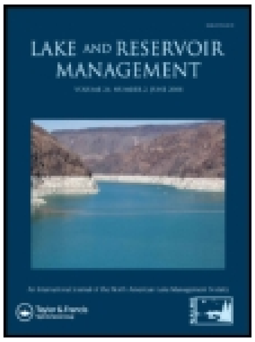

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Effects of repeated, early season, herbicide treatments of curlyleaf pondweed on native macrophyte assemblages in Minnesota lakes

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Abstract

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We examined the response of native aquatic macrophyte communities to spring herbicide treatments of curlyleaf pondweed (*Potamogeton crispus*) from 2006 through 2009. Eleven lakes were examined during our study; 8 were treated in May with endothall at 0.75–1.00 mg active ingredient per liter (ai/L) and 3 were used as untreated reference lakes. Macrophyte communities were assessed for frequency of occurrence in the littoral zone with the point intercept method in the summer after each treatment. During each survey, we collected biomass samples from 40 random locations in each lake. In the reference lakes, curlyleaf persisted at moderate to high frequencies over the 4 years, and no consistent changes in native macrophyte frequency of occurrence were seen. In most treated lakes, overall native macrophyte frequency of occurrence and species richness changed little over the 4 consecutive years of treatment, although shifts in the abundance of some species were observed. In untreated lakes, biomass varied between years, whereas in many treated lakes, biomass generally increased; however, these increases were usually not significant. The most substantial increases in biomass were attributed to single species in each treatment lake. Likewise, we observed substantial but insignificant increases of *Chara* sp. frequency and biomass in many treated lakes. Multiple years of treatment may be needed to see significant increases in overall native macrophyte abundance because significant changes in abundance were not observed within 4 years of treatment; however, consecutive early season, lakewide endothall treatments of curlyleaf pondweed can control curlyleaf pondweed without substantial harm to native macrophytes.

Key words: *Ceratophyllum demersum*, *Chara*, curlyleaf pondweed, *Elodea canadensis*, endothall, native macrophytes, *Potamogeton crispus*

Curlyleaf pondweed (*Potamogeton crispus*) is an aggressive invasive aquatic macrophyte found in Minnesota and the northern United States. The timing of curlyleaf's annual life cycle is a major factor that allows it to be a successful invader (Bolduan et al. 1994). In Minnesota and much of North America, curlyleaf sprouts in the fall as well as under ice cover during winter months (Bolduan et al. 1994). When the water starts to warm in early spring, curlyleaf exhibits rapid growth toward the water's surface (Sastroutomo 1981, Jian et al. 2003), where it begins to form a dense mat that can block sunlight (Sastroutomo 1981). This early sprouting, rapid, cold-water growth and dense canopy formation all occur before native macrophytes begin actively growing and allow curlyleaf to out-compete and displace native macrophytes in Minnesota (Madsen and Crowell 2002). Biomass in monotypic curlyleaf stands is often much higher than in indigenous aquatic macrophyte stands (Kunii 1984, Bolduan et al. 1994). These dense monotypic stands of curlyleaf can impair recreational use of lakes (Bolduan et al. 1994) and have been shown to displace native submersed macrophytes (Catling and Dobson 1985). In addition, upon senescence in early summer, decaying curlyleaf releases nutrients into the water column that can perpetuate phytoplankton growth

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and degrade water quality (Rogers and Breen 1982, Bolduan et al. 1994).

As a result of these detrimental effects, there is considerable interest in improved approaches to control curlyleaf infestations. Previous mesocosm studies have suggested that herbicide treatment in the early spring can selectively target curlyleaf without harming native macrophytes (Getsinger et al. 1997, Netherland et al. 2000). Specifically, these past studies suggest that early season endothall treatments could benefit native macrophytes by reducing the formation of dense, surface-matted curlyleaf growth, thus increasing light availability. Although peak curlyleaf abundance occurs in mid to late spring in Minnesota, the peak abundance of most native macrophytes typically occurs later in the summer months (Crow and Hellquist 2000). Reducing curlyleaf abundance may also lead to reductions in phosphorus release and subsequent algal blooms after curlyleaf senescence (James et al. 2002), which could allow more native macrophyte growth in deeper water due to increases in water clarity. Recent research further suggests that early season, low-dose endothall treatments can be an effective method for control of curlyleaf pondweed in Minnesota lakes (Skogerboe et al. 2008, Johnson et al. 2012).

It is important that any control of curlyleaf pondweed not damage native macrophytes, but rather maintain or increase their abundance. Abundant and diverse native macrophytes communities host a variety of epiphytic organisms (Carpenter and Lodge 1986), increase macroinvertebrate abundance and diversity (Gilinsky 1984, van den Berg et al. 1997), and provide food and shelter for fishes (Smart et al. 1996, Shoup et al. 2003, Valley et al. 2004). In addition, a diverse macrophyte community can help sustain water clarity by providing a refuge for zooplankton, sequestering pelagic nutrients, reducing sediment resuspension, and decreasing phytoplankton abundance (Jeppesen et al. 1998).

