EVALUATION OF LAKE-WIDE, EARLY-SEASON HERBICIDE TREATMENTS FOR CONTROLLING INVASIVE CURLYLEAF PONDWEED (*POTAMOGETON CRISPUS*) IN MINNESOTA LAKES

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DEDICATION

To my wife, Chris, and sons, Aidan and Alec,

for their steadfast support and patience over the last four years

COMPREHENSIVE ABSTRACT

Submersed aquatic plants play an important role in freshwater systems, affecting nutrient dynamics, trophic interactions, biological assemblages, and fish productivity. However, waters infested with non-native invasive aquatic plants often experience severe impairment of ecological and recreational quality due to excessive plant growth. Curlyleaf pondweed (*Potamogeton crispus* L.), one such exotic submersed aquatic plant, has become a widespread nuisance in temperate regions of North America. Curlyleaf's early-season growth, propensity to form dense surface mats, and ability to out-compete native aquatic plants allow it to degrade the ecological and recreational quality of lakes. Consequently, there has been a great deal of interest in adopting lake-wide management strategies that can reduce the negative impacts of curlyleaf and provide some degree of long-term control.

We collaborated with the Minnesota Department of Natural Resources in 2006, 2007, and 2008 to evaluate lake-wide, early-season herbicide treatments for curlyleaf management. Six curlyleaf-infested lakes were treated with herbicide (endothall or fluridone) for at least three consecutive years. Three additional lakes with established curlyleaf infestations were selected to serve as untreated reference lakes during the same period. For all study lakes, we annually assessed the frequency and biomass of curlyleaf in May and June, documented the production of new curlyleaf turions (reproductive buds) on standing plants, and tracked changes in the abundance and viability of turions in lake sediments.

Previous studies have shown that biomass is a key metric for evaluating aquatic plant management projects. We used a boat-based rake method for collecting biomass samples rather than the standard diver (SCUBA) quadrat method because it allowed us to collect a greater number of samples in each lake and provided a higher degree of safety than the diver quadrat method. However, this boat-based rake method had not been thoroughly evaluated to determine whether it produced biomass estimates that were comparable to the diver quadrat method. Consequently, we conducted a separate study to compare the vertical rake sampling method to the diver quadrat method. Results of this study showed that biomass estimates from rake samples were comparable to diver quadrat samples for most individual plant taxa. However, the rake method produced substantially higher estimates than the quadrat method when sampling in dense stands of aquatic plants, particularly in areas dominated by coontail (*Ceratophyllum demersum* L.). Although rake estimates of plant biomass were significantly less precise than quadrat estimates, we determined that the rake method allowed us to collect a sufficiently greater number of samples to offset the method's lower precision. Consequently, we concluded that the biomass data we collected were precise enough to make meaningful relative comparisons in our study lakes.

After three to four consecutive years of herbicide treatment, curlyleaf frequency, biomass, turion production, and sediment turion abundance were all reduced and were all significantly lower in treated lakes than in untreated reference lakes. However, viable turions remained in lake sediments after three consecutive years of treatment. These results suggest that serial lake-wide, early-season herbicide treatments can effectively decrease the negative impacts of curlyleaf infestation and reduce the abundance of curlyleaf turions in lake sediments, but ongoing management will likely be required to maintain long-term control of curlyleaf in infested lakes.

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CHAPTER 1

THESIS OVERVIEW

Background

Submersed aquatic plants play an important role in freshwater systems, affecting nutrient dynamics, trophic interactions, biological assemblages, and fish productivity (Jeppeson et al. 2008; Scheffer 2004). However, waters infested with non-native invasive aquatic plants often experience severe impairment of ecological and recreational quality due to excessive plant growth. Curlyleaf pondweed (*Potamogeton crispus* L.) is one such exotic submersed aquatic plant that has become a widespread nuisance in temperate regions of North America (Bolduan et al. 1994; Catling and Dobson 1985). It typically forms dense surface-matted growth that interferes with recreational water use and often displaces native vegetation (Bolduan et al. 1994; Catling and Dobson 1985; Wehrmeister and Stuckey 1992).

In the past, management of curlyleaf has generally been limited to the use of mechanical harvesting and localized herbicide treatments to reduce areas of nuisance curlyleaf growth. These strategies provided effective short-term control of curlyleaf in localized areas, but were rarely applied on a lake-wide scale because native plants would also be negatively impacted by such activities. Furthermore, these strategies were usually implemented in the later spring and early summer after curlyleaf had already formed nuisance surface matted growth and had already begun to produce reproductive structures. Consequently, these curlyleaf management projects generally resulted in short-term, localized control immediately after treatment or harvest, but did not reduce the overall level of curlyleaf infestation or the need for intensive management in subsequent years. However, several studies have suggested that large-scale selective control of curlyleaf growth and reproduction may be feasible with early-season, lake-wide, low-dose herbicide applications (Netherland et al. 2000; Poovey et al. 2002). As a result, there has been increasing pressure on regulating agencies, such as the Minnesota Department of Natural Resources (MNDNR), to allow such treatments as a standard management practice. However, managers and regulators needed to gain a better understanding of the short-term and long-term impacts of such treatments before permitting their widespread use.

We collaborated with the MNDNR in 2006, 2007, and 2008 to evaluate the effectiveness of early-season, lake-wide, low-dose herbicide treatments for managing curlyleaf in Minnesota lakes (Chapter 3). To assess curlyleaf growth in our study lakes, we adopted sampling methodologies used previously by the MNDNR: point-intercept surveys to assess curlyleaf frequency of occurrence, sediment sampling to measure annual curlyleaf reproduction, and a boat-based vertical rake sampling method to collect curlyleaf biomass samples. The vertical rake biomass sampling method allowed for rapid sample collection throughout the vegetated area of each study lake while avoiding the inherent safety concerns, equipment maintenance needs, and formal training associated with SCUBA sampling. However, this boat-based rake method had not been thoroughly evaluated to determine whether it produced biomass estimates that were comparable to the diver quadrat method. Consequently, we conducted a separate study to compare the two biomass sampling methods (Chapter 2).

Organization of Thesis

This thesis focuses on two manuscripts (Chapters 2 and 3) to be submitted to appropriate journals for publication. After further editing, I will submit these manuscripts to appropriate journals with Raymond M. Newman as a co-author on the manuscript presented in Chapter 2, and Raymond M. Newman and Ajay R. Jones as co-authors on the manuscript presented in Chapter 3. However, I wrote this entire thesis document and conducted all of the research and analyses described herein.

In Chapter 2, I present a comparison study to evaluate the boat-based vertical rake method we used to assess plant biomass in our study lakes, and compare its performance to the diver (SCUBA) quadrat sampling method. For this study, we compared three aspects of these two sampling methods: detection rates for individual taxa, comparability of biomass estimates, and precision of biomass estimates. The two sampling methods detected most individual plant taxa at comparable rates. Similarly, rake and quadrat biomass estimates were relatively comparable in magnitude and precision for most individual plant taxa. However, rake estimates of coontail and flat-stemmed pondweed biomass were significantly higher than quadrat estimates. Our samples were dominated by coontail, so these sampling differences were also reflected in estimates of total biomass (all taxa combined). Although the results of this comparison suggest that the rake method is not a perfect surrogate for the quadrat method, particularly in systems dominated by dense coontail growth, the increased sample collection afforded by the vertical rake method would be expected to offset the method’s inherently lower precision in most studies. In addition, the rake method may allow for improved detection of rare taxa, and greater geographical coverage. Consequently, we concluded that it should be considered an acceptable alternative to the diver quadrat method, particularly for large-scale studies requiring high sampling intensity.

In Chapter 3, I present a detailed overview of my evaluation of early-season, lake-wide herbicide applications for controlling curlyleaf pondweed (*Potamogeton crispus* L.) in Minnesota lakes over 3 years. The specific objectives of this project were to determine: i) if early-season, low-dose herbicide treatments reduce curlyleaf frequency and biomass within the year of treatment; ii) if curlyleaf frequency and biomass decrease from year to year with consecutive annual treatments; iii) the degree to which the production of new curlyleaf turions is reduced by treatment; and iv) the degree to which the abundance and viability of turions in sediments are reduced by multiple consecutive years of treatment.

For this project, I monitored nine curlyleaf-infested lakes over a period of three consecutive years. During this time, six of these lakes received repeated annual herbicide treatments and three lakes served as untreated reference lakes. For all study lakes, we annually assessed the frequency and biomass of curlyleaf in May and June, documented the production of new curlyleaf turions (reproductive buds) on standing plants, and tracked changes in the abundance and viability of turions in lake sediments. We also assessed the effects on native plants with sampling in May, June and August, but do not present the results in this thesis. The response of native plants will be covered in an MS thesis to be completed by Ajay R. Jones. After three to four consecutive years of herbicide treatment, curlyleaf frequency, biomass, turion production, and sediment turion abundance were all reduced and were all significantly lower in treated lakes than in untreated reference lakes. However, viable turions remained in lake sediments after three consecutive years of treatment. These results suggest that serial lake-wide, early-season herbicide treatments can effectively decrease the negative impacts of curlyleaf infestation and reduce the abundance of curlyleaf turions in lake sediments, but ongoing management will likely be required to maintain long-term control of curlyleaf in infested lakes (Deschenes and Ludlow 1993).

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CHAPTER 2

A COMPARISON OF TWO METHODS FOR SAMPLING BIOMASS OF AQUATIC PLANTS

Planned submission to the Journal of Aquatic Plant Management in 2010
with Raymond M. Newman as second author

Chapter Summary

We evaluated the performance of a boat-based vertical rake method for sampling aquatic plant biomass to determine whether it was a suitable alternative to diver quadrat sampling. Boat-based methods are generally considered to be safer and require less time per sample than diver quadrat sampling, but may be less accurate and less precise. We compared three aspects of the two sampling methods: detection rates for individual taxa, comparability of biomass estimates, and precision of biomass estimates. Detection rates for the two sampling methods were comparable for all evaluated plant taxa. Similarly, rake and quadrat biomass estimates were comparable in magnitude and precision for most individual plant taxa. However, rake estimates of coontail and flat-stemmed pondweed biomass were significantly higher ($P<0.05$) than quadrat estimates. Linear regression of rake yields against quadrat yields indicated significant positive relationships for all individual taxa evaluated, and generally showed slopes that did not differ significantly from 1. However, rake estimates of total biomass (all taxa combined) were significantly higher and less precise than quadrat estimates in areas with dense plant growth (>200 dry g/m^2). Although our results suggest that the rake method is not a perfect surrogate for the quadrat method, the increased sample collection afforded by the vertical rake method may offset the method’s inherently lower precision. In addition, the rake method may allow for improved detection of rare taxa, and greater geographical coverage. Consequently, it should be considered an acceptable alternative to the diver quadrat method, particularly for large-scale studies requiring high sampling intensity.

Introduction

Submersed aquatic plants play an important role in freshwater systems, affecting nutrient dynamics, trophic interactions, biological assemblages, and fish productivity (Jeppeson et al. 2008; Scheffer 2004). Consequently, aquatic plant communities are often monitored as a part of large-scale lake management and restoration projects. Although plant communities can be evaluated in many different ways (species composition, distribution of plant growth, maximum depth of colonization, etc.), plant abundance is generally a key metric in aquatic plant studies. Plant abundance has been evaluated using measures of biovolume, plant height, and density ratings, but biomass per unit area is the standard measure (Madsen 1993; Nichols 1984; Valley and Drake 2005).

Diver quadrat sampling has been widely accepted as the most accurate and precise method for collecting aquatic plant biomass samples (Downing and Anderson 1985; Madsen 1993; Sheldon and Boylen 1978; Wetzel and Likens 2000). However, boat-based methods that use mechanical dredges, coring devices, or rakes have been adopted by many researchers to address concerns over the safety of diving or to reduce the time and effort required to collect numerous samples (Capers 2000; Crowell et al. 1994; Kenow et al. 2007; McCauley 1975; Rodusky et al. 2005; Schloesser and Manny 1984; Skogerboe et al. 2008).

When designing aquatic vegetation studies, researchers strive to incorporate methods that will provide the most accurate estimates, with the greatest precision, for the least amount of effort (Canfield et al. 1990; Downing and Anderson 1985; Madsen 1993; Nichols 1982). For this reason, diver quadrat sampling has generally been favored in small-scale studies that required high precision from a relatively small number of samples. However, large-scale vegetation studies may achieve better system-wide estimates of plant biomass by using a boat-based method that allows for more rapid collection of many samples, provided that the level of bias and loss of precision relative to the quadrat method are sufficiently small.

Previous evaluations of boat-based dredge samplers (Rodusky et al. 2005; Schloesser and Manny 1984; Sliger et al. 1990; Westlake 1969) and large coring devices (Madsen et al. 2007; McCauley 1975) suggested that these samplers could achieve levels of accuracy and precision that were fairly comparable to the diver quadrat method. However, these boat-based methods generally required cumbersome field equipment and a substantial amount of time and effort per sample (Madsen et al. 2007; McCauley 1975; Schloesser and Manny 1984). Consequently, these mechanical samplers may be suitable alternatives to quadrat sampling in studies where safety concerns far outweigh the need for rapid sample collection, but do not provide sufficient reductions in sampling time to be favored in large-scale studies that require numerous samples.

Alternatively, boat-based rake methods allow for very rapid sample collection with simple equipment, making them attractive for large-scale studies that require the collection of many samples. Consequently, several researchers have recently adopted a vertical rake method for sampling aquatic plant biomass in large-scale vegetation management studies (Crowell and Asmus 2008; Crowell et al. 1994; Skogerboe et al. 2008). For this method, samples are collected by lowering a long-handled rake vertically to the bottom and turning it three times on its axis before retrieving. Biomass is then estimated as the amount of plant material collected from within the circular area sampled by the rotated rake head. This differs from previously used rake methods that collected plant biomass by dragging a rake over a defined sampled area. Although the vertical rake method has been used to conduct over 150 lake-wide surveys of aquatic plant biomass in Minnesota and Wisconsin, little is known about its comparability to diver quadrat sampling. Consequently, the aim of our study was to evaluate the comparability of these two sampling methods across a wide range of plant taxa and growth densities. The specific objectives were to:

1. Compare the ability of the vertical rake method and diver quadrat method to detect the presence of different plant taxa

2. Determine whether the vertical rake method produces biomass estimates that are similar to quadrat estimates for individual taxa and total biomass (all taxa combined)

3. Compare the precision of biomass estimates produced by the two sampling methods for individual taxa and total biomass

Methods

Sample Collection

We selected a total of 38 vegetated sampling sites from four lakes in the Twin Cities metropolitan area of Minnesota. The lakes ranged from 60 to 130 ha surface area and from 6 to 16 m maximum depth, but all of the sampling sites were located in areas between 0.5-m and 2.5-m deep. Sites were selected to provide a range of biomass density and plant diversity. At each site, we placed marker buoys at three points around the front of an anchored boat (starboard, bow, and port) and collected paired samples (1 rake sample and 1 quadrat sample) at each point from opposite sides but within 0.25 m of the marker buoy, resulting in a total of 114 samples for each sampling method.

Rake samples were collected using a 0.33-m wide, 14-tine, single-headed rake attached to a 3-m long pole. We lowered the rake into the water with the handle perpendicular to the water surface until the rake head reached the sediment. We then rotated the rake on its axis three full turns while maintaining contact with the sediment and then retrieved the rake while continuing to turn it slowly to prevent the loss of collected plants. Each sample collected plants from approximately 0.09 m^2 of sediment. Upon retrieval, we placed all of the collected plants into a labeled plastic bag, drained out excess water, and stored the samples on ice while in the field.

