Effects of Repeated Early Season Herbicide Treatments of Curlyleaf Pondweed on Native Macrophyte Assemblages in Minnesota Lakes.

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DEDICATION

I dedicate my work to my wife Kristin for her unconditional support and understanding throughout my graduate career. Without her this achievement would have been infinitely more difficult.

COMPREHENSIVE ABSTRACT

Non-native invasive species have the potential to cause various problems in small isolated ecosystems, these constraints are exemplified in the small, shallow, lakes of Minnesota. Curlyleaf pondweed (*Potamogeton crispus* L.) is one of the common occurring and most influential non-native invasive species in Minnesota. Characterized by its early spring growth and early summer senescence, curlyleaf has the ability to negatively affect native macrophyte growth by forming dense monotypic canopies in late spring and releasing nutrients in early summer. Because of the negative impact on natural ecosystems along with the problems curlyleaf can cause for people, there is serious interest in the management of curlyleaf. One management practice is the early season application of herbicides, where curlyleaf is targeted before it is capable of producing turions (propagules) and native plants are unlikely to be affected.

We examined the response of native plant communities to spring herbicide treatments of curlyleaf pondweed from 2006 through 2009. Thirteen lakes were examined during our study; ten were treated with herbicide and three were used as non-treatment reference lakes. Plant communities were assessed in the littoral zone with the point intercept method in early spring (before treatment), late spring (after treatment) and mid summer (peak native plant growth). For each survey, approximately 40 random biomass samples were taken in each study lake to estimate plant biomass. To determine changes in native plant frequency and biomass throughout the course of consecutive annual treatments, we compared differences between treated and untreated lakes within years for August surveys and compared August surveys between years for treated or untreated lakes. Additionally, we examined inter-seasonal changes, comparing differences within and between treated or untreated lakes from May to June and June to August in every year.

In the reference lakes, curlyleaf persisted at moderate to high frequencies over the four years, and no consistent changes in native plant frequencies were seen. Herbicide treatments proved effective for controlling curlyleaf, which decreased in frequency within 1 month following treatment for each year of the study. The total frequency of occurrence of native plants in August did not decrease in most of the treatment lakes between years. Native macrophyte species richness also showed little change with continued treatment, although shifts in abundance of some species were observed. August native plant biomass increased between 2006 and 2009 in most treatment lakes, whereas native biomass varied in untreated lakes. Much of the change in biomass was attributed to a single species in most treatment lakes. We observed increases of *Chara spp.* frequency and biomass in most treated lakes. However, multiple years of treatment may be needed to obtain increases in native plant abundance as the largest increases occurred after 3 years of treatment. Early-season lake-wide herbicidal treatments of curlyleaf pondweed can reduce curlyleaf occurrence and density without major harm to native plants.

Significant inter-seasonal changes in frequency and biomass were observed in both treated and untreated lakes although differences were not observed between treated and untreated lakes. Native species richness increased from May to June and June to August in both treated and untreated lakes. The frequency of native plants increased between May and June but less so between June and August in both treated and untreated lakes. Conversely, native plant biomass increased from June to August more so than from May to June for both treated and untreated lakes. These findings suggest that early season

growth primarily manifests in distribution and colonization, while late season growth results in increased biomass. Similarly, many plant species follow the same patterns as the overall native plants, although other plant species may have different frequency and biomass regimes throughout a single season. Despite the differences in native plant species, we did not see any major inter-seasonal detriment caused by the presence of curlyleaf pondweed in untreated lakes, or positive influence of herbicide treatment in treated lakes. These findings suggest that increases in native plant growth may occur only after several annual treatments.

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Prologue

This thesis is organized into four chapters, two of which are manuscripts that are to be submitted to scientific journals. These manuscripts are based on the work done by James A. Johnson, Raymond M. Newman and myself (Ajay R. Jones) on early season herbicide control of the invasive macrophyte curlyleaf pondweed (*Potamogeton crispus* L.). This thesis primarily focuses on native plant response to early season herbicide treatment over four consecutive years. A prior manuscript on the response of curlyleaf pondweed to early season herbicide treatment was produced as a chapter in a thesis by James A. Johnson; the chapter, entitled *Evaluation of Lake-wide, Early-season Herbicide Treatments for Controlling Invasive Curlyleaf Pondweed (Potamogeton crispus) in Minnesota Lakes,* listed Raymond M. Newman and myself (Ajay R. Jones) as coauthors. The research was conducted from 2006 to 2010 and I was involved in data collection from 2008 to 2010.

Chapter 1 gives an overview of native macrophytes, their benefits and role in structuring lake ecosystems. In Chapter 2, I analyzed the inter-seasonal variation of native plant communities in treated and untreated lakes. I used data from each of the four years and analyzed native plant community changes throughout each year. With these data I was able to determine the effects that curlyleaf has on native plants during the growing season. I tested the difference between each survey (May and June and June and August) in treated and untreated lakes. By testing treated lakes against untreated lakes, we were able to determine if there are inhibitory effects within the growing season by curlyleaf (in untreated lakes) or negative effects on native plant growth by herbicides.

Additionally, we were able to determine overall patterns in native plant frequency, richness, diversity (natives per point) and biomass in each season. Frequency and biomass of single taxa were also assessed to understand growth patterns of individual macrophyte species throughout a growing season.

Chapter 3 is a manuscript aimed at determining the amount of change in native aquatic plant species over time as a result of control of curlyleaf by herbicides. In this chapter, I examined differences in native plant growth between years, for native plants combined and common individual plant taxa. Four years of data were used from both treatment and reference lakes. Only August surveys were used in this analysis, because August is the primary period for maximum native plant growth. By August, curlyleaf is generally absent from lakes in Minnesota and native plants are at peak abundance, thus changes of native plant growth are more likely to be observed during this time period versus May or June. Peak native plant growth is an indicator of whether curlyleaf was inhibiting native plant growth or if herbicide treatments hindered native plant growth in that year. If the treatment and subsequent reduction of curlyleaf is effective, native plants would be expected to increase. Conversely, if herbicides are inhibiting native plant growth we would see a reduction in native plants in August. Either consecutive increases or decreases among years would indicate some effect with repeated herbicide treatment. Native plants in untreated lakes should either not change or should decrease due to competition with curlyleaf.

Chapter 4 is a general summary of both manuscripts presented in this thesis. Additionally, concluding observations and management recommendations are provided to the reader.

Chapter 1

Importance of Native Macrophytes

Native macrophytes are known to benefit the natural ecology of lake ecosystems by adding littoral zone complexity and hosting a variety of epiphytic organisms, thus resulting in increased abundance and diversity of aquatic wildlife (Carpenter and Lodge, 1986; Gilinsky, 1984; Van den Berg et al., 1997). Vegetated areas provide shelter for many organisms compared to un-vegetated areas, hosting up to four times the biomass of associated inhabitants than does silt and 15 times that of rocky substrates (Sculthorpe, 1967). Macrophyte stands have also shown to increase survivability of prey fish (Shoup et al., 2003) and fish species diversity often correlates with macrophyte abundance and complexity (Smart et al., 1996; Valley et al., 2004).

Macrophytes also play a large role in water quality. Macrophytes provide shelter for large cladocerans, which graze on pelagic algae, thus resulting in increased water clarity (Lampert et al., 1986; Norlin et al., 2005). Macrophytes also act as nutrient sinks, both competing with planktonic algae for resources and creating denitrifying conditions (van Donk and van de Bund, 2002). Nutrient removal from the water column is a growth mechanism for many aquatic plants and has even been used to treat wastewater (Reddy and DeBusk, 1985). *Chara spp*. in particular has been shown to act as a nutrient sink in shallow lakes due to the sequestering of pelagic phosphorus and nitrogen and because of its long senescence period, in which the resulting discharge of nutrients is slow (Kufel and Kufel, 2002). Plant detritus also provides fuel for denitrifying bacteria in the form of organic carbon (Weisner et al., 1994).

In addition to nutrient competition, alleopathic effects have been hypothesized to hinder phytoplankton communities (van Donk and van de Bund, 2002). A final benefit plants have on water quality is the physical alteration of habitat structure. Macrophytes reduce the likelihood of sediment and nutrient suspension by decreasing water movement and flow velocity (Madsen et al., 2001). Suspension of benthic nutrients provides energy for phytoplankton blooms and results in water turbidity from subsequent algal production. Mat-forming macrophytes act as a mitigating net, blocking horizontal movement of sediment (Barko and James, 1998) and impeding the suspension of the substrate they cover (Sand-Jensen and Mebus, 1996). Horppila and Nurminen (2003) found that submerged macrophytes reduced sediment resuspension by as much as 50% and chlorophyll-a levels were also reduced in areas containing macrophytes versus open water, especially in mid summer months where macrophytes are in high densities. The roots and rhizomes of macrophytes may also reduce rates of phosphorus dissolution in the surface sediment layer due to oxidation when O_2 is released as a byproduct of lacunar metabolic processes (Kemp and Murray, 1986; Sand-Jensen et al., 1982).

Given all the positive effects in lakes, native macrophytes are a key component in lake health. The threat that non-native invasive macrophyte species pose to native aquatic plants is a major concern. Curlyleaf pondweed (*Potamogeton crispus* L.) is one of the most widespread and detrimental invasive species in Minnesota (Bolduan et al., 1994). Due to curlyleaf's impact on native macrophytes and lake health as a whole, there is considerable interest in the management of this species. Many curlyleaf management programs also aim at restoring native macrophyte populations as well.

In this thesis, native plant community response to spring herbicide treatments of curlyleaf pondweed was examined from 2006 through 2009. Thirteen lakes were examined during our study; ten were treated with herbicide and three were used as non-treatment reference lakes. In 8 treated lakes the herbicide endothall was used. It's generally believed that endothall's mode of action is to inhibit protein synthesis in a wide variety of aquatic plants (Skogerboe and Getsinger 2006). In one lake, fluridone was applied, which inhibits phytoene desaturase, an enzyme used in the production of carotene. In plants affected by fluridone, the reduction of carotene results in the photooxidization of chlorophyll, effectively killing the plant (Sprecher et al. 1998). Additionally, one lake was treated with 2, 4-D and triclopyr, both auxin mimics that induce rapid cell growth in target dicotyledons like Eurasion watermilfoil (Sprecher and Stewart 1995; Skogerboe and Getsinger 2006). Plant communities were assessed in the littoral zone ($\leq$ 4.6 m depth) using a throw rake with the point intercept method in early spring (before treatment), late spring (after treatment) and late summer (peak native plant growth). During each survey we used a vertical rake method to collect approximately 40 random biomass samples of $0.33 m^2$ to estimate plant biomass.

We examined inter-seasonal differences between treated and untreated lakes for May, June and August surveys and compared differences between treated or untreated lakes from May to June and June to August (Chapter 2). Using these methods I sought to obtain an accurate depiction of the long term effects of consecutive low-dose, early-season herbicide treatment on native plant communities. By assessing native aquatic plant communities I provided a general assessment of major changes (both long and short term) in native macrophyte assemblages as a result of herbicide treatments to control curlyleaf pondweed. In addition, to determine changes in native plant frequency and biomass throughout the course of consecutive annual treatments, we compared differences between treated and untreated lakes within years for August surveys and compared August surveys between years for treated or untreated lakes (Chapter 3).

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Chapter 2

Phenology of Native Macrophytes in Herbicide Treated and Non-Treated Lakes Infested with *Potamogeton crispus*

Planned submission to The Journal of Aquatic Plant Management in 2010 with James A. Johnson as second author and Raymond M. Newman as third author.

Summary

Non-native invasive aquatic macrophytes are a major detriment to both human activities and the health of lake ecosystems. One of the earliest and most wide-spread non-native macrophyte invasions in North America is that of curlyleaf pondweed. Curlyleaf pondweed (*Potamogeton crispus* L.) was first recorded in Minnesota waters in 1910; since then curlyleaf has been recorded in over 740 lakes and rivers in Minnesota. In Minnesota, curlyleaf grows rapidly in early spring due to its ability to tolerate cold temperatures and its low compensation point. In Late spring, curlyleaf becomes a nuisance due to its propensity to form large monotypic surface mats. Furthermore, curlyleaf senesces in early summer, releasing nutrients, thus providing fuel for algal blooms. Crowding, canopy formation and turbidity throughout the growing season can affect native plant phenology. Herbicide application has been shown to be an effective management method for curlyleaf pondweed. Over time, herbicide treatments have the potential to target curlyleaf early in the growing season without harming native plant communities; however, changes in inter-seasonal patterns of native plant growth in response to these methods are not well understood.

Over the course of four years, 10 lakes were treated with herbicide to control curlyleaf pondweed and three untreated lakes with curlyleaf infestations were investigated as well. We used the point intercept method to examine inter-seasonal changes native plant frequency in herbicide treated lakes and reference lakes with curlyleaf infestations. Concurrent with the point intercept surveys, we collected biomass samples to determine native plant density over the course of four years. Surveys were taken at ≤ 4.6 m depth and included 100 to 400 points per lake. Surveys were conducted three times per year: pretreatment (April/May), post-treatment (June) and when native plants reached peak abundance (August). We observed little difference between herbicide treated and untreated lakes in the timing of native plant frequency and biomass and no change over time for any sampled periods. Furthermore, there was little difference between the number of species present in treated and untreated lakes. Although we did not see differences between treated or untreated lakes, we did observe changes in frequency and biomass across individual seasons. Frequency typically increased between April/May and June surveys but not between June and August surveys. Conversely, biomass and species richness increased in each survey period (May to August) within each studied year. Additionally, we saw differences in phenology between individual plant taxa, but no phenological difference in individual species between treated and untreated lakes. Although herbicide treatment of curlyleaf pondweed may be effective at enhancing native plant communities, these enhancements are likely to occur after repeated treatments and between specific periods across years.

Introduction

Variation in inter-seasonal aquatic plant growth in Minnesota is not well documented, with evaluations typically limited to periods of peak abundance. Less is known about the active growing period of macrophytes prior to peak abundance and associations between distribution and biomass. Some macrophytes in Minnesota are annuals, germinating by seeds when light conditions, water temperature, or nutrient availability are favorable for growth (Bonis et al., 1995). Other macrophytes are perennials that remain dormant throughout the winter and some aquatic plants persist as evergreens year round, even during ice cover (Boylen and Sheldon, 1976; Sculthorpe, 1967). Differences in the life histories of aquatic plants may result in changes of abundance throughout the growing season and growth rate may vary among species.

Competition with non-native invasive macrophytes can further complicate the timing of aquatic plant growth by competition for space, resources and light (Barrat-Segretain and Elger, 2004; McCreary, 1991). Curlyleaf pondweed (*Potamogeton crispus* L.) is one of the most invasive non-native aquatic plants in Minnesota and much of the northern United States (Bolduan et al., 1994). This plant has a variety of properties that make it an aggressive colonizer in northern latitudes. The ability to persist under ice cover, rapid growth at low temperatures and a low compensation point are all factors that allow this non-native to colonize cold climate locales like Minnesota. Curlyleaf has the ability to grow earlier than many annual and perennial plants in Minnesota (Madsen and Crowell, 2002). The ability to tolerate cold water allows curlyleaf to grow rapidly in the early spring, taking advantage of the clear early spring waters (Tobiessen and Snow, 1984). When most native macrophytes begin growing in spring, curlyleaf has already formed large surface mats that not only compete for space, but also limit available light (Bolduan et al., 1994; Skogerboe et al., 2008). Studies have shown that native macrophyte phenology is greatly affected by light resources (Spencer and Ksander, 1990); competition by large infestations of curlyleaf pondweed may inhibit timing or overall growth of native macrophyte species by reducing light availability. Other complicating factors include the senescence of curlyleaf. Curlyleaf senesces in early summer and some evidence suggest that the senescence of curlyleaf may result in phytoplankton blooms, due to the release of nutrients (Rogers and Breen, 1982).