Endothall, the herbicide used to control curlyleaf in our study, is a useful contact herbicide due to its short persistence time and potential to target only actively growing macrophytes during the time of application (Langeland and Warner 1986). Most native macrophyte species are dormant at the time of early spring treatments, and many emergent, floating-leaf species and charophytes are relatively tolerant of endothall, even when actively growing. However, certain submersed native macrophyte species have been shown to be sensitive to endothall if present during the time of treatment (Skogerboe and Getsinger 2001, 2002, Skogerboe et al. 2008). In particular, many macrophytes in the genus *Potamogeton* show high sensitivity to endothall, whereas coontail (*Ceratophyllum demersum*) shows only a moderate sensitivity. Despite the sensitivity of some native macrophytes to herbicide treatments, the positive effects resulting from removal of curlyleaf pondweed may enhance the overall native macrophyte community composition and abundance. Although consecutive, annual, early season endothall treatments have been shown to be effective for controlling curlyleaf pondweed (Skogerboe et al. 2008, Johnson et al. 2012), the long-term effects of such treatments on native macrophytes have not been examined across multiple lakes.

The primary goal of this study was to assess the effects of consecutive, early season endothall treatments on native macrophyte communities. Specifically, our objective was to determine if the frequency, biomass, species richness, and diversity of native macrophytes were maintained or enhanced in lakes that received successive years of endothall treatment. A companion paper by Johnson et al. (2012, this issue) assessed the response of curlyleaf pondweed to the endothall treatments.

Methods

Study lakes

In cooperation with the Minnesota Department of Natural Resources (MNDNR), we selected 11 curlyleaf-infested lakes in Minnesota for this study (8 treated and 3 reference; Table 1). These lakes ranged in trophic status from mesotrophic to hypereutrophic, but most were eutrophic; mean Secchi depth ranged from 0.6 to 3.8 m (Table 1). The 3 reference lakes were chosen to represent levels of curlyleaf infestation, location, size and trophic status similar to the treated lakes. Study lakes varied in size from 60 to 290 ha, and all lakes were moderately shallow with maximum depth <10 m. All lakes were sampled by the University of Minnesota, although surveys prior to June 2008 for Clear, Blueberry, and Long lakes were conducted by the MNDNR. Study lake locations are provided in Johnson et al. (2012).

Herbicide treatments

Staff from the MNDNR delineated treatment areas and supervised all herbicide applications in 2006, 2007, 2008, and 2009 (Table 2). All treatment lakes were treated exclusively with endothall to achieve concentrations of 0.75–1.0 mg active ingredient per liter (ai/L) in the treated areas. In 2009, treatment was stopped on 2 of the study lakes (Crookneck and Fish), and 1 of the previously untreated reference lakes (Rebecca) was treated with endothall. Endothall applications were limited to areas of early spring curlyleaf growth; MNDNR staff delineated these areas and monitored herbicide applications. Endothall was applied by a boat-mounted tank injection system with 1 m drop hoses, allowing precise dosing and coverage. Endothall treatments were composed of a liquid formulation of the dipotassium salt of endothall and were applied when surface water

Table 1.-Characteristics of treated and untreated reference lakes, ecoregion within Minnesota and Division of Waters identifying number (DOW).

Lake (DOW)	Trophic[a] Status	Mean Secchi (m)[b]	Ecoregion[c]	Size (ha)	% Littoral	Maximum Depth (m)	Mean Depth (m)	Survey Points (≤4.6m)
Treated Lakes								
Blueberry (80-0034)	E	0.8	NLF	211	100	4.2	1.7	400
Clear (47-0095)	H	0.6	WCBP	201	83	5.5	2.8	225
Crookneck (49-0133)	M	2.9	NLF	74	80	6.7	3.3	166
Fish (70-0069)	E	1.6	CHF	70	40	8.5	4.6	128
Julia (71-0145)	E	0.6	CHF	62	100	4.6	2.1	106
Long (30-0072)	H	1.0	CHF	158	100	4.2	1.9	408
Lower Mission (18-0243)	M	3.8	NLF	292	60	8.5	3.9	220
Rush (71-0147)	E	0.6	CHF	65	100	3.4	1.3	112
Untreated Lakes								
Coal (77-0046)	M	2.4	NLF	69	40	8.2	4.7	101
Rebecca (27-0192)	E	1.9	CHF	105	50	9.2	4.2	159
Vails (73-0151)	E	1.6	CHF	64	80	6.1	2.7	174

[a] Mesotrophic (M), Eutrophic (E), Hypereutrophic (H) (Minnesota Department of Natural Resources).
[b] May-September mean Secchi (Minnesota Pollution Control Agency).
[c] Central Hardwood Forests (CHF), Northern Lakes and Forests (NLF), Western Corn Belt Plains (WCBP).

temperatures were between 10 and 15 C. The rate of application was continuously adjusted based on the water depth to achieve target concentrations in the area of treatment.

The reference lakes did not receive lakewide herbicide treatments during the years of monitoring. In addition to the experimental treatments discussed above, several of the study lakes received herbicide treatments prior to 2006. The MNDNR supervised lakewide treatments on Fish Lake in 2005, but previous shoreline endothall treatments on Julia and Rush lakes from 2000 to 2005 and on Lower Mission in 2005 were not supervised by the MNDNR.