Quadrat samples were collected by divers using scuba equipment and a 0.10-m^2 square PVC frame. The quadrat frame was placed on the sediment surface and all plant shoots within the frame were collected manually after breaking stems at the sediment surface. Collected plants were placed into a labeled sealable plastic bag while underwater. In the lab, all collected samples were rinsed to remove sediment and belowground plant structures were removed. Remaining above-ground plant shoots were sorted by taxonomic group (Crow and Hellquist 2000a,b), dewatered in a salad-spinner, and dried at 105°C (Wetzel and Likens 2000) for at least 48 hours prior to weighing. Results were converted to dry mass per square meter (g/m^2).

Data Analysis

To reduce variability due to the inherent patchiness of aquatic plant growth at the sampled scale, we calculated the mean of the triplicate samples collected at each site with each method (site-average yield). These site-average yields (henceforth referred to as "yields") were then log$_{10}$-transformed to reduce skewness and stabilize variance. All statistical analyses were conducted using R statistical software version 2.8.1 (R Development Core Team 2008).

We evaluated whether the rake method detected individual plant taxa with the same frequency as the quadrat method by using McNemar's χ^2 test of symmetry (with a continuity correction) on the presence or absence of each taxon at each site (Agresti 1990; Zar 2010). This test compared the number of sites where only the rake method detected a given taxon to the number of sites where only the quadrat method detected the same taxon, and then tested whether this ratio departed significantly from 1:1. Only taxa with more than five total occurrences were evaluated (17 taxa).

We assessed the overall comparability of the rake and quadrat yields (log$_{10}$-transformed) by calculating Lin's concordance correlation coefficients (Lin 1989, Zar 2010) for both total biomass and individual taxa. Concordance correlation measures the degree to which pairs of observations fall on the line of perfect concordance (y=x), thus incorporating measures of both bias and precision; a concordance correlation coefficient of 1 indicates perfect agreement between measurements. Only taxa that were encountered by both sampling methods at more than five in-common sites were evaluated for concordance (nine taxa).

To further evaluate the comparability of the two methods, we used linear regression on $\log_{10}$-transformed rake yields (y) and quadrat yields (x) of total biomass and individual taxa. Only taxa that were encountered by both sampling methods at more than five in-common sites were evaluated (nine taxa), and regressions for each given taxon only included data from sites where both methods encountered that taxon. Each regression was evaluated to determine whether the slope deviated from 1, and whether the intercept deviated from zero. We also examined normal quantile-quantile plots and residual plots for all regressions to verify assumptions of linearity, normality, and stable variance. In addition, we used mixed-effects models (Pinheiro and Bates 2000) to verify that the nested sample collection in our study (samples clustered within lakes and sites) did not significantly affect the lack of fit or bias the slope of our regression models.

To compare the precision of yields from the two sampling methods, we first calculated the coefficient of variation on non-transformed yields for each of the 12 taxa encountered at more than five sites for each method (Table 1). We then tested for equality of variance between $\log_{10}$-transformed rake and quadrat yields using Levene's test (Levene 1960; Zar 2010). Only taxa that were encountered by both sampling methods at more than five in-common sites were evaluated using Levene's test (nine taxa).

Results

We encountered plant biomass at all 38 sampled sites and identified a total of 29 aquatic plant taxa in our samples. Of these 29 taxa, 17 were encountered at more than five total sites with either sampling method, 12 were encountered at more than five sites for each method, and nine were encountered by both sampling methods together at more than five in-common sites (Table 1).

Results from McNemar's tests indicated that the vertical rake method detected all but one of the 17 evaluated taxa at rates that were similar to the quadrat method (Table 2). The only exception was Eurasian watermilfoil (*Myriophyllum spicatum* L.), which was detected significantly more frequently (*P*=0.04) by the rake method.

Lin's concordance correlation coefficients (CCC) for the nine evaluated taxa ranged from 0.73 to 0.92, with most taxa exhibiting CCC's between 0.82 and 0.85 (Table 3). This indicates that although there was not perfect agreement between rake and quadrat yields, the level of comparability was generally high and consistent across taxa. Northern watermilfoil (*Myriophyllum sibiricum* Komarov) and wild celery (*Vallisneria americana* Michx.) exhibited the highest CCC's (0.92), while coontail (*Ceratophyllum demersum* L.) exhibited the lowest CCC (0.73). However, the CCC for total biomass (0.74) was lower than all of the individual taxa except coontail.

Linear regressions also indicated that vertical rake yields were comparable to quadrat yields for most of the nine evaluated taxa (Figure 1, Table 4). The relationship of rake yield (y) to quadrat yield (x) was significant ($P<0.025$) and positive for all of the evaluated taxa, and explained 61% to 89% of biomass variability. Furthermore, most of the taxa exhibited regression slopes that did not deviate significantly from 1 and intercepts that did not differ significantly from zero (Table 4), indicating that rake and quadrat yields were statistically equivalent for most taxa. However, there were several notable exceptions. Regression intercepts indicated that flat-stemmed pondweed (*Potamogeton zosteriformis* Fern.) and sago pondweed (*Stuckenia pectinata* (L.) Börner) were consistently over-sampled by the rake across the measured range of biomass relative to quadrat estimates. A slope < 1 indicated that water stargrass (*Zosterella dubia* (Jacq.) Small) was under-sampled by the rake, particularly when present at high biomass, and significantly different slopes and intercepts indicated that northern watermilfoil was over-sampled by the rake relative to the quadrat when present at low biomass, but under-sampled by the rake at high biomass (Figure 1, Table 4).

When all plant taxa were considered collectively (total biomass), the vertical rake method consistently produced higher estimates than the quadrat method at biomass densities greater than 200 g/m^2 ($\log_{10}$=2.3). Although the relationship between rake yields (y) and quadrat yields (x) for total biomass was significant (P<0.001), positive, and explained 78% of the variability in biomass (Figure 2, Table 4), the regression slope (1.39) was significantly greater than 1 (P=0.003) and the intercept (-0.66) was significantly less than zero (P=0.026). Closer inspection of the regressions for total biomass and for individual taxa suggested that this over-sampling of total biomass by the rake at high plant density was almost entirely attributable to over-sampling of two taxa, coontail and flat-stemmed pondweed. Coontail was the most commonly encountered taxon in our study and was the largest contributor to total biomass at 53% of sites for rake samples (519 ±138 g/m^2; $\bar{x}$ ± 1SE) and 55% of sites for quadrat samples (134 ± 25 g/m^2). Consequently, the over-sampling of coontail by the rake likely had a substantial effect upon total biomass estimates.

Our evaluations of the precision of rake and quadrat estimates indicated that both methods produced estimates of similar precision for each of the evaluated taxa. The coefficient of variation (CV) for non-transformed rake yields were substantially different than quadrat yield CV's for flat-stemmed pondweed (+61%), coontail (+35%), northern watermilfoil (+29%), and southern water nymph (*Najas guadalupensis*; -40%), but all eight of the other evaluated taxa had rake CV's that were within 20% of the quadrat CV's (Table 5). Despite these differences in rake and quadrat CV's, tests for equality of variance (Levene's test) on $\log_{10}$-transformed biomass estimates indicated that the variance of rake and quadrat yields were statistically similar ($P>0.05$) for each of the evaluated taxa (Table 6). However, rake yields of total biomass were significantly less precise than quadrat yields. The CV for rake total biomass (1.33) was 1.7 times larger than the CV for quadrat total biomass (0.79) (Table 5). Similarly, the variance of $\log_{10}$-transformed rake estimates for total biomass was significantly greater ($P<0.008$; Levene's test) than the variance of $\log_{10}$-transformed quadrat estimates for total biomass (Table 6).

Discussion

Our results indicate that the vertical rake method was very comparable to the diver quadrat method in its ability to detect a wide range of aquatic plant taxa. This finding contrasts with results presented by Capers (2000) and Wood (1963) who reported substantially lower species richness estimates and lower frequency of occurrence for select taxa when using dragged rakes and grapnels relative to diver quadrat sampling. This discrepancy suggests that the vertical rake method we tested was more effective at snagging and retrieving plants than previously used rake and grapnel methods. The vertical rake method's ability to detect taxa may be enhanced due to the multiple passes of the rake head (three rotations) over the defined sample area. These multiple passes may have resulted in more thorough removal of plant shoots than the single pass of a dragged rake or grapnel. However, it should be noted that Capers (2000) and Wood (1963) both reported that grapnels and rakes often failed to snag and retrieve small or firmly-rooted plants, namely *Zannichellia* and *Isoetes*, which were not found at our sites by either method. Consequently, caution should be exercised when using the vertical rake method to assess plant biomass in waters with small, firmly-rooted taxa.

Our study also showed that rake and quadrat estimates of biomass were fairly comparable for individual taxa, but the rake did not sample all taxa equally well. Rake estimates of northern watermilfoil, slender water nymph (*Najas flexilis* Willd.), and wild celery were very similar to quadrat estimates in both magnitude and precision. However, the rake consistently over-sampled flat-stemmed pondweed across the range of sampled growth densities, and severely over-sampled coontail when present at high density. This over-sampling of coontail by the rake was likely due to the coontail's strong intertwining stems and lack of roots, which allowed coontail biomass to be pulled from outside of the intended sample area as the rake was turned.

Rodusky et al. (2005) also reported that different taxa showed differing levels of comparability between rake and quadrat biomass estimates. In particular, they found that relative to quadrat sampling, their rake method (hinged oyster-tong rake) under-sampled dense stands of wild celery and *Potamogeton* species, and over-sampled moderately dense stands of *Hydrilla*. These observed differences in rake and quadrat comparability among taxa suggests that the overall comparability of the two methods for estimating total biomass is dependent upon the dominant taxa present at the monitored locations.

For total biomass estimates (all taxa combined), our results indicated that rake estimates were significantly higher and less precise than quadrat estimates, particularly in samples collected from areas with dense plant growth. Crowell et al. (1994) similarly reported substantially higher estimates of total biomass in rake samples ($\bar{x}$ ± 1SE; 1669±748 g/m^2) than in quadrat samples (799±379 g/m^2), but concluded that the precision of the rake method they tested was sufficiently comparable to the quadrat method (coefficient of variation; rake=116%, quadrat=110%) to allow for meaningful relative comparisons of total biomass. Rodusky et al. (2005) similarly reported that there was little difference between the precision of their rake estimates and quadrat estimates, but unlike Crowell et al. (1994), found no consistent pattern to over-sampling or under-sampling of total biomass by the rake method relative to quadrat samples. Both of these previous studies were based upon a relatively small number of samples collected from nearly monotypic stands of plants, and only compared estimates for a few taxa. However, the reported results further suggest that the vertical rake method may provide substantially more precise estimates of total biomass at sites not dominated by dense coontail growth. Given the substantial differences we observed in rake performance for sampling individual taxa, further evaluation of the vertical rake method should be conducted prior to adoption in systems dominated by plant taxa that were not evaluated in our study.

Although our results indicate that the vertical rake method was not a perfect surrogate for diver quadrat sampling, the rake method would allow for greater sampling frequency and the collection of substantially more samples than the quadrat method without requiring more effort. This would increase the likelihood of detecting rare plant taxa, increase the resolution and geographic coverage of data within the study area, and could offset the rake method's inherently lower precision relative to the diver quadrat method for estimating total biomass.

To estimate the number of rake samples needed to achieve the same level of precision as the quadrat method, we used a standard formula for estimating sample size based upon the anticipated sample variance and mean (Zar 2010).

$$n = \frac{s^2 t^2_{0.05,n-1}}{(E \times \bar{x})^2} \tag{1}$$

n = number of samples
s^2 = anticipated sample variance
t = two-tailed critical value of Student's t
E = acceptable % error (0.2 = 20%)
$\bar{x}$ = anticipated mean biomass

We used the variances and means observed in our samples for individual taxa to estimate the requisite number of rake (n_{rake}) and quadrat ($n_{quadrat}$) samples. We then calculated the ratio of n_{rake}:$n_{quadrat}$ (rake factor) for each taxon (Table 7). Rake factors for individual taxa were quite variable, ranging from 0.4 to 2.6, but had a median value of 1.0. The rake factor for total biomass was 2.8, indicating that roughly three times more rake samples would be needed to achieve the same level of precision for total biomass estimates as for quadrat samples in our study. Although we did not record the time needed to collect samples with each method, rake samples required substantially less than one-third of the time to collect than quadrat samples. This suggests that the vertical rake method could achieve a similar level of precision as the quadrat method while maintaining, or possibly decreasing, the total time required for sampling.

Despite its shortcomings, the vertical rake method provides key advantages over the diver quadrat method: improved safety, increased sampling intensity (better detection of rare taxa and increased geographic coverage), and inexpensive equipment with no special training requirements (SCUBA). Given these advantages and the potential to achieve a similar level of precision through increased sample collection, the vertical rake method should be considered a viable alternative to the quadrat method, particularly for large-scale studies requiring the collection of many samples over expansive areas.

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Table 1. Taxa encountered in samples collected from a total of 38 sites using the vertical rake method and diver quadrat method; *N* is the number of occurrences for each method.

Taxon	*Common Name*	$N_{Quadrat}$	N_{Rake}
Total Biomass (all taxa)	-	38	38
Bidens beckii	Water marigold	3	1
Ceratophyllum demersum [abc]	Coontail	34	31
Chara spp.[abc]	Muskgrass	9	14
Eleocharis acicularis	Needle spikerush	3	1
Elodea canadensis [abc]	Canadian waterweed	11	12
Fontinalis antipyretica	Fontinalis moss	0	1
Lemna minor	Lesser duckweed	2	3
Lemna trisulca [a]	Star duckweed	6	4
Myriophyllum sibiricum [abc]	Northern watermilfoil	25	25
Myriophyllum spicatum [a]	Eurasian watermilfoil	3	9
Najas flexilis [abc]	Slender water nymph	9	8
Najas guadalupensis [ab]	Southern water nymph	10	6
Nuphar variegata	Spatterdock	1	0
Nymphaea odorata	White waterlily	1	2
Potamogeton amplifolius	Large-leaf pondweed	2	2
Potamogeton crispus (sprouts) [ab]	Curlyleaf pondweed	7	6
Potamogeton foliosus	Leafy pondweed	1	1
Potamogeton gramineus [a]	Variable-leaf pondweed	5	7
Potamogeton nodosus	Long-leaf pondweed	1	1
Potamogeton praelongus [ab]	White-stemmed pondweed	7	9
Potamogeton richardsonii [a]	Clasping-leaf pondweed	5	3
Potamogeton robbinsii [a]	Robbins' pondweed	6	2
Potamogeton zosteriformis [abc]	Flat-stemmed pondweed	15	14
Ranunculus longirostris	Longbeak buttercup	1	2
Stuckenia pectinata [abc]	Sago pondweed	15	14
Utricularia vulgaris	Bladderwort	1	1
Vallisneria americana [abc]	Wild celery	18	19
Zannichellia palustris	Horned pondweed	4	1
Zosterella dubia [abc]	Water stargrass	22	24

[a] Taxa that were encountered more than five total times

[b] Taxa that occurred at more than five sites for each sampling method (not necessarily the same sites)

[c] Taxa that occurred in both rake and quadrat samples at more than five in-common sites

Table 2. Comparison of detection rate for individual taxa by quadrat and rake biomass sampling methods; McNemar's χ^2 test for symmetry on presence (+) or absence (-) by sampling method (Q=quadrat, R=rake) at the 38 sample sites. *McNemar's* χ^2 is the test statistic for the test of Q+/R- vs. Q-/R+ (df=1), and *P* is the associated p-value.