Some studies suggest that there are within year differences between native plant phenology in lakes that are treated for invasive macrophytes versus those with large untreated infestations (Kovalenko et al., 2010). Furthermore, invasive macrophytes can have impacts on native plant species by negatively affecting fecundity between and within years (Santos et al. 2009). Despite these findings, most observations were limited to individual lakes and varied in response. Due to the life cycle of curlyleaf in Minnesota, its necessary to asses the early season growth of native plants to fully gauge the effects caused by curlyleaf infestations. It's likely that the non-native curlyleaf may interrupt native plant growth cycles or hinder overall native plant growth, especially earlier in the growing season. (Daehler, 2003)

Due to curlyleaf's propensity to compete with native plants, and alter native ecosystems, some studies have been conducted on the effectiveness of herbicides to reduce curlyleaf abundance in spring, killing the plant before it reaches maximum growth (Poovey et al., 2002; Skogerboe et al., 2008). Johnson (2010) found that treating curlyleaf in the early spring with the herbicide endothall significantly reduces *P. crispus* with each treatment. Furthermore, by treating curlyleaf in early spring, when its actively growing, its possible to eliminate or reduce the effects that the herbicide has on native plants (Poovey et al., 2002). Jones (Chapter 3 of this thesis) found that early season herbicide treatments did not decrease native plant frequency or diversity and native plant biomass increased throughout the course of four years of treatment.

Our study focused on the growth of native plants throughout a growing season and examined differences in the response of specific plant species in herbicide treated and untreated lakes infested with curlyleaf pondweed.

Methods

Study Lakes

The treated study lakes initially had significant curlyleaf infestations and were treated with herbicide for four consecutive years. Treatment lakes were chosen by the Minnesota Department of Natural Resources (MNDNR) and ranged in trophic status from mesotrophic to hyper-eutrophic; most were eutrophic. We chose three reference lakes based on similar levels of curlyleaf infestations, proximity, size and trophic status compared to treated lakes (Table 1). Water clarity corresponded with trophic status; average Secchi depth ranged from 3.8m in mesotrophic lakes to 0.6m in hypereutrophic lakes (Table 1). Lakes varied in size from 29 to 292 ha and all lakes, with the exception of Weaver, were moderately shallow. All lakes were sampled by the University of Minnesota, although surveys prior to June 2008 for Silver, Clear, Blueberry, and Long Lakes were conducted by the MNDNR.

Procedures

Herbicide Treatments

All delineations of curlyleaf infestations and supervision of herbicide applications were conducted by the MNDNR. Applications of a liquid formulation of endothall (0.75-1.0 mg active ingredient (ai)/L) were completed in eight of the lakes (Lower Mission, Crookneck, Julia, Rush, Fish, Blueberry, Long, and Clear). Weaver Lake was treated with liquid fluridone in 2006 and 2007 with a target concentration of 2-4 μg ai/L; in 2008 and 2009 fluridone treatments were replaced by endothall (0.75-1.0 mg ai/L). To combat

Eurasian watermilfoil (*Myriophyllum spicatum*) as well as *P. crispus,* Silver Lake received a combined liquid formulation of 2,4-D (0.5-1.0 mg ai/L) and endothall (0.75-1.0 mg ai/L) in 2006 and 2007, a combined liquid formulation of triclopyr (0.5 mg ai/L) and endothall (0.75-1.0 mg ai/L) in 2008 and an endothall only treatment in 2009 (0.75-1.0 mg ai/L). In 2009, treatment was stopped on two of the study lakes (Crookneck and Fish). Lake Rebecca, which was an untreated reference lake in 2006-2008, was treated in 2009 with endothall (0.75-1 mg ai/L).

All herbicides were applied by a boat-mounted tank injection system with 1-m drop hoses, allowing for precise dosing and coverage. Endothall was applied as the dipotassium salt of endothall, triclopyr as the triethylamine (TEA) salt and 2, 4-D as the butoxethyl ester or a dimethylamine salt formulation. All three of these herbicides were applied when surface water temperatures were between 10°C and 15°C. The rate of application of endothall, triclopyr and 2, 4-D was continuously adjusted based upon the water depth to achieve target concentrations. In Weaver Lake, liquid fluridone was applied uniformly over the entire lake by means of transects. Fluridone concentrations were periodically monitored in the following weeks after application using an enzyme-linked immunosorbent assay (FastTEST®) developed by SePRO Corporation, Carmel, Indiana (Netherland et al., 2002). If target concentrations of fluridone (> 2µg ai/L) were not maintained for at least 60 days a second application of fluridone was applied to reach the target concentration.

Plant Frequency

We employed the point-intercept method (Madsen 1999) to survey aquatic vegetation in the study lakes. The sample sites (points) were located using the ArcMap GIS regularly spaced grid generating software extension. The distance between sample points ranged from 50 to 80 m depending on lake size. Due to the variability of size and littoral area in each lake, lakes contained 106-408 points (Table 1). Lakes were sampled three times each year: early season (April-May), early-midseason (June), and during peak native plant abundance (August). May surveys were completed to determine native plant abundance before the application of herbicides in treated lakes and to indicate plant frequency early in the growing season. June surveys were conducted to assess native plant abundance with the absence of curlyleaf pondweed in treated lakes, and in competition with curlyleaf in untreated lakes. June surveys would also detect negative changes in plant abundance due to early season application of herbicides in treated lakes. August surveys were conducted to examine peak native plant abundance in treated and untreated lakes, and to assess differences between treatment factors.

At each survey point a 0.33 m wide double headed rake, weighed and tethered to a rope, was used to sample plants. The rake was tossed and then dragged for 3 meters along the bottom before retrieving for analysis, essentially representing 1 m^2 of sampling area. Floating leaf and emergent plants that were not easily sampled by the throw rake were given ratings based on their proximity to the boat (within a 3 meter radius). Rare taxa, or taxa not easily sampled by the throw rake, either due to size or infrequency, were noted as present when observed submerged or floating anywhere within the water column if not present in a sample. The littoral frequency of plants was calculated as the number of sites with plants present divided by the total number of sites ≤ 4.6-m depth sampled.

Biomass

Plant biomass was sampled in conjunction with each point intercept survey. Biomass was collected at 40 sampling sites randomly selected from the point intercept sites using the MNDNR random sample generator extension to ArcView. Biomass was collected using a single headed, 0.33 m wide, 14-tine rake (Johnson 2010). The rake had an adjustable arm to facilitate sampling in depths of up to 4.6m. Samples were acquired by placing the tines of the rake flush with the lake bed and rotating 3 times on the axis of the rake's handle. The rake was rotated slowly as it was retrieved, thus retaining plant material on the rake. The collected plants were then bagged and stored in an ice-filled cooler while in the field. Upon arrival to the lab, samples were stored in industrial coolers at 4 ˚C until they could be sorted.

Plants from the biomass samples were separated by taxon and spun in a salad spinner for one minute to remove excess water. Individual taxa were recorded and placed in a pre-weighed paper bag and weighed. Plants were dried for at least 48 h at 105 ˚C and then weighed and recorded. Plant biomass (g dm/m^2) was converted to m^2 based on the area sampled by the rake (0.09 m^2). Mean biomass was determined as the average mass of samples collected from depths less than or equal to the maximum depth of biomass samples collected in each study lake for each corresponding year.

Statistical Analysis

All statistical analyses were completed using R statistical software version 2.10.1 (The R Foundation for Statistical Computing, 2009). Changes in native vegetation were assessed by statistical differences in frequency, species richness or biomass samples between sample dates (May, June or August) and between years. A Welch's paired t-test was conducted to determine whether changes in frequency or species richness of native plants between May and June or June and August surveys were significant in any given year. An unpaired Welch's t-test was used to determine differences in frequency or species richness for months and years between treated and untreated lakes. Additionally, a Chi-squared analysis was used to evaluate changes in frequency between months in individual lakes. To evaluate differences between periods for average biomass, values for treated or untreated lakes were log transformed and analyzed using an exact paired Wilcoxon non-parametric rank sum test. To evaluate differences in average biomass between treated and untreated lakes, a Wilcoxon two sample rank sum test was conducted. To fully gauge differences in phenology of individual taxa, data from all four years was combined when analyzing frequency of occurrence and biomass.

Results

There were no significant differences in any given year in the average number of species present between May and June or June and August surveys; however, the number of native plant species increased throughout the growing season (May to August) each year, for both treated and untreated lakes (Figure 1). Additionally, there were no significant differences between the species present in treated or untreated lakes in any survey date for each year and no discernable trend across years in either treated or untreated lakes.

Native plant frequency of occurrence increased from May to June, in most lakes. Of the 31 surveyed periods of treated lakes, plants in 27 increased and 17 of those increased significantly. Similarly, in untreated lakes native plants increased in 10 survey periods, 7 of which were significant (Table 2). Unlike May to June frequency, many treated lakes decreased in frequency between June and August. Of the 31 studied periods there were 13 decreases of plant frequency, 8 of which were significant and most decreases were observed in light limited lakes (Clear, Long, Julia, and Rush). In the other treated lakes, we saw slight increases between June and August. Untreated lakes followed the same pattern, where of the 3 periods with decreasing plant frequency, 2 were significant (Table 2). We also did not see any pattern in frequency between years of treatment and date (May, June or August) in treated or untreated lakes. Overall, frequency was generally higher in treated lakes in 3 of the 4 studied years, but was highly variable based on year and not significant (Figure 2).

Apart from the first year of study, average native biomass was highest in June and August surveys (Table 3). Treated lakes also had higher average biomass in every year of the study; except for year 1, although no significant differences were found between any two periods. Additionally, August surveys produced considerably higher average native biomass in year 4 compared to year 1 in treated lakes ($P = 0.1$). Average biomass in untreated lakes did not increase between years and biomass between untreated lakes was highly variable (Table 3).

Overall, biomass increased consistently from May to August, and increased significantly from June to August in both treated ($P = 0.003$) and untreated lakes ($P = 0.01$). Conversely, overall frequencies of native plants increased significantly between May and June in treated ($P < 0.001$) and untreated lakes ($P = 0.002$), but were constant from June to August (Figure 3).

Native Plant Taxa

Chara spp. frequency increased from May to June in treated lakes ($P<0.001$) but did not further increase in August (Table 4). Average *Chara* spp. biomass in treated lakes increased throughout the seasons from 12 g/m^2 in May to 25 g/m^2 in June and 73 g/m^2 in August, although none of these increases were significant (May-June $P=0.13$, June-August $P=0.18$; Table 5). There were no significant changes in untreated lakes between sampling dates for either frequency or biomass. *Chara* spp. had low abundance in untreated lakes and it was not found in one of the untreated lakes (Vails). There were significant differences in *Chara* spp. frequency between treated and untreated lakes for each survey date (P<0.001) and significantly higher August biomass in treated lakes (P=0.02; Table 5).

Unlike *Chara* spp., *Ceratophyllum demersum* (L.) did not show significant changes in frequency in treated lakes between May and June or June and August and changed little over the growing season unlike other plant taxa (Table 4). Biomass of *C. demersum* increased throughout the growing season in both treated and untreated lakes, although none of the increases were significant. In untreated lakes *C. demersum* frequency increased significantly between May and June (P=0.004) but the increase in August was not significant. Similar to treated lakes, the average biomass of *C. demersum* in untreated lakes increased throughout the growing season, although only the June and August surveys were significantly different (P=0.03). Frequency and biomass of *C. demersum* varied in treated and untreated lakes throughout the study and were not significantly different (Table 4 & 5).

Similar to *Chara* spp., we saw significant increases between May and June frequencies of *E. canadensis* (Michx.) in treated lakes (P=0.001), but not in untreated lakes (Table 4). June surveys in treated lakes also had significantly higher frequencies of *E. canadensis* when compared to untreated lakes (P=0.01), although no other dates were significantly different. Average *E. canadensis* biomass in treated lakes rose significantly from June to August (P=0.002; Table 5), and was significantly higher in May when compared to untreated lakes (P=0.02). *E. canadensis* biomass in untreated lakes increased only slightly between dates but none of these increases were significant.

Unlike the other aforementioned plants in the study, *Vallisneria americana* (Michx.) and *Myriophyllum sibiricum* (Komarov) were either present in low quantities or not found in May surveys. *M. sibiricum* occurred in about half of our study lakes and *V. americana* in many of the lakes; however, *V. americana* occurred in low frequencies (Table 4) and biomass (Table 5) overall. However, *V. americana* increased in frequency from May to June (P=0.002) and June to August (P=0.002) in treated lakes; similar patterns were seen in untreated lakes, although not significant. Furthermore, biomass of *V. americana* was higher in August than in June in both treated (P=0.004) and untreated lakes (P=0.16). We did not find significant differences of *V. americana* between treated and untreated lakes in any given period for either biomass and frequency.

Similar to *V. americana*, *M. sibiricum* was found in low quantities in May, although *M. sibiricum* increased in frequency and biomass later in the growing season (June & August) in both treated and untreated lakes (Table 4). In treated lakes, we observed significant increases of *M. sibiricum* frequency between June and August (P=0.03) and in untreated lakes, we observed large increases, however these were not significant (Table 4). Biomass followed the same pattern (Table 5), increasing marginally in treated lakes and considerably in untreated lakes. The major increases of *M. sibiricum* in untreated lakes were due to one or two dates and therefore not significant.

Discussion

Our findings indicate that the treatment of curlyleaf pondweed with herbicides did not significantly alter species richness, frequency, or biomass of native plants between treated and untreated lakes. Both treated and untreated lakes were similar in the average number of species present in each lake although variation was present between years. Despite small differences between years, we saw increases in both May to June and June to August surveys (Figure 1). Light availability is considered the major determinant of native macrophyte growth, especially in areas where nutrients are abundant (Barko and Smart, 1981; Best et al., 2001). We found that species richness increased in a linear pattern from May to August, suggesting that light is not the only determining factor of species richness. Some plant species rely on factors other than just light to initially sprout (Smolders et al., 1995). Temperature may have various effects on increasing seed, tuber, or turion germination by removing the outer coating (Haag, 1983). Increased temperature results in increased microbial activity, acidity levels and chance of seed coat rupture (Teltscherová and Hejný, 1973). Although not all of the plants in our study reproduce by seeds, many do, resulting in higher species richness when temperatures are warmer.

In both treated and untreated lakes increases in native plant frequency between May and June were greater than between June and August (Figure 2). This was most evident in eutrophic and hypereutrophic lakes where light was limited (Clear, Julia, Long and Rush; Table 2). We also saw significant increases in native plant frequency between May and June across all years (Figure 3). This increase in plant frequency is best explained by the colonization patterns of plants and light availability. Many macrophytes do not persist year-round in Minnesota due to the lack of light during ice cover conditions

(Lotter and Bigler, 2000). A spring clear water phase is common in many lakes throughout Minnesota; during this time period, macrophytes have the opportunity to colonize habitable areas altered by the onset of winter conditions (Lampert et al., 1986; Sommer, 1983). Because of the clear water phase and subsequent light availability, we noted increased frequencies from May to June, but not from June to August, when maximum plant depth had already been attained and water clarity was poorer (Canfield et al., 1985). In late summer, macrophytes are more likely to increase in biomass or fecundity (Doust and Laporte, 1991). Similar results have been found in other studies, where algal species compete for light and resources later in the growing season (Berglund et al., 2003). Curlyleaf pondweed is one of the more exploitive plants during the clear water phase, reaching peak frequency and biomass by late spring (Johnson, 2010). Despite competition by curlyleaf, we did not see differences in native plant frequency between treated or untreated lakes.

Elodea canadensis and *Chara* spp. frequencies both increased in June and then declined in August in treated lakes, either due to competition with other plants or from reduction in water clarity. In untreated lakes, frequency of *E. canadensis* and *Chara* spp. did not increase but rose only moderately between months. Likewise, *C. demersum* frequency increased moderately throughout the growing season in both treated and untreated lakes. In plants such as *C. demersum* and *E. canadensis*, moderate increases would be likely because these plants persist under ice cover and are already in an actively growing state when ice cover subsides (Nichols and Shaw, 1986; Spencer and Wetzel, 1993). Similarly, *Chara* spp., an annual macroalgae, is a rapid colonizer and grows quickly, sprouting from oospores early in the year (Anderson, 1984; Bonis and Grillas, 2002). Why *E. canadensis* and *Chara* spp. frequencies decreased in August and *C.demersum* did not is unknown, but may be due to *C. demersum's* ability to float near the surface while *E. canadensis* and *Chara* spp. remain rooted on the bottom.

Unlike frequency of occurrence, native plant biomass increased each month throughout the growing season in every year for both treated and untreated lakes, with little difference between the two treatments. Considering that the time period between June and August was one month longer than May and June, we would expect the biomass increases to be larger.