Table 2.-Number of consecutive years lakes were treated with endothall and years of treatments.

Lake	Years Treated	Years of Treatments
Blueberry	1, 2, 3	2007, 2008, 2009
Clear	1, 2, 3	2007, 2008, 2009
Crookneck	1, 2, 3	2006, 2007, 2008
Fish	1, 2, 3, 4	2005, 2006, 2007, 2008
Long	1, 2, 3	2007, 2008, 2009
Lower Mission	1, 2, 3, 4	2006, 2007, 2008, 2009
Julia	1, 2, 3, 4	2006, 2007, 2008, 2009
Rush	1, 2, 3, 4	2006, 2007, 2008, 2009

Native macrophyte frequency

We employed the point intercept method (Madsen 1999) to survey aquatic vegetation in the study lakes. The sample sites (points) were located using the MNDNR Random Sample Generator extension for ArcView or the ArcMap GIS regularly spaced grid generating software extension. The distance between sample points ranged from 50 to 80 m depending on lake size. To determine the maximum depth of macrophyte colonization, we sampled beyond depths where macrophytes were found, although only depths of ≤4.6 m (littoral area defined by MNDNR) were analyzed for frequency and biomass to provide consistency across lakes and between years (Johnson et al. 2012). Due to the differences in the amount of littoral area among lakes, between 101 and 408 points were ≤4.6 m deep in each lake (Table 1). To examine native macrophyte response subsequent to spring endothall treatments, surveys for native macrophytes were conducted in August, the time of peak native macrophyte abundance. We also surveyed the plant communities in our study lakes in May and June of each year; results from these surveys are given in Jones (2010) and Johnson et al. (2012).

At each survey point, we measured water depth and sampled macrophytes with a weighted, double-headed, 0.33 m wide rake attached to a rope. The rake was tossed and then dragged for 3 m along the bottom before retrieving for analysis. Macrophytes retrieved on the rake at each point were identified and recorded as present. Floating and emergent macrophytes that were not easily sampled by the throw rake were rated based on their visible density within a 3 m radius of the boat. Rare taxa, or taxa that were not easily sampled by the throw rake due to small size or firm rooting, were noted as present within the lake when observed submerged or floating anywhere within the water column. The littoral frequency of occurrence of macrophyte species was calculated as the number of sites with the species present divided by the total number of sampled sites ≤4.6 m deep.

Native macrophyte biomass

Macrophyte biomass was sampled in conjunction with each point intercept survey. Biomass was sampled at 40 sample sites randomly selected from the point intercept sites using the MNDNR Random Sample Generator extension for ArcView. Biomass was collected using a single-headed, 0.33 m wide, 14-tine rake (Johnson and Newman 2011). The rake had an extendable pole to facilitate sampling in depths of up to 4.6 m. Samples were acquired by placing the tines of the rake flush with the lake bed and rotating 3 times on the axis of the rake's handle. The rake was rotated slowly as it was retrieved to keep macrophyte material on the rake. The collected macrophytes were then bagged and stored in an ice-filled cooler while in the field. Upon arrival to the lab, samples were stored at 5 C until they could be sorted.

Macrophytes from the biomass samples were separated by taxon and spun in a salad spinner to remove excess water. Individual taxa were placed into a preweighed paper bag, and fresh weight biomass was recorded. Bagged macrophytes were then dried for at least 48 h at 105 C and reweighed. Macrophyte biomass was converted to grams of dry mass (dm/m^2; rake sample area = 0.09 m^2). Mean biomass was determined as the mean mass of samples from ≤4.6 m depths for all native macrophytes collectively as well as each individual taxon.

Statistical analysis

All statistical analyses were completed using R statistical software version 2.10.1 (R Development Core Team 2008). Unless stated otherwise, differences were considered significant if $P < 0.05$. Given the categorical nature of frequency data (presence or absence), we used a chi-squared analysis to test between-year differences in native macrophyte frequency (collectively and for select individual taxa) in each individual lake. Additionally, a Wilcoxon 2-sample rank sum test was used to test differences in native macrophyte biomass (collectively and for select individual taxa) between years in individual lakes.

Results

Native macrophytes (all taxa combined)

Overall, there was no clear pattern to changes in native macrophyte frequency in treated or untreated lakes during our study (Table 3). Looking at individual lake responses, native macrophyte frequency in treated lakes did not change significantly between consecutive years (chi-squared; $P > 0.05$) after 15 of the 18 lake treatments, with the remaining comparisons showing 2 cases of increased native macrophyte frequency and 1 case of decreased frequency after treatments. Similarly, native macrophyte frequency in the untreated lakes did not change significantly between consecutive years in 7 of the 8 cases, with the remaining 1 case

Table 3.-Frequency (% occurrence) of native macrophytes in each study lake for treated and untreated lakes. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after 2005. "*" indicates the first year of endothall treatment. "♦" indicates significant change between years ($P < 0.05$, chi-squared).