Taxon	*Q+/R+*	*Q+/R-*	*Q-/R+*	*Q-/R-*	*McNemar's* χ^2	*P*
Total Biomass (all taxa)	38	0	0	0	0.00	1.00
Ceratophyllum demersum	29	5	2	2	0.57	0.45
Chara spp.	7	2	7	22	1.78	0.18
Elodea canadensis	10	1	2	25	0.00	1.00
Lemna trisulca	2	4	2	30	0.17	0.68
Myriophyllum sibiricum	23	2	2	11	0.00	1.00
Myriophyllum spicatum	3	0	6	29	4.17	0.04
Najas flexilis	6	3	2	27	0.00	1.00
Najas guadalupensis	4	6	2	26	1.13	0.29
Potamogeton crispus	4	3	2	29	0.00	1.00
Potamogeton gramineus	3	2	4	29	0.17	0.68
Potamogeton praelongus	5	2	4	27	0.17	0.68
Potamogeton richardsonii	2	3	1	32	0.25	0.62
Potamogeton robbinsii	1	5	1	31	1.50	0.22
Potamogeton zosteriformis	14	1	0	23	0.00	1.00
Stuckenia pectinata	11	4	3	20	0.00	1.00
Vallisneria americana	16	2	3	17	0.00	1.00
Zosterella dubia	19	3	5	11	0.13	0.72

Table 3. Lin's concordance correlation coefficient (*CCC*) for paired rake and quadrat site average yields of total biomass and individual taxa. Results listed in order of increasing concordance.

Taxon	*CCC*	*95% CI*	*N (pairs)*
Total Biomass (all taxa)	0.74	0.62 – 0.83	38
Ceratophyllum demersum	0.73	0.56 – 0.84	29
Zosterella dubia	0.78	0.51 – 0.91	18
Elodea canadensis	0.82	0.52 – 0.94	10
Potamogeton zosteriformis	0.82	0.61 – 0.92	14
Chara spp.	0.84	0.40 – 0.97	7
Najas flexilis	0.84	0.29 – 0.97	6
Stuckenia pectinata	0.84	0.57 – 0.95	11
Myriophyllum sibiricum	0.92	0.82 – 0.96	22
Vallisneria americana	0.92	0.80 – 0.97	16

Table 4. Regression statistics for models of $\log_{10}$ site-average biomass for quadrat (x) vs. rake (y) samples (Figures 1 and 2). Taxa are listed in order of decreasing regression slope. *T* is the t-test statistic for the regression, *P* is the p-value associated with the regression, $P_{1:1}$ is the p-value associated with the test for slope=1, P_{Int} is the p-value associated with the test for y-intercept=0.

Taxon	*N*	R^2	*P*	*Slope*	$P_{1:1}$	*Intercept*	P_{Int}
Total Biomass (all taxa)	38	0.78	<0.001	1.39	0.003	-0.66	0.026
Ceratophyllum demersum	29	0.70	<0.001	1.21	0.176	-0.12	0.711
Elodea canadensis	10	0.85	<0.001	1.09	0.607	0.24	0.231
Potamogeton zosteriformis	14	0.89	<0.001	1.04	0.708	0.38	0.038
Vallisneria americana	16	0.86	<0.001	1.04	0.715	-0.04	0.814
Najas flexilis	6	0.76	0.024	1.00	0.989	0.11	0.572
Stuckenia pectinata	11	0.83	<0.001	0.88	0.383	0.44	0.049
Myriophyllum sibiricum	22	0.87	<0.001	0.83	0.028	0.27	0.009
Chara spp.	7	0.78	0.008	0.74	0.209	0.10	0.684
Zosterella dubia	18	0.61	<0.001	0.70	0.045	0.24	0.109

Table 5. Comparison of coefficient of variation (standard deviation ÷ mean) for site-averaged biomass estimates yielded by quadrat and rake sampling methods at 38 sites. Only taxa that occurred at more than five sites for each method are listed. *N* = number of sites with occurrence of taxon, $\bar{x}$ is the mean biomass, *CV* = coefficient of variation, and *% Difference* is $[(CV_{rake} - CV_{quadrat}) \div CV_{quadrat}]$.

	Quadrat			Rake			
Taxon	*N*	$\bar{x}$	*CV*	*N*	$\bar{x}$	*CV*	*% Difference*
Total Biomass (all taxa)	38	274	0.79	38	215	1.33	+ 69
Potamogeton zosteriformis	15	49	1.19	14	223	1.91	+ 61
Ceratophyllum demersum	34	142	1.07	31	602	1.44	+ 35
Myriophyllum sibiricum	25	33	1.34	25	33	1.74	+ 29
Zosterella dubia	22	17	2.11	24	17	2.50	+ 18
Vallisneria americana	18	26	1.44	19	30	1.59	+ 11
Elodea canadensis	11	30	2.40	12	74	2.43	+ 1
Potamogeton crispus (sprouts)	7	2	1.66	6	3	1.67	+ 1
Najas flexilis	9	3	1.69	8	5	1.65	- 2
Stuckenia pectinata	15	50	2.20	14	99	2.06	- 6
Potamogeton praelongus	7	56	1.50	9	70	1.27	- 15
Chara spp.	9	32	2.03	14	13	1.64	- 19
Najas guadalupensis	10	2	1.66	6	9	1.00	- 40

Table 6. Results from Levene's test for equality of variance (Levene 1960; Zar 2010) for site-average biomass estimates ($\log_{10}$-transformed) by quadrat and rake methods. Only taxa that were encountered by both sampling methods at more than five in-common sites were evaluated; listed in order of increasing p-value. *W* is Levene's test statistic, *DF* is the degrees of freedom, *P* is the associated p-value.

Taxon	*W*	*DF*	*P*
Total Biomass (all taxa)	7.5	74	0.008
Ceratophyllum demersum	3.3	56	0.074
Myriophyllum sibiricum	0.9	42	0.339
Zosterella dubia	0.8	34	0.381
Elodea canadensis	0.4	18	0.559
Vallisneria americana	0.1	30	0.717
Potamogeton zosteriformis	0.1	26	0.724
Najas flexilis	<0.1	10	0.803
Chara spp.	<0.1	12	0.808
Stuckenia pectinata	<0.1	20	0.946

Table 7. Rake factors ($n_{rake} \div n_{quadrat}$) based upon the requisite number of rake (n_{rake}) and quadrat samples ($n_{quadrat}$) needed to achieve the same level of precision when estimating mean biomass ($\bar{x}$) of individual taxa. Requisite n_{rake} *and* $n_{quadrat}$ calculated using a standard formula for estimating sample size as described by Zar (2010); based upon observed mean ($\bar{x}$) and standard deviation (*s*) of biomass for individual taxa, assuming an acceptable error of 20% of the mean. Note that the listed means and standard deviations were from 38 paired samples collected from several lakes.

	Quadrat			Rake			*Rake Factor*
Taxon	*s*	$\bar{x}$	$n_{quadrat}$	*s*	$\bar{x}$	n_{rake}	($n_{rake} \div n_{quadrat}$)
Total Biomass (all taxa)	216	274	62	1103	215	173	2.8
Potamogeton zosteriformis	58	49	137	426	223	353	2.6
Ceratophyllum demersum	151	142	112	865	602	200	1.8
Myriophyllum sibiricum	45	33	176	58	33	292	1.7
Zosterella dubia	36	17	433	42	17	604	1.4
Vallisneria americana	37	26	202	47	30	247	1.2
Elodea canadensis	71	30	555	181	74	571	1.0
Potamogeton crispus (sprouts)	3	2	267	5	3	271	1.0
Najas flexilis	5	3	275	9	5	264	1.0
Stuckenia pectinata	109	50	466	203	99	411	0.9
Potamogeton praelongus	84	56	217	89	70	157	0.7
Chara spp.	65	32	400	21	13	261	0.7
Najas guadalupensis	4	2	267	9	9	98	0.4

Figure 1. Relationship between rake and quadrat yields (log$_{10}$-transformed) for individual taxa encountered by both sampling methods together at more than five in-common sites (Table 1). Solid lines indicate fitted regressions and dashed lines indicate 1:1 correspondence. Rejected outliers indicated by "+". Regression statistics given in Table 4.

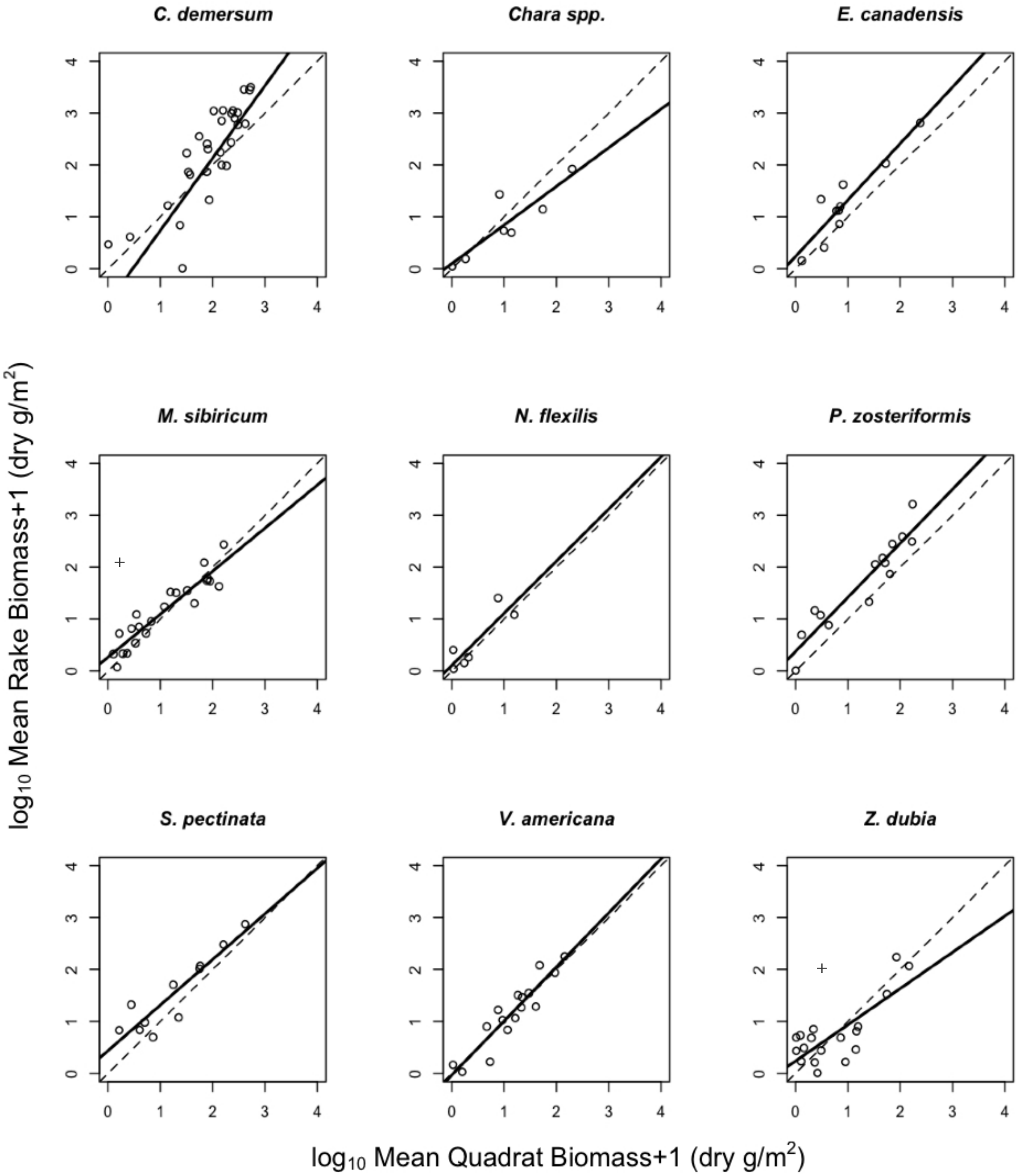
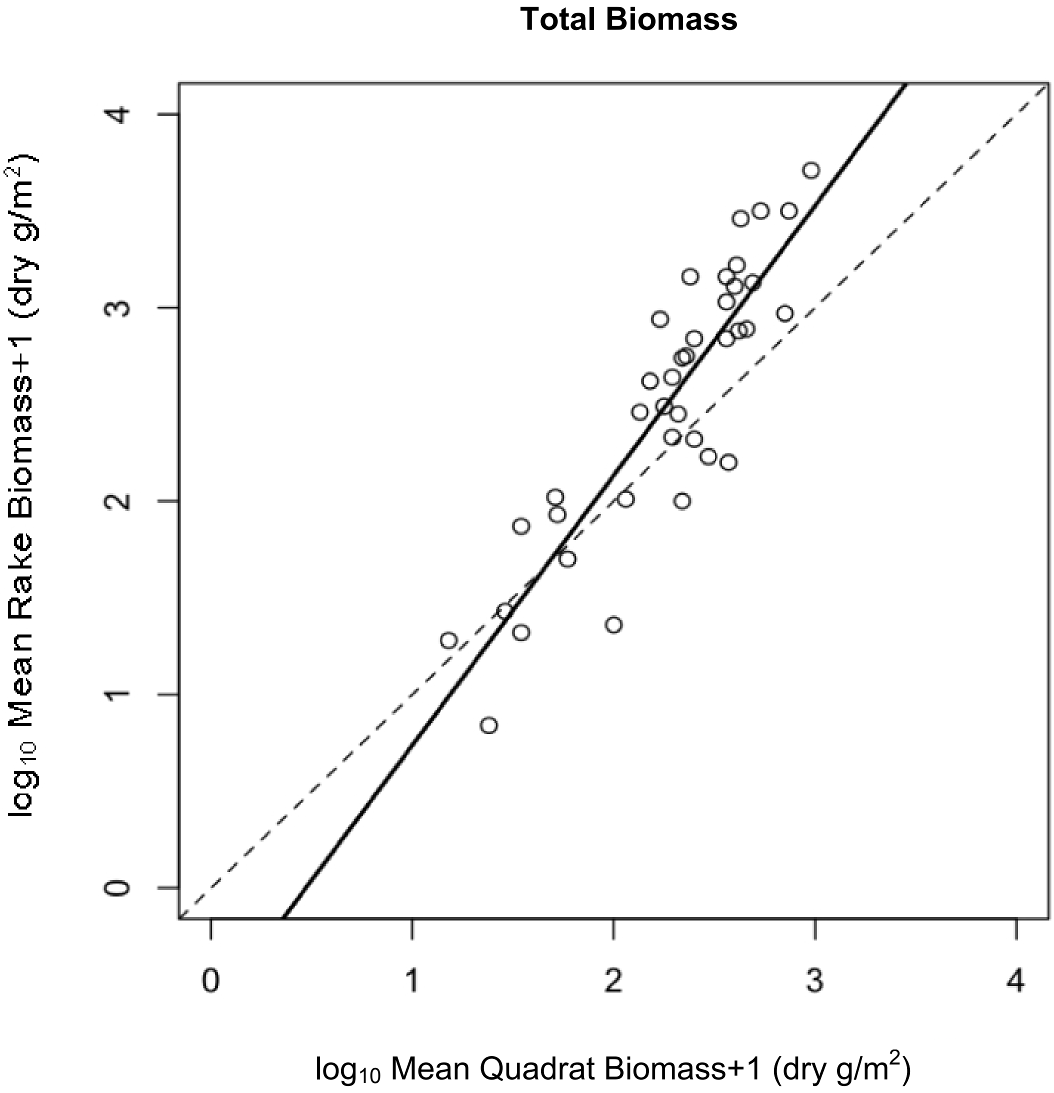


Figure 2. Relationship between rake and quadrat yields ($\log_{10}$ transformed) for total biomass (all plant taxa combined). Solid line indicates fitted regression and dashed line indicates 1:1 correspondence. Regression statistics given in Table 4.



