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Early Season Applications of Endothall and 2,4-D for Selective Control of Eurasian Watermilfoil and Curlyleaf Pondweed in Minnesota Lakes: Year Two Evaluations of Submersed Plant Communities

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PURPOSE: This study evaluates early spring application of low doses of the herbicides endothall (1 mg/L active ingredient (ai)) combined with 2,4-D (0.5 mg/L acid equivalent (ae)), applied annually in early spring to selectively control the invasive aquatic species curlyleaf pondweed (*Potamogeton crispus* L) and Eurasian watermilfoil (*Myriophyllum spicatum* L), in two Minnesota lakes.

BACKGROUND: The invasive submersed plants Eurasian watermilfoil (*Myriophyllum spicatum* L.) and curlyleaf pondweed (*Potamogeton crispus* L.) have become widespread problems in many northern lakes. Both species begin growing toward the water surface in early spring when ice first disappears from lakes, thereby forming canopies before many native submersed plants begin to emerge. These canopies adversely affect navigation, recreation, and water quality. Early season growth of these invasive plants may present managers with a window to employ treatment strategies when many native plants remain dormant. Selective removal of invasive species can improve recreational use of the lake, stabilize water quality, and increase native plant diversity (Getsinger et al. 1997). The potential for protection of native aquatic plant communities using herbicides has been documented by numerous small-scale research studies (Netherland et al. 1997; Sprecher et al. 1998; Skogerboe and Getsinger 2001, 2002; Poovey et al. 2002) and field demonstrations (Madsen et al. 2002; Poovey et al. 2004).

The contact herbicide endothall (7-oxabicyclo[2.2.1]heptane-2-3dicarboxylic acid) provides effective control of a wide range of aquatic plants including monocotyledons (monocots) and dicotyledons (dicots) (Westerdahl and Getsinger 1988, Madsen 1997). This herbicide is effective at controlling Eurasian watermilfoil and curlyleaf pondweed (Poovey et al. 2002). Native aquatic plant sensitivity to endothall varies greatly among species (Skogerboe and Getsinger 2001, 2002). Species such as Illinois pondweed (*Potamogeton illinoensis* Morong.), southern naiad [*Najas guadalupensis* (Sprengel) Magnus], and sago pondweed (*Stukenia pectinata* L.) are sensitive to endothall, while coontail (*Ceratophyllum demersum* L.) is moderately sensitive. Other plants such as elodea (*Elodea canadensis* Michx), wild celery (*Vallisneria americana* L.), water stargrass [*Zosterella dubia* (Jacq.) MacM.], and many floating-leaf and emergent species are more tolerant of endothall.

Generally, dicots such as Eurasian watermilfoil are susceptible to 2,4-D, while monocots such as curlyleaf pondweed are less so. Although variable control of Eurasian watermilfoil using 2,4-D has been reported (Elliston and Steward 1972; Lim and Lozoway 1976; Hoeppel and Westerdahl 1983), good control was achieved when 2,4-D exposure times exceeded 24 hr (Green and Westerdahl 1990). As 2,4-D is more specific to dicots, it can frequently be used for selective control of Eurasian watermilfoil, where native plant populations are dominated by monocots such as wild celery and elodea, including species in the *Potamogeton, Stukenia, and Zannichellia* genera. In situations where native dicots are abundant in submersed plant communities, selectivity of 2,4-D may be greatly limited.

Previous outdoor mesocosm studies showed that applying endothall (1 mg ai/L) to curlyleaf pondweed in low water temperatures (12 to 15 °C) resulted in better control compared to applications in warmer temperatures ($\geq$ 20 °C) (Poovey et al. 2002). In addition, results from small-scale greenhouse evaluations showed that combining endothall (1 mg ai/L) with a low rate of 2,4-D (0.5 mg ae/L) provided improved control of Eurasian watermilfoil. This herbicide combination may therefore improve control of both invasive species in a single treatment event. Combining low rates of two herbicides to target two invasive species could result in reduced environmental loading of herbicides to aquatic sites, improved native plant recovery, and savings in labor and costs.

Early season applications are designed to control Eurasian watermilfoil and curlyleaf pondweed before many susceptible native plants, such as pondweeds, are actively growing. Some native plant species such as coontail and elodea may be actively growing during early spring applications; however, they are more tolerant at the recommended herbicide combination application rates (Skogerboe et al. 2004). Treatment of Eurasian watermilfoil and curlyleaf pondweed early in the growing season can provide temporal selectivity by preventing the exposure of sensitive native plants to the herbicides during periods of active growth. Early removal of the invasive plants allows native species to grow with greatly reduced competition. Also, the early removal of curlyleaf pondweed prevents the formation of new turions critical for the continued survival of that plant in future years.

While endothall applied at 1 mg/L ai is effective at controlling curlyleaf pondweed, endothall alone would require application rates of 2 to 3 mg ai/L to control Eurasian watermilfoil. While many native species that are sensitive to endothall (e.g., pondweeds and naiads) remained dormant until that herbicide had sufficiently degraded or dissipated, some species such as coontail would have been at risk at the higher rates. Other herbicides such as 2,4-D applied alone at 2 to 4 mg ae/L to control Eurasian watermilfoil would not have controlled curlyleaf pondweed and would have risked damage to spatterdock (*Nuphar advena*) and fragrant water lily (*Nymphaea odorata*), which are protected by Minnesota Department of Natural Resources (MNDNR) from general herbicide treatments. Failure to treat both invasive species could result in replacement of one invasive species with the other, resulting in further degradation of the native plant community.