Vallisneria americana was uncommon or rare prior to June surveys in treated lakes and not present during this period in untreated lakes. Both the frequency and biomass of *V. americana* increased significantly from June to August in treated lakes. Increases in biomass and frequency were observed in untreated lakes from June to August, although these increases were not significant. Titus and Stephens (1983) found similar inter-seasonal variation of *V. americana*, with rapid growth later in the growing season. *Vallisneria americana* has been reported to be a poor competitor with canopy forming adventive macrophytes in other studies (Haller and Sutton, 1975; Smart et al., 1994; Titus and Adams, 1979) primarily because of its late growth and low stature. Differences between growth in treated and untreated lakes was not clear in our study.

In both treated and untreated lakes, we saw increases of *C. demersum* biomass from May to August, which increased substantially in both untreated and treated lakes (Table 5), but because the high biomass of *C. demersum* was not observed in all study periods, increases between dates were not significant.

Unlike changes in frequency of occurence, *E. canadensis* and *Chara* spp. biomass increased throughout each monitored period. Along with other macrophytes in our study, increasing biomass patterns throughout the growing season can best be explained by the positive relationship between temperature and plant growth. Temperature and light are correlated, where increased summer photoperiods and light intensity result in higher water temperatures (Barko and Smart, 1981). Barko et al. (1982) found that interactions between temperature and light can result in higher biomass increases than either factor alone. Other studies have shown that in some species, temperature can increase Q^{10} values (Madsen and Brix, 1997; Rooney and Kalff, 2000). Despite the influence of light and temperature on macrophyte growth, summer water clarity is often much lower compared to spring in Minnesota, primarily due to high pelagic algal biomass (Sommer, 1985; Wehr and Sheath, 2003). We saw higher rates of colonization (frequency) of some plants early in the growing season and this is largely dependent on light availability. Later in the growing season, pelagic algae limit light availability, although photoperiod and temperatures are still high. Because of these inter-seasonal factors; we observed increased macrophyte frequency early in the season, but not later and increased biomass throughout the entire growing season.

Despite curlyleaf pondweed being a major nuisance throughout the early macrophyte growing season, we did not see large differences in the patterns of native macrophyte growth in any given year or for any specific species. Overall differences in macrophyte growth as a result of curlyleaf control may either be negligible or may only be observed over consecutive years of removal and at particular intervals of native plant growth. Further assessment during the period of peak native plant growth may be needed to experience overall changes in native plant communities.

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Table 1. Study lake characteristics, ecoregion within Minnesota, Division Of Waters identifying number (DOW) and number of littoral (≤ 4.6 m) points sampled.

Lake (DOW)	**Trophic† Status**	**Avg. Secchi Depth(m)**	**Ecoregion***	**Size (hectares)**	**% Littoral**	**Maximum Depth (m)**	**Survey Points (Littoral)**
Blueberry (80-0034)	E	0.9	NLF	211	100	4.2	400
Clear (47-0095)	HE	0.6	WCBP	201	83	5.5	265
Coal ♦ (77-0046)	M	2.4	NLF	69	40	8.2	118
Crookneck (49-0133)	M	2.9	NLF	74	80	6.7	195
Fish (70-0069)	E	1.6	CHF	70	40	8.6	127
Long (30-0072)	HE	1.0	CHF	158	100	4.2	408
Lower Mission (18-0243)	M	3.8	NLF	292	60	8.5	265
Julia (71-0145)	E	0.6	CHF	62	100	4.6	106
Rebecca ♦ (27-0192)	E	1.9	CHF	105	50	9.2	186
Rush (71-0147)	E	0.6	CHF	65	100	3.4	112
Silver (62-0001)	E	2.6	CHF	29	99	6.2	150
Vails ♦ (73-0151)	E	1.6	CHF	64	80	6.1	185
Weaver (27-0117)	E	2.3	CHF	62	50	17.4	125

♦ Untreated Reference Lake

* Central Hardwood Forests (CHF), Northern Lakes and Forests (NLF), Western Corn Belt Plains (WCBP)

† Mesotrophic (M), Eutrophic (E), Hypereutrophic (HE)

Table 2. Frequency of occurrence (%) of native plants in treated and untreated lakes. Year represents the consecutive year of treatment where herbicide was used. Chi-square P-values for May vs. June and June vs. August between the respective year and period (α = * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

Treated Lakes	Year	May		June		Aug
Blueberry †	2	32	**	41	***	53
Clear †	3	39		37	**	23
	4	24	***	40	**	24
Crookneck ‡	1	99		99		99
	2	91		96		98
	3	94	*	99		100
Fish ‡	1	51	*	65		76
	2	72		72		69
	3	65		70		76
Julia	1	37	***	64	*	48
	2	42		52		51
	3	49		54		50
	4	27		37		42
Long †	2	4		7		7
	3	9	***	24	***	11
	4	5	***	18		15
Lower Mission	1	91	***	98	***	87
	2	86		91		88
	3	75		72	***	90
	4	74		83		87
Rush	1	15	***	47	**	29
	2	32	***	66	*	50
	3	36	*	50	*	63
	4	30	***	53		42
Silver †	2	85	*	94		95
	3	89	*	72	***	27
	4	32	***	51		52
Weaver	1	30	***	59		69
	2	24	*	42		42
	3	30		28	***	51
	4	32	***	59		70
Untreated Lakes						
Coal	1	83	*	92		85
	2	79		80		88
	3	78		82		88
	4	85		78		86
Rebecca ‡	1	15	***	33		36
	2	15	*	27		36
	3	12	*	24	***	46
Vails	1	59		68	***	40
	2	17	*	27		30
	3	7	***	36	**	21
	4	3	***	21		23

† Lakes with limited data prior to 2008
‡ Lakes with herbicide treatment status changes in 2009

Table 3. Average native plant biomass (g dm/m^2) in treated and untreated lakes for each of the studied years followed by one standard error of the mean.

	Year 1	Year 2	Year 3	Year 4
Treated				
May	130 ± 73	138 ± 111	97 ± 77	62 ± 34
June	119 ± 58	175 ± 83	154 ± 70	90 ± 38
Aug	99 ± 57	216 ± 87	215 ± 98	313 ± 107
Untreated				
May	113 ± 44	42 ± 32	64 ± 47	84 ± 83
June	198 ± 74	76 ± 60	89 ± 54	61 ± 59
Aug	164 ± 92	185 ± 131	165 ± 84	184 ± 70

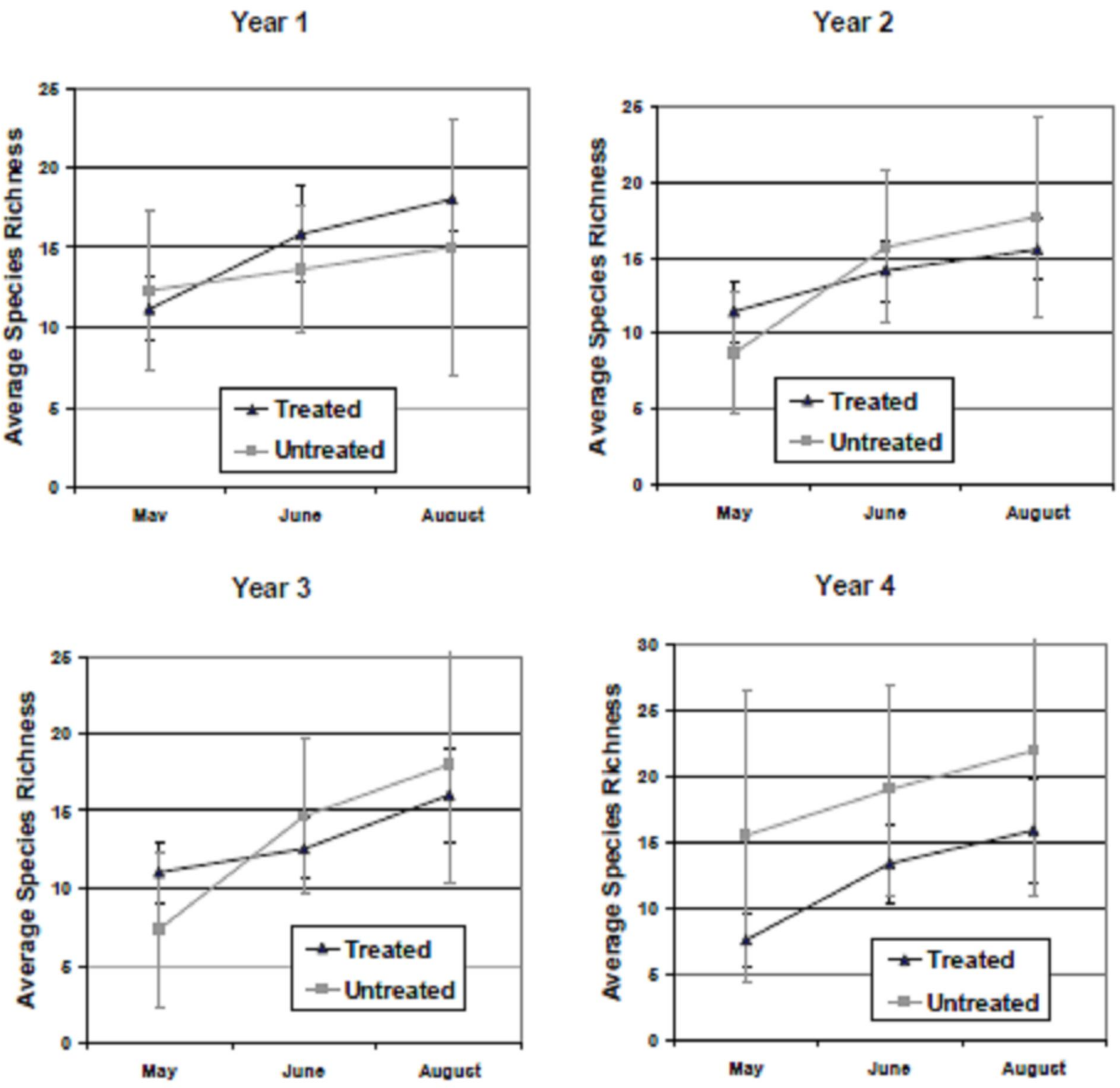
Table 4.) Mean and standard error for frequency (% occurrence) of common macrophyte taxa found in our study.

	Chara spp.	*C. demersum*	*E. canadensis*	*M. sibiricum*	*V. americana*
Treated Lakes					
May	7 ± 1	30 ± 6	16 ± 3	4 ± 1	<1 ± <1
June	13 ± 2	32 ± 5	25 ± 5	5 ± 1	5 ± 1
August	12 ± 2	33 ± 5	20 ± 3	8 ± 2	10 ± 2
Untreated Lakes					
May	1 ± <1	31 ± 7	8 ± 3	5 ± 2	0
June	1 ± 1	38 ± 6	3 ± 11	10 ± 4	2 ± 1
August	2 ± 1	41 ± 6	11 ± 4	18 ± 3	7 ± 4

Table 5. Mean biomass (g dm/m^2) and standard error of common macrophyte taxa found in our study.

	Chara spp.	*C. demersum*	*E. canadensis*	*M. sibiricum*	*V. americana*
Treated Lakes					
May	12 ± 6	58 ± 19	4 ± 1	1 ± <1	0
June	25 ± 10	66 ± 21	10 ± 3	2 ± 1	1 ± <1
August	73 ± 31	96 ± 27	30 ± 12	3 ± 1	4 ± 1
Untreated Lakes					
May	1 ± <1	63 ± 33	1 ± <1	1 ± <1	0
June	2 ± 1	68 ± 28	4 ± 1	3 ± 1	2 ± 1
August	5 ± 3	90 ± 40	6 ± 2	18 ± 16	5 ± 4

Figure 1. Mean number of native plant species in treated and untreated lakes in each year of treatment. Error bars represent standard error among lakes.


Year 1

Year 2

Year 3

Year 4

Figure 2. Mean frequency of native plant species in treated and untreated lakes in each year of treatment. Error bars represent standard error among lakes.

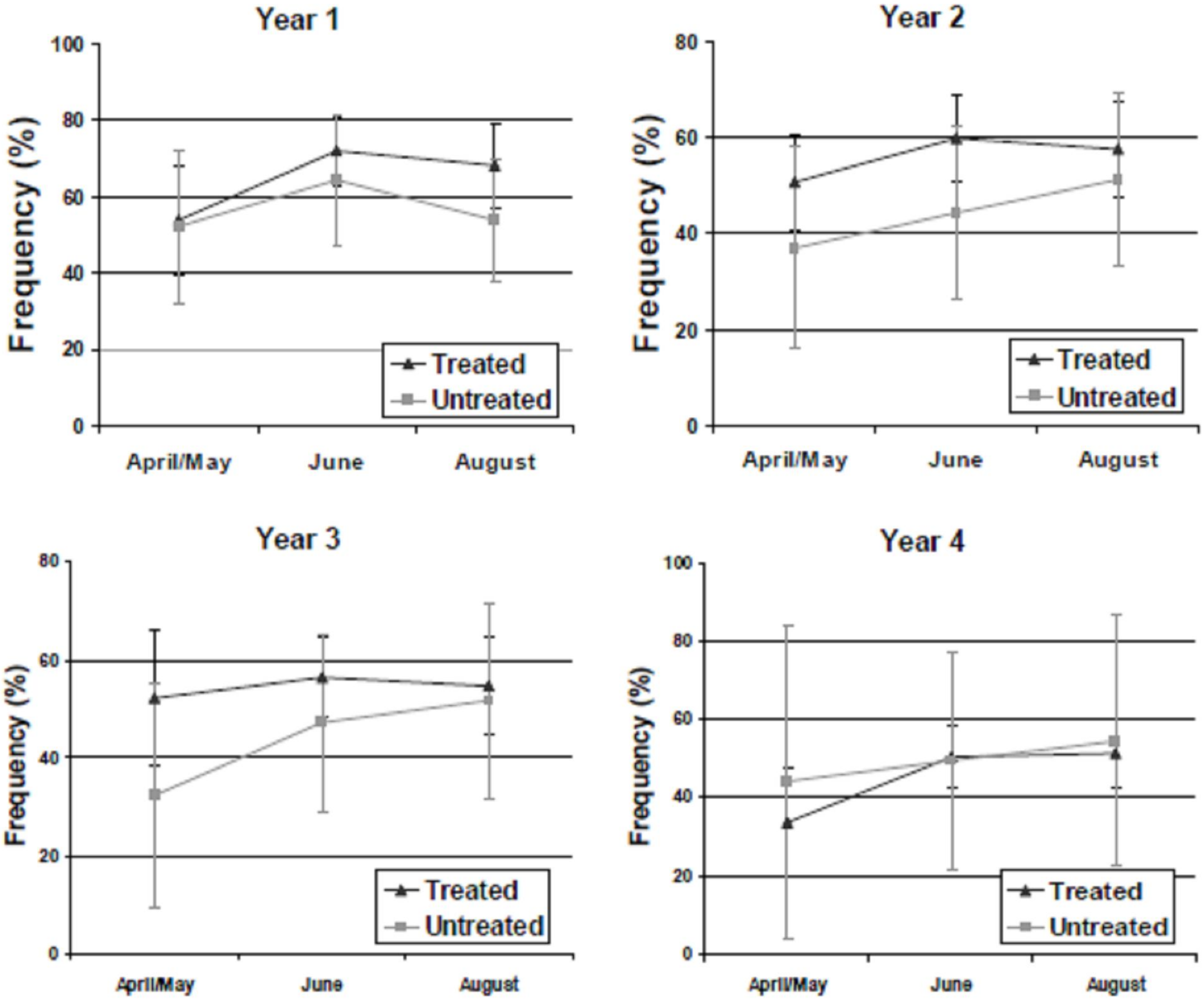
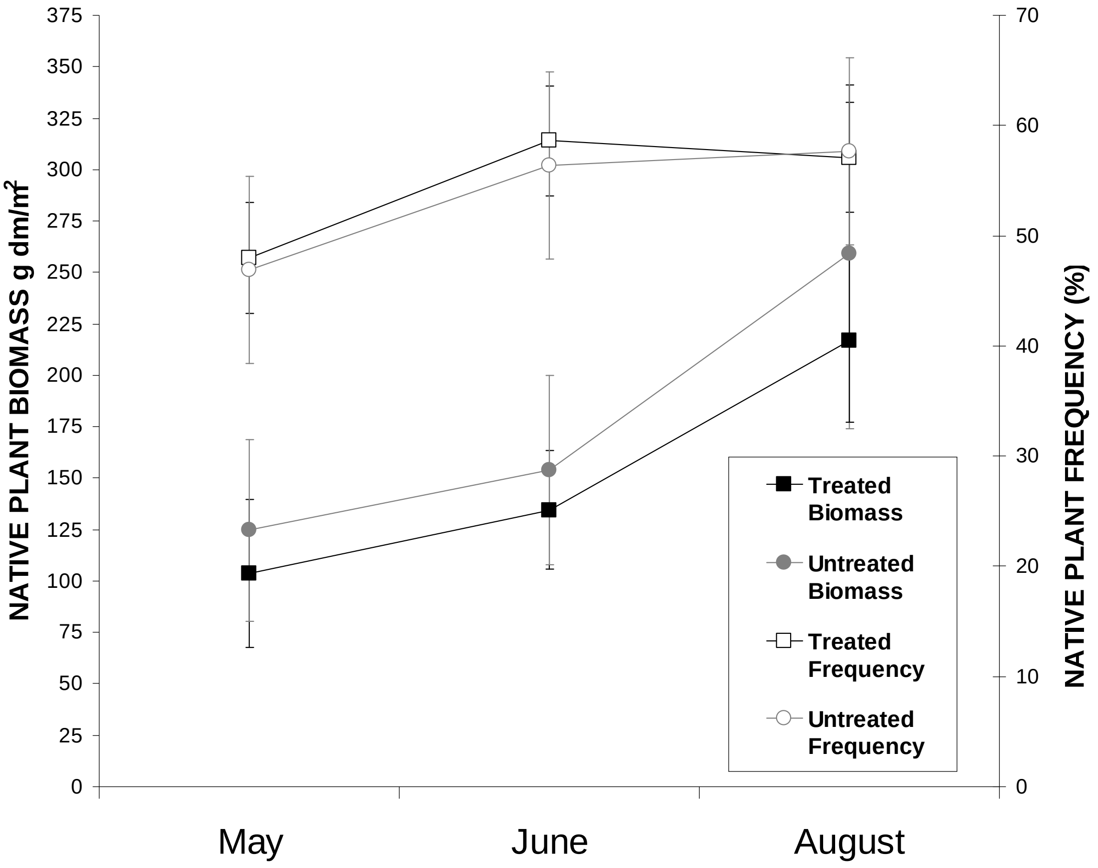