CHAPTER 3

EVALUATION OF LAKE-WIDE, EARLY-SEASON HERBICIDE TREATMENTS FOR CONTROLLING INVASIVE CURLYLEAF PONDWEED (*POTAMOGETON CRISPUS*) IN MINNESOTA LAKES

Planned submission to Lake and Reservoir Management in 2010
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Chapter Summary

Non-native curlyleaf pondweed (*Potamogeton crispus* L.) is widespread throughout temperate regions of North America. Its early-season growth, propensity to form dense surface mats, and ability to out-compete native aquatic plants allow it to degrade the ecological and recreational quality of lakes. Consequently, there is great interest in adopting lake-wide management strategies that can reduce the negative impacts of curlyleaf and provide some degree of long-term control. We collaborated with the Minnesota Department of Natural Resources in 2006, 2007, and 2008 to evaluate lake-wide, early-season herbicide treatments for curlyleaf management. Six curlyleaf-infested lakes were treated with endothall at 0.75 to 1.00 mg/L active ingredient (ai), or fluridone at 2 to 4 μg ai/L for at least three consecutive years. Three additional lakes with established curlyleaf infestations were selected to serve as untreated reference lakes. For all study lakes, we annually assessed the frequency and biomass of curlyleaf in May and June, documented the production of new curlyleaf turions (reproductive buds) on standing plants, and tracked changes in the abundance and viability of turions in lake sediments. Curlyleaf frequency, biomass, turion production, and sediment turion abundance were reduced after treatment and were all significantly lower in treated lakes than in untreated reference lakes. However, viable turions remained in lake sediments after three consecutive years of treatment. These results suggest that serial lake-wide, early-season herbicide treatments can effectively decrease the negative impacts of curlyleaf and reduce the abundance of turions in lake sediments, but ongoing management will likely be required to maintain long-term control of curlyleaf in infested lakes.

Introduction

Curlyleaf pondweed (*Potamogeton crispus* L.) is an exotic submersed aquatic plant that has become a widespread nuisance in temperate regions of North America (Bolduan et al. 1994; Catling and Dobson 1985). It typically forms dense surface-matted growth that interferes with recreational water use and often displaces native vegetation (Bolduan et al. 1994; Catling and Dobson 1985; Wehrmeister and Stuckey 1992). Consequently, there is great interest in adopting management strategies that reduce these negative impacts on a lake-wide scale and decrease the need for intensive management in subsequent years.

Curlyleaf's ability to dominate the plant community in northern lakes is enhanced by its novel life-cycle (Tobiessen and Snow 1984); although curlyleaf is a perennial species, it behaves as a winter annual in northern lakes (Madsen and Crowell 2002; Netherland et al. 2000). This winter annual cycle begins in the fall with new curlyleaf sprouts emerging from previously deposited vegetative propagules (turions) in lake sediments. These new sprouts over-winter as small shoots during the period of ice-cover, but grow rapidly as water temperatures warm in the early spring (Kunii 1982; Tobiessen and Snow 1984). Rapidly growing shoots typically reach the water surface by late spring and begin producing new turions. After several weeks of turion production, curlyleaf plants rapidly senesce and deposit their newly produced turions to lake sediments. These newly deposited turions, together with unsprouted turions remaining from previous years, serve as the source of new sprouting in the subsequent fall (Sastroutomo 1981). Curlyleaf also produces seeds, but these are much less important than turions for maintaining the plant's annual cycle of regrowth under most conditions (Bolduan et al. 1994; Rogers and

Breen 1980). Consequently, there is great interest in management strategies that can interrupt the production of new turions for multiple consecutive years and thus reduce the abundance of turions in lake sediments. Although the link between turion abundance and the level of curlyleaf infestation is not well understood, previous studies have shown that turions older than one year are less viable than newly produced turions (Sastroutomo 1981). Thus, inhibition of turion production for multiple consecutive years may allow the most viable turions to be removed from the sediments of treated lakes (through sprouting without replacement), leaving only turions of lower viability (Netherland et al. 2000).

Mesocosm tank studies have shown that curlyleaf growth and turion production can be selectively managed with low-dose herbicide treatments, specifically fluridone (1-methyl-3-phenyl-5-[3- (trifluoromethyl)phenyl]-4(1H)-pyridinone) and endothall (7-oxabicyclo[2.2.1]hep-tane-2-3dicarboxylic acid) (Netherland et al. 1997; Netherland et al. 2000; Poovey et al. 2005; Poovey et al. 2006; Poovey et al. 2002; Skogerboe and Getsinger 2002). Both of these herbicides were found to be fairly selective for curlyleaf at low concentrations (Netherland et al. 1997; Poovey et al. 2005; Poovey et al. 2006), but required very different contact times for effective control of turion formation. Fluridone inhibited turion production at concentrations of 4 to 5 µg ai/L, but required a contact time of over 50 days at this low concentration (Poovey et al. 2005; Poovey et al. 2006). At higher concentrations, fluridone required less contact time to inhibit turion formation (Poovey et al. 2005), but resulted in reduced selectivity for curlyleaf (Netherland et al. 1997). The long contact time needed for effective control of turion formation with fluridone limits this herbicide's use to whole-basin treatments, as it would not be feasible to maintain the target concentration in smaller "spot" treatment areas for

50 days. Alternatively, endothall has been shown to inhibit turion production at concentrations between 0.75 and 1.00 mg ai/L with only 12 to 24 hours of contact time (Netherland et al. 2000).

The shorter contact time required by endothall allows for spatial and seasonal targeting of treatments, further increasing its selectivity for curlyleaf and minimizing potential impacts on native plants (Netherland et al. 2000; Skogerboe and Getsinger 2002). Endothall treatments can be targeted spatially by treating only areas where curlyleaf is found, and targeted seasonally by treating during the cold-water periods when curlyleaf is growing more actively than most native aquatic plants (Barko et al. 1982; Nichols and Shaw 1986) and is thus more susceptible to herbicide damage (Netherland et al. 2000). Curlyleaf shoots do not begin to grow actively until water temperatures approach 10°C (Kunii 1982; Tobiessen and Snow 1984); this represents the coolest temperature at which endothall treatments are likely to be effective. Although endothall effectiveness increases in warmer water, curlyleaf begins forming turions when temperatures reach 15°C to 20°C (Bolduan et al. 1994; Chambers et al. 1985; Kunii 1982). Given these critical temperatures, Netherland et al. (2000) recommended that endothall treatments should occur when water temperatures were between 10°C and 18°C to minimize turion production while maximizing selectivity for curlyleaf through seasonal targeting. Although this range of water temperatures occurs in both the fall and spring of each year in northern lakes, Woolf and Madsen (2003) suggested that spring treatments would likely be more effective because curlyleaf’s carbohydrate storage, and thus its capacity to recover from herbicide damage, is lowest in the winter and early spring.

Recent evaluations of early-season, low-dose herbicide treatments in curlyleaf-infested lakes have shown dramatic reductions in curlyleaf biomass within the year of treatment and limited recurrence of curlyleaf sprouting after multiple consecutive years of treatment (Skogerboe et al. 2008). Although these results suggest that treatments reduced the number of curlyleaf turions in lake sediments, turion production and the abundance of turions in lake sediments were not directly measured. A more complete understanding of the effects of early-season herbicide treatments on curlyleaf growth, turion production, and the abundance and viability of turions in lake sediments is needed to further evaluate the effectiveness of early-season treatments for managing curlyleaf.

We collaborated with the Minnesota Department of Natural Resources (MNDNR) in 2006, 2007, and 2008 to evaluate the effectiveness of early-season, low-dose herbicide treatments for managing curlyleaf in Minnesota lakes. The specific objectives of our project were to determine: i) if early-season, low-dose herbicide treatments reduce curlyleaf frequency and biomass within the year of treatment; ii) if curlyleaf frequency and biomass decrease from year to year with consecutive annual treatments; iii) the degree to which the production of new curlyleaf turions is reduced by treatment; and iv) the degree to which the abundance and viability of turions in sediments are reduced by multiple consecutive years of treatment.

Methods

Study Lakes

Six Minnesota lakes were selected by the MNDNR to receive lake-wide early-season endothall or fluridone treatments (Table 1, Figure 1) in multiple consecutive years. These lakes were selected based upon the availability of pretreatment data and the potential for support from local management organizations. To assess whether observed changes in treated lakes were larger than natural background variation, we selected three additional curlyleaf-infested lakes to serve as untreated reference lakes during the study. All nine study lakes were located in east-central Minnesota (Figure 1) between 46.8°N and 44.6°N (230 km north-south distance) and represented a range of trophic states (mesotrophic to eutrophic) and degrees of pre-treatment curlyleaf infestation (30% to 80% littoral area infested; Table 1).

Herbicide Treatments

The six treated lakes received early-season, low-dose applications of herbicide annually for three to four consecutive years between 2005 and 2008 (Table 1). These treatments were intended to control curlyleaf lake-wide. Staff from the MNDNR delineated areas of curlyleaf growth in each treated lake just prior to each treatment and supervised herbicide applications. Five of the six study lakes were treated exclusively with endothall, and one lake (Weaver) was treated with fluridone annually from 2005 to 2007 before treatment was switched to endothall in 2008. All herbicide applications were conducted using a boat-mounted tank injection system with 1-m drop hoses to allow for precise dosing and coverage. For endothall treatments, a liquid formulation of the dipotassium salt of endothall was applied when surface water temperatures were between

10°C and 15°C. The rate of endothall application was continuously adjusted based upon the water depth at treated locations to achieve a target in-lake concentration of 0.75 to 1.00 mg ai/L. In-lake endothall concentrations were not monitored. For fluridone treatments, liquid fluridone was applied uniformly over several widely-spaced, deep-water transects at a rate that was sufficient to achieve a target concentration of 2 to 4 μg ai/L once fully mixed into the observed epilimnetic volume of the lake. 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 needed, a second application of fluridone was conducted to maintain the target concentration of 2 to 4 ai μg/L for at least 60 days.

Assessment of Curlyleaf Frequency

We conducted point-intercept aquatic vegetation surveys (Madsen 1999) on each lake in May and June of each year. These surveys assessed the presence of curlyleaf growth at a minimum of 100 sample points arranged in a regularly-spaced grid for each lake. We used a greater number of sample points on lakes with more littoral area or more complex shorelines. We sampled vegetation at each established point with one toss of a 0.33-m wide, weighted, double-headed rake attached to a rope. On each toss, the rake was dragged for approximately three meters while in contact with lake sediments to sample vegetation in an area of approximately one square meter at each location. Retrieved plants were inspected in the field and all occurrences of curlyleaf were recorded. In addition, we recorded occurrences of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and native aquatic plant taxa in May, June, and August of each year, but those results are the focus of a separate paper.

We calculated curlyleaf frequency (% occurrence) for each survey by dividing the number of sampled littoral locations (<4.6 m depth) where curlyleaf was found by the total number of littoral sites sampled on each lake. Within-year changes in curlyleaf frequency (May to June) for each lake were evaluated using a chi-square test (Zar 2010). We compared curlyleaf frequency in treated and reference lakes within each survey period (i.e. May 2006, June 2007) using a nonparametric exact Wilcoxon rank sum test (Hollander and Wolfe 1973; Zar 2010). The nonparametric Wilcoxon test was determined to be more appropriate for these comparisons than standard parametric tests for equal proportion because of the small sample sizes being compared (6 treated, 3 reference) and the resulting uncertainty of whether curlyleaf frequencies were normally distributed within each group.

For between-year comparisons of curlyleaf frequency, we focused on frequency data collected in May of each year. May frequencies were more appropriate for between-year comparisons, because in treated lakes, June frequencies were heavily impacted by treatments within each year. This made it difficult to differentiate within-year effects from the effects of previous treatments. Alternatively, May data were collected just prior to each year's herbicide treatment, so any between-year decreases in May frequency that were significantly greater than seen in reference lakes would indicate carryover effects from treatments in previous years. In addition, we compared the degree of change in curlyleaf frequency between years (May to May) in reference and treated lakes using the exact Wilcoxon rank sum test.

Assessment of Curlyleaf Biomass

We used a boat-based vertical rake method (Chapter 2) to collect biomass at approximately 40 locations during each point-intercept plant survey. Biomass sample locations were randomly selected from the point-intercept survey points using desktop GIS software. At each selected location, we lowered a 0.33-m wide, 14-tine, single-headed rake vertically until it reached the lake sediment. We then rotated the rake on its axis three full turns, and then retrieved it vertically while continuing to turn it slowly to prevent the loss of collected plant fragments. Each sample collected plants from approximately 0.09 m^2 of sediment. Upon retrieval, we rinsed off any sediment, placed all of the collected plants into a labeled plastic bag, drained out excess water, and stored the samples at 5 °C until they could be processed. In the lab, curlyleaf in each sample was sorted from other taxa, and roots and rhizomes were removed and discarded. Sorted curlyleaf plants were then washed, dewatered in a salad spinner, dried for at least 48 hours at 105 °C, and weighed. In addition, we determined biomass of Eurasian watermilfoil and native aquatic plant taxa collected in May, June, and August of each year, but those results are the focus of a separate paper.

Dry curlyleaf biomass values from each sample were divided by the rake sample area (0.09 m^2) to yield dry g/m^2. For subsequent statistical tests, these biomass values were $\log_{10}$-transformed to reduce skewness, stabilize variance within groups (reference and treated lakes), and reduce the difference in variance between groups. Although the log transformation of biomass values greatly improved the comparability of variance both within and between lakes, subsequent F-tests indicated that variances were still not sufficiently comparable for standard parametric tests. Consequently, we chose to use tests that did not assume equal variance: Welch's t-test (Welch 1947; Zar 2010) and a nonparametric exact Wilcoxon rank sum test (Hollander and Wolfe 1973; Zar 2010). We used Welch's t-test to evaluate within-year changes in curlyleaf biomass (May to June) in each lake, and the exact Wilcoxon test to evaluate differences in mean curlyleaf biomass between reference and treated lakes within each survey period. We also used the exact Wilcoxon rank sum test to compare the degree of between-year (May to May) change in curlyleaf biomass in reference and treated lakes.

