio)</th>
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<td>denDF</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trt</td>
<td>2</td>
<td>6</td>
<td>0.83</td>
<td>2</td>
</tr>
<tr>
<td>year</td>
<td>1</td>
<td>192</td>
<td>p&lt;0.01</td>
<td>2</td>
</tr>
<tr>
<td>BACI</td>
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<td>0.52</td>
<td>2</td>
</tr>
<tr>
<td>BACI comparison</td>
<td>estimate</td>
<td>SE</td>
<td>p-value</td>
<td>estimate</td>
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<td>0.83</td>
<td>26.04</td>
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#### b.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>log(Chlorophyll-a)</th>
<th>log(Phytoplankton)</th>
<th>log(Cyanobacteria)</th>
<th>log(Microcystin)</th>
<th>log(Zooplankton)</th>
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<td>denDF</td>
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<td>year</td>
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<td>-8.96E+05</td>
<td>1.51E+07</td>
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</table>
Table 3. ANOVA table for chemical (panel a) and biological (panel b) water quality variables. Each response variable was modeled using linear mixed-effects (LMER) models with a random variable of (1|lake).

**Panel a: Chemical Water Quality Variables**

<table>
<thead>
<tr>
<th>week*BACI effect</th>
<th>DO</th>
<th>log(TN)</th>
<th>TP</th>
<th>log(Ratio)</th>
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</table>

**Panel b: Biological Water Quality Variables**

<table>
<thead>
<tr>
<th>week*BACI effect</th>
<th>log(Chlorophyll-a)</th>
<th>log(Phytoplankton)</th>
<th>log(Cyanobacteria)</th>
<th>log(Microcystin)</th>
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<td>1.20</td>
<td>0.72</td>
<td>1.04E+07</td>
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Figure 1. Map of Fremont Lakes State Recreation Area with designated treatment lakes. Each treatment group includes one lake on the north and two lakes on the south side of the park.
Figure 2. BACI analysis for chemical response variables of dissolved oxygen (panel a), total nitrogen (panel b), total phosphorus (panel c), and the ratio of total nitrogen:phosphorus (panel d). Each line represents a treatment group over time such as control (black, solid line, circle symbol), alum (dark grey, dashed line, triangle), and dual treatment (light grey, dotted line, square symbol). Error bars represent the 95% confidence interval for each treatment.
Figure 3. BACI analysis for chemical response variables of chlorophyll-\textit{a} (panel a), phytoplankton (panel b), cyanobacteria (panel c), microcystin (panel d), and zooplankton (panel e). Each line represents a treatment group over time such as control (black, solid line, circle symbol), alum (dark grey, dashed line, triangle), and dual treatment (light grey, dotted line, square symbol). Error bars represent the 95% confidence interval for each treatment.
Figure 4. Chemical water quality metrics over time pre- (2012 left column) and post-treatment (2013 right column) by treatment (n=3 per treatment). Treatments include control (solid line), alum (dashed line) and dual treatment (dotted line) for 2013. Water quality metrics of interest include: dissolved oxygen (panel a-b), total nitrogen (panel c-d), total phosphorus (panel e-f), and the ratio of nitrogen to phosphorus (panel g-h). Lines represent generalized linear models (GLM) of each metric over time, with shaded 95% confidence intervals.
Biological water quality metrics over time pre- (2012 left column) and post-treatment (2013 right column) by treatment (n=3 per treatment). Treatments include control (solid line), alum (dashed line) and dual treatment (dotted line) for 2013. Water quality metrics of interest include: phytoplankton density (panel a-b), cyanobacteria density (panel c-d), zooplankton density (panel e-f), chlorophyll-α (panel g-h), and microcystin (panel i-j). Lines represent generalized linear models (GLM) of each metric over time, with shaded 95% confidence intervals.
Figure 6. Phytoplankton (panels a, b, c) and zooplankton (panels d, e, f) rank-abundance curves comparing pre (solid line) and post-treatment (dashed line) community structures for each treatment group. Only post-treatment data was available for control lake zooplankton (panel d).
Figure 7. Conceptual model linking lake management techniques to possible influences on water quality results. Also, we predict that control lakes the most eutrophic and dual treatment lakes are the most clear post restoration due to several possible mechanisms.
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