This study was initiated in 2003, and the first herbicide treatment was conducted in 2004 (Skogerboe and Getsinger 2006). The study was continued in 2005 to assess long-term effects of manipulating plant communities on water quality and fisheries. In 2004 and 2005, combinations of 2,4-D and endothall were applied in early spring to maintain Eurasian watermilfoil and curlyleaf pondweed populations below nuisance levels, while protecting native plant communities. Plant, fish, and invertebrate communities and water quality were monitored to determine the long-term effects of lake management using aquatic herbicides on these biotic organisms. This document will summarize results of the plant community changes through the 2005 growing season. Results of changes to fish and invertebrates are reported elsewhere.

MATERIALS AND METHODS: Four eutrophic to mesotrophic lakes in Minnesota (Auburn, Pierson, Bush, and Zumbra) were selected for this long-term evaluation based on the following criteria: a) 50 to 100 hectares (125 to 250 acres) and similar morphometry; and b) a large percentage of the littoral zone (≥ 50 percent) occupied by submersed plants, and dominated by Eurasian watermilfoil and curlyleaf pondweed (≥ 60 percent) coverage). Auburn and Pierson were chosen as untreated references, and Bush and Zumbra were designated for herbicide treatments. All of these water bodies are within the Minneapolis/St. Paul, MN, metropolitan area and serve as recreational and fisheries resources for local residents.

Assessment of plant communities. Plant species diversity (percent occurrence) was evaluated in the littoral zones (≤ 4.5 m deep) using a quantitative point intercept method developed by Madsen (1999). A sampling grid (50 m × 50 m) was employed for each lake using Garmin MapSource© U.S. Topographic software, downloaded to a Garmin global positioning system (GPS) accurate to 4 m. At each sample point, a double metal rake head (30 cm long) attached to a rope was thrown twice (in opposite directions), approximately 3 to 6 m away from the sampling boat, and retrieved slowly along the bottom. Plants harvested by the rake toss were identified to species, as were plants that could be visually identified growing below the water surface.

Percent occurrence of plant species was calculated by dividing the number of points where a particular species was present by the total number of sample points in the littoral zone (≤ 4.6 m [15 ft]). Over 150 sampling points were used to calculate percent occurrence data on each lake. June and August posttreatment data were compared to the same pretreatment months using Chi Square analysis ($p \leq 0.05$). The total number of all species (invasive and native) and of native species alone per sample point was determined and means were calculated.

Relative plant biomass was evaluated by randomly selecting 30 to 35 sample points from species diversity evaluations and quantifying the amount of plant material retrieved from each point. Samples were collected using a 36-cm-wide rake attached to the end of a 3-m pole. At each sample point, the rake was lowered from the boat perpendicular to the bottom and then raised up to the water surface with a slow clockwise twisting motion. Plant species from each sample were separated and oven-dried at 70 °C to a constant weight. Data were log transformed to preserve the assumptions of normality and equal variance, and posttreatment data were compared to pretreatment data using analysis of variance (ANOVA). Means were separated using the least significant difference method (LSD, $p \leq 0.05$).

Pretreatment evaluations (species occurrence and plant biomass) were conducted from 10 to 30 June and from 15 to 31 August 2003 on all lakes, and in mid April 2004 and 2005 on herbicide-treated lakes (Bush and Zumbra) prior to chemical applications. Posttreatment plant evaluations (species diversity and plant biomass) were conducted during the same periods in June and August of 2004 and 2005 on all lakes.

Herbicide applications. Based on pretreatment plant evaluations in 2003, and on 1-year posttreatment evaluations in 2004, Bush and Zumbra Lakes were divided into various herbicide treatment zones consisting of distinct blocks and narrow strips (Figures 1 through 4). Treatment