Assessment of Curlyleaf Turions

We estimated the annual production (number and biomass) of new curlyleaf turions in each lake by assessing turions collected in June curlyleaf biomass samples. Turions were manually sorted from dried biomass samples, counted, re-dried at 105 °C for 24 hours, and weighed. Counts of newly produced turions in each littoral sample were divided by the sampled area ($0.09m^2$) to yield littoral turion production in turions/m^2, and results from all littoral samples were averaged for each lake and year. In addition, we determined the average dry mass of individual turions produced at each littoral sample site, and calculated the percent mass contribution of turions to total curlyleaf biomass. We used the exact Wilcoxon rank sum test to compare the average number of turions produced (turions/m^2) each year within the littoral region of treated and reference lakes, and to compare the degree of between-year change in turion abundance in reference and treated lakes. We used Welch's t-test to compare the average mass of individual turions (dry mg/turion) and the biomass of turions produced (g/m^2) at each site in treated and reference lakes.

In the fall of each year (October), we measured the abundance of curlyleaf turions in littoral sediments collected from each lake. We used a petite Ponar grab (225 cm^2 basal area) to collect one sediment sample at the same sites where we collected peak biomass within each lake. Upon retrieving each sample, we removed any material from the outside of the closed Ponar dredge, emptied its contents into a sifting bucket (1-mm screen), and gently sifted the sample in the field to remove fine sediment. The contents remaining in the bucket after sifting were placed into a labeled plastic bag and stored in a cooler while in the field. In the lab, we manually sorted turions from other debris and recorded total turion counts for each sample. Small turion fragments (those that did not included a portion of a central turion stem) and severely decayed turions (those that did not retain their shape when lightly squeezed) were discarded and were not included in the final turion counts. Turion counts from each sediment sample were divided by the sampled area (0.0225 m^2) to yield sediment turion abundance (turions/m^2) for each sampled site.

We assessed the viability of all turions collected in sediment samples from each lake in each year. Turions that were found to be sprouted at the time of sample processing were tallied as viable and then discarded. Remaining unsprouted turions from each lake were placed into clear sealable plastic bags with a small amount of water and stored in a dark refrigerator at 5 °C for 30 days to simulate typical fall conditions in surface sediments of Minnesota lakes in order to break turion dormancy (Sastroutomo 1981). During this period of cold storage, bagged turions were inspected weekly, and any sprouted turions were tallied and discarded. After this period of cold storage, remaining unsprouted turions were incubated for an additional 90 days at 20 °C with 14 hours of full-spectrum light per day from fluorescent grow lamps. Two different methods of warm incubation were used. In 2006, turions were incubated in shallow trays with one cm of sandy sediment and three to five cm of water. After several weeks of warm incubation, many of the trays became covered with dense filamentous algae that made it difficult to assess sprouting. In subsequent years, turions were incubated in sealed clear plastic bags with a small amount of water, little air-space, and no sediment; this reduced the amount of algae growth and made assessments of sprouting easier. During the period of warm incubation, samples were inspected every two weeks and sprouted turions were tallied and discarded. After 90 days of warm incubation, we calculated final turion viability (% viable) by dividing the total number of sprouted turions (in-lake + cold-storage + warm incubation) by the total number of turions collected (sprouted + unsprouted) from each lake, and calculated the abundance of viable turions (turion abundance x % viable ÷ 100; N/m^2) in each lake for each year. It is important to note that although we exposed turions to cold and light to break their dormancy (Rogers and Breen 1980), some of the remaining unsprouted turions may have been dormant rather than inviable.

For evaluations of turion viability, we used the exact Wilcoxon rank sum test to compare mean turion viability in treated and reference lakes within each year, and to compare between-year changes in mean turion viability within each group of lakes (treated and reference). For evaluations of the abundance of turions (total and viable) in study lakes, we used the exact Wilcoxon rank sum test lakes for within-year comparisons of mean turion abundance in treated and reference lakes, and Welch’s t-test for evaluations of between-year changes in individual lakes. We also used the exact Wilcoxon test to compare the amount of between-year change in mean turion abundance in reference and treated lakes. All analyses were conducted using R statistical software (R Development Core Team 2008).

Results

Curlyleaf Frequency

Comparing within years, May (pre-treatment) curlyleaf frequencies in treated lakes were similar to those in reference lakes in all three years ($P=0.11$ to 0.76), while June (post-treatment) frequencies were significantly lower ($P<0.025$) in treated lakes than in reference lakes (Figure 2). Within each year, curlyleaf frequencies in treated lakes generally decreased by more than one-third between May and June. Chi-squared analysis of these within-year decreases in treated lakes indicated that most of them (14 out of 18) were significant ($P<0.05$). Two of the three untreated reference lakes (Rebecca and Vails) also exhibited within-year decreases in curlyleaf frequency ($P<0.02$) in 2006 and 2007, but June frequencies in these reference lakes remained substantially higher than in the treated lakes despite the observed within-year decreases. Overall, June curlyleaf frequency in all three reference lakes remained relatively high (40 to 85 percent occurrence) in each of the three monitored years, while June frequencies in all six treated lakes remained below 40 percent occurrence in 2006 and 2007, and below 20 percent occurrence in 2008.

Comparing between years, there was no difference in the degree to which curlyleaf frequency changed (May to May) in treated and reference lakes between 2006 and 2007 ($P=0.91$) or between 2007 and 2008 ($P=0.71$). However, we observed high between-year variability in both treated and reference lakes (Figure 2). In treated lakes, between-year changes in May curlyleaf frequency ranged from -23 to +28 percent occurrence (-2 ± 8 percent occurrence; $\bar{x}$ ± 1SE, used henceforth) between 2006 and 2007 and from -51 to -12 percent occurrence (-27 ± 13 percent occurrence) between 2007 and 2008. Similarly, between-year changes in reference lakes ranged from -31 to +8 percent occurrence (-8 ± 12 percent occurrence) between 2006 and 2007 and from -46 to +0 percent occurrence (-21 ± 13 percent occurrence) between 2007 and 2008.

Curlyleaf Biomass

Comparing within years, treated lakes had significantly lower curlyleaf biomass ($P<0.025$) than reference lakes during all surveys except in May of 2008 ($P=0.095$), when all nine study lakes exhibited low curlyleaf biomass (Figure 3). In all three years, biomass remained low (<20 g/m^2) in treated lakes from May (3.6 ± 1.2 g/m^2) to June (1.0 ± 0.4 g/m^2). However, curlyleaf biomass in reference lakes increased between May (50.7 ± 14.5 g/m^2) and June (163 ± 45 g/m^2) in all years ($P<0.025$), with mean June curlyleaf biomass ranging from 45 to 350 dry g/m^2 in all reference lakes in all years with the exception of Coal Lake in 2006 (26 dry g/m^2).

Comparing between years (May to May), all lakes showed similar patterns in curlyleaf biomass (Figure 3). Between 2006 and 2007 ($P=0.55$) curlyleaf biomass did not change significantly in treated ($P>0.9$; +4.0 ± 3.3 g/m^2) or in reference lakes ($P>0.7$; +7.4 ± 26.9 g/m^2). However, between 2007 and 2008, May curlyleaf biomass decreased in all nine study lakes, with a greater degree of decrease ($P=0.024$) in reference lakes (-73 ± 3.5 g/m^2) than in treated lakes (-5.6 ± 2.5 g/m^2). It should be noted that June curlyleaf biomass was relatively stable in both treated and reference lakes, but was consistently one to two orders of magnitude higher in reference lakes (163 ± 45 g/m^2) than in treated lakes (1.0 ± 1.8 g/m^2). Furthermore, there were no significant between-year changes in June curlyleaf biomass in either group of lakes.

Curlyleaf Turions

Turion production was nearly eliminated in herbicide-treated lakes, with much lower production ($P<0.025$) in treated lakes than in reference lakes in all three years of the study (Table 2). The average number of turions produced per m^2 in treated lakes (0.4 ± 0.2 turions/m^2) was less than 0.1% of the average production observed in reference lakes (523 ± 182 turions/m^2). Additionally, the few turions that were produced in treated lakes were smaller ($P=0.004$; 27 ± 4 dry mg/turion) than those produced in reference lakes (43 ± 2 mg/turion). Furthermore, the average turion biomass produced in treated lakes (0.012 ± 0.006 g/m^2) was significantly lower than in reference lakes (19 ± 2 g/m^2).

We also observed that the number of turions produced at individual sampling sites in reference lakes was related to curlyleaf shoot biomass (Figure 4). Regression of turion production (turions/m^2) against curlyleaf shoot biomass from these samples indicated that roughly three turions (2.9 ± 0.1 turions) were produced for every gram (dried) of curlyleaf shoot biomass in untreated reference lakes. On average, dry turion biomass accounted for 9.6% ± 0.7% of total dry curlyleaf biomass in reference lakes.

Total turion abundance in lake sediments was generally lower in treated lakes than in reference lakes in all three years (Figure 5). In 2006, total turion abundance in treated lakes (126 ± 28 turions/m^2) was not significantly different ($P=0.17$) than in reference lakes (347 ± 164 turions/m^2). However in 2007 and 2008, abundance in treated lakes (91 ± 25 turions/m^2; 98 ± 24 turions/m^2) was significantly lower ($P<0.025$) than in reference lakes (478 ± 163 turions/m^2; 615 ± 196 turions/m^2). However, the greater differences observed between treated and reference lake in 2007 and 2008 were not due to decreased turion abundance in treated lakes, but rather, increased turion abundance in all reference lakes.

Comparing between years, total turion abundance remained relatively stable in most of the study lakes through all three years of the study, with only one treated lake (Fish) and one reference lake (Vails) showing any significant between-year changes (Figure 5). Fish Lake (treated) showed a decrease in turion abundance ($P=0.006$) between 2006 (190 ± 48 turions/m^2) and 2007 (44 ± 15 turions/m^2), and Vails Lake (reference) showed an increase in turion abundance ($P<0.05$) between 2006 (254 ± 70 turions/m^2) and 2007 (531 ± 121 turions/m^2). Furthermore, of the nine study lakes, only Vails Lake showed a significant cumulative change in turion abundance between 2006 and 2008 ($P=0.011$), increasing by 511 turions/m^2 during that period.

We did not monitor turion abundance prior to the initial treatments. Consequently, these results do not include reductions of turion abundance that may have occurred in treated lakes during the initial year of treatment. Only one treated lake (Weaver) had available pre-treatment turion abundance data (collected in 2004). These pretreatment data showed that turion abundance in Weaver Lake decreased by 51% ($P=0.006$) in the initial year of treatment (2005), and then remained fairly constant ($P>0.17$) in subsequent years of treatment (Figure 5).

Turion viability (% sprouted) was significantly lower ($P=0.048$) in treated lakes than in reference lakes in both 2006 and 2007 (Table 3). In 2006, turion viability was 55% ± 8% in treated lakes, and 83% ± 4% in reference lakes. Similarly, in 2007, turion viability was 58% ± 6% in treated lakes, and 82% ± 3% in reference lakes. However, in 2008 turion viability in treated lakes (60% ± 7%) was not significantly different ($P=0.095$) than in reference lakes (82% ± 6%). Turion viability was highly variable among lakes during our study (Table 3), ranging from 29% to 83% in treated lakes and from 71% to 91% in untreated reference lakes, but was relatively stable within each individual lake. Furthermore, we found no difference ($P>0.35$) in the degree to which turion viability changed in treated and reference lakes between 2006 and 2007 or between 2007 and 2008. However, it is important to note that turion viability data were not available for years prior to 2006, so we were not able to assess changes in turion viability relative to pretreatment levels in treated lakes.

The average abundance of viable turions in treated lakes (Table 3) remained low in all three years (50 to 62 turions/m^2) and exhibited consistent and low variability (SE =12 to 15 turions/m^2). This contrasts with the average abundance of viable turions in reference lakes, which increased from 275 ± 119 turions/m^2 in 2006, to 401 ± 143 turions/m^2 in 2007, and to 522 ± 181 turions/m^2 in 2008. Within-year comparisons indicated that the average abundance of viable turions in treated lakes was significantly lower ($P<0.025$) than in reference lakes in all three years. Between-year comparisons indicated that turion viability changed less ($P<0.025$) in treated lakes (-12 to +3 viable turions/m^2) than in reference lakes (+121 to +126 viable turions/m^2). However, the abundance of viable turions did not change significantly ($P>0.4$) within either group of lakes (treated and reference) over the course of our study.

Discussion

Curlyleaf Frequency

Our results show that lake-wide, early-season, endothall and fluridone treatments substantially reduced curlyleaf frequency within each year of treatment (May to June). These results agree with those of Skogerboe et al. (2008), who also reported dramatic within-year (April to June) lake-wide decreases in curlyleaf frequency in two Minnesota lakes during each of the first three years of endothall treatment. Skogerboe et al. (2008) also noted that curlyleaf plants collected from treated lakes in June were often small and still attached to the turion from which they sprouted, suggesting that these plants had emerged after the early-season treatment in each year. It is important to note that as a metric, frequency of occurrence is relatively insensitive to reductions in curlyleaf unless the changes are quite dramatic. This insensitivity is due to the fact that curlyleaf is recorded as present at a site whether hundreds of individual plants are found or just a small fragment of one plant. In light of this insensitivity, the decreased curlyleaf frequency in our treated lakes indicates that within-year effects of herbicide treatments on curlyleaf were large.

In comparing between years, we were interested in evaluating whether serial treatments resulted in cumulative effects on curlyleaf. Such cumulative effects over multiple years would indicate a potential for improved long-term control of curlyleaf via repeated annual treatments. Although we observed some significant between-year (May to May) decreases curlyleaf frequency in individual treated lakes, these changes were not substantially larger than the natural year-to-year variation observed in untreated reference lakes. Much of this between-year variation in reference lakes was attributable to decreases in May curlyleaf frequency that occurred between 2007 and 2008. Nearly all of the study lakes, including the reference lakes, showed decreased curlyleaf frequency during this period, suggesting that regional climatic changes limited or delayed curlyleaf growth during the winter and spring of 2008.

Curlyleaf Biomass

We also found that early-season herbicide treatments had a substantial effect upon curlyleaf biomass within each year of treatment. Curlyleaf biomass in treated lakes remained very low (<20 g/m^2) throughout each year of our study, while curlyleaf biomass in reference lakes generally increased to much higher levels by June ($\approx$40 to 350 g/m^2). Furthermore, we observed no surface-matted curlyleaf growth in treated lakes but encountered widespread dense surface-matted growth in reference lakes in June of all three years. These results agree strongly with those reported by Skogerboe et al. (2008), who also found that curlyleaf biomass was controlled in treated lakes during each year of treatment while biomass in untreated reference lakes increased to nuisance levels by June. Similarly, Netherland et al. (2000) reported near complete within-year control of curlyleaf biomass in small pond cells treated with endothall.

Although we observed strong within-year herbicide treatment effects on curlyleaf biomass, our results did not provide a clear indication of cumulative between-year reductions in biomass over sequential years of treatment. We observed some substantial between-year reductions of May curlyleaf biomass in treated lakes (particularly between 2007 and 2008), but these reductions were not greater than the natural background variation we observed in reference lakes. Much of the between-year variability in May curlyleaf biomass occurred between 2007 and 2008. During this period, all nine of our study lakes exhibited decreased curlyleaf biomass, with reference lakes showing particularly large decreases. However, by June of 2008 curlyleaf biomass in reference lakes had returned to levels that were statistically similar to those seen in previous years. Skogerboe et al. (2008) similarly reported high between-year variability in April curlyleaf biomass in both treated and reference lakes, but concluded that reductions in treated lakes were sufficiently different from reference lakes to suggest carry-over effects from previous years of treatment.