	2005	2006		2007		2008		2009
Treated Lakes								
Eutrophic								
Blueberry	—	—		—*		38	♦	53
Clear	—	—		22*		23		24
Fish	—*	76		69		76		—
Julia	—	48*		51		50		42
Long	—	—		7*		11		15
Rush	—	29*	♦	50		63	♦	42
Mesotrophic								
Crookneck	—	99*		98		100		—
Lower Mission	—	87*		88		90		87
Untreated Lakes								
Eutrophic								
Rebecca	—	36		36	♦	46		—
Vails	—	40		30		21		23
Mesotrophic								
Coal	—	85		88		88		86

Table 4.-Mean August biomass (dry g/m^2) ± 2 SE of native macrophytes in each study lake. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after 2005. "*" indicates the first year of endothall treatment. There were no significant differences in biomass between years in any study lakes.

	2005	2006	2007	2008	2009
Treated Lakes					
Eutrophic					
Blueberrry	—	—	—*	150 ± 40	293 ± 318
Clear	—	—	—*	198 ± 60	98 ± 100
Fish	—*	370 ± 182	209 ± 114	645 ± 320	—
Julia	—	43 ± 26*	101 ± 64	263 ± 182	794 ± 604
Long	—	—	—*	59 ± 40	33 ± 36
Rush	—	1 ± 2*	60 ± 72	32 ± 34	21 ± 18
Mesotrophic					
Crookneck	—	371 ± 226*	650 ± 228	630 ± 190	—
Lower Mission	—	111 ± 54*	179 ± 102	185 ± 100	327 ± 250
Untreated Lakes					
Eutrophic					
Rebecca	—	44 ± 46	64 ± 36	128 ± 106	—
Vails	—	21 ± 18	1 ± 2	2 ± 2	57 ± 4
Mesotrophic					
Coal	—	336 ± 128	410 ± 104	266 ± 80	247 ± 98

showing increased frequency. Furthermore, we did not see changes in native macrophyte maximum depth of colonization in any lake.

Native macrophyte biomass varied substantially both within individual lakes (between years) and among lakes (Table 4). Mean native macrophyte biomass in many of our treated lakes was substantially higher in the final year of our study when compared to treatment year 1; however, this higher mean biomass coincided with greater variability. Consequently, the observed changes in mean treated lake biomass over the years of treatment were not significant ($P > 0.05$; Table 4). The most notable changes in native macrophyte biomass occurred in 2 of the study lakes between the last 2 years of treatment. In Lower Mission, mean native biomass increased by 75% ($P = 0.15$) while in Julia, biomass increased by 300% ($P = 0.33$) compared to the previous year (Table 4). Biomass also increased noticeably between years 1 and 2 of treatment in Crookneck (371 ± 226 to 650 ± 228 g/m^2, $P = 0.45$) and Rush (1 ± 2 to 60 ± 72 g/m^2, $P = 0.14$) and between years 2 and 3 in Blueberry (150 ± 40 to 293 ± 318 g/m^2, $P = 0.33$), but declined appreciably between years 2 and 3 in Clear Lake (198 ± 60 to 98 ± 100 g/m^2, $P = 0.15$; Table 4). Although some lakes experienced large biomass increases in the final year of treatment, most of these changes were due to a few species in each lake, and large increases in biomass were typically observed at a relatively small number of points as isolated patches of dense growth.

We observed no changes in native macrophyte species richness (number of taxa; Table 5) or the mean number of native taxa per point (Table 6) between consecutive years of treatment or between years 1 and 4 of treatment. Similarly, richness and taxa per point did not change between survey years in untreated lakes. Findings were similar when analyses were restricted to submersed taxa (floating and emergent taxa excluded).

Looking at lakes by trophic status (eutrophic and mesotrophic), there was no difference in the observed pattern of change in mean frequency (Table 3), biomass (Table 4), species richness (Table 5), or native macrophytes per point (Table 6) over time; however, mesotrophic lakes consistently had higher native macrophyte frequency, biomass, native species richness, and native taxa per point than eutrophic lakes.

Ceratophyllum demersum

C. demersum was present every year in all study lakes; however, its mean frequency and mean biomass varied greatly both within lakes (between years) and among lakes. Overall, there was no significant change of *C. demersum* frequency or biomass between years in any of the treated or untreated lakes, although biomass increased dramatically in several treated lakes (Table 7).

Elodea canadensis

Elodea canadensis was found in 7 of the 8 treatment lakes and 2 of the 3 untreated lakes. Overall, there was no pattern to changes in *E. canadensis* mean frequency or mean biomass

Table 5.-Annual species richness in each study lake and list of all macrophyte taxa found in our study lakes. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after the first treatment year. "*" indicates the first year of endothall treatment.