Figure 3. Mean Frequency (% occurrence) and biomass (g dm/m^2) of all native plants combined from May to August. Frequency scale appears on the right Y-axis and biomass on the left Y=axis. Open squares represent frequency, whereas solid squares represent biomass. Error bars represent one standard error among lakes.



Chapter 3

Effects of Repeated Early Season Herbicide Treatments of Curlyleaf Pondweed on Native Macrophyte Assemblages in Minnesota Lakes.

Planned submission to Lake and Reservoir Management in 2010 with James A. Johnson as second author and Raymond M. Newman as third author.

Summary

Within the Midwestern United States, curlyleaf pondweed (*Potamogeton crispus* L.) is one of the most invasive aquatic plants. Curlyleaf invasions are characterized by dense mats of nuisance growth, causing extensive obstruction to boat traffic and negatively impacting the natural ecology of a lake. Monotypic curlyleaf stands supply excess nutrients upon senescence and may hinder native macrophyte growth. Lake-wide, early-season herbicide treatments of curlyleaf pondweed may provide an effective control strategy that would also reduce harm to native macrophyte communities.

We examined the response of native plant communities to spring herbicide treatments of curlyleaf pondweed from 2006 through 2009. Thirteen lakes were examined during our study; ten were treated with herbicide and three were used as non-treatment reference lakes. Plant communities were assessed in the littoral zone (≤ 4.6 m depth) with the point intercept method (100 to 400 points per lake) in early spring (before treatment), late spring and late summer. For each survey, approximately 40 random biomass samples ($0.33m^2$) were taken throughout each study lake to estimate plant biomass. In the reference lakes, curlyleaf persisted at moderate to high frequencies over the four years, and no consistent changes in native plant frequency of occurrence were seen. Herbicide treatments proved effective for controlling curlyleaf and curlyleaf decreased in occurrence within 1 month following treatment. In response to consecutive herbicide treatments over the course of four years, overall native plant frequency of occurrence did not decrease in most treatment lakes. Native macrophyte species richness also indicated little change with continued treatment, although shifts in abundance of some species were observed. Native plant biomass increased between 2006 and 2009 in most treatment

lakes, while native biomass varied in untreated lakes. Much of the change in biomass was attributed to a single species in most treatment lakes. We observed consecutive increases of *Chara spp.* frequency and biomass throughout most treated lakes. However, multiple years of treatment may be needed to obtain increases in native plant abundance as the greatest increases occurred after 3 years of treatment. Consecutive early-season, lake-wide herbicide treatments of curlyleaf pondweed can potentially reduce curlyleaf occurrence and density in each year of application without harm to native plants.

Introduction

Curlyleaf pondweed (*Potamogeton crispus* L.) is one of the most detrimental invasive macrophytes found in Minnesota and the northern United States. Curlyleaf has been recorded in Minnesota since 1910 (Bolduan et al., 1994; Moyle and Hotchkiss, 1945). Biomass in curlyleaf stands is often much higher than in indigenous aquatic plant stands (Bolduan et al., 1994; Kuni, 1984). The homogonous macrophyte habitat of invasive species has been shown to reduce the foraging success of predatory fishes (Valley and Bremigan, 2002), displace native submersed macrophytes (Catling and Dobson, 1985) and inhibit recreation (Valley et al., 2004). In addition, curlyleaf sequesters phosphorus from lake sediments (Rogers and Breen, 1980; Rogers and Breen, 1982). Upon senescence, curlyleaf releases nutrients into the water column from decaying tissue and the resultant nutrient loading has the potential to perpetuate phytoplankton growth and inhibit water quality (Bolduan et al., 1994; Rogers and Breen, 1982).

The timing of curlyleaf's annual life cycle is one of the major factors that allows curlyleaf to be a successful invader (Bolduan et al., 1994). In Minnesota and much of North America, curlyleaf multiplies by vegetative propagules called turions, whereas sexual reproduction by seeds accounts for as little as 3% of new pants (Woolf and Madsen, 2003). Turions are produced at the end of the curlyleaf growing season (June) and are formed asexually as modified basal leaf structures (Sastroutomo, 1981). Turions commonly sprout in the fall, as well as under ice cover during winter months (Bolduan et al., 1994). At the onset of early spring, curlyleaf exhibits rapid growth towards the water surface (Jian et al., 2003; Sastroutomo, 1981). Early sprouting and accelerated growth before the native macrophyte growing season, make curlyleaf a successful competitor among aquatic plants in Minnesota (Madsen and Crowell, 2002). After reaching the water surface, curlyleaf plants begin to form a dense mat and produce turions. During the time it takes to produce turions (about 2 weeks), carbohydrates are allocated to turion production and make up to 30-66% of the turion biomass (Bolduan et al., 1994; Woolf and Madsen, 2003). By early summer, curlyleaf plants senesce and turions drop to the lake substrate, remaining dormant throughout the rest of the summer season to sprout in fall and winter (Bolduan et al., 1994; Kunii, 1982; Sastroutomo, 1981).

There is considerable interest in improved approaches to manage invasive aquatic plants. Due to the timing of growth that curlyleaf pondweed exhibits in Minnesota lakes, herbicide treatments can be an effective method for control (Johnson 2010; Skogerboe et al., 2008). Although maximum peak curlyleaf growth occurs in the late spring months of Minnesota, native plant growth typically occurs later, in mid summer (Crow and Hellquist, 2005). By treating curlyleaf in spring, before maximum biomass is attained and before turions are formed, herbicides can selectively target curlyleaf without harming native plants (Getsinger et al., 1997; Netherland et al., 2000). Disrupting turion formation for consecutive years by herbicide may reduce reoccurrence of curlyleaf by reducing the sediment turion bank (Madsen and Crowell, 2002; Poovey et al., 2002). Reducing curlyleaf levels may also lead to reductions in phosphorus release and subsequent algal blooms (James et al., 2002). Without the dense canopy forming stands and reduced water clarity, light availability is likely to increase, thus benefiting native plant growth. The potential to control curlyleaf while not harming native plants makes lake-wide early season herbicide treatment an attractive method for control.

Native Macrophytes

Native macrophyte communities have numerous positive effects on lake ecology. Habitat complexity provided by a vegetated littoral zone benefits a variety of species, providing a baseline for productivity. Not only do macrophytes create food and shelter for aquatic animals, they host a variety of epiphytic organisms (Carpenter and Lodge, 1986). Macrophytes and epiphytes alike are known to increase macroinvetebrate abundance and diversity (Gilinsky, 1984; Van den Berg et al., 1997). Likewise, a complex macrophyte community provides food and shelter for fishes (Jeppesen et al., 1998), increasing diversity (Smart et al., 1996) and survivability of various fish species (Shoup et al., 2003; Valley et al., 2004).

In addition to providing habitat, the environment produced by macrophytes has a role in water quality. Macrophytes provide shelter for large cladocerans, which graze on pelagic algae, thus resulting in increased water clarity (Lampert et al., 1986; Norlin et al., 2005). Kuczy ska-Kippen and Nagengast (2006) found that two major algal consuming groups of zooplankton, the Rotifera and Cladocera, were nearly twice as diverse in macrophyte beds versus open water. There is strong evidence that macrophytes also act as nutrient sinks, both competing with planktonic algae for resources and hosting denitrifying epiphytic bacteria within their roots (van Donk and van de Bund, 2002; Weisner et al., 1994). Some macrophytes have been shown to improve water quality in shallow lakes by sequestering pelagic nutrients, reducing sediment suspension (Horppila and Nurminen, 2003), increasing oxidation (Sand-Jensen et al., 1982) and from alleopathic effects (Kufel and Kufel, 2002).

Due to the role that native macrophytes have in the ecology of lakes, it is important that control of invasives not result in damage to native macrophytes, but rather enhance them. Although long-term control of curlyleaf pondweed may be a successful treatment method (Sokoberboe et al. 2008, Johnson 2010), the long term effects on native plants from either the lack of curlyleaf growth or repeated herbicidal treatments has not been examined across a multitude of lakes. Some native aquatic plants have been shown to be sensitive to herbicides that are intended to control curlyleaf pondweed (Skogerboe et al., 2008). Endothall, the primary herbicide used to control curlyleaf in our study, is a useful contact herbicide due to it's short persistence time (Langeland and Warner, 1986). Skogerboe and Getsinger (2001, 2002) found that some native species, particularly many in the genus *Potamogeton*, are sensitive to endothall. Other macrophytes such as coontail (*Ceratophyllum demersum* L.) show moderate sensitivity to endothall, whereas members of the Characeae family, emergent macrophytes and floating leaf species show little sensitivity to endothall (Skogerboe and Getsinger, 2001; Skogerboe and Getsinger, 2002). Despite the sensitivity of some macrophytes to herbicide treatments, the positive effects as a result of curlyleaf pondweed removal may enhance the overall native macrophyte community composition.

Early season herbicide treatments are likely to improve native plant growth due to reductions in curlyleaf, although it is important to determine whether these treatments have a negative effect on native plants as well. In this manuscript, we evaluate the effects of repeated annual herbicide applications to control curlyleaf pondweed on native plants over the course of four years. Native plant biomass, percent occurrence, diversity and richness were analyzed following each year of treatment. In addition, patterns of specific aquatic plant species that either increased or decreased were assessed to better understand the effects of herbicide treatment as a control mechanism for curlyleaf pondweed.

Methods

Study Lakes

All of our treated study lakes had significant levels of curlyleaf infestation and were chosen by the Minnesota Department of Natural Resources (MNDNR). Treatment lakes ranged in trophic status from mesotrophic to hyper-eutrophic, but most treated lakes were eutrophic (Table 1). We chose three reference lakes based on similar levels of curlyleaf infestations, location (Figure 1), size and trophic status compared to treated lakes. Average Secchi depth ranged from 3.8m in mesotrophic lakes to 0.6m in hypereutrophic lakes (Table 1). Lakes varied in size from 29 to 292 ha, and all lakes, with the exception of one (Weaver), were moderately shallow with maximum depth <10 m. All lakes were sampled by researchers from the University of Minnesota, although surveys prior to June 2008 for Silver, Clear, Blueberry, and Long Lakes were conducted by MNDNR staff.

Procedures

Herbicide Treatments

Staff from the MNDNR delineated treatment areas and supervised all herbicide application to the study lakes in 2006, 2007, 2008 and 2009 (Table 2). Eight of the lakes (Lower Mission, Crookneck, Julia, Rush, Fish, Blueberry, Long, and Clear) were treated exclusively with endothall (0.75-1.0 mg active ingredient (ai)/L); Weaver Lake was treated with fluridone in 2006 and 2007 (target concentration of 2-4 μg ai/L) and endothall in 2008 and 2009 (0.75-1.0 mg/L); Silver Lake received a combined 2,4-D (0.5-1.0 mg ai/L)/endothall (0.75-1.0 mg/L) treatment in 2007, a combined triclopyr (0.5

µg ai/L)/endothall (0.75-1.0 mg/L) treatment in 2008 and an endothall only treatment in 2009 (0.75-1.0 mg/L). In addition to lakes receiving consecutive treatments, treatment was stopped in the fourth year (2009) on two of the study lakes (Crookneck and Fish) to examine the effects of discontinued herbicide treatments. Rebecca, which was an untreated reference lake in 2006-2008, was treated in 2009 with endothall (0.75-1 mg/L).

Endothall, 2, 4-D and tryclopyr applications were limited to areas of early-spring *P. crispus* or Eurasian watermilfoil (*Myriophyllum spicatum* L.) growth; these areas were delineated and application monitored by MNDNR staff. All herbicides were applied by a boat-mounted tank injection system with 1-m drop hoses, allowing for precise dosing and coverage. Endothall was composed of a liquid formulation of the dipotassium salt of endothall, triclopyr as liquid formulation of triethylamine (TEA) salt and 2, 4-D as a butoxethyl ester or a dimethylamine salt formulation. All three of these herbicides were applied when surface water temperatures were between 10°C and 15°C. The rate of application of endothall, triclopyr and 2, 4-D was continuously adjusted based upon the water depth to achieve target concentrations. For fluridone treatments, liquid fluridone was applied uniformly over several widely-spaced, deep-water transects at a rate that was sufficient to achieve the target concentration (2-4 µg ai/L). Fluridone concentrations were periodically monitored in the weeks following application using an enzyme-linked immunosorbent assay (FastTEST®) developed by SePRO Corporation, Carmel, Indiana (Netherland et al., 2002). If target concentratons of fluridone were not maintained for at least 60 days, a second application of fluridone was applied to maintain the target concentration.

The reference lakes did not receive lake-wide herbicide treatments during the years of monitoring. In addition to the experimental treatments discussed above, several of the study lakes received lake-wide treatments prior to 2006. The MNDNR supervised lake-wide treatments on Fish (endothall) and Weaver Lakes (fluridone) in 2005, but earlier shoreline endothall treatments on Julia and Rush lakes from 2000 to 2005 and on Lower Mission in 2005, were not supervised by the MNDNR.

Plant Frequency

We employed the point-intercept method (Madsen 1999), to survey aquatic vegetation in the study lakes. The sample sites (points) were located using the ArcMap GIS regularly spaced grid generating software extension. The distance between sample points ranged from 50 to 80 m depending on lake size. To determine the maximum depth of macrophyte colonization we sampled beyond depths where plants were found, although for our study, depths of ≤4.6m were analyzed to provide continuity. Due to the variability of size and littoral area in each lake, lakes contained between 93 and 408 points, covering a range of depths (Table 1). Lakes were sampled three times each year: pre-treatment (April-May), curlyleaf peak growth (June), and peak native plant growth (August). May surveys were completed to determine the level of infestation before treatment each year and the level of curlyleaf reoccurrence as a response to the previous year's treatment. June surveys were primarily conducted to determine the level of peak curlyleaf growth in untreated lakes and to establish the response of curlyleaf to herbicide treatments that were conducted prior to the June survey. Native plant occurrence and density was also recorded in the May and June surveys although native plant response could not be fully gauged until later in the growing season. August surveys were conducted during the time of peak native plant biomass primarily to examine the native plant reaction to the spring herbicide treatments.