The reduced curlyleaf biomass in all of our study lakes in 2008 further suggests that regional climatic conditions limited curlyleaf growth during the winter and early spring of 2008. Previous studies have reported that although curlyleaf is able to grow in cold, low-light conditions (Nichols and Shaw 1986), its growth is very slow below 10°C (Kunii 1982) and may be further slowed in dark conditions under deep snow cover and ice (Trudeau 1982). Climate records from the Minneapolis–St. Paul metropolitan area (State Climatology Office 2010) indicated that in 2008, east central Minnesota had deeper snow cover from January to March (11 to 19 cm) and experienced a cooler April and May (-1.6°C from normal) than in either 2006 (6 to 9 cm; +3.9°C) or 2007 (7 to 17 cm; +2.4°C). This suggests that early-season curlyleaf growth in our study lakes may have been limited by lower light penetration during the period of ice cover in 2008 and by cooler water temperatures in April and May of 2008 than in 2006 or 2007. However, we did not monitor in-lake conditions during the winter or early spring.

The high amount of year-to-year variability we observed in May curlyleaf biomass and the strong within-year effects of treatments on June curlyleaf biomass made it difficult to discern any carry-over or cumulative effects of repeated lake-wide herbicide treatments on curlyleaf biomass. Accordingly, future evaluations of such treatments should consider collecting several years of pretreatment and post-treatment data in study lakes to better assess any cumulative effects. It is important to note that pretreatment curlyleaf biomass data were not available for any of our study lakes. Consequently, our analysis of between-year changes in curlyleaf biomass did not include potential reductions that occurred in the initial year of lake-wide treatment.

Curlyleaf Turions

Lake-wide, early-season, herbicide treatments nearly eliminated the production of new turions (>99% reduction compared to reference lakes). Poovey et al. (2002) and Netherland et al. (2000) reported similar effects on turion production in cool-water (18°C) endothall tank studies (>90% reduction) and in treated pond cells (86% reduction). Although turion production rates in our study were much higher in reference lakes than in treated lakes, we observed a large amount of variability in turion production both within and among reference lakes. This variability appeared to be related to differences in the extent of curlyleaf growth. Coal Lake generally had less extensive curlyleaf growth within the littoral area than either of the other two reference lakes, and thus had fewer sites with high turion production. As a result, littoral averages of turion production in Coal were substantially lower than the other reference lakes. Conversely, Rebecca Lake supported dense curlyleaf growth throughout most of its littoral area, and thus had higher average turion production rates.

Overall, our estimates of turion production in untreated reference lakes (≈100 to

1900 turions/m^2) were generally lower than those reported by Bolduan et al. (1994) (≈900 to 1000 turions/m^2) and Woolf and Madsen (2003) (≈700 to 2700 turions/m^2). Similarly, the percentage contribution of turions to total curlyleaf biomass in our reference lakes (9.6%) was substantially lower than the percentages reported by Rogers and Breen (1980) (23% of total biomass), Kunii (1982) (42%), and Woolf and Madsen (2003) (22 to 58%) from untreated lakes. Moreover, our estimates of the number of turions produced per dry gram of curlyleaf biomass in reference lakes (2.9 turions/dry g) were roughly one-half of that reported by Kunii (1982) from untreated lakes (≈6 turions/dry g, estimated from plotted data). These discrepancies in turion production can be largely explained by considering that the values reported in these other studies reflected turion production in dense stands of curlyleaf growing in shallow water (<2 m), whereas our values represented littoral averages. Our littoral averages incorporated data from a wide range of curlyleaf densities and included samples from sites with no curlyleaf growth and deeper sites with little or no turion production on standing plants (Sastroutomo 1980). Despite these differences, the average dry mass of individual newly-produced turions in our reference lakes (43 mg/turion) was similar to the 53 mg/turion reported by Kunii (1982).

Based upon the observed lack of turion production and continued annual sprouting of turions in treated lakes, we expected to see substantial reductions in the abundance of turions in sediments of treated lakes. Instead, our results indicated that turion abundance within each of the treated lakes was relatively stable during all three years of the study, with no clear indication of declining turion abundance in treated lakes. However, turion abundance in treated lakes was generally much lower than in reference lakes, suggesting that undocumented reductions may have occurred in the initial year of treatment. It is important to note that reductions in turion abundance may have been masked to some degree by fragmentation of older turions, resulting in higher turion counts. We observed some fragmentation of older turions in most samples, however, most of turions we encountered appeared to be intact. In future studies, turion samples should be handled carefully to prevent turion fragmentation during sifting and counting. Furthermore, future studies should consider recording the total mass of turions in each sample in addition to the total number to help account for elevated turion counts due to fragmentation.

Pretreatment estimates of turion abundance were available for only one of our study lakes (Weaver). Consequently, our analysis of observed changes in turion abundance in treated lakes between 2006 and 2008 did not include reductions that may have occurred during the initial year of treatment. Pretreatment (2004) sediment turion abundance in Weaver Lake (426 ± 70 turions/m^2) was comparable to the levels seen in our reference lakes (Figure 5). However, Weaver Lake experienced a dramatic decrease in turion abundance (-51% to 207 turions/m^2) during the initial year of treatment, followed by relatively stable turion abundance in subsequent years of treatment. This suggests that the other treated lakes in our study may have experienced similar large reductions in turion abundance in the initial year of treatment that were not documented by our monitoring. Furthermore, this may explain why we observed consistently lower turion abundance in treated lakes relative to reference lakes without detecting significant decreases in turion abundance in treated lakes between 2006 and 2008. Given the apparent importance of turion reductions in the first year of treatment, future evaluations of such treatments should measure turion abundance in the fall prior to treatment or in the early spring just prior to herbicide application.

The average turion abundance in our reference lakes (≈120 to 880 turions/m^2) was substantially lower than reported by others for untreated lakes: Rogers and Breen (1980) (1320 turions/m^2), Sastroutomo (1981) (2100 turions/m^2), Bolduan et al. (1994) (1130 turions/m^2), Woolf and Madsen (2003) (1150 to 3030 turions/m^2). However, as we noted in our assessment of turion production, the values reported in these other studies generally depicted turion abundance in sediments from shallow areas (<2 m) that had previously supported dense stands of curlyleaf with high turion production and deposition. In contrast, our estimates of turion abundance represented littoral averages, and thus included samples from areas with little or no curlyleaf growth and fewer deposited turions.

In our assessment of turion viability, we found that turions collected from the sediments of treated lakes were generally less viable than those from reference lakes, even when removed and placed into favorable sprouting conditions. The documented inhibition of turion production in our treated lakes means that almost all of the turions we collected from treated lakes were produced prior to the initial year of herbicide treatment. These turions had not sprouted in previous years, suggesting that they were either intrinsically different (lower viability or more quiescent dormancy) from those that had sprouted, or that their immediate environment was not favorable to sprouting, possibly due to deep burial in sediments or highly anoxic conditions (Wu et al. 2009). Turion viability was relatively stable within each lake over the three years of the study, but we observed a high amount of variability among lakes. This suggests that conditions within each lake, such as sediment texture, sediment deposition rate, or the degree of anoxia (Wu et al. 2009), affected the ability of each lake to harbor and accumulate viable turions.

Sastroutomo (1981) reported that older (brown) turions were less viable than newly produced (green) turions. This suggests that these older unsprouted turions were either innately less viable from the start, or were fully viable when produced but became less viable over time. Our results did not indicate decreasing viability in treated lakes over the three years of the study (Table 3), but turions from treated lakes were consistently less viable than those from reference lakes. This suggests that the overall viability of the turion bank in treated lakes decreased during the initial year of treatment (as seen with turion abundance in Weaver Lake) due to removal (sprouting without replacement) of the most viable turions. However, we do not have pretreatment turion viability data to verify this. Furthermore, the lack of additional decreases in turion viability in treated lakes during the three years of our study indicates that either the rate of decline in viability of buried turions is extremely slow, or that turion viability remains fairly constant until turions either sprout or decay.

The use of herbicides to control curlyleaf pondweed is not a new idea, however, the goal of such treatments in the past has generally been to reduce the biomass of curlyleaf within the year of treatment (Netherland et al. 2000). Although our study was based upon observations from a small sample of lakes, our results indicate that early-season, lake-wide herbicide treatments can effectively accomplish this goal of reducing biomass while also providing the added benefits of inhibiting the production of new turions and reducing the abundance of viable turions in lake sediments. However, the link between the abundance of viable turions in lake sediments and the level of curlyleaf sprouting after lake-wide herbicide treatments stop is not currently well understood. Additional work is needed to determine how long viable turions can persist in lake sediments, and to assess the rate of curlyleaf reestablishment from different levels of viable turion abundance in treated lakes.

It is important to note that the goal of these early-season, lake-wide herbicide treatments was to control curlyleaf while simultaneously protecting or enhancing the native plant community. The response of the native plant community in our study lakes will be addressed a separate paper, but preliminary assessments indicated that most native taxa were not affected substantially by lake-wide, early-season endothall treatments. However, fluridone appeared to reduce the frequency and biomass of coontail (*Ceratophyllum demersum* L.) in Weaver Lake.

Lake-wide control of curlyleaf has also been proposed as an in-lake nutrient reduction strategy. Bolduan et al. 1994 and James et al. 2002 suggested that the early-summer senscensce and subsequent decay of curlyleaf may increase internal nutrient loading (particularly phosphorus) in some lakes, potentially leading to increased algae and decreased water clarity. However, water clarity in our treated lakes generally did not change more than in untreated reference lakes, and there was no clear trend of increasing water clarity in most of our treated lakes (Table 4, Figure 6). Weaver Lake was a notable exception, showing a 150% increase in late summer (July and August) water clarity (0.8 m pretreatment to ≈2.0 m post-treatment). Although curlyleaf senescence and decay did not appear to have a large effect upon summer water clarity in most of our study lakes, we did not assess water column nutrient concentrations or account for external nutrient loading to our study lakes. Additional studies are needed to determine the relative contribution of curlyleaf decay to overall lake nutrient budgets and to further explore the impacts of curlyleaf infestation on water clarity.

Despite the substantial within-year reduction of curlyleaf biomass, inhibition of turion production, and reduced abundance of viable turions in treated lakes, relatively high numbers of viable turions remained in lake sediments even after three to four consecutive years of herbicide treatment. Furthermore, we found no clear indication of decreasing turion viability or substantial cumulative depletion of turion abundance after the initial year of treatment. Although the remaining turions in our treated lakes were likely insulated from environmental sprouting cues due to deep burial (Jian et al. 2003; Sastroutomo 1981), or inhibited by sediment anoxia (Wu et al. 2009), our results showed that many of these turions would sprout if brought to the sediment surface. The persistence of these viable turions and the possibility of additional sporadic sprouting from deposited curlyleaf seeds suggests that eradication of curlyleaf is not feasible using herbicides. However, our results indicate that early-season, lake-wide herbicide treatments should be considered an effective tool for reducing severe infestations of curlyleaf to a point where less intensive control measures, such as localized “spot” herbicide treatments and small-scale harvesting or cutting, may be able to maintain effective long-term control (Deschenes and Ludlow 1993).

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Table 1. Lake identifiers, herbicide application details, lake attributes, and pretreatment conditions for the study lakes. Untreated reference lakes shown in bold.

Lake	MN Lake ID	Herbicide [a]	First Treated	Secchi [b] *(m)*	Area *(ha)*	% Littoral [c]	% Littoral Infested [d]
Coal	77-0046	None	-	2.4	69	40	45
Rebecca	27-0192	None	-	1.9	105	50	95
Vails	73-0151	None	-	1.6	64	80	75
Crookneck	49-0133	E	2006	2.9	74	80	40
Fish	70-0069	E	2005	1.6	70	40	60
Julia	71-0145	E	2006	0.6	62	100	50
Lower Mission	18-0243	E	2006	3.8	292	60	60
Rush	71-0147	E	2006	0.6	65	100	80
Weaver	27-0117	F/E[e]	2005	2.3	62	50	80

[a] E = Endothall (0.75 to 1.00 mg ai/L), F = Fluridone (2 to 4 μg ai/L)

[b] Pre-treatment Secchi depth data from the Minnesota Pollution Control Agency (May-September average)

[c] % Littoral calculated as [(area of lake ≤ 4.6m) ÷ total lake area]

[d] % Littoral Infested is maximum littoral % occurrence of curlyleaf pondweed from all available surveys

[e] Weaver Lake was treated with fluridone in 2005, 2006, and 2007, and with endothall in 2008

Table 2. Production of new curlyleaf pondweed turions (N produced/m^2) in June within the littoral area (≤4.6 m depth) of herbicide-treated lakes and untreated reference lakes 2006 to 2008; presented as littoral mean ($\bar{x}$) with standard errors (*SE*) in parentheses.

		HERBICIDE-TREATED LAKES						UNTREATED REFERENCE		
		Crookneck	Fish [a]	Julia	L. Mission	Rush	Weaver [a]	Coal	Rebecca	Vails
2006	$\bar{x}$	<1	3	0	0	0	0	96	1871	360
	(SE)	(<1)	(2)	-	-	-	-	(47)	(589)	(144)
2007		<1	<1	0	<1	0	0	312	778	509
		(<1)	(<1)	-	(<1)	-	-	(93)	(210)	(124)
2008		2	<1	0	0	0	0	87	313	379
		(2)	(<1)	-	-	-	-	(46)	(93)	(124)

[a] Fish and Weaver were treated for four consecutive years (2005 to 2008)

Table 3. Viability (%) and abundance of viable curlyleaf pondweed turions (N/m^2) collected from herbicide-treated lakes and untreated reference lakes, 2006 to 2008.

	HERBICIDE-TREATED LAKES						UNTREATED REFERENCE		
	Crookneck	Fish [a]	Julia	L. Mission	Rush	Weaver [a]	Coal	Rebecca	Vails
TURIONS TESTED (N)									
2006	102	129	26	125	49	361	77	525	219
2007	147	38	26	137	29	140	164	738	550
2008	88	93	18	164	43	135	179	787	637
% VIABLE [b]									
2006	73	29	81	59	43	42	91	76	82
2007	74	50	77	45	62	40	76	85	85
2008	76	40	83	52	63	45	71	85	89
ABUNDANCE OF VIABLE TURIONS (N/m^2) [c]									
2006	85	55	27	102	25	79	111	505	208
2007	115	22	22	67	20	54	131	620	452
2008	74	38	17	88	29	71	161	725	681

[a] Fish Lake and Weaver Lake were treated for four consecutive years (2005 to 2008)

[b] P-values (exact Wilcoxon rank sum test) for within-year comparisons of turion viability in reference and treated lakes: 2006=0.048, 2007=0.048, 2008=0.095

[c] Calculated as [total turion abundance x (% viable ÷ 100)], $P<0.025$ (exact Wilcoxon rank sum test) for all within-year comparisons of viable turion abundance in reference and treated lakes, total turion abundance presented in Figure 5

Table 4. Water clarity (Secchi depth) for herbicide-treated lakes and untreated reference lakes, 2006 to 2008. Values represent average of available Secchi data from each month. Data provided by the Minnesota Pollution Control Agency[a], with additional data collected by the University of Minnesota during vegetation surveys.