	2005	2006	2007	2008	2009
Treated Lakes					
Eutrophic					
Blueberrry	—	—	—*	19	23
Clear	—	—	12*	14	11
Fish	—*	16	19	16	—
Julia	—	14*	8	12	10
Long	—	—	4*	8	12
Rush	—	9*	11	10	11
Mesotrophic					
Crookneck	—	21*	22	26	—
Lower Mission	—	29*	29	32	37
Untreated Lakes					
Eutrophic					
Rebecca	—	9	11	10	—
Vails	—	7	11	7	11
Mesotrophic					
Coal	—	30	31	33	33

Note. Macrophyte Taxa Found:

Bidens beckii
Brasenia schreberi
Eleocharis acicularis
Chara sp.
Ceratophyllum demersum
Elodea canadensis
Equisetum fluviatile
Fontinalis antipyretica
Hippuris vulgaris
Lemna minor
Lemna trisulca
Myriophyllum sibiricum
Myriophyllum spicatum
Najas flexilis
Najas guadalupensis
Nitella sp.
Nymphaea odorata
Nuphar variegata
Potamogeton amplifolius
Phragmites australis
Potamogeton crispus
Potamogeton foliosus
Potamogeton friesii
Potamogeton gramineus
Potamogeton illinoensis
Potamogeton spp.
Potamogeton nodosus
Potamogeton praelongus
Potamogeton pusillus
Potamogeton richardsonii
Potamogeton robbinsii
Potamogeton strictifolius
Potamogeton zosteriformis
Ranunculus longirostris
Scirpus acutus
Sparganium eurycarpum
Sagittaria graminea
Stuckenia pectinata
Spirodella polyrhiza
Typha sp.
Utricularia vulgaris
Vallisneria americana
Wolffia columbiana
Zosterella dubia
Zizania sp.
Zannichellia palustris

Table 6.-Mean number of native macrophyte taxa per point in treated and untreated lakes. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after the first treatment year. "*" indicates the first year of endothall treatment.

	2005	2006	2007	2008	2009
Treated Lakes					
Eutrophic					
Blueberrry	—	—	—*	0.7	1.5
Clear	—	—	0.5*	0.1	0.7
Fish	—*	1.3	1.3	1.5	—
Julia	—	0.9*	1	1.1	0.8
Long	—	—	0.1*	0.1	0.2
Rush	—	0.4*	0.8	1	0.8
Mesotrophic					
Crookneck	—	2.5*	2.4	2.5	—
Lower Mission	—	2.3*	2.2	2.4	2.8
Untreated Lakes					
Eutrophic					
Rebecca	—	0.4	0.4	0.6	—
Vails	—	0.2	0.2	0.4	0.2
Mesotrophic					
Coal	—	3.8	2.9	3.6	3.7

in treated lakes between treatment years (Table 8); however, 3 treated lakes showed significant changes in *E. canadensis* frequency between years. *E. canadensis* frequency tripled between years 1 and 2 of treatment in Rush Lake ($P < 0.001$) and between years 2 and 3 of treatment in Blueberry Lake ($P < 0.001$). By contrast, *E. canadensis* frequency in Julia Lake decreased by a factor of 4 between years 1 and 4 of treatment ($P < 0.001$).

Potamogeton spp.

Broadleaf *Potamogeton* spp. were found throughout a moderate number of lakes in the study at fairly low frequency and consisted of the following taxa: *P. amplifolius*, *P. gramineus*, *P. illinoensis*, *P. nodosus*, *P. praelongus*, and *P. richardsonii.* Due to the sparse distribution of these native *Potamogeton* species throughout the study lakes, the frequency and biomass of a combination of these 6 broadleaf *Potamogeton* taxa were analyzed as if they were a single species (collectively referred to as *Potamogeton* spp. hereafter). Overall mean frequency of the native broadleaf *Potamogeton* species did not change significantly in any treated lakes between any of the years of treatment. Similarly, mean *Potamogeton* spp. biomass in most treated lakes was highly variable between years and showed no clear pattern of change after treatments. Untreated lakes also showed variability in mean *Potamogeton* spp. between years, but only 1 untreated lake (Coal) had considerable amounts of *Potamogeton* spp. present.

Chara sp.

The native macroalga *Chara* was found in all of our study lakes, with the exception of one untreated lake (Vails). In most treated lakes, the mean frequency of *Chara* sp. changed little over the treated years (Table 9). Mean biomass of *Chara* sp. in many treated lakes increased noticeably but insignificantly between years, however, particularly between the first and final year of treatment (Table 9). Similarly, overall *Chara* sp. biomass increased as a proportion of total native biomass from 7% in year 1 of treatment to 45% in year 4. Despite these increases between years 1 and 4 of treatment, we did not see significant increases between other years in individual lakes due to the high variability among biomass samples. In the 2 untreated lakes where *Chara* sp. occurred (Coal and Rebecca), its frequency and biomass (≤ 1 g/m^2 in any given year) remained low throughout the study period and did not change between years.

Table 7.-Frequency and mean biomass (± 2) SE of *Ceratophyllum demersum* in treated and untreated lakes. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after the first treatment year. There was no significant ($P > 0.05$) difference in frequency or biomass between years in individual lakes. "*" indicates the first year of endothall treatment.