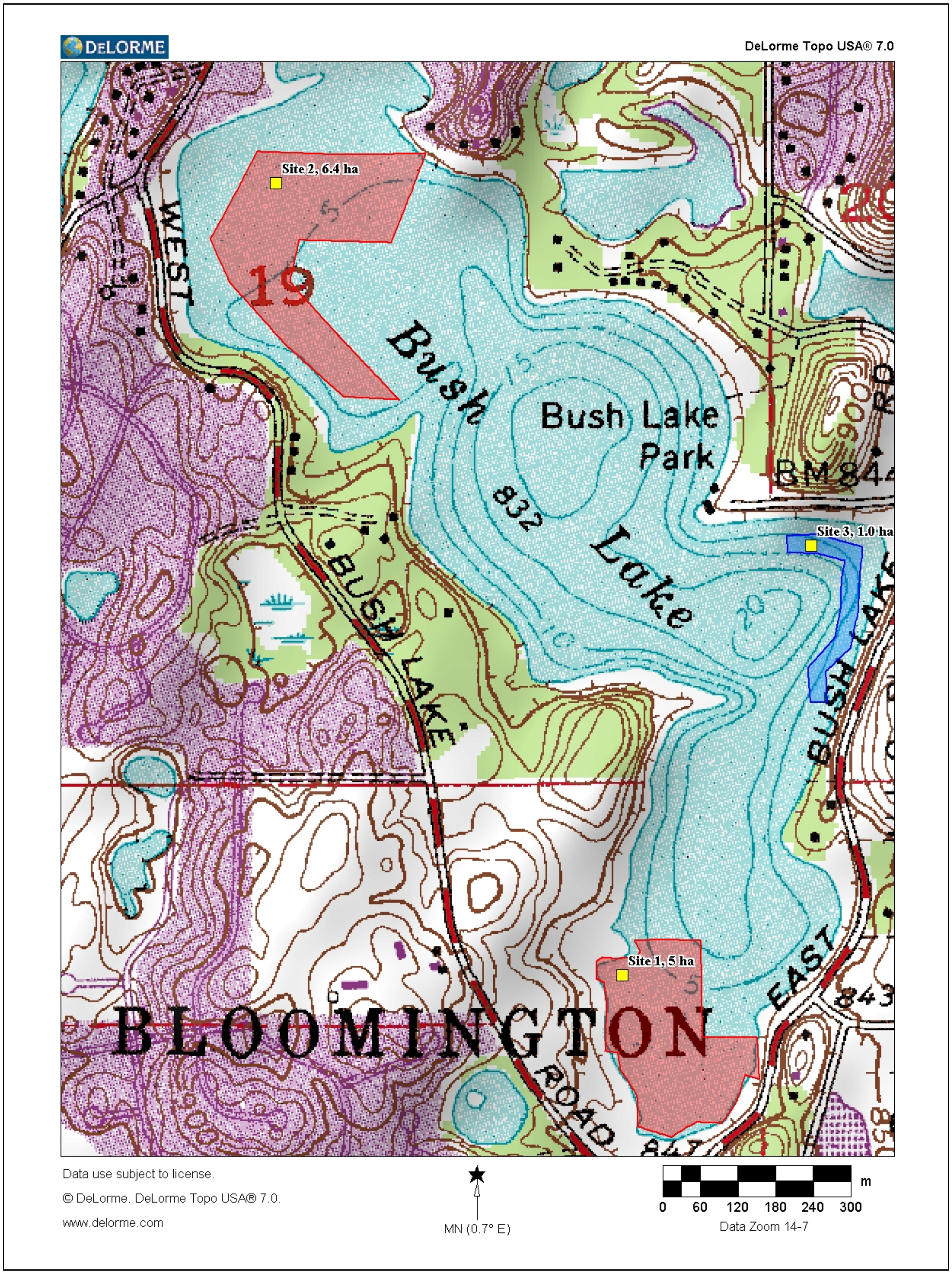

Figure 1. Bush Lake 2004 herbicide treatments. Liquid endothall and 2,4-D were applied to blocks (red), and granular endothall was applied to strips (blue).

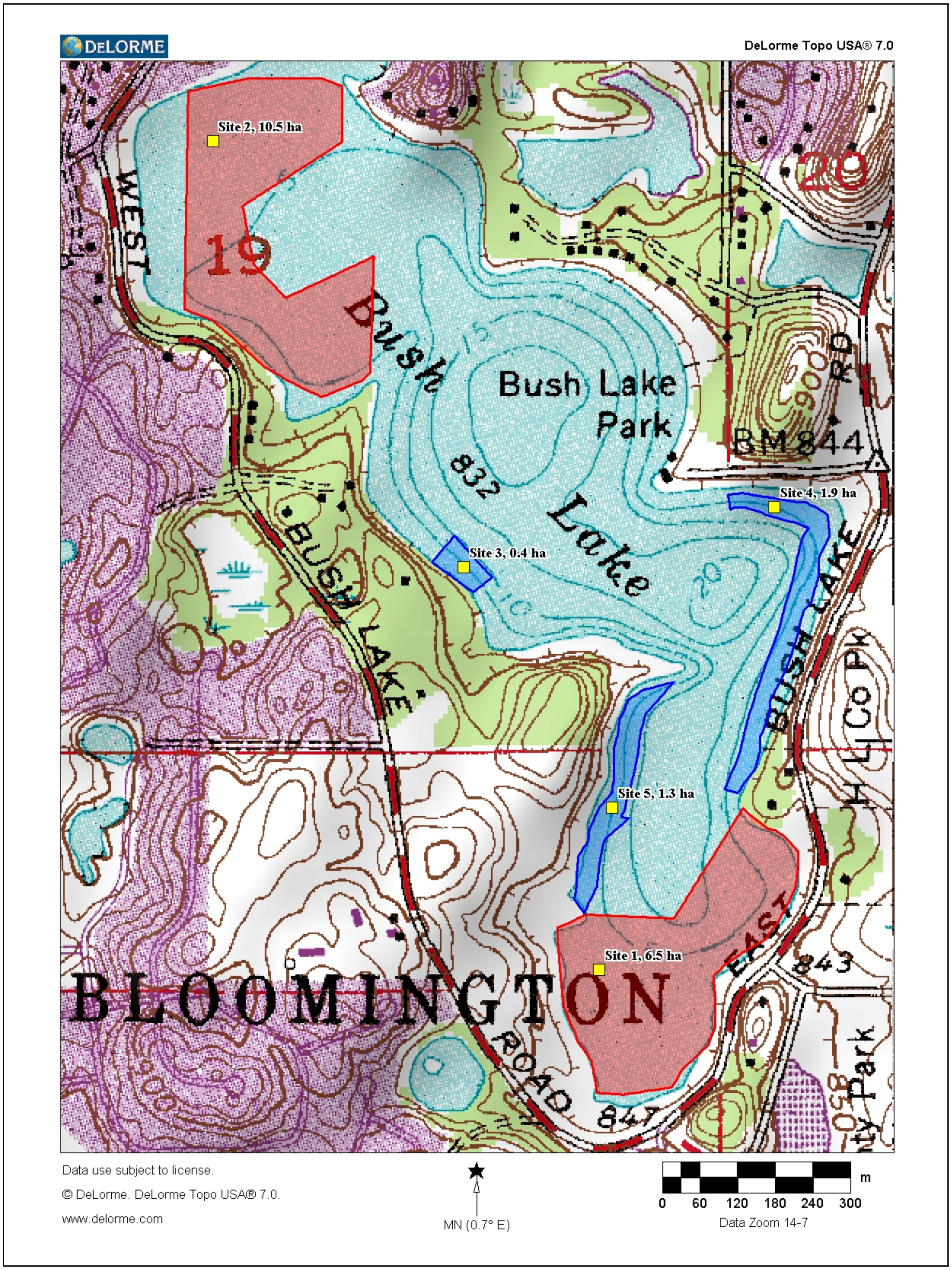

Figure 2. Bush Lake 2005 herbicide treatments. Liquid endothall and 2,4-D were applied to blocks (red), and granular endothall was applied to strips (blue).