At each survey point, depth was measured and a 0.33m wide double headed rake, weighed and tethered to a rope was used to sample plants. The rake was tossed and then dragged for 3 meters along the bottom before retrieving for analysis. Plants were identified and recorded if present on the rake. Floating leaf and emergent plants that were not easily sampled by the throw rake were rated based on their proximity to the boat (within a 3 meter radius). Rare taxa, or taxa not easily sampled by the throw rake, either due to size or infrequency, were noted as present within the lake when observed submerged or floating anywhere within the water column. The littoral frequency of plant species sampled was calculated as the number of sites present divided by the total number of sites ≤ 4.6-m deep.

Biomass

Plant biomass was sampled in conjunction with each point intercept survey. Biomass was sampled at 40 randomly selected sample sites from the point intercept sites using the MNDNR random sample generator extension to ArcView. Biomass was collected using a single headed, 0.33-m wide, 14-tine rake (Johnson 2010). The rake had an adjustable arm to facilitate sampling in depths of up to 4.6m. Samples were acquired by placing the tines of the rake flush with the lake bed and rotating 3 times on the axis of the rake’s handle. The rake was rotated slowly as it was retrieved, to keep plant material on the rake. The collected plants were then bagged and stored in an ice-filled cooler.

Upon arrival to the lab, samples were stored in industrial coolers at 5 ˚C until they could be sorted.

Plants from the biomass samples were separated by taxon and spun in a salad spinner for one minute to remove excess water. Individual taxa were recorded and placed in a pre-weighed paper bag and weighed. Plants were dried for at least 48 h at 105 ˚C and then weighed and recorded. Plant biomass (g dm/m^2) was converted to m^2 based on the area sampled by the rake (0.09 m^2) and mean biomass was determined as the average mass of samples from ≤4.6m depths.

Statistical Analysis

All statistical analyses were completed using R statistical software version 2.10.1 (The R Foundation for Statistical Computing, 2009). Changes in native vegetation were estimated by comparing differences in August frequency or biomass between each year of treatment and between the first and final year of treatment. Welch’s paired t-test was conducted to determine whether the change in frequency, species richness, or plants per point between samples of native plants in treated lakes or untreated lakes was significant between years. An unpaired Welch’s t-test was used to compare treated and untreated lakes for each year. To determine differences in average biomass between years in treated or untreated lakes, average biomass was log transformed and analyzed using an exact paired Wilcoxon non-parametric rank sum test. To test average biomass differences between treated and untreated lakes, average biomass was log transformed and a Wilcoxon two sample rank sum test was conducted. Chi-square analysis was also used to compare plant frequency within single lakes in two separate years.

Results

Native Macrophytes

In treated lakes, overall native plant frequency neither increased nor decreased during the time of peak native vegetation (August) throughout the four years of herbicide treatment. Likewise, no significant yearly changes in native plant frequency were observed in untreated lakes. There was no difference in native plant frequency of occurrence throughout all four years between treated and untreated lakes (Figure 2). Despite no overall difference over time in treated lakes, there was variation in frequency between years of treatment and individual lakes (Table 3). Chi-square analysis indicated that two lakes (Rush and Blueberry) had significant increases in frequency of native plants from year one to year two ($P<0.05$) of treatment, whereas native plants in Silver and Weaver decreased from year two to three and year one to two respectively ($P<0.05$). In addition, Weaver and Silver increased significantly between the third and fourth year of treatment ($P<0.05$). Alternatively, Rush Lake experienced decreased native plant frequency from year three and four ($P<0.05$). Untreated lakes showed similar annual variation in native plant frequencies; Vails decreased from year one to three whereas Rebecca increased from year two to three ($P<0.05$).

The diversity of native plants in August remained unchanged throughout the study period. The number of species present in treated lakes did not differ significantly from untreated lakes in any single year and untreated lakes remained relatively unchanged in the number of species present throughout the four years of study (Figure 3). Similarly, treated lakes did not differ in the overall number of species present between the first and fourth year or within other consecutive years with the exception of year three to year four, where increases in the number of species present in Long, Lower Mission, and Silver Lake were observed ($P < 0.02$). Similar to richness, there was no distinctive overall pattern in the number of native plants per point throughout the four years of treatment (Figure 4). However, slight increases from year three to four (Table 4) were observed in treatment lakes ($P<0.04$) although major changes were only seen in Clear, Silver and Weaver Lakes. The increased native plants per point in Silver Lake between year 3 and 4 were largely due to a decrease in year 3 where native plants per point were lower than previous years. The native plants per point in untreated lakes remained relatively unchanged throughout the study period and showed little difference compared to treated lakes.

We observed an overall increasing trend in native plant biomass in August within the treated lakes, although these trends were not significant (Figure 5). Biomass increased each consecutive year and the greatest increase of native biomass was between year one (155 ± 59 g/m^2) and two (220 ± 79 g/m^2). There was no pattern in average native biomass in untreated lakes (Figure 5), and no significant difference between untreated and treated lakes in any of the four years of study ($P=0.67$ to 0.83).

Within each lake there was variation in biomass between years, although biomass in most treated lakes increased through the course of the study. Within the second year of treatment native plant biomass increased noticeably in Blueberry, Crookneck and Rush, while in year four, native plants in Clear Lake declined appreciably (Table 5). All three untreated lakes showed similar variation in average native biomass throughout the study period (Table 5). The most notable change in native plant biomass occurred in four of the study lakes in the last year of treatment: in one lake (Lower Mission) average native biomass increased by as much as 75%, while in three others (Silver, Julia, Weaver) biomass increased by 200-900% compared to the previous year (Table 5). Although some lakes experienced large biomass increases in the fourth year of treatment, most of these changes are due to a single taxon in each lake.

Ceratophyllum demersum

C. demersum was present every year in all study lakes, both treated and untreated. There was no significant change in *C. demersum* over the course of the study in treated lakes, although a decrease in average frequency was observed between each year (Figure 6). Compared to the first year, the fourth year of treatment produced much lower *C. demersum* frequencies, although this change was not significant. Individual lakes varied in the frequency of *C. demersum* present throughout each year. Silver and Weaver Lakes were the only lakes that had appreciable decreases in *C. demersum* in 2008 and 2007 respectively. In these years, herbicides other than endothall were used (Table 2). In untreated lakes, there was no overall pattern of increase or decrease of *C. demersum* throughout the study and little difference compared to treated lakes (P=0.45 to 0.80).

Treated lakes showed no patterns in average *C. demersum* biomass between years (P=0.25 to 0.83), ranging from as little as 55 ± 30 g/m^2 in year 4 to as much as 112 ± 48 g/m^2 in year three. Although *C. demersum* was present throughout the study period in every lake, biomass in individual lakes varied between years. Weaver Lake experienced high *C. demersum* biomass in year four of treatment (224 g/m^2), while prior to year four, *C. demersum* biomass was low (1 to 9 g/m^2). We observed similar variation in untreated lakes between years, and differences in average *C. demersum* biomass between treated and untreated lakes in every year of the study

Elodea canadensis

Elodea canadensis was found in eight of the ten treatment lakes and two of the three untreated lakes. There was no observable pattern in *E. canadensis* frequency for treated (P= 0.66 to 0.87) or untreated lakes (P=0.24 to 0.77) between years (Figure 6) and little difference between treated and untreated lakes in each year of study (P= 0.26 to 0.95). Despite the lack of significant changes in lakes as a group, four lakes had large differences in *E. canadensis* frequencies between years. In two lakes (Blueberry and Rush) frequencies of *E. canadensis* were observed to triple between the first and second year of treatment (Chi-squared; P< 0.001). In Weaver, *E. canadensis* was present in 17% of the littoral zone in year four, while in other years, *E. canadensis* was not found in more than 2% of the littoral zone (P<0.001). In contrast to the increases observed in some lakes, *E. canadensis* in Julia decreased significantly by a factor of four between the first and fourth year of study (P< 0.001).

E. canadensis biomass also varied across the four years of study. There was no pattern of change across each year in both treated (P=0.36 to 0.63) and untreated lakes (P= 0.33 to 0.67) and between both treated and untreated lakes (P= 0.40 to 0.89) (Figure 7). In individual lakes, *E. canadensis* biomass, both increased (Blueberry, Coal, Lower Mission, Silver, Weaver) and decreased (Julia, Rush) over the four years of study.

Potamogeton spp.

Due to sparse distribution of several native *Potamogeton* taxa throughout the lakes in our study, we analyzed the frequency and biomass of a combination of broadleaf *Potamogeton* taxa as if they were a single species. Broadleaf *Potamogeton spp.* were found throughout a moderate number of lakes in the study and consisted of the following taxa: *Potamogeton amplifolius, Potamogeton gramineus, Potamogeton illinoensis, Potamogeton nodosus, Potamogeton praelongus* and *Potamogeton richardsonii.* Overall frequencies of *Potamogeton* spp. did not significantly change in treated lakes between any of the years of treatment (P=0.27 to 0.93). Similarly, untreated lakes showed little change throughout any of years of study (P=0.17 to 0.46), however, untreated lakes had much higher frequencies compared to treated lakes (P= 0.06 to 0.09) (Figure 6). The difference between treated and untreated lakes was due to extremely high frequencies of *Potamogeton* spp. in one untreated lake (Coal). Average *Potamogeton* spp. frequency ranged from 12.5% ± 4.5% to 16.2% ± 6.2% in untreated lakes to 2% ± 0.9% to 2.5% ± 1.2% in treated lakes.

Potamogeton spp. biomass in treated lakes responded similar to frequency, as there was high variation between years and no distinguishable pattern throughout the four years (Figure 7). Biomass ranged from 0.2 ± 0.1 g/m^2 to 2.0 ± 1.1 g/m^2. Untreated lakes showed similar patterns of variation, with biomass ranging from 4.8 ± 2.3 g/m^2 to 7.4 ± 5.0 g/m^2. Similar to frequencies of *Potamogeton* spp., biomass of *Potamogeton* spp. was higher in the untreated lakes and was significantly higher in year one (P=0.01), although almost all biomass from any given year in untreated lakes was found in one lake (Coal).

Chara spp.

The native macroalga genus *Chara* was found in every lake with the exception of Vails, an untreated lake. In treated lakes, the average frequency of *Chara* spp. increased between each year of treatment (Figure 6), although none of these increases were significant. The most noticeable increase was between year one and four ($P=0.07$), when the average frequency of *Chara* spp. tripled from 6% ± 3% to 21% ± 6% (Figure 6). Among individual lakes, the largest increase occurred in Silver Lake between year three and four (Chi-Squared $P<0.001$). Most other lakes had marginal increases between years, and showed slight increases of *Chara* spp. frequency over time. Of the two untreated lakes where *Chara* spp. did occur (Coal and Rebecca), frequencies were low and did not increase over the study period. Due to low frequencies and the lack of *Chara* spp. in Vails, we were unable to statistically compare between year differences within untreated lakes. Treated lakes had higher average frequencies of *Chara* spp. than untreated lakes, although comparisons between untreated and treated lakes were only possible in year three ($P=0.07$) and four ($P=0.01$), in which there were significantly higher frequencies in treated lakes.

Average August *Chara* spp. biomass increased dramatically in treated lakes between years (Figure 7), nearly doubling from year one (8 ± 5 g/m^2) to two (21 ± 11 g/m^2) and two to three (43 ± 25 g/m^2) ($P=0.08$, $P=0.59$). There was also a significant increase from year three to four (140 ± 89 g/m^2) in treated lakes ($P=0.02$), where *Chara* spp. increased nearly four fold (Figure 7). Average *Chara* spp. biomass increased in every lake in year four, with the largest contributions from Julia, Lower Mission and Silver Lake. Similarly, over the four consecutive years of treatment, average *Chara* spp.

biomass increased as a proportion of average native biomass (Figure 8). In the untreated lakes, *Chara* spp. biomass was only found in Coal, which did not have *Chara* spp. biomass higher than 1 g/m^2 in any given year. Thus, biomass comparisons between treated and untreated lakes could not be completed.

Discussion

Johnson et al. (2010) found that curlyleaf pondweed was successfully controlled by early season herbicide treatments and that there was some evidence that repeated treatments decreased curlyleaf infestations over time. In our study, we found that consecutive herbicide treatments over the course of four years did not have an overall negative impact on native aquatic plants. Similar native plant response to herbicides have been found in other studies (Getsinger et al., 1997; Madsen et al., 2002; Skogerboe et al., 2008; Skogerboe and Getsinger, 2002). Skogerboe et al. (2008) found that early-season low dose herbicide treatments reduced curlyleaf occurrence and densities while not harming native plants and subsequently increasing the occurrence and biomass of the native plants *E. canadensis* and *C. demersum.* Although we did not see overall increases in native plant occurrence or the abundance of *E. canadensis* and *C. demersum*, there was an overall increase in native plant biomass throughout the four years of herbicide treatment. Similar increases in native biomass were not observed in the reference lakes, suggesting that the removal of curlyleaf pondweed by repeated annual herbicide treatments was beneficial to native plant biomass.

Throughout our study, native plant species richness and diversity did not increase or decrease dramatically in either treated or untreated lakes. These findings lead us to believe that early season herbicide treatments over the course of multiple years did not result in the loss of species within our treated study lakes. Although it may be difficult to quantify the presence of more obscure or rare species, we did not witness the loss of a single species throughout the course of our study. Likewise, untreated reference lakes did not decline in species richness over the course of study, suggesting that the number of species present was not affected by the amount of curlyleaf present, at least over the four years of our study.

We did not note an increase in native plant taxa per point in treated lakes across August surveys (the time of peak native plant abundance). The number of plants found per point was not expected to decline throughout surveys, as removal of monotypic curlyleaf stands by herbicide treatment would allow for colonization by native macrophytes. Some aquatic plants are more likely to colonize new habitats faster than other plants due to pressure from seed, propugule or fragment dispersal, overall hardiness and increased fecundity compared to other plants (Barrat-Segretain et al., 1999; Barrett et al., 1993). Rapid growth and colonization by one or two species could result in little change of the number of plants per point. Colonization by multiple species would seem unlikely within a short period of time (Grahn, 1977; Ray et al., 2001). Although increases in native plant diversity were not observed, we did not see a decrease either. Similar to treated lakes, untreated lakes did not show a change in native plants per point throughout our study. These observations seem typical as curlyleaf has long been established in untreated lakes and may not result in further decreases of native plants per point.

Our observations did not indicate that overall native plant frequency was declining due to herbicide treatment or lack of treatment in untreated lakes. Plants in many lakes increased in frequency, while plants in some (Silver and Weaver) decreased in certain years but not others. Rapid increases in native plant frequencies are not likely in many of the eutrophic lakes due to factors other than curlyleaf presence. In many eutrophic lakes (which compose the majority of our study), frequency is dependent on maximum depth of growth. The deeper a plant is capable of growing, the larger its habitable area. In most lakes, maximum depth of growth is dependent on light availability and in lakes with high levels of nutrients, light is limited by phytoplankton abundance (Barko and Smart, 1981; Best et al., 2001). Due to the low clarity in many of our study lakes, we did not expect to see major increases in frequency due to curlyleaf control, but rather increases in biomass.

We noted an overall increase in native plant biomass in treated lakes (Figure 5), which indicated that early season application of herbicides did not hinder native plant growth and decreases in early season curlyleaf abundance (Johnson, 2010) resulted in increased growth of native plants. Untreated lakes did not show any overall increase in native plant biomass, but rather stayed at similar levels. We saw an increase of native plant biomass in the treated lakes throughout the course of our study, with the exception of Clear and Long Lakes (Table 5). Despite decreased plant biomass in these two lakes, biomass increased noticeably in most other lakes. We attribute these increases of native plant biomass to decreases in curlyleaf abundance throughout consecutive years of treatment (Johnson, 2010). Although the increases in native plant biomass were not significant, we deduced that curlyleaf was unable to achieve dense canopies in treated lakes; consequently, more light was likely available for actively growing native plants during their early stages of growth. Light limitation caused by dense canopies of invasive plants is the most detrimental influence on native plants in lakes with competition between native and non-native macrophytes (Madsen et al., 1991). Through annual consecutive herbicide treatments, native plant biomass was able to increase each year, likely due to both the reduced levels of curlyleaf and the greater success of native plant propagules the previous year. Although overall native plant biomass increased, not all native plants showed the same response to treatments and the increases were generally not rapid or dramatic.