	Frequency					Biomass g/m2				
	2005	**2006**	**2007**	**2008**	**2009**	**2005**	**2006**	**2007**	**2008**	**2009**
Treated Lakes										
Eutrophic										
Blueberry	—	—	—*	16	16	—	—	—*	60 ± 13	46 ± 13
Clear	—	—	24*	17	19	—	—	—*	14 ± 6	62 ± 56
Fish	—*	76	67	73	—	—*	352 ± 285	198 ± 137	616 ± 187	—
Julia	—	7*	3	11	8	—	1 ± 1*	1 ± 1	3 ± 1	1 ± 1
Long	—	—	0*	0	2	—	—	—*	10 ± 9	1 ± 1
Rush	—	8*	6	5	4	—	1 ± 1*	1 ± 1	0	0
Mesotrophic										
Crookneck	—	78*	77	76	—	—	109 ± 23*	331 ± 78	384 ± 16	—
Lower Mission	—	78*	64	64	40	—	39 ± 9*	36 ± 9	35 ± 11	63 ± 15
Untreated Lakes										
Eutrophic	—									
Rebecca		35	33	40	—	—	87 ± 56	62 ± 34	87 ± 54	—
Vails	—	36	24	20	4	—	48 ± 23	2 ± 2	4 ± 2	41 ± 13
Mesotrophic										
Coal	—	52	40	49	44	—	19 ± 5	19 ± 13	28 ± 12	31 ± 16

Discussion

Johnson et al. (2012) found that curlyleaf pondweed was successfully controlled by early season endothall treatments. Our study found that repeated, early season endothall treatments in these same lakes did not have an overall negative impact on native aquatic macrophytes. Other studies have similarly reported a lack of negative effects from endothall on nontarget native macrophytes (Skogerboe and Getsinger 2002, Skogerboe et al. 2008). Skogerboe et al. (2008) found that early season, low-dose endothall treatments reduced

Table 8.-Frequency and mean biomass (± 2 SE) of *Elodea canadensis* in treated and untreated lakes. "♦" indicates significant change between years ($P < 0.05$, chi-squared). A dash (–) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after the first treatment year. "*" indicates the first year of endothall treatment.

	Frequency					Biomass g/m2				
	2005	**2006**	**2007**	**2008**	**2009**	**2005**	**2006**	**2007**	**2008**	**2009**
Treated Lakes										
Eutrophic										
Blueberry	—	—	—*	13	♦ 38	—	—	—*	16 ± 6	248 ± 165
Clear	—	—	24*	17	19	—	—	—*	6 ± 1	22 ± 14
Fish	—*	—	—	—	—	—*	—	—	—	—
Julia	—	40*	42	22	♦ 9	—	45 ± 12*	43 ± 14	7 ± 1	4 ± 1
Long	—	—	0*	0	0	—	—	—*	1 ± 1	0
Rush	—	16*	♦ 46	56	36	—	0*	77 ± 17	39 ± 12	6 ± 3
Mesotrophic										
Crookneck	—	3*	2	5	—	—	1 ± 1*	1 ± 1	1 ± 1	—
Lower Mission	—	17*	19	33	24	—	1 ± 1*	1 ± 1	24 ± 12	36 ± 4
Untreated Lakes										
Eutrophic	—									
Rebecca		—	—	—	—	—	—	—	—	—
Vails	—	13	2	5	5	—	0	6 ± 2	1 ± 1	7 ± 2
Mesotrophic										
Coal	—	33	19	25	32	—	10 ± 2	2 ± 1	2 ± 1	9 ± 3

Table 9.-Frequency and mean biomass (± 2 SE) of *Chara* sp. in treated and untreated lakes with means ± 2 SE. A dash (—) indicates that the data were unavailable either due to change in lake treatment or because the lake was added after the first treatment year. There was no significant ($P > 0.05$) difference in frequency or biomass between years in individual lakes. "*" indicates the first year of endothall treatment.

	Frequency					Biomass g/m2				
	2005	2006	2007	2008	2009	2005	2006	2007	2008	2009
Treated Lakes										
Eutrophic										
Blueberry	—	—	—*	6	14	—	—	—*	33 ± 15	40 ± 12
Clear	—	—	4*	1	1	—	—	—*	4 ± 3	13 ± 12
Fish	—*	3	1	3		—*	1 ± 1	4 ± 2	3 ± 2	—
Julia	—	13*	23	24	20	—	1 ± 1*	51 ± 24	224 ± 145	697 ± 337
Long	—	—	1*	7	9	—	—	—*	15 ± 12	12 ± 10
Rush	—	1*	7	16	10	—	0*	1 ± 1	1 ± 1	14 ± 8
Mesotrophic										
Crookneck	—	9*	7	9	—	—	4*	2 ± 2	3 ± 2	—
Lower Mission	—	27*	26	25	37	—	44*	52 ± 16	44 ± 11	157 ± 26
Untreated Lakes										
Eutrophic										
Rebecca	—	0	0	6	—	—	0	0	0	—
Vails	—	—	—	—	—	—	—	—	—	—
Mesotrophic										
Coal	—	5	4	2	0	—	0	0	0	0

curlyleaf frequency and biomass while not harming native macrophytes. They also reported that *E. canadensis* and *C. demersum* subsequently increased in some treated lakes. Although we did not see many significant increases in native macrophyte frequency or biomass over the 4 years of endothall treatment, biomass increased substantially in many of the treated lakes. By contrast, in the untreated lakes, native macrophyte biomass varied from year to year and did not show a clear pattern of change.