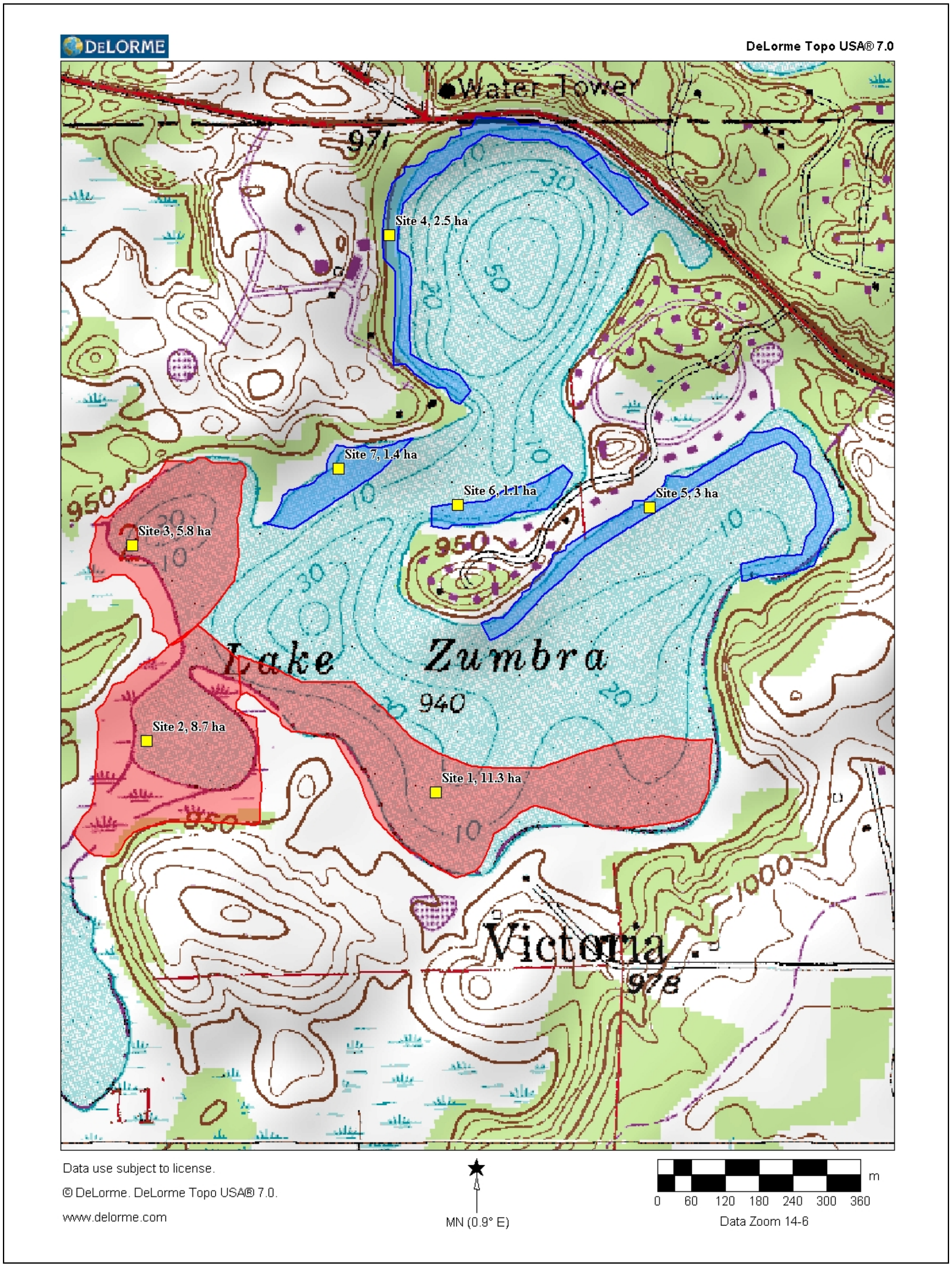

Figure 3. Zumbra Lake 2004 herbicide treatments. Liquid endothall and 2,4-D were applied to blocks (red), and granular endothall was applied to strips (blue).