Ceratophyllum demersum

The function of early season treatment is to target curlyleaf when it is actively growing. Because curlyleaf's main competitive advantage over native plants in Minnesota is it's early season growth, early season herbicide treatments were not expected to damage native macrophytes that do not actively grow until later in the season (Skogerboe et al., 2008). Although many native macrophytes in Minnesota do not grow until late spring, when water temperatures are warmer, some macrophytes persist throughout the year (Barko et al., 1982). *Ceratophyllum demersum* persists throughout the year in Minnesota and has the potential to be affected by early season herbicide applications (Spencer and Wetzel, 1993). The average frequency of *C. demersum* decreased in treated lakes in each consecutive year over the course of our study. In untreated lakes, *C. demersum* showed no pattern of change, remaining at similar frequencies throughout all years. Skogreboe and Getsinger (2002) found that *C. demersum* exhibits moderate sensitivity to endothall treatments, although on a lake wide survey, decreases due to endothall treatments may be hard to discern. Overall decreases of *C. demersum* frequency in treated lakes were primarily due to major decreases of the plant in two lakes. Weaver Lake experienced decreases of *C. demersum* in 2007-2008 and Silver Lake experienced decreased frequency in 2008-2009. In both of these lakes, herbicides other than endothall were used. Weaver Lake was treated with fluridone in

2006 and 2007, while Silver Lake was treated with a combination of 2, 4-D and endothall in 2006-2007 and triclopyr in 2008.

C. demersum frequency and biomass decreased in Weaver Lake from 2006 to 2007, but once fluridone treatments were stopped, we saw resurgence in *C. demersum* frequencies from 19% to 47% and biomass from 9.4 ± 7 g/m^2 to 223.4 ± 38.4 g/m^2 in 2008 and 2009 respectively. Valley et al. (2006) found that coontail is susceptible to fluridone and that fluridone treatments can greatly reduce coontail biovolume in some lakes.

Decreases in *C. demersum* frequency were most pronounced in Silver Lake in year three (2008) when triclopyr was used to combat Eurasian watermilfoil (*Myriophyllum spicatum* L.). Prior to 2008, the frequency of *C. demersum* was higher than 90% in Silver Lake. After combined triclopyr and endothall treatments, frequencies were 11% in 2008 and 1% in 2009. *Ceratophyllum demersum* biomass was not sampled prior to 2008, but was <1g/m^2 in 2008 and coontail was not found in biomass samples in 2009. Studies have shown that triclopyr is effective at systemically targeting invasive dicotyledons such as Eurasian watermilfoil; the native *C. demersum* is also a dicot (Sprecher and Stewart, 1995) and has additionally been shown to have moderate sensitivity to endothall (Getsinger et al., 1997; Skogerboe and Getsinger, 2001; Skogerboe et al., 2006). Our results suggest that the combination of triclopyr and endothall treatments in 2008 had a negative impact on *C. demersum* in Silver Lake.

In lakes treated only with endothall, there were slight decreases of *C. demersum* frequency over the course of our study; however, these decreases were very small and not significant. Contrary to frequency, *C. demersum* increased in biomass in most lakes over the course of the study. Increased biomass may be due to the lack of curlyleaf growth in June, although *C. demersum* biomass varied considerably between years (Figure 7).

Elodea canadensis

Elodea canadensis was found in 12 of the 13 study lakes. Overall frequency of *E. canadensis* did not increase or decrease during the four years of our study in either treated or untreated lakes. Increases were observed in several lakes while a decrease was observed in one. We observed a large increase in the frequency of *E. canadensis* in Blueberry Lake between year one and two of treatment. Biomass of *E. canadensis* was also much higher in Blueberry in year two (248.3 ± 36.4 g/m^2) compared to year one (15.5 ± 3.1 g/m^2). Increased frequency and biomass of *E. canadensis* was likely due to lack of curlyleaf, primarily because large mats of *E. canadensis* were found in the same area that curlyleaf was abundant the previous year. Other major increases in *E. canadensis* frequency were found in Rush Lake from 16% in year one of treatment to 26% in year two.

Decreases in *E. canadensis* frequency from year one to year four were found in Julia Lake and were primarily due to competition by the other native macrophytes *Chara* spp. and *Najas guadalupensis* (Spreng.). From 2006 to 2009, *N. guadalupensis* biomass increased linearly each year (7.6 ± 2.2 g/m^2 in 2006 to 99.7 ± 7.7 g/m^2 in 2009), while *E. canadensis* biomass decreased linearly during the same time period (45.4 ± 4.3 g/m^2 in 2006, 3.5 ± 0.9 g/m^2 in 2009). Evidence of native plant dominance shifts were not recorded in any other lakes, although reductions in *E. canadensis* due to fluridone treatments were noted.

In Weaver Lake, *E. canadensis* was absent in 2006 and 2007 as a result of fluridone treatment and found in frequencies of 2 % in 2008 and 17% in 2009 with a biomass of 69.3 ± 19.1 g/m^2 in 2009. Similar to *C. demersum*, *E. canadensis* persists year round (Cavers et al., 2003). We believe that due to the year-round persistence of *E. canadensis,* fluridone treatments in 2006 and 2007 effectively reduced the plant to undetectable levels; these results are consistent with Crowell et al. (2006), where *E. canadensis* was drastically reduced subsequent to fluridone treatments. When endothall treatments were applied in 2008 and 2009 *E. canadensis* increased in frequency and biomass. Skogerboe and Getsinger (2002) found that *E. canadensis* is not sensitive to endothall treatments if applied at low dosages.

In 2008 Silver Lake was treated with triclopyr and endothall and *E. canadensis frequencies* decreased to 12%, whereas in 2007 it was 31%. In 2009, when triclopyr was not used, *E. canadensis* frequencies increased to 30% and biomass increased from 6 ± 1 g/m^2 in 2008 to 17 ± 5 g/m^2 in 2009. However, Sprecher and Stewart (1995) concluded that triclopyr has relatively little effect on *E. canadensis,* which leads us to believe that declines of *E. canadensis* in Silver Lake were likely due to low water clarity in 2008 as a result of severely decreased *C. demersum* levels.

Potamogeton spp.

The low density and occurrence of *Potamogeton* spp. made it difficult to distinguish any changes in treated lakes throughout our study, although we did not see decreases in any native *Potamogeton* taxon. Laboratory studies on *Potamogeton praelongus* (Wulf.), *Potamogeton nodosus* (Poir.) and *Potamogeton illinoensis* (Mur.)

have shown that *Potamogeton* species are highly sensitive to endothall treatments (Skogerboe and Getsinger, 2001; Skogerboe and Getsinger, 2002). Because many native *Potamogeton* taxa were not present during early spring herbicide treatment, we did not see reductions of native *Potamogeton* taxa.

The untreated lake that contained abundant *Potamogeton* species (Coal) did not show any declining trends in biomass or frequency in *Potamogeton* taxa. These results suggest that curlyleaf presence did not cause continued declines in native *Potamogeton* taxa. Water clarity and native plant growth in Coal was very high and not typical of lakes where large curlyleaf invasions occur, which may have allowed *Potamogeton* species to coexist with curlyleaf.

Chara spp.

The frequency of *Chara* spp. increased during the study (Figure 6). Many lakes also had large increases of *Chara* spp. biomass. Unlike other dominant native taxa (*E. canadensis* and *C. demersum*), *Chara* spp. did not decline in frequency or biomass in any of the studied lakes, suggesting that none of the herbicides used had a negative effect on the macrophyte.

Charaphytes are green macroalgae which differ greatly in physiology compared with aquatic angiosperms and sensitivity to the same herbicides is not likely. Herbicide resistance in Charaphytes is thought to be caused by a thick calcium and magnesium coating, which may offer some resistance to the effects of chemicals (Wade, 1990). Furthermore, propagule pressure from the spores of charphytes (called oospores) that are found in sediment in large densities and sprout annually (Bonis and Grillas, 2002), promote the growth of *Chara* spp.. Wade (1990) found that in lakes where herbicides have been used to treat angiosperms, *Chara* spp. oospores were found to be resistant to chemical treatment and as a result, colonized areas previously inhabited by angiosperms. Similar results have been found in Minnesota where increases of *Chara* spp. were observed shortly after fluridone treatments in Lac Lavon (Crowell et al., 2006) and in Lake Minnetonka (Skogerboe and Netherland, 2008) *Chara* spp. doubled in frequency within two years of treatment by the herbicides 2, 4-D, triclopyr and endothall.

In addition to herbicide resistance and the possibility of absence during herbicide application, there are other reasons why *Chara* spp. may be increasing in our study lakes. Some charaphytes have been recorded to grow in areas of low light intensity due to a low compensation point (Casanova and Brock, 1999; Shilla and Dativa, 2008). Charaphytes are also rapid colonizers. Meijer et al. (1999) found that after the biomanipulation of three eutrophic lakes (removal of fishes), charaphytes had colonized 50% of the littoral area within two months. Other lake-wide changes such as increasing habitat (Beltman and Allegrini, 1997) and aquatic pollutant removal (Simons et al., 1994) have been followed by explosive growth of charaphytes.

Despite the quick growth and rapid colonization of new and disturbed habitable area, charaphytes are poor competitors with other macrophytes (Wade, 1990). High densities of other macrophytes limit the growth and germination of charaphytes, whereas low densities of vegetation provide opportunities for *Chara* spp. to quickly establish thick mats (Bonis and Grillas, 2002).

The increases of native biomass in the treated lakes of our study were likely due to significant reductions of curlyleaf pondweed (Johnson, 2010). Through four years of study we witnessed some increases in native plant biomass as a result of decreased curlyleaf frequency and biomass. Despite the increase in native biomass, there were major decreases of some native species in two lakes that were treated with herbicides other than endothall. Early season treatment with herbicides other than endothall may have a negative effect on macrophytes that are present during the period of treatment. Early season herbicide treatments may have the most benefit for macrophyte species that do not actively grow during the time of herbicide application. Macrophytes, such as *Chara* spp., which germinate annually from seeds or propagules are not likely to be harmed from early season herbicide treatments. Early season endothall treatments of curlyleaf pondweed can be effective at restoring native plant communities, but caution should be used when applying these methods to lakes where native plants persist year round.

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Table 1. Study lake characteristics, ecoregion within Minnesota and Division Of Waters identifying number (DOW).

Lake (DOW)	**Trophic† Status**	**Avg. Secchi Depth(m)**	**Ecoregion***	**Size (hectares)**	**% Littoral**	**Maximum Depth (m)**	**Survey Points (≤4.6m)**
Blueberry (80-0034)	E	0.9	NLF	211	100	4.2	397-400
Clear (47-0095)	HE	0.6	WCBP	201	83	5.5	213-225
Coal ♦ (77-0046)	M	2.4	NLF	69	40	8.2	93-101
Crookneck (49-0133)	M	2.9	NLF	74	80	6.7	152-166
Fish (70-0069)	E	1.6	CHF	70	40	8.6	126-128
Long (30-0072)	HE	1.0	CHF	158	100	4.2	407-408
Lower Mission (18-0243)	M	3.8	NLF	292	60	8.5	207-220
Julia (71-0145)	E	0.6	CHF	62	100	4.6	106
Rebecca ♦ (27-0192)	E	1.9	CHF	105	50	9.2	154-159
Rush (71-0147)	E	0.6	CHF	65	100	3.4	112
Silver (62-0001)	E	2.6	CHF	29	99	6.2	147-149
Vails ♦ (73-0151)	E	1.6	CHF	64	80	6.1	150-174
Weaver (27-0117)	E	2.3	CHF	62	50	17.4	110-114

♦ Untreated Reference Lake
* Central Hardwood Forests (CHF), Northern Lakes and Forests (NLF), Western Corn Belt Plains (WCBP)
† Mesotrophic (M), Eutrophic (E), Hypereutrophic (HE)

Table 2. Number of consecutive years lakes were treated, year of treatments and herbicides used (E=Endothall, F=Fluridone, Tri=Triclopyr, and 2,4-D)

Treated Lakes	Years Treated	Year of Treatments	Herbicide Used
Blueberry	1,2	2008, 2009	E
Clear	1,2,3,4	2006, 2007, 2008, 2009	E
Crookneck	1,2,3	2006, 2007, 2008	E
Fish	1,2,3	2006, 2007, 2008	E
Long	1,2,3,4	2006, 2007, 2008, 2009	E
Lower Mission	1,2,3,4	2006, 2007, 2008, 2009	E
Julia	1,2,3,4	2006, 2007, 2008, 2009	E
Rush	1,2,3,4	2006, 2007, 2008, 2009	E
Silver	1,2,3,4	2006, 2007, 2008, 2009	E/2, 4-D (2006,2007), E/Tri(2008), E(2009)
Weaver	1,2,3,4	2006, 2007, 2008, 2009	F(2006,2007), E(2008,2009)

Table 3. Frequency of native plants in August in each study lake along with the average for all treated and for all untreated lakes. Not applicable (N/A) means that the data were unavailable either due to change in lake treatment or because the lake was added after the first study year.

TREATED LAKES	Year 1	Year 2	Year 3	Year 4
Blueberrry	38	53	N/A	N/A
Clear	20	22	23	24
Crookneck	99	98	100	N/A
Fish	76	69	76	N/A
Julia	48	51	50	42
Long	3	7	11	15
Lower Mission	87	88	90	87
Rush	29	50	63	42
Silver	99	95	27	52
Weaver	69	42	51	70
Average	**57**	**58**	**55**	**47**
UNTEATED LAKES				
Coal	85	88	88	86
Rebecca	36	36	46	N/A
Vails	40	30	21	23
Average	**54**	**51**	**52**	**55**

Table 4. Native plant taxa per point and standard error in each study lake in August and the average for all treated and untreated lakes. Not applicable (N/A) means that the data was unavailable either due to change in lake treatment or because the lake was added after the first study year.

TREATED LAKES	Year 1	Year 2	Year 3	Year 4
Blueberrry	0.7 ± 0.1	1.5 ± 0.2	N/A	N/A
Clear	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.7 ± 0.1
Crookneck	2.5 ± 0.2	2.4 ± 0.2	2.5 ± 0.3	N/A
Fish	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	N/A
Julia	0.9 ± 0.2	1 .0 ± 0.2	1.1 ± 0.3	0.8 ± 0.2
Long	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Lower Mission	2.3 ± 0.2	2.2 ± 0.2	2.4 ± 0.2	2.8 ± 0.3
Rush	0.4 ± 0.1	0.8 ± 0.1	1 .0 ± 0.1	0.8 ± 0.1
Silver	2.1 ± 0.4	1.9 ± 0.3	0.4 ± 0.1	0.8 ± 0.2
Weaver	1.5 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	2.1 ± 0.4
Average	**1.2 ± 0.3**	**1.3 ± 0.2**	**1.1 ± 0.3**	**1.2 ± 0.3**
UNTEATED LAKES				
Coal	3.8 ± 0.3	2.9 ± 0.2	3.6 ± 0.3	3.7 ± 0.4
Rebecca	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	N/A
Vails	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Average	**1.5 ± 1.1**	**1.2 ± 0.9**	**1.5 ± 1.0**	**2.0 ± 1.8**

Table 5. Mean biomass in August (dry g/m^2) and standard error of native plants in each study lake and the average biomass of all treated and untreated lakes and associated standard deviation. Not applicable (N/A) means that the data was unavailable either due to change in lake treatment or because the lake was added after the first study year.

TREATED LAKES	Year 1	Year 2	Year 3	Year 4
Blueberrry	150 ± 20	293 ± 159	N/A	N/A
Clear	N/A	N/A	198 ± 30	98 ± 50
Crookneck	371 ± 113	650 ± 114	630 ± 95	N/A
Fish	370 ± 91	209 ± 57	645 ± 160	N/A
Julia	43 ± 13	101 ± 32	263 ± 91	794 ± 302
Long	N/A	N/A	59 ± 20	33 ± 18
Lower Mission	111 ± 27	179 ± 51	185 ± 50	327 ± 125
Rush	1 ± 1	60 ± 36	32 ± 17	21 ± 9
Silver	N/A	N/A	33 ± 20	272 ± 165
Weaver	38 ± 15	49 ± 29	38 ± 13	388 ± 146
Average	**155 ± 59**	**220 ± 79**	**231 ± 82**	**276 ± 102**
UNTEATED LAKES				
Coal	336 ± 64	410 ± 52	266 ± 40	247 ± 49
Rebecca	44 ± 23	64 ± 18	128 ± 53	N/A
Vails	21 ± 9	1 ± 1	2 ± 1	57 ± 2
Average	**133 ± 101**	**158 ± 127**	**132 ± 76**	**152 ± 95**

Figure 1. Location of Minnesota study lakes. Treated Lakes represented by black print, untreated-reference lakes in grey print.