Throughout our study, lakewide native macrophyte species richness (Table 5) and the number of species per point (Table 6) did not change significantly in most treated or untreated lakes. This indicates that repeated early season endothall treatments generally did not result in an overall loss of the number of native macrophyte species or decreased diversity of the native plant community in treated lakes. The lack of an increase in the frequency of native macrophytes after curlyleaf control is also not unexpected, particularly for eutrophic lakes where phytoplankton were abundant and light availability was likely the main factor limiting macrophyte distribution (Barko and Smart 1981, Best et al. 2001). In these lakes, removing curlyleaf did not generally improve water clarity (Johnson et al. 2012; Table 10), and we did not see a change in the maximum depth of native macrophyte colonization. Similarly, mesotrophic lakes did not show increases in frequency or maximum depth of colonization. The most notable effects we observed in treated lakes were changes in the abundance (biomass) of macrophytes; however, frequency is based on macrophyte presence or absence and is thus not particularly sensitive to changes in macrophyte abundance.

The lack of a significant decrease and the substantial but insignificant increase of overall native macrophyte biomass over 4 treatment years (Table 4) suggest that

Table 10.-Mean July/August Secchi depth in treated and untreated lakes from 2006 to 2009. Pretreatment data (PRE) include mean July/August Secchi depth 2 to 3 years prior to endothall treatment. Data provided by the Minnesota Pollution Control Agency. (http://www.pca.state.mn.us).

	PRE	2006	2007	2008	2009
Eutrophic Treated					
Blueberry	0.5	ˆ	ˆ	0.8	0.7
Clear	0.5	ˆ	ˆ	0.4	0.3
Fish[a]	1.0	1.8	1.1	1.4	1.3[a]
Julia	0.4	0.6	0.4	0.5	0.5
Long	0.3	ˆ	ˆ	0.3	0.4
Rush	0.4	0.4	0.3	0.4	0.3
Eutrophic Untreated					
Rebecca[b]	—	0.6	0.6	0.8	0.6[b]
Vails	—	0.6	0.4	0.5	0.6
Mesotrophic Treated					
Crookneck[a]	2.9	2.4	2.7	2.2	2.6[a]
Lower Mission	1.1	1.3	1.6	2.6	2.5
Mesotrophic Untreated					
Coal	—	2.2	2.7	2.7	3.0

[a] Not treated in 2009
[b] Treated in 2009
ˆIncluded in average pretreatment Secchi data

early season application of endothall did not hinder native macrophyte sprouting or growth and that the effective control of curlyleaf (Johnson et al. 2012) may have promoted increased abundance of some native macrophytes. Native macrophyte species that actively grow during the early spring or that persist throughout the year may be most susceptible to endothall treatments (Skogerboe et al. 2008). *E. canadensis* has the ability to grow quickly after ice cover recedes (Cook and Urmi-König 1984), and *C. demersum* persists year-round in Minnesota lakes (Spencer and Wetzel 1993); however, we did not see major decreases in mean frequency of *C. demersum* (Table 7) or *E. canadensis* (Table 8) in most treated lakes. Curlyleaf pondweed has low carbohydrate reserves during the time of endothall application (Woolf and Madsen 2003), making recovery after endothall treatments difficult, whereas native macrophytes like *E. canadensis* and *C. demersum* do not exhaust carbohydrate reserves during this time and have also been shown to recover after endothall treatments (Skogerboe and Getsinger 2002). Furthermore, endothall has been shown to target metabolically active macrophytes. Although curlyleaf pondweed actively grows immediately after ice-out, the majority of native macrophytes in our study are not thought to be metabolically active until water temperatures exceed 15 C (Westerdahl and Getsinger 1988).

Considering that both *C. demersum* and *E. canadensis* were present in many of our study lakes during early season endothall treatments, positive effects due to reduced competition with curlyleaf for light or nutrients may possibly have been masked by nonlethal damage from endothall contact. In untreated lakes, *C. demersum* and *E. canadensis* frequency showed no clear pattern of change; however, *C. demersum* biomass increased dramatically in several treated lakes between years 1 and 4 of treatment (Table 7). Similarly, *E. canadensis* biomass remained unchanged or increased slightly in every treated lake with the exception of Julia and Blueberry, and we observed a substantial increase of *E. canadensis* biomass in Blueberry Lake between year 2 (15.5 ± 12.4 g/m^2) and year 3 (248.3 ± 154.4 g/m^2) of treatment. In addition, large mats of *E. canadensis* were found in the same areas of Blueberry Lake where curlyleaf was abundant the previous year. This suggests that the increased biomass of *E. canadensis* in Blueberry was associated with reductions of curlyleaf. In Lake Julia, *E. canadensis* frequency decreased between year 1 and year 4, possibly due to competition with other native macrophytes, particularly *Chara* sp. and *Najas guadalupensis*. The biomass of N. guadalupensis in Julia increased each year from 7.6 ± 8.8 g/m^2 in year 1, to 99.7 ± 15.4 g/m^2 in year 4, while *E. canadensis* biomass decreased each year during the same time period (Table 8), although both these changes were insignificant.