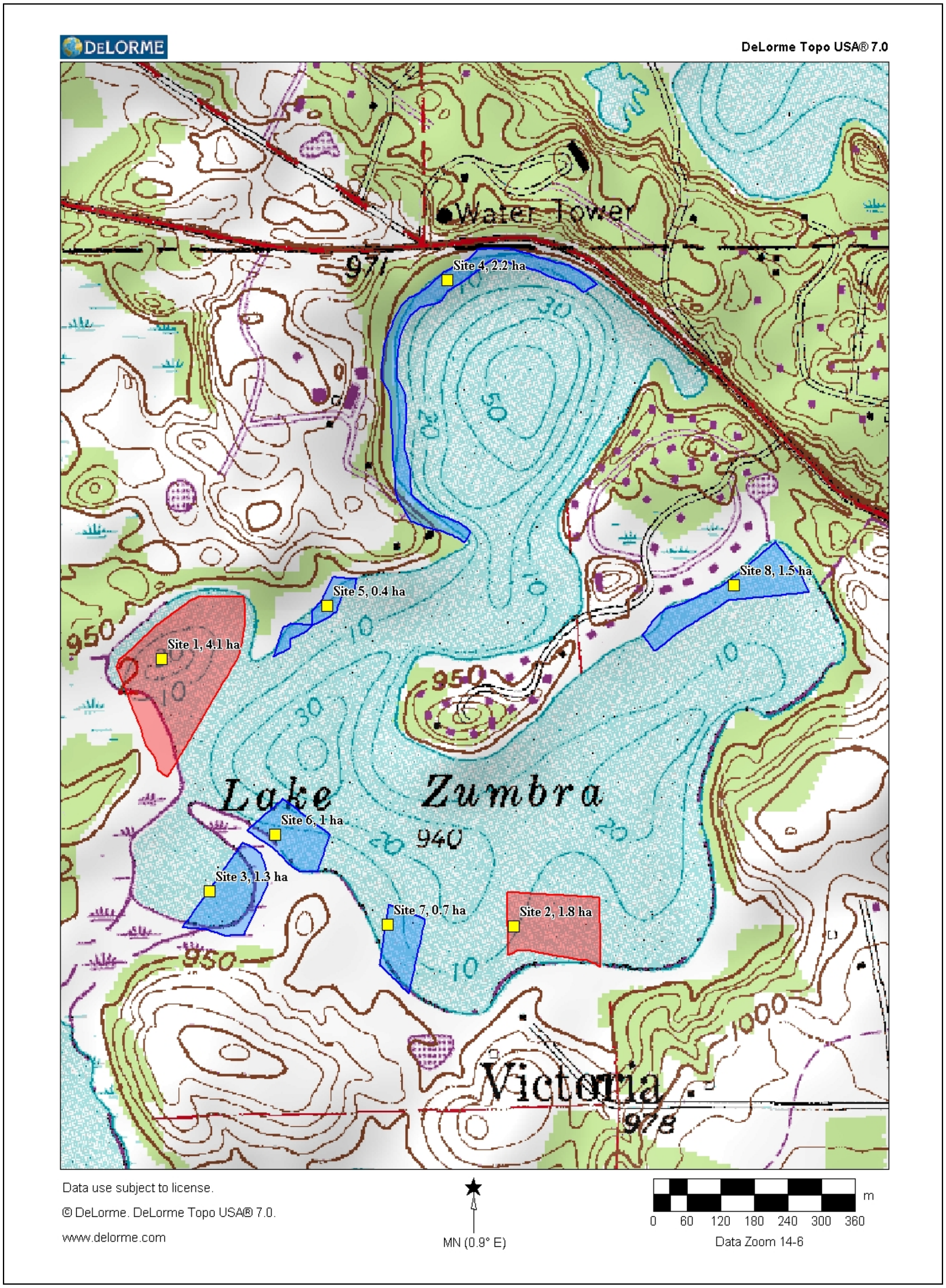

Figure 4. Zumbra Lake 2005 herbicide treatments. Liquid endothall and 2,4-D were applied to blocks (red), and granular endothall was applied to strips (blue).

blocks (5.0 to 11.3 ha) were treated with liquid formulations, since it was expected that adequate herbicide exposure times could be maintained in these areas. Treatments consisted of endothall (Aquathol® K, United Phosphorus Inc., King of Prussia, PA) at 1 mg /L ai and 2,4-D (DMA 4 IVM®, Dow AgroSciences, Indianapolis, IN) at 0.5 mg ai/L using a dual-tank injection system with 3-m drop hoses mounted on the stern of a boat. The maximum label use rates for endothall and 2,4-D are 5.0 mg/L ai and 4.0 mg/L ae, respectively. To avoid product compatibility issues, herbicide concentrates were not directly mixed, and were kept in separate tanks. In addition to block treatments, narrow strips were targeted for treatment up to 100 m long (1.0 to 6.4 ha) and were located in areas parallel to the shoreline where water depth increased quickly and vegetation extended only 15 m out from the bank. To maintain adequate herbicide exposure times, these narrow plant stands required the use of granular endothall (Aquathol Super® K, United Phosphorus Inc., King of Prussia, PA) at 1.5 mg ai/L. This granular formulation was applied using an electric cyclone fertilizer spreader mounted on the bow of a boat.

Bush Lake. Herbicides were initially applied to designated treatment zones on 23 April 2004 (Figure 1). Winds were southerly at 8 to 9 kph and water temperature was 14.8 °C. Treatment zones consisted of two separate blocks (6.4 and 5.0 ha) and one narrow strip (1.0 ha). Total treated area was 12.4 ha, which comprised 27 percent of the littoral zone and 18 percent of the entire lake.

Based on plant evaluations of 7 April 2005, additional herbicides were applied to designated treatment zones on 6 May 2005 (Figure 2). Winds were northerly at 8 to 9 kph and water temperature was 15.2 °C. Treatment zones consisted of two separate blocks (10.5 and 6.5 ha) and three narrow strips (0.4, 1.9, and 1.3 ha). Total treated area was 20.6 ha and comprised 46 percent of the littoral zone and 31 percent of the entire lake. The total treatment area in 2005 was approximately 61 percent greater than the total area treated on the lake in 2004 because additional areas of the lake became severely infested with Eurasian watermilfoil.

Zumbra Lake. Herbicides were initially applied to designated treatment zones on 1 May 2004 (Figure 3). Winds were southerly at 6 to 8 kph and water temperature was 15.5 °C. Treatment zones consisted of three separate blocks (11.3, 8.7, and 5.8 ha) and four narrow strips (2.5, 3.0, 1.1 and 1.4 ha). Total treated area was 33.8 ha, which comprised 91 percent of the littoral zone and 52 percent of the entire lake.