		HERBICIDE-TREATED LAKES						UNTREATED REFERENCE		
		Crookneck	Fish	Julia	L. Mission	Rush	Weaver	Coal	Rebecca	Vails
SECCHI DEPTH (m)										
PRE[b]	MAY	3.3	2.5	na	6.0	1.1	5.7	-	-	-
	JUNE	3.4	1.4	1.0	4.8	0.9	2.6	-	-	-
	JUL/AUG	2.9	1.0	0.4	2.5	0.4	0.8	-	-	-
2006	MAY	4.4	2.1	2.2	3.6	2.2	7.2	6.8	3.0	3.2
	JUNE	3.2	1.2	1.7	2.2	0.9	4.7	4.2	0.8	1.9
	JUL/AUG	2.4	1.8	0.6	1.1	0.4	2.0	2.2	0.6	0.6
2007	MAY	5.8	1.7	1.5	5.7	1.4	5.0	4.7	2.1	0.7
	JUNE	4.0	1.3	1.1	3.7	0.7	2.6	4.1	0.8	0.7
	JUL/AUG	2.7	1.1	0.4	1.3	0.3	2.2	2.7	0.6	0.4
2008	MAY	4.1	1.5	1.4	4.3	1.9	3.2	6.1	2.0	1.4
	JUNE	3.5	1.4	1.0	3.8	1.9	3.7	3.5	3.1	3.7
	JUL/AUG	2.2	1.4	0.5	1.6	0.4	1.8	2.7	0.8	0.5

[a] MPCA - Water Quality Data; http://www.pca.state.mn.us/data/edaWater/index.cfm

[b] Pretreatment water clarity - average of Secchi depth data collected in each month during the two to three years prior to the initial lake-wide treatment

Figure 1. Map of Minnesota showing the locations of the six herbicide-treated lakes (●) and three untreated reference lakes (■).

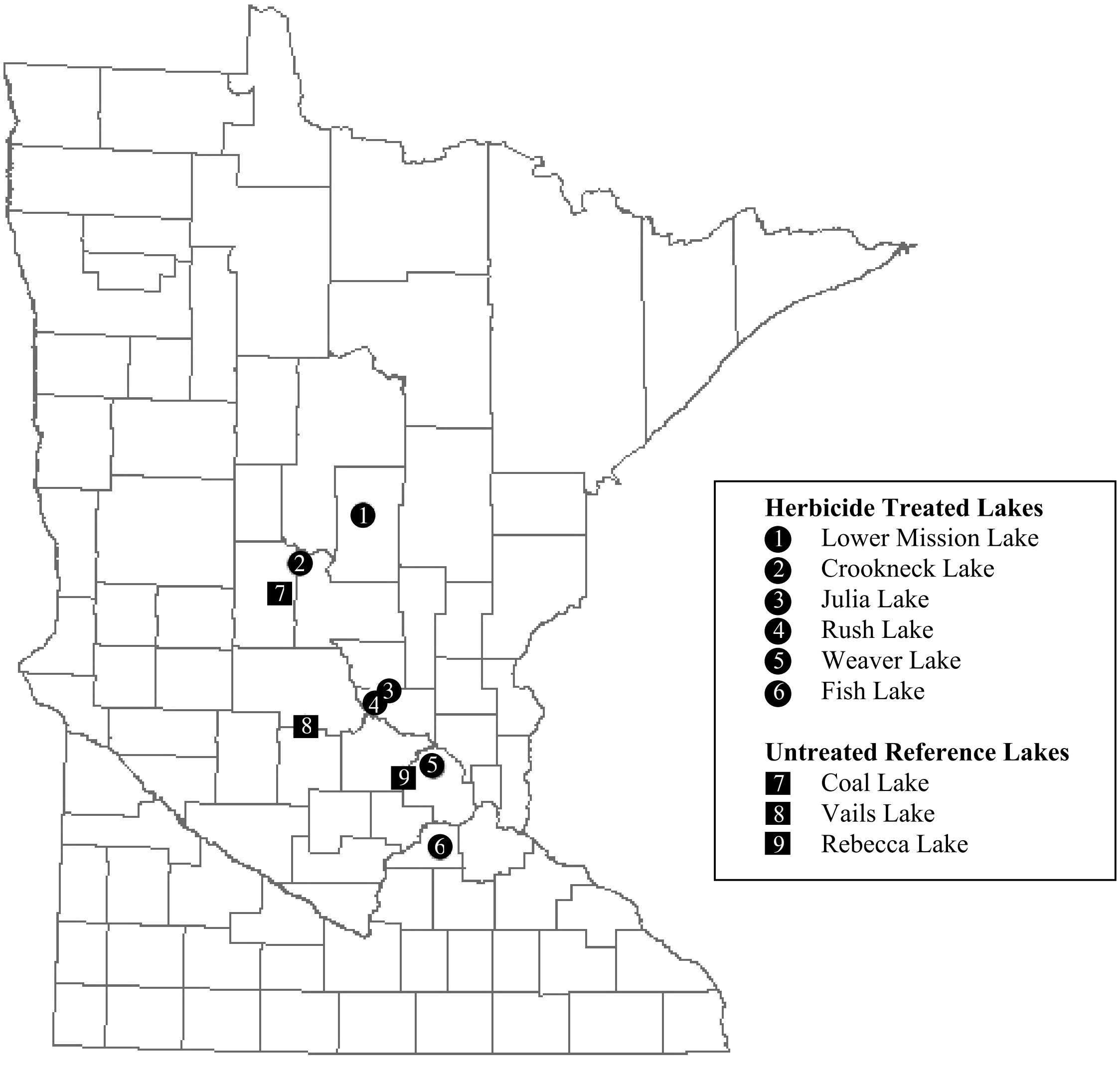


Figure 2. Littoral (≤ 4.6 m) frequency of curlyleaf pondweed in untreated reference lakes (black lines), and herbicide-treated lakes (gray lines). Number of sampled littoral locations given in parentheses. Significant (χ-squared; P>0.05) within-year changes in frequency indicated by solid lines; non-significant changes indicated by broken lines. P-values for within-survey comparisons of frequency in reference and treated lakes (exact Wilcoxon rank sum test) are reported along the top margin.

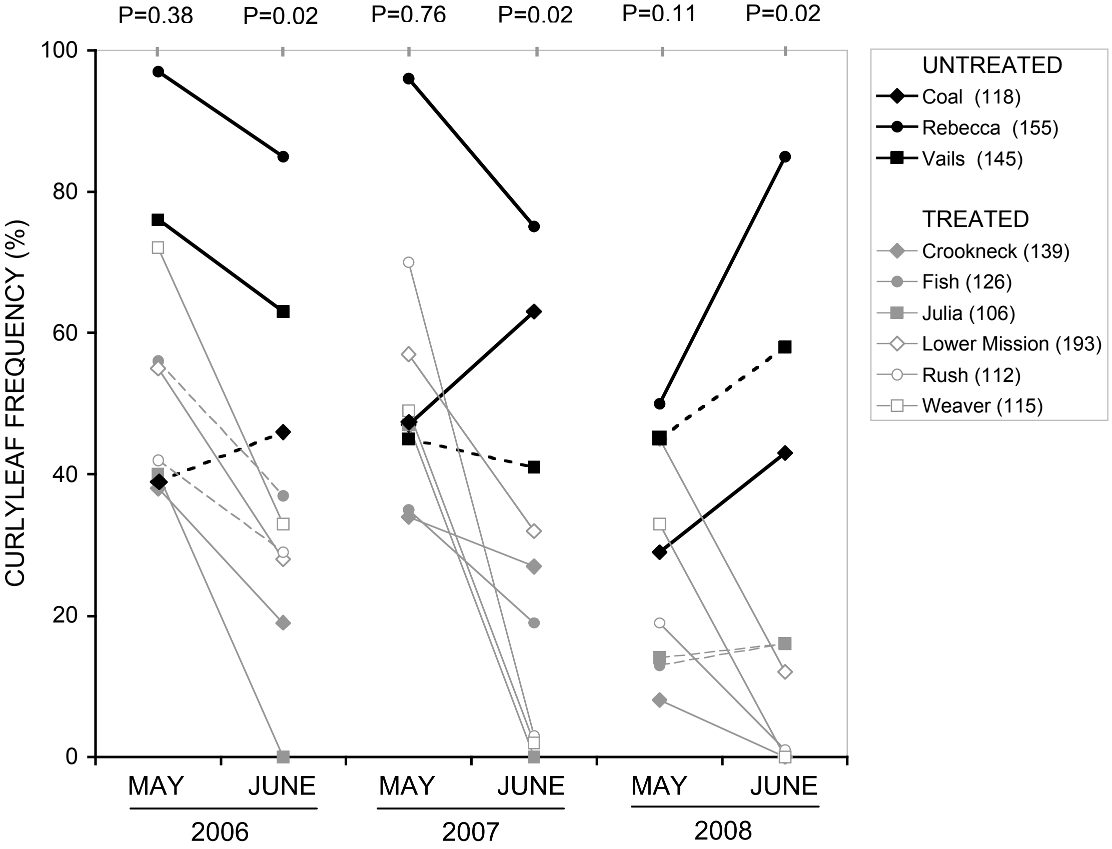


Figure 3. Littoral (≤ 4.6 m) curlyleaf pondweed dry biomass (g/m^2) in untreated reference lakes (black), and herbicide-treated lakes (gray). Error bars represent ± 1 SE. Within-year changes (May to June) in biomass (log$_{10}$-transformed) were tested using Welch's t-test; significant within-year changes ($P<0.05$) indicated by solid lines; non-significant ($P>0.05$) by broken lines. P-values for within-survey comparisons of biomass in untreated reference lakes and treated lakes (exact Wilcoxon rank sum) given along the top margin.

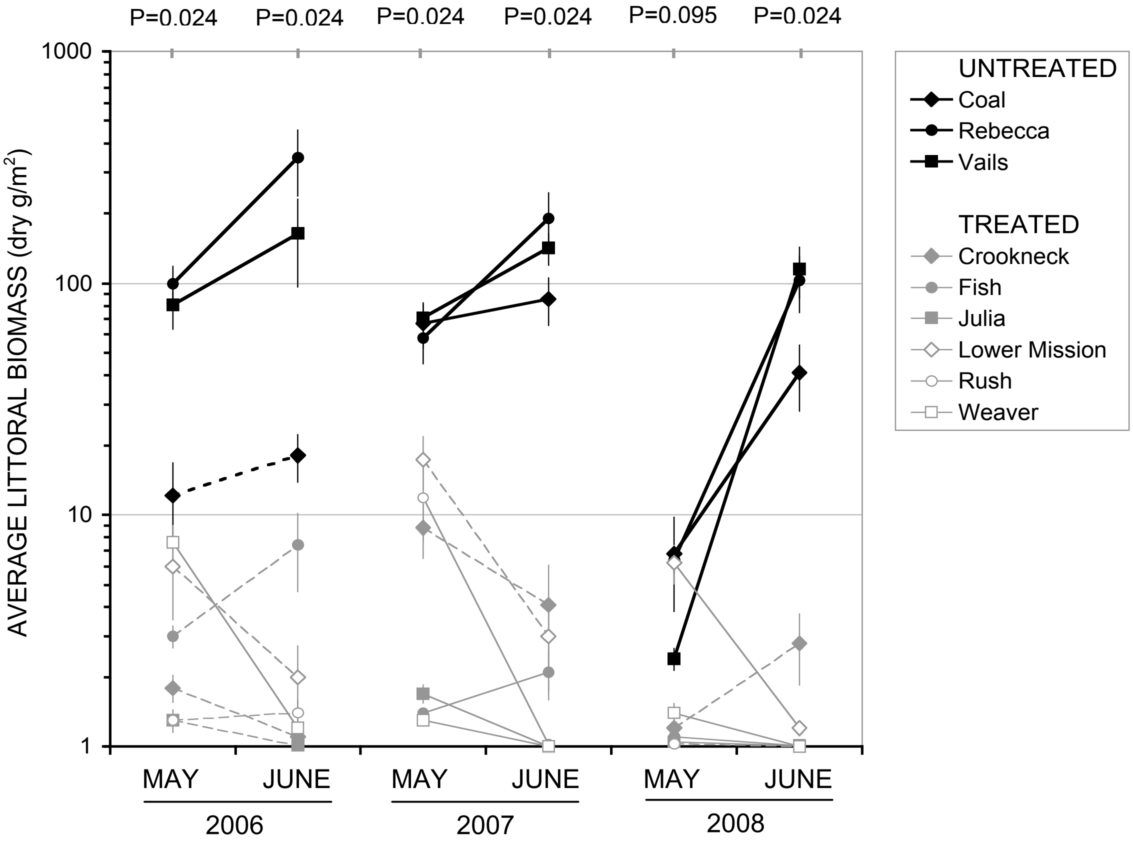


Figure 4. Relationship of turion production to curlyleaf shoot biomass (June) in untreated reference lakes (2006 to 2008).