We expected that various native *Potamogeton* spp. would be affected by endothall treatments because many *Potamogeton* species are sensitive to endothall. Laboratory studies have shown that *P. praelongus*, *P. nodosus*, and *P. illinoensis* are highly sensitive to endothall treatments (Skogerboe and Getsinger 2001, 2002); however, many of these native *Potamogeton* species do not actively grow during the time of early season treatment in Minnesota. The abundance of native *Potamogeton* spp. was also very low in our study lakes, making it difficult to detect any effects. We did not see major decreases in any native *Potamogeton* species in our study lakes, suggesting that there were few nontarget effects of the endothall treatments. The untreated lake that contained abundant native *Potamogeton* species (Coal) also did not show any trends of declining biomass or frequency for *Potamogeton* spp., suggesting that the curlyleaf infestation in that lake was not causing continued declines in native *Potamogeton* taxa. Water clarity (Table 10) and native macrophyte growth in Coal Lake were high and not typical of lakes where large curlyleaf invasions occur, which may have allowed *Potamogeton* species to coexist with curlyleaf in this lake.

Endothall treatments did not have a negative effect on *Chara* sp. and may have been associated with enhanced growth. Extremely large increases of *Chara* sp. were observed in some of our treatment lakes; however, the rake method for biomass collection may possibly overestimate high densities of macrophytes (Johnson and Newman 2011), and the increases of *Chara* sp. biomass may have been magnified due to the collection methods. Despite possible overestimates of biomass, it is likely that our estimates accurately reflect relative changes in biomass.

Our finding that *Chara* sp. persisted or increased in our treated lakes is not unexpected. Charophytes are green macroalgae that differ greatly in physiology compared to aquatic angiosperms; thus, sensitivity to the same herbicides is not likely. Herbicide tolerance in charophytes may be due to a thick calcium and magnesium coating that may act as a barrier to chemicals (Wade 1990). Furthermore, charophytes produce spores (oospores) that are released in large numbers, sprout annually (Bonis and Grillas 2002), and may result in high recruitment. Wade (1990) found that in lakes where herbicides have been used, *Chara* sp. colonized areas previously inhabited by angiosperms. Similar results have been found in Minnesota where increases of *Chara* sp. were observed shortly after fluridone treatments in Lac Lavon (Crowell et al. 2006) and after treatment with 2,4-D, triclopyr, and endothall in Lake Minnetonka, where *Chara* sp. doubled in frequency within 2 years of treatment (Skogerboe and Netherland 2008).

In addition to herbicide tolerance and dormancy during application, there are other reasons why *Chara* sp. may have increased in our study lakes. Some charophytes are able to grow in areas of low light intensity due to a low compensation point (Casanova and Brock 1999, Shilla and Dativa 2008). Charophytes are also rapid colonizers; Meijer et al. (1999) found that after the removal of fishes from 3 eutrophic lakes, charophytes had colonized 50% of the littoral area within 2 months. Although *Chara* sp. is a rapid colonizer that can grow quickly in disturbed habitats, charophytes have been known to be poor competitors with other established macrophytes (Wade 1990). High densities of other macrophytes limit the growth and germination of charophytes, whereas low densities of vegetation provide opportunities for *Chara* sp. to quickly establish thick mats (Bonis and Grillas 2002). In our study, *Chara* was able to inhabit areas where curlyleaf was controlled by herbicide over consecutive years.

Our finding of a lack of negative effects on native macrophytes is restricted to early season, low-dose endothall treatments; lakewide treatment with other herbicides may harm native macrophytes. Results from 2 other Minnesota lakes showed that consecutive years of early season treatment with fluridone resulted in dramatic declines in *C. demersum* in one lake, and in another lake, combined treatments with 2,4-D and endothall or tricolopyr and endothall resulted in declines of *C. demersum* and *E. canadensis* (Jones 2010).

Although native species richness, taxa per point, and frequency of occurrence for native macrophytes were all substantially higher in mesotrophic lakes than in eutrophic lakes, there was no evidence of differential response to treatment. Thus early season control of curlyleaf with endothall seems suitable for both eutrophic and mesotrophic lakes, but such treatments will likely not result in rapid increases in native macrophytes in either lake type.

Increased native macrophyte biomass in some of our treated lakes may have been associated with the significant reductions of curlyleaf pondweed documented by Johnson et al. (2012). However, early season endothall treatments may have provided the most benefit for macrophyte species that do not actively grow during the time of herbicide application. Macrophytes, such as *Chara* sp., which germinate annually from seeds or propagules, are not likely to be harmed by early season herbicide treatments, whereas macrophytes that persist over the winter and actively grow in the early spring may be affected by early season endothall treatments. Overall, our results show that early season, low-dose endothall treatments do not cause substantial damage to native macrophyte communities and may promote increased abundance of some taxa after several years of treatment through effective control of curlyleaf.

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