Based on plant evaluations conducted on 5 April 2005, additional herbicides were applied to designated treatment zones on 21 April 2005 (Figure 4). Winds were southerly at 6 to 7 kph and water temperature was 14.5 °C. Treatment zones consisted of two separate blocks (4.1 and 1.8ha) and six narrow strips (1.3, 2.2, 0.4, 1.0, 0.7, and 1.5 ha). Total treated area was 13 ha, which comprised 35 percent of the littoral zone and 20 percent of the entire lake. The total treatment area in 2005 was less than one half of the total area treated on the lake in 2004 because infested areas were reduced by the 2004 treatment.

RESULTS AND DISCUSSION: Size, littoral zones, and pretreatment plant communities for the four study lakes are shown in Table 1. Lakes ranged from 65.6 to 106.0 ha in size, and littoral zones ranged from 50 to 66 percent of the respective lake areas. Eurasian watermilfoil had formed nuisance-level surface canopies in all lakes, with infestations ranging from 36 to 80 percent. Portions of the littoral zones occupied by curlyleaf pondweed ranged from 8 to 37 percent, but this amount of coverage was not considered a serious nuisance level based on plant biomass. As coontail was the predominant native plant in all lakes in 2003, native species data were separated into three categories: a) total native species, b) coontail alone, and c) native species other than coontail.

Table 1. Physical characteristics and submersed vegetation of Minnesota study lakes, June 2003.

Lake	Lake Area (hectares)	Littoral Zone (hectares)	% Littoral Zone	Trophic Status	% Native Plants[1]	% Eurasian Watermilfoil[1]	% Curlyleaf Pondweed[1]
Auburn	105.6	63.9	61%	Eutrophic	89%	77%	8%
Bush	69.6	46.1	66%	Mesotrophic	91%	36%	24%
Pierson	95.1	47.8	50%	Eutrophic	73%	62%	25%
Zumbra	65.6	37.1	57%	Eutrophic	90%	80%	37%

[1] Value represents the percentage of the littoral zone (depth ≤ 4.5 m).

While variations in plant species occurrence and biomass were expected in lakes subjected to herbicide treatments, variations in plant community also occurred in the untreated reference lakes. Variations in species occurrence and biomass in lakes that are not under active aquatic plant management are most likely due to differences in annual weather conditions and phenological cycles of individual plant species. For instance, the growing season of 2004 was considerably cooler than in 2003, and native species including some of the pondweeds, wild celery, and water stargrass were slow to break dormancy in 2004. Some of these species were abundant in the lakes by early to mid July, based on visual observations, but were not present in June when the quantitative evaluations were conducted. Seasonal variation also occurred because some species, such as pondweeds, began to senesce in mid to late summer. Other species such as Eurasian watermilfoil may grow all summer and spread to new areas in a lake. Plant community changes measured in each lake are summarized below.

Auburn Lake – untreated reference. Auburn Lake is 105.6 ha in size with a littoral zone of 63.9 ha, representing 61 percent of the total surface area. It is classified as eutrophic based on Carlson's Trophic Status Index (Moore and Thornton 1988). In June of 2003, Eurasian watermilfoil was measured in 77 percent of sample points in the littoral zone, while only 8 percent of sample points contained curlyleaf pondweed. Native plants were found in 89 percent of sample points. Secchi disk readings averaged 2 m from 1999 to 2003, 2.8 m in 2004, and 2.3 m in 2005.

Eurasian watermilfoil biomass declined from ca. 20 to 3 g dry weight (DW)/m^2 between June and August in 2003 (Table 2); however, percent occurrence was not significantly different during this same period (Table 3). This decline in biomass was driven by the necrosis and disappearance of older leaf whorls along the lower stems (apical leaves were healthy) due to unexplained natural causes. Moreover, inspection of injury symptoms suggested that this leaf necrosis was not indicative of damage by milfoil weevils (*Eurhychiopsis lecontei* [Dietz]) or known milfoil pathogens,[1] or phytotoxicity from aquatic herbicides. By 2004, biomass recovered to levels greater than those measured in 2003, comprising > 50 percent of total plant biomass, and remained at that level through August 2005. Occurrence of Eurasian watermilfoil was significantly greater in August 2005 compared to August 2003.

[1] Personal communications. 2003. R. Newman, Professor, University of Minnesota, Minneapolis, MN, and J. Shearer, Research Plant Pathologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Table 2. Mean (± 1 SE) biomass (g dry weight) of aquatic plants in two untreated reference lakes located in Minnesota, June and August 2003 to 2005. Numbers followed by the same letter are not significantly different for a given plant species and lake (LSD, p≤0.05).

Auburn Lake	Jun 03	Aug 03	Jun 04	Aug 04	Jun 05	Aug 05
Eurasian watermilfoil	20.4 ± 4.4 b	2.9 ± 1.2 c	42.0 ± 7.9 a	29.9 ± 6.6 ab	44.5 ±8.8 a	36.9 ± 9.5 a
Curlyleaf pondweed	0.0 ± 0.0 b	0.0 ± 0.0 b	1.5 ± 1.1 a	0.0 ± 0.0 b	0.3 ±0.2 a	0.0 ± 0.0 b
Coontail	29.4 ± 6.0 b	52.4 ± 8.7 a	25.3 ± 4.7 bc	31.0 ± 6.2 b	10.2 ± 3.7 c	12.6 ± 2.7 c
Other native species1	0.3 ± 0.2 a	1.7 ± 0.7 a	3.6 ± 1.8 a	0.7± 0.7 a	1.2 ± 0.3 a	0.8 ± 0.4 a
Pierson Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Eurasian watermilfoil	25.2 ± 7.2 b	34.7 ± 7.2 b	25.5 ± 6.1 b	49.7 ± 9.9 a	26.3 ± 8.0 b	53.6 ± 9.8 a
Curlyleaf pondweed	0.1 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 a	0.2 ± 0.2 a	0.1 ± 0.1 a	0.0 ± 0.0 a
Coontail	25.2 ± 8.8 ab	48.5 ± 17.8 a	13.6 ± 4.0 b	13.2 ± 4.7 b	26.8 ± 3.8 ab	47.7 ± 13.3 a
Other native species1	4.9 ± 2.7 a	3.9 ± 2.2 a	0.8 ± 0.4 a	5.5 ± 3.1 a	3.2 ± 0.6 a	2.0 ± 0.7 a