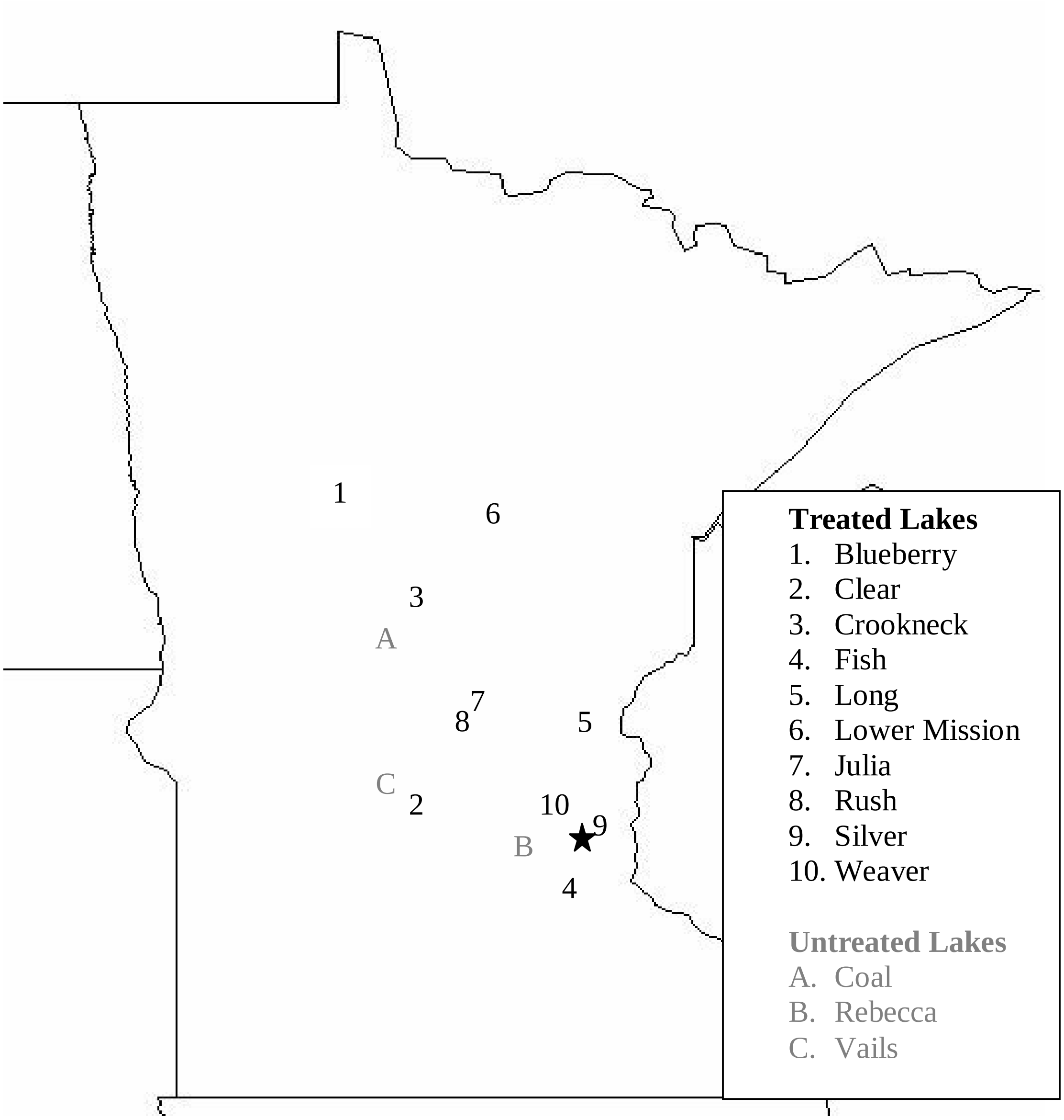


Figure 2. Average littoral (≤ 4.6 m) frequency of native plants in August throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines). Error bars represent one standard error of the mean. P-values for comparisons of frequency between reference and treated lakes each year (Welch 2 sample t-test) are reported along the top margin.

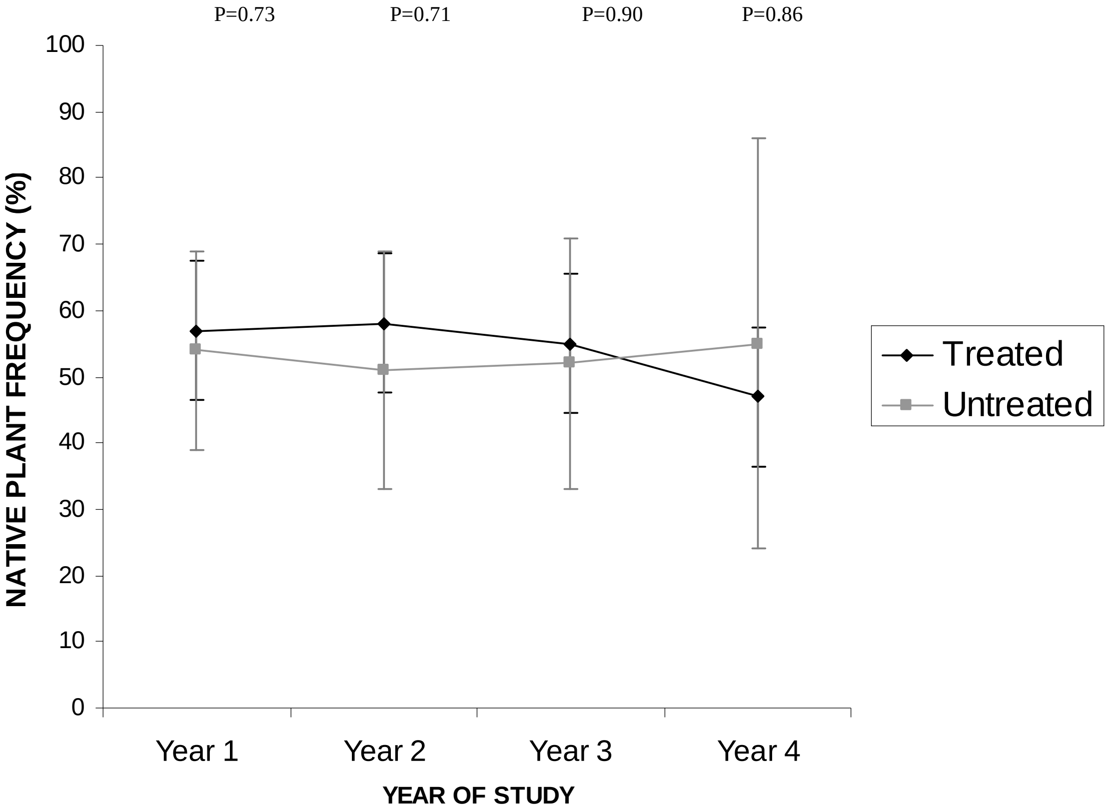


Figure 3. Average native plant species richness in August throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines). Error bars represent one standard error of the mean. P-values for comparisons of species richness between reference and treated lakes each year (Welch 2 sample t-test) are reported along the top margin.

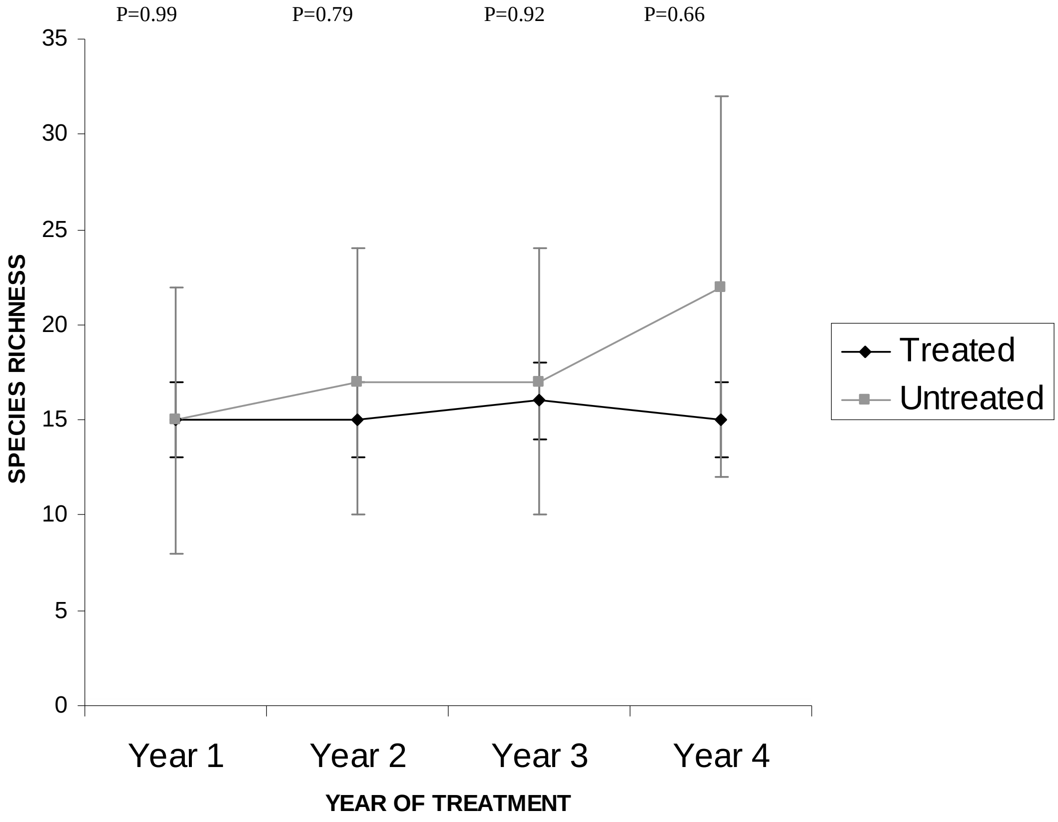


Figure 4. Average number of native plant taxa per point in August throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines). Error bars represent one standard error of the mean. P-values for comparisons of plants per point between reference and treated lakes each year (Welch 2 sample t-test) are reported along the top margin.

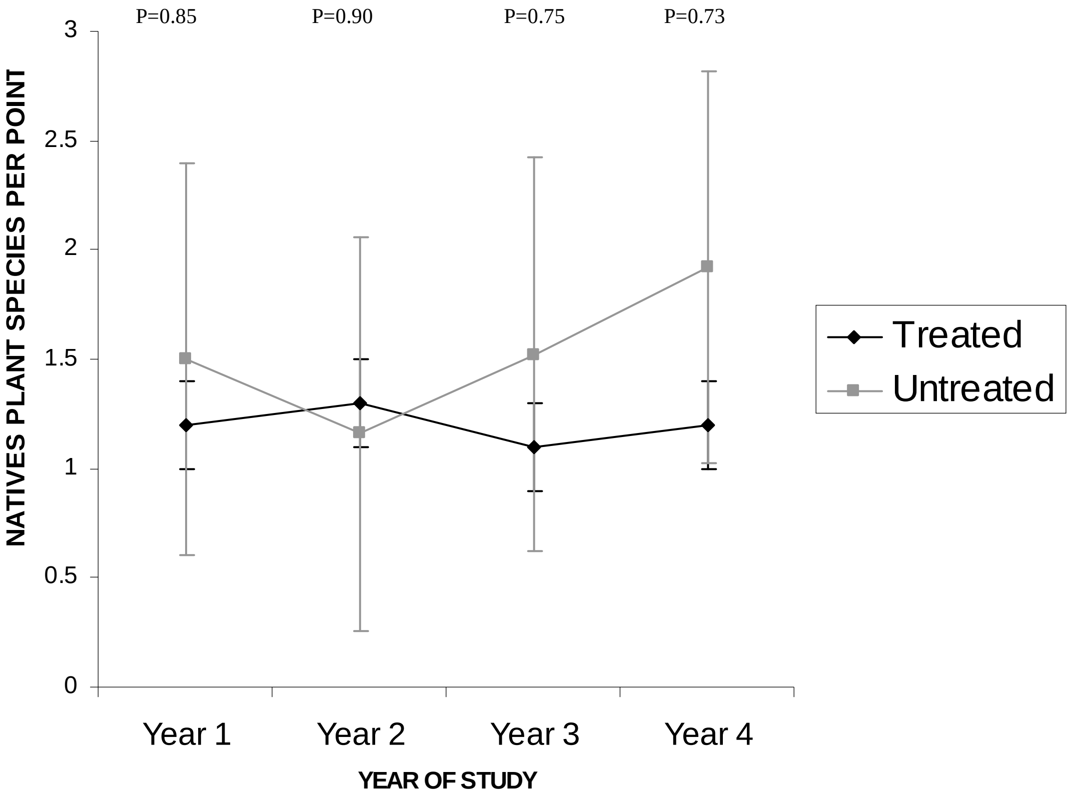


Figure 5. Average August biomass of native plants throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines). Error bars represent one standard error of the mean. P-values for comparisons of biomass between reference and treated lakes each year (Wilcoxn rank-sum test) are reported along the top margin.

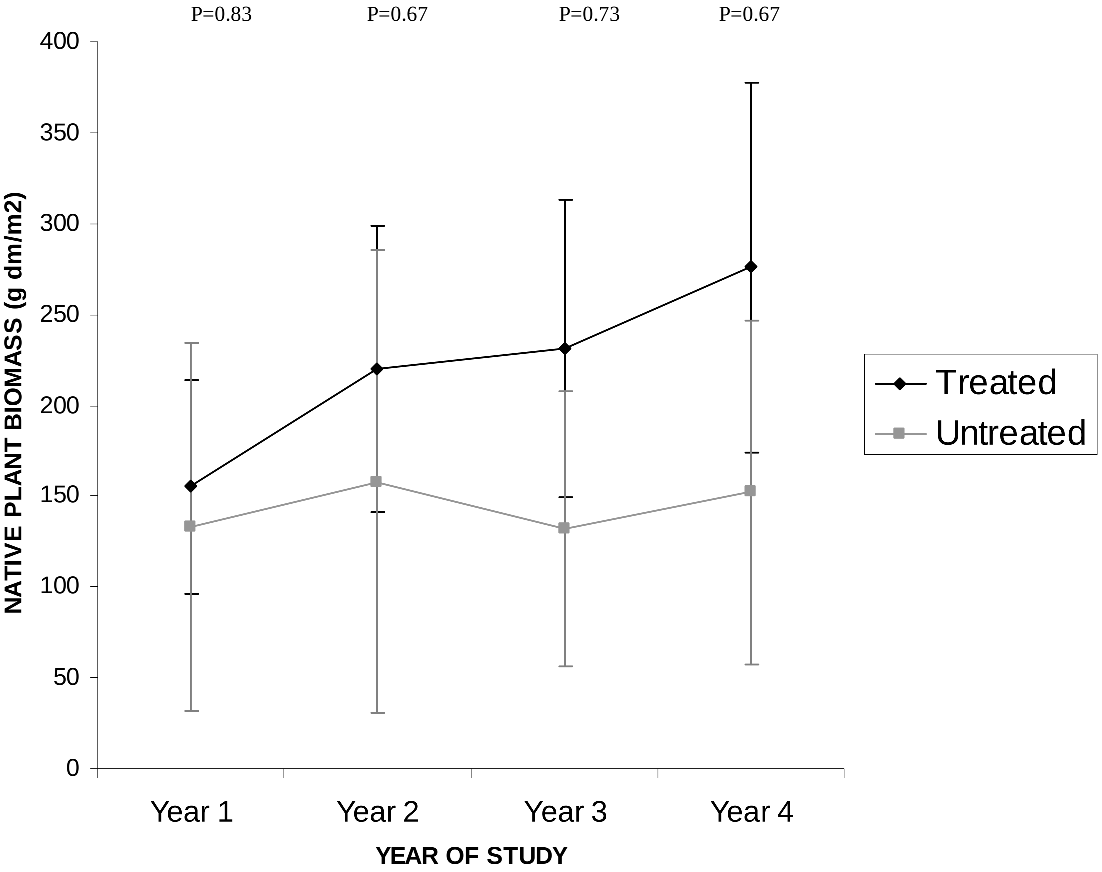


Figure 6. Average littoral (≤ 4.6 m) frequency of *Potamogeton* spp., *Chara* spp., *Ceratophyllum demersum* and *Elodea canadensis* in August throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines).