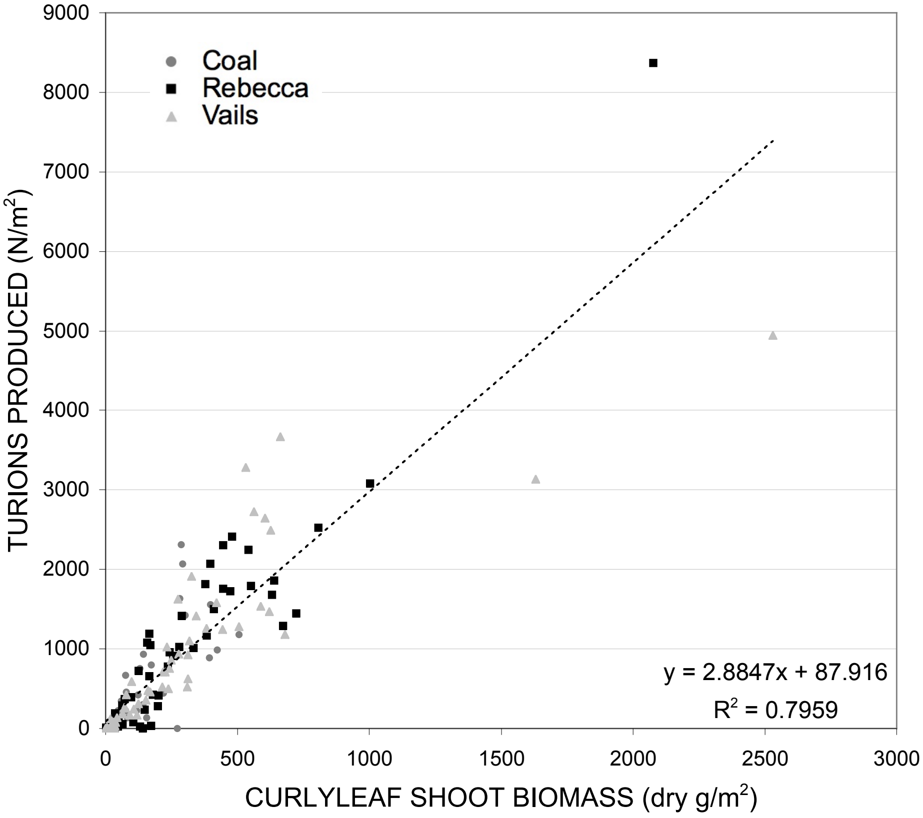
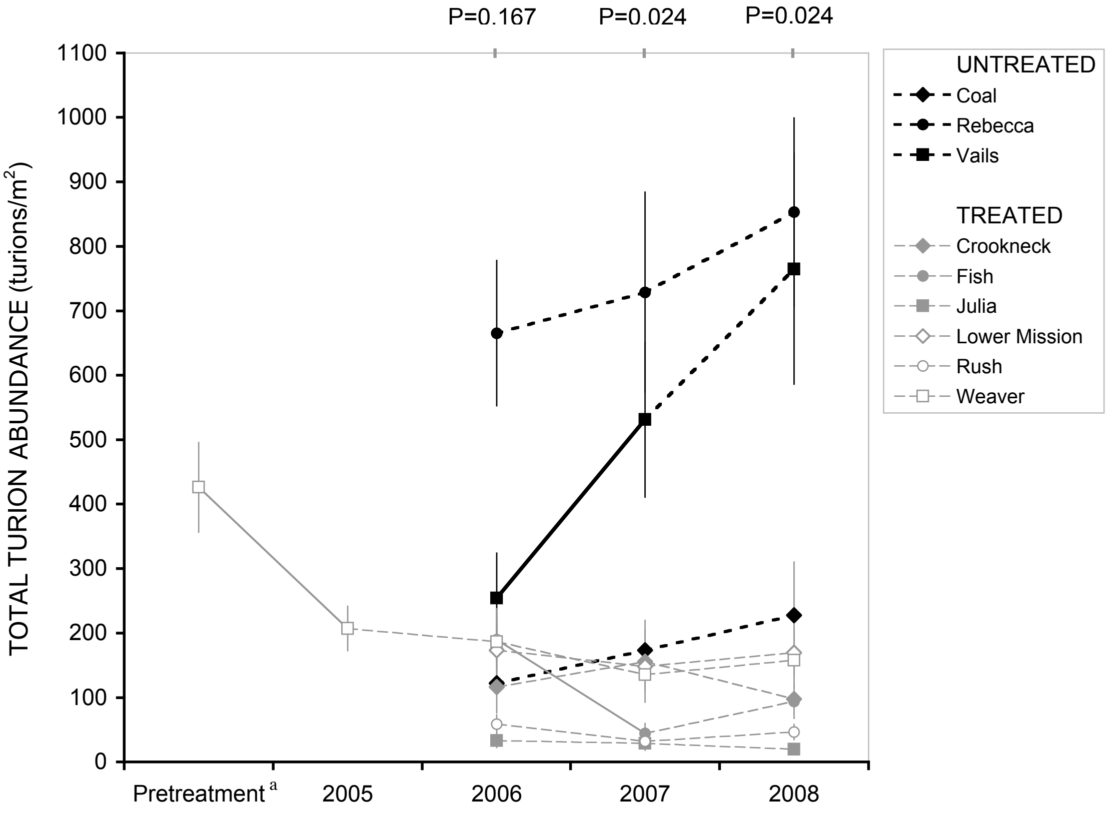


Figure 5. Abundance of curlyleaf pondweed turions (sprouted + unsprouted) in littoral (≤ 4.6m) sediments of herbicide-treated lakes and untreated reference lakes; Error bars represent ± 1SE. Between-year differences for each lake were tested using Welch's t-test; significant changes ($P<0.05$) indicated by solid lines; non-significant ($P>0.05$) by broken lines. P-values for within-year comparisons of average turion abundance in untreated reference lakes and treated lakes (exact Wilcoxon rank sum) given along the top margin.



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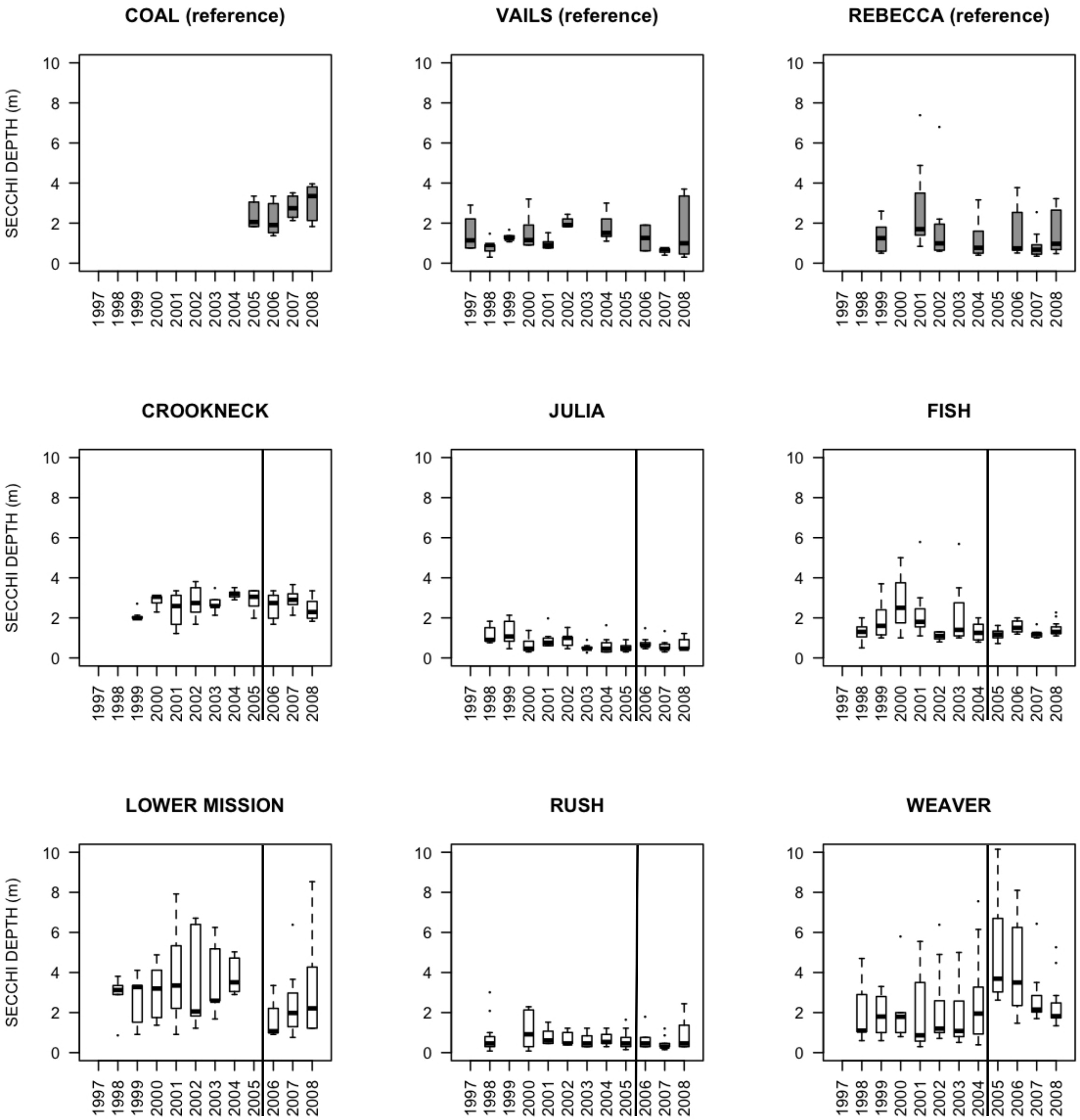
[a] Pretreatment turion abundance data only available for Weaver Lake (2004); collected by Freshwater Scientific Services, LLC – Maple Grove, MN (N=58)

Figure 6. Secchi depth (m) boxplots (median, interquartile range, 95% CI) for treated lakes and untreated reference lakes; May to September, 1997 to 2008. Data provided by the Minnesota Pollution Control Agency [a], with additional data collected by the University of Minnesota during vegetation surveys. Initiation of annual treatments denoted by solid vertical line in treated lake plots.



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[a] MPCA - Water Quality Data; http://www.pca.state.mn.us/data/edaWater/index.cfm

CHAPTER 4

CONCLUDING REMARKS

Invasive aquatic plants, such as curlyleaf pondweed, have become a major focus of overall lake management activities throughout the United States. Past curlyleaf management strategies have generally used biomass removal (harvesting) and small-scale herbicide treatments to maintain navigational channels and minimize the effects of dense canopy growth on recreational use of waters. However, these approaches have proven to be short-term solutions that do little to reduce the overall level of infestation. Moreover, these actions usually need to be repeated at least annually to control nuisance growth of curlyleaf (Madsen and Crowell, 2002). Consequently, citizen lake groups, local governmental units, and state agencies have been very interested in identifying new, effective management tools to reduce the impacts of invasive plants in recreational waters.

In Chapter 3, I showed that lake-wide, early-season, low-dose herbicide treatments are an effective way to dramatically reduce curlyleaf biomass and inhibit the production of new turions in severely infested lakes. However, viable turions still remained in treated lakes after three to four consecutive years of treatment. Based upon these findings, early-spring, lake-wide treatments may be particularly useful in managing severely infested systems where less intensive management strategies would have little impact. Multiple consecutive treatments would likely reduce these severe curlyleaf infestations to a point where less intensive control measures, such as localized “spot” herbicide treatments and small-scale harvesting or cutting, may be able to maintain fairly effective long-term control (Deschenes and Ludlow 1993). However, the effectiveness and ecological impact of lake-wide treatments cannot be fully evaluated without continued assessments for several years after lake-wide treatments are stopped. It is essential that monitoring be continued in these previously treated lakes to determine whether initial (post-treatment) turion abundance affects the rate at which curlyleaf reestablishes after prolonged periods of treatment, to assess the rate at which turion abundance increases after treatments cease, and to document changes in the native plant community.

The rate of curlyleaf reestablishment after lake-wide treatments is not currently well documented. However, widespread but very sparse sprouting was observed in most of our study lakes after three or four successive years of treatment. This suggests that if maintenance control strategies are not implemented in the years immediately after lake-wide treatments cease, curlyleaf may rapidly reestablish. I could find no reports in the literature that described patterns or rates of curlyleaf pondweed spread within individual lakes, however, I speculate that curlyleaf reestablishment after lake-wide treatments would likely follow a very different pattern than seen in new infestations. New curlyleaf infestations typically begin at a single introduction site, such as a boat launch. I hypothesize that from this single site, curlyleaf would typically spread as an expanding patch of moderate to dense growth with fairly localized dispersal of turions. Some turions would drift to distant areas and create sporadic new patches of curlyleaf growth in previously uninfested areas. Over several years, these separate patches would continue to expand and merge to form a continuous area of nuisance growth (Figure 1). In most lakes, I believe that this process would take many years, with the rate of spread being relatively slow and highly dependent upon the spread of turions to new uninfested areas. Conversely, in treated lakes, moderate to dense curlyleaf growth has usually already spread throughout much of the lake (hence triggering the desire to conduct a lake-wide treatment). This results in large areas of lake sediment having moderate to high turion abundance prior to treatment. Although I have shown that multiple years of lake-wide treatment reduces the abundance of viable turions to some degree, remaining turions resulted in widespread, but very sparse, sprouting. If left unchecked, each of these new sprouts would likely deposit new turions in the first year after treatment and would likely form a small, localized patch of curlyleaf growth within two years. If hundreds of these patches were uniformly distributed throughout the lake, these patches would likely result in a much more rapid reestablishment of curlyleaf throughout previously infested areas than seen in new invasions (Figure 1).

Although a resurgence in the frequency (% occurrence) of curlyleaf after lake wide treatments would indicate a potential for rapid reestablishment of nuisance curlyleaf growth to pretreatment levels, increased frequency may not necessarily translate into increased nuisance growth. Consequently, curlyleaf biomass should be a much better metric for evaluating long-term control in treated lakes. Perceptions of nuisance curlyleaf growth are generally associated with the amount of dense, surface-matted growth rather than whether growth is simply widespread. If curlyleaf growth after treatment is widespread, but does not form expansive areas of dense growth, then the treatments should be judged as a successful long-term control strategy. Furthermore, the intensity of continued curlyleaf control programs after lake-wide treatments should be adjusted to minimize a resurgence in the biomass of curlyleaf. Consequently, post-treatment monitoring of curlyleaf growth should incorporate biomass sampling methods that allow for the collection of numerous samples that cover the entire littoral area of treated lakes.

In Chapter 2, I showed that the vertical rake method was an acceptable alternative to diver quadrat sampling that allowed for rapid, safe collection of samples in large-scale studies while maintaining an acceptable level of precision. This method has already been adopted as a standard sampling method by researchers from the Minnesota Department of Natural Resources – Division of Ecological Resources (Crowell and Asmus 2008) and the United States Army Corps of Engineers – Engineer Research and Development Center (Skogerboe et al. 2008), and has been used to assess aquatic plant biomass in well over 100 lake-wide surveys to date.

In this thesis, I have advanced our understanding of early-season, lake-wide herbicide treatments for curlyleaf control, and have validated a simplified method for assessing aquatic plant biomass in large-scale studies. Furthermore, this work has provided insights into the effects of lake-wide treatments on curlyleaf turion production and the abundance of turions in lake sediments. Collectively, these works will allow for the development of more effective management plans for curlyleaf infested waters, and will improve the ability to document and evaluate effects on curlyleaf pondweed and native aquatic plants in treated lakes.

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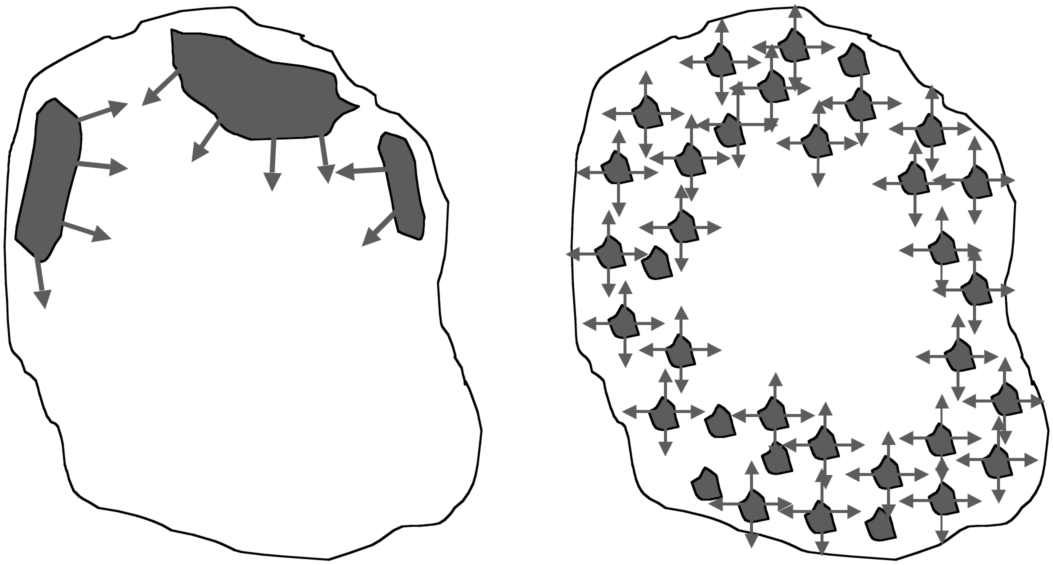
Figure 1. Graphical representation of hypothesized differences in the pattern of curlyleaf colonization in a newly infested lake (left) and in a treated lake with a previously established lake-wide curlyleaf infestation with sparse but widespread post-treatment sprouting.



Hypothesized Spread of
New Infestation

Reestablishment from
Widespread Sparse Growth
(post-treatment)

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