[1] Native species other than coontail

Table 3. Percent occurrence of aquatic plants in two untreated reference lakes located in Minnesota, June and August 2003 to 2005. Asterisks denote significant changes in percent occurrence post-treatment compared to the corresponding pre-treatment month (Jun 03 or Aug 03, Chi square, p≤0.05).

Auburn Lake	Jun 03	Aug 03	Jun 04	Aug 04	Jun 05	Aug 05
Eurasian watermilfoil	77	69	69	80	80	84*
Curlyleaf pondweed	8	0	15	0	16	0
Native species	89	82	89	87	87	87
All species	89	85	94	91	94	89
Pierson Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Eurasian watermilfoil	62	75	69	73	64	62
Curlyleaf pondweed	25	2	10*	1	11*	1
Native species	73	63	64	70	60	59
All species	77	86	82	86	70	72

Biomass of curlyleaf pondweed was low (Table 2) and biomass represented < 2 percent of total plant biomass. Biomass was significantly higher in June of 2004 and 2005, but was not considered to be at a nuisance level. Percent occurrence of curlyleaf pondweed (Table 3) declined in Auburn Lake between June and August for 2003 through 2005. This spring-to-summer decline is typical with curlyleaf pondweed, which begins its annual senescence in mid to late June.

The mean number of all plant species per sample point ranged from 2.1 to 2.6 and the mean number of all native plant species per sample point ranged from 1.1 to 1.8 (Table 4). The total number of native species identified in Auburn Lake during the August evaluations was 8 in 2003, 9 in 2004, and 11 in 2005. Coontail was the dominant native species on the lake, comprising > 95 percent of the total native plant biomass in August 2003 (Table 2). Coontail biomass declined significantly in 2004, and again in 2005, when biomass declined by more than 75 percent compared to 2003. Occurrence of all native species ranged from 82 to 89 percent, and no significant differences were

Table 4. Mean (±1 SE) number of aquatic plant species per sample point in four Minnesota lakes, June and August, 2003 to 2005. Auburn and Pierson were untreated reference lakes; Bush and Zumbra were herbicide-treated lakes.

Auburn Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Native species	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.8 ±0.1
All species	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.1 ±0.1	2.6 ± 0.1
Pierson Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Native species	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
All species	2.1 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
Bush Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Native species	2.6 ± 0.2	2.9 ± 0.2	1.7 ± 0.1	3.2 ± 0.2	3.7 ± 0.2	3.7 ± 0.2
All species	3.7 ± 0.2	3.3 ± 0.2	1.8 ±0.1	3.5 ± 0.2	3.8 ± 0.2	3.8 ± 0.2
Zumbra Lake	**Jun 03**	**Aug 03**	**Jun 04**	**Aug 04**	**Jun 05**	**Aug 05**
Native species	1.8 ± 0.1	1.8 ± 0.1	1.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
All species	2.9 ± 0.1	2.4 ± 0.1	1.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1

measured between years (Table 3). Only fragrant water lily was significantly higher in June 2005 compared to June 2003. Likewise, softstem bulrush and sago pondweed occurrence was higher in August 2005 compared to August 2003 (Table 5).

Pierson Lake – untreated reference. Pierson Lake is 95.1 ha with a littoral zone of 47.8 ha, or 50 percent of the total surface area. It is classified as eutrophic based on Carlson's Trophic Status Index (Moore and Thornton 1988). In June 2003, Eurasian watermilfoil was measured in 62 percent of sample points, while only 25 percent of sample points contained curlyleaf pondweed. Native plants were found at 73 percent of sample points. Secchi disk readings averaged 2.2 m from 1999 to 2003, 2.0 m in 2004, and 2.2 m in 2005.

There were no significant changes in the seasonal biomass of Eurasian watermilfoil from June to August in 2003 (Table 2). However, a significant increase was measured between June and August in 2004 and 2005. Biomass nearly doubled from June (26 percent) to August (53 percent) in 2005. There was no significant change in occurrence of Eurasian watermilfoil between post-treatment samples compared to the same pretreatment month (Table 3).

Since trace amounts of curlyleaf pondweed were found in the lake, biomass (Table 2) was quite low (< 0.5 g DW), with no significant differences measured between months or years. Occurrence of curlyleaf pondweed ranged from 1 to 25 percent and was significantly greater in June 2003 compared to June 2004 and 2005 (Table 3). As in the other untreated reference lake, percent occurrence of curlyleaf pondweed declined between June and August for all years.

The mean number of plant species per sample point in the littoral zone ranged from 1.8 to 2.3 and the mean number of native plant species per sample point ranged from 1.2 to 1.6 (Table 4). The total numbers of native plants recorded in Pierson Lake during the August plant evaluations were 19 in 2003, 19 in 2004, and 19 in 2005. As with Auburn Lake, coontail was the dominant native species, comprising > 90 percent of the total native plant biomass in August 2003 (Table 2). Coontail

Table 5. Auburn Lake percent occurrence data.