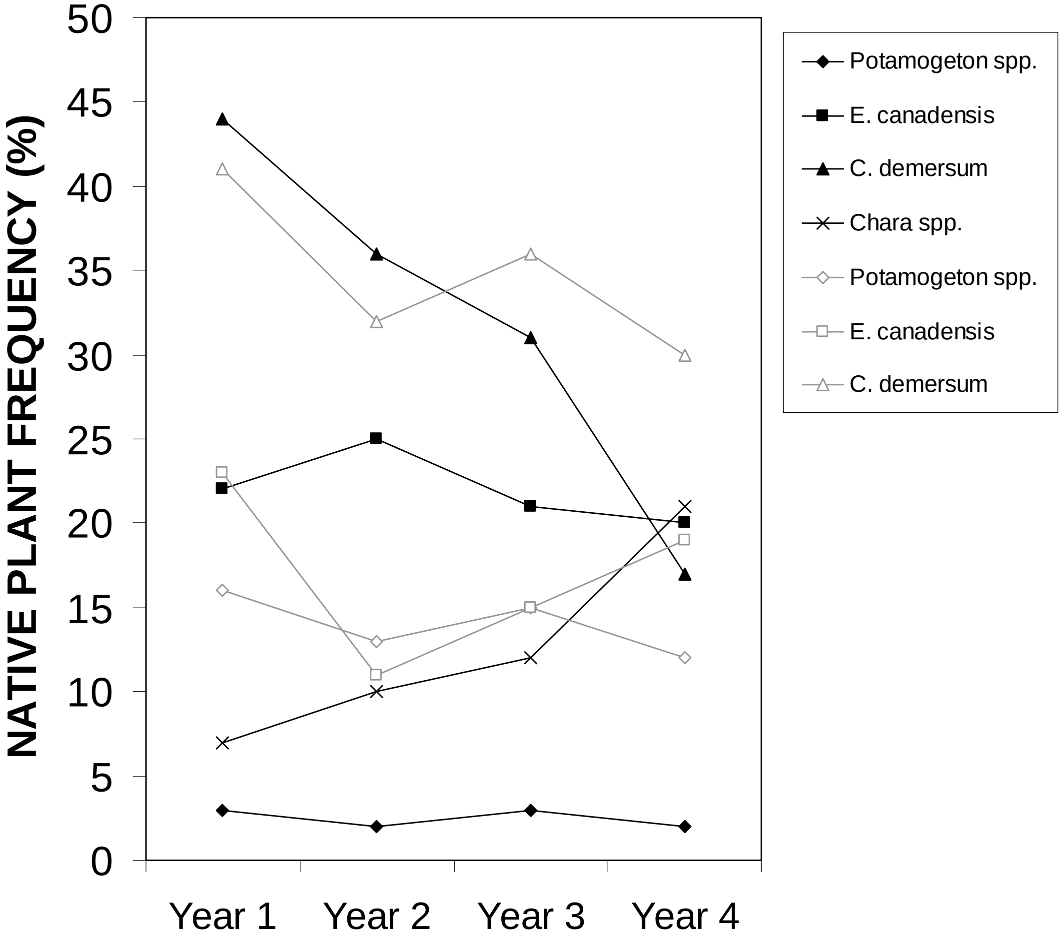


Figure 7. Average biomass of *Potamogeton* spp., *Chara* spp., *Ceratophyllum demersum* and *Elodea canadensis* in August throughout the four years of study in treated lakes (black lines) and untreated lakes (grey lines).

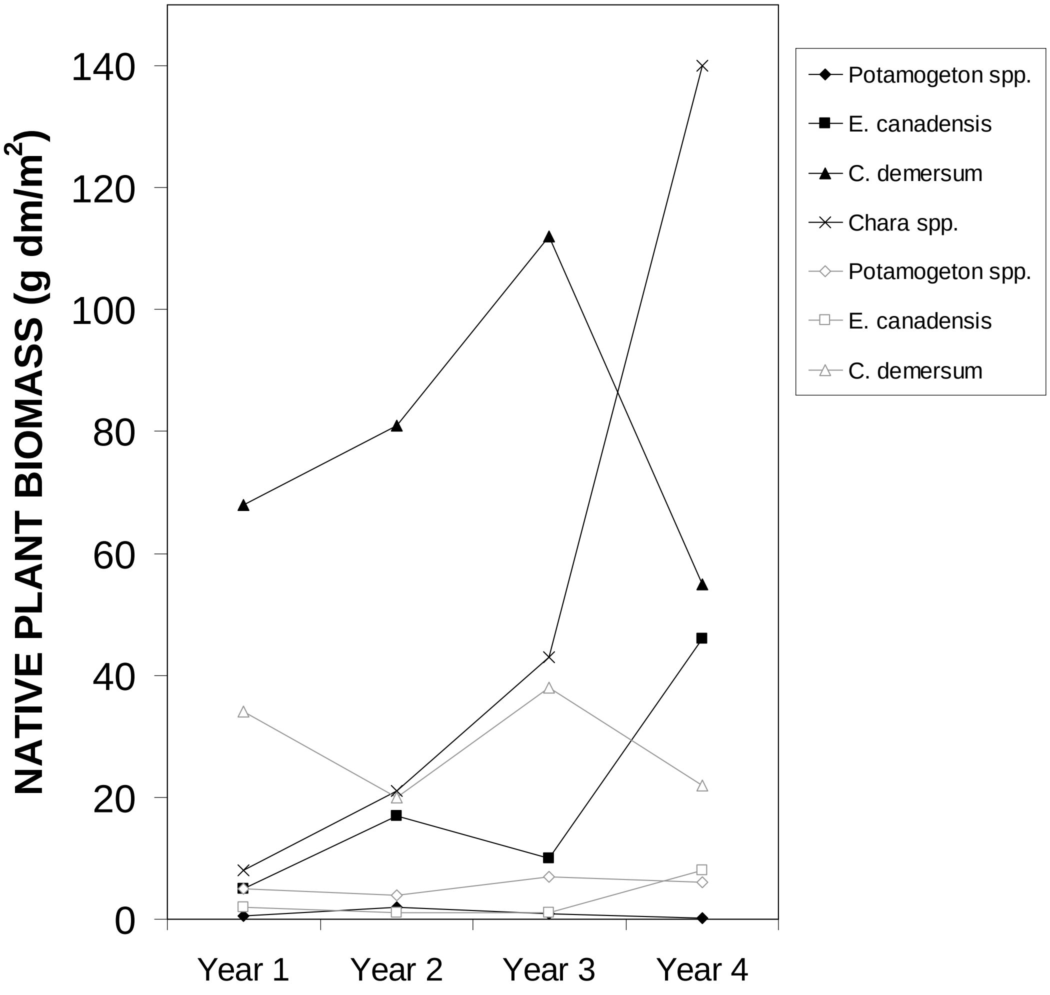
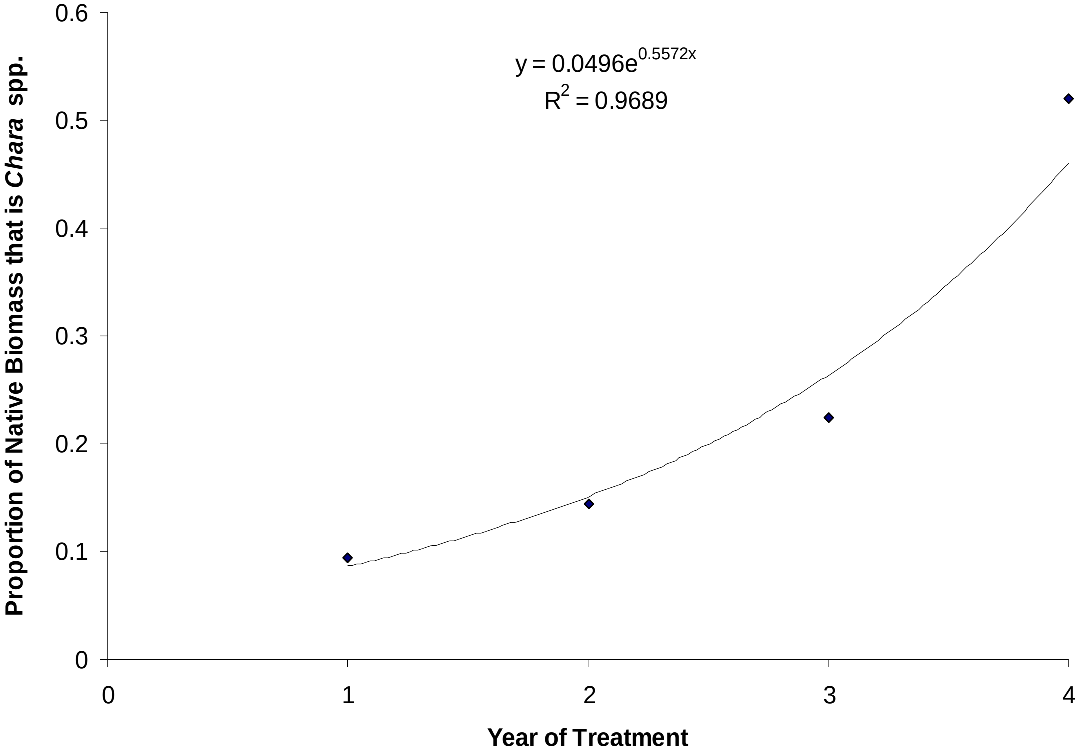


Figure 8. Proportion of mean total August biomass that is *Chara* spp. throughout four years of herbicide treatment.



Chapter 4

Concluding Remarks

Invasive species are becoming more and more of a concern, primarily because of climate change and from increased transcontinental travel (Maki and Galatowitsch, 2004). Exotic macrophytes have the potential to invade aquatic habitat and out-compete native macrophytes, resulting in damage to other facets of lake ecosystems as well (Valley et al. 2004). As the number of invasive species increase, so does the need for safe and effective control mechanisms. Goals for invasive species management often include rehabilitation of native species as well as noticeable reductions in non-native infestations (Madsen et al. 2002). Likewise, it is important to find control methods that not only reduce macrophyte invasions in an efficient way (both conventionally and financially), but that also do not harm native species. Our study has shown that early season, low-dose, endothall treatments of curlyleaf pondweed did not have negative impacts on overall native plant communities over the course of four years. Furthermore, some native macrophyte species responded favorably to repeated herbicide treatments.

In chapter 2, seasonal patterns of macrophyte growth were analyzed. We found little difference between herbicide-treated and untreated lakes in the timing of frequency and biomass of overall native plants. These results suggest that native plants do not show major inhibition by curlyleaf or herbicide treatments throughout a growing season, although we do not have results from lakes that are both absent of curlyleaf and herbicide treatments. Positive changes in native plant growth following herbicide treatments may only be seen when comparing between years and significant increases of native plants may be more likely to be seen over multiple years of herbicide treatment as we discussed in chapter 3. We also saw that throughout the growing season, species richness increased in both treated and untreated lakes. Increases in species richness and plants per point can best be explained by our analysis of single plant taxa. While some plants are present year-round, others sprout in spring when light intensity is high and some require higher temperature along with light to sprout. These phenologic factors likely result in a more comprehensive community of macrophytes later in the growing season.

In both treated and untreated lakes, biomass increased from May to June and June to August, but more so from June to August, likely because of the longer duration between surveys. Conversely, frequency typically increased from May to June, but not from June to August. The increases from May to June are likely due to light availability and the recruitment ability of native plants. Water clarity and thus light penetration, declines later in the native plant growing season as phytoplankton becomes more abundant, preventing increased colonization of aquatic plants (Sommer, 1985; Wehr and Sheath, 2003)

In chapter 3, we evaluated changes in native plant communities over four years of study comparing herbicide treated and untreated lakes. The diversity of native plants did not decrease nor increase over the four years of our study. Overall, average native plants per point remained relatively unchanged from 2006 to 2009 in both treated and untreated lakes. Similarly, the species richness of native plants did not change in treated or untreated lakes. Overall, the decline in curlyleaf in each year (Johnson 2010) did not allow a diverse array of plant species to colonize new areas, or to become more diverse in previous inhabited areas. We attribute this lack of increase in native plant diversity to increases of a select few native plant species that may be more assertive when colonizing new habitat; therefore, new habitable areas would not be expected to have a diverse array of native plant species but more likely a few rapid colonizers. Despite this conclusion, the occurrence of all native plants did not increase, although some particular species did.

Overall frequency of native plants did not increase in treated lakes and did not decrease in untreated lakes; thus we have little evidence that the control of curlyleaf will increase native plant frequency as a whole. However, we also did not see decreases in native plant occurrence in lakes treated with only endothall. In Silver Lake, we saw large decreases in 2008 due to the application of triclopyr and endothall, but once endothall only treatments were used, we saw resurgence in native plant frequency. Increases in native plant frequency were also observed in Weaver Lake when fluridone treatments were discontinued and endothall treatments applied in 2008. Although increases in Silver and Weaver were observed, these increases were due to a few plant species that were sensitive to the particular herbicides used prior to switching to endothall. *Elodea canadensis* increased in Silver Lake, due to the near complete eradication of the previously dominant *Ceratophyllum demersum* after the application of triclopyr in 2008. In Weaver Lake, *E. canadensis* was observed to increase after fluridone treatments were discontinued in 2008, likely due to its sensitivity to fluridone. Our findings suggest that fluridone and triclopyr, or a combination of triclopyr with endothall can have detrimental effects on native plant communities, as well as curlyleaf. Although we did not see large increases or decreases in overall native plant frequencies in endothall-only treated lakes, we did see a large increasing trend in the frequency of *Chara* spp., as frequency increased within each year of treatment. Increases in *Chara* spp. were likely due to reductions of curlyleaf, resulting in more habitable area for native plant growth, as well as *Chara's* resistance to herbicides and the rapid colonization patterns characteristic of charaphytes.

Unlike frequency, we saw a consistent pattern of increase in native plant biomass over the four years of our study, with the greatest increase coming in the fourth year of treatment. In untreated lakes, we did not see the same increasing pattern of biomass, although we did not observe any decreasing patterns either. Increases of native plant biomass suggest that the reductions in curlyleaf in each year of treatment had a positive effect on overall biomass. This was likely due to decreased competition with curlyleaf for space and increased light availability due to the lack of curlyleaf canopies during the early growing season. With each annual decrease in curlyleaf, native plants have the ability to grow more densely and propagate in greater numbers.

Although overall native plant biomass increased in each year of the study, this increase was largely due to increases of *Chara* spp., which increased significantly from 8 ± 5 g dm/m^2 in the first year of treatment to 140 ± 89 g dm/m^2 in the last year of treatment. Likewise, the proportion of total biomass consisting of *Chara* spp. increased from about 10% in the first year of treatment to around 50% in the final year of treatment. Evidence for increasing patterns of other native plant species are lacking and variable between years. These findings suggest that early season herbicide treatments have little effect on the biomass of most plants, but can result in large increases of *Chara* spp. biomass.

This thesis has provided a clear understanding of the response of native macrophyte communities to early season herbicide treatments for curlyleaf pondweed. Through our study we have concluded that there is no overall major negative consequence on native Minnesota macrophytes when using low-dose early-season endothall treatments to control curlyleaf pondweed. We have also determined that only using endothall to treat curlyleaf pondweed does not have a major negative impact on any particular species of native macrophyte but other herbicides prove to be more risky. Furthermore, there is little difference between seasonal changes of native macrophytes in lakes that contain curlyleaf pondweed and lakes that are treated with herbicide for curlyleaf pondweed. Thus, early season lake-wide herbicide treatment of curlyleaf pondweed with endothall may be an effective management tool to control curlyleaf pondweed and maintain native plants.

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