Survey Points	June Percent Occurrence					August Percent Occurrence				
	June 2003#	June 2004	Significance p =	June 2005	Significance p =	August 2003	August 2004	Significance p =	August 2005	Significance p =
Total survey points	171	162		157		180	168		157	
All native species	89	89	0.60	87	0.54	82	87	0.24	87	0.07
Myriophyllum spicatum	77	69	0.65	80	0.28	69	80	0.08	84*	0.01
Potamogeton crispus	8	15	0.10	16	0.05	0	0		0	
Brasenia schreberi	0	1	0.30	0		0	1	0.30	1	0.28
Ceratophyllum demersum	78	85	0.21	80	0.33	80	86	0.21	84	0.10
Elodea canadensis	1	0	0.33	1	0.95	1	2	0.52	2	0.48
Nuphar advena	3	2	0.70	2	0.72	2	5	0.22	7	0.06
Nymphaea odorata	17	20	0.48	38*	0.01	29	31	0.56	48*	0.01
Potamogeton natans	0	0		1	0.29	1	0	0.33	3	0.25
Potamogeton nodosus	1	1	0.97	1	0.95	1	0	0.33	0	0.35
Potamogeton zosteriformis	2	3	0.61	5	0.21	3	7	0.16	9*	0.04
Ranunculus longirostris	1	1	0.97	0	0.34	0	0		0	
Scirpus validus	4	1	0.20	4	0.90	0	5*	0.02	5*	0.02
Stukenia pectinata	2	3	0.61	5	0.21	3	14*	0.01	14*	0.01
Zosterella dubia	0	0		0		0	0		1	0.28

** or red number indicate a statistically significant decrease of post-treatment data compared to pre-treatment data (Chi-square, p < 0.05)
* or blue number indicate a statistically significant increase of post-treatment data compared to pre-treatment data (Chi-square, p < 0.05)
# 2003 data are pretreatment and 2004-2005 data are post-treatment

biomass declined significantly (ca. 75 percent) in 2004, but returned to initial levels by June 2005. Although percent occurrence of all native species did not significantly change in 2004 and 2005 compared to 2003 data (Table 3), elodea and spatterdock did show a significant decline in 2004 and 2005 compared to the same month in 2003 (Table 6).

Bush Lake - herbicide treated. Bush Lake is 69.6 ha with a littoral zone of 46.1 ha, or 66 percent of the total surface area (Table 1). It is classified as mesotrophic based on Carlson's Trophic Status Index (Moore and Thornton 1988). In June 2003, Eurasian watermilfoil was measured in 36 percent of sample points, while 24 percent of sample points contained curlyleaf pondweed. Native plants were found in 91 percent of sample points. Secchi disk readings averaged 2.4 from 1999 to 2003, 2.5 in 2004, and 2.6 in 2005.

Table 6. Pierson Lake percent occurrence data.

Survey Points	June Percent Occurrence					August Percent Occurrence				
	June 2003#	June 2004	Significance p =	June 2005	Significance p =	August 2003	August 2004	Significance p =	August 2005	Significance p =
Total survey points	206	193		190		206	206		189	
All native species	73	64	0.63	60	0.42	63	70	0.46	59	0.67
Myriophyllum spicatum	62	69	0.23	64	0.44	75	73	0.84	62	0.17
Potamogeton crispus	25	10**	0.01	11**	0.03	2	1	0.56	1	0.56
Ceratophyllum demersum	41	44	0.48	42	0.59	45	56	0.21	47	0.81
Elodea canadensis	22	8**	0.01	6**	0.01	19	5**	0.01	7**	0.01
Myriophyllum verticillatum	1	1	0.96	1	0.95	1	1	1.00	1	1.00
Najas flexilis	0	6*	0.01	8*	0.01	11	14	0.54	11	1.00
Nuphar advena	4	6	0.46	3	0.78	8	5	0.40	2**	0.05
Nymphaea odorata	19	20	0.70	20	0.66	26	34	0.26	30	0.56
Potamogeton amplifolius	3	2	0.71	1	0.35	0	5*	0.02	2	0.16
Potamogeton graminea	1	1	0.96	1	0.95	1	0	0.32	1	1.00
Potamogeton natans	0	1	0.30	1	0.30	1	1	1.00	1	1.00
Potamogeton pusillus	1	3	0.28	1	0.95	1	1	1.00	0	0.32
Potamogeton richardsonii	2	9*	0.02	1	0.61	1	2	0.56	3	0.31
Potamogeton robbinsii	0	0		1	0.30	0	1	0.32	0	
Potamogeton zosteriformis	7	6	0.87	3	0.25	11	10	0.82	5	0.13
Ranunculus longirostris	4	2	0.46	1	0.21	1	1	1.00	1	1.00
Scirpus validus	2	0	0.17	1	0.61	1	2	0.56	1	1.00
Stukenia pectinata	1	8	0.01	3	0.28	5	8	0.40	6	0.76
Vallisneria americana	2	1	0.60	1	0.61	1	2	0.56	1	1.00
Utricularia vulgaris	7	5	0.64	4	0.43	5	6	0.76	3	0.47
Zannichellia palustris	1	1	0.96	0	0.34	1	0	0.32	2	0.56
Zosterella dubia	0	1	0.30	1	0.30	1	1	1.00	2	0.56
Chara sp	7	5	0.64	3	0.25	2	1	0.56	1	0.56

** or red number indicate a statistically significant decrease of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
* or blue number indicate a statistically significant increase of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
# 2003 data are pretreatment and 2004-2005 data are posttreatment.

Eurasian watermilfoil biomass was reduced by 68 percent between the June and August 2003 plant evaluations (Table 7) because of an unscheduled 2,4-D treatment (112 kg/ha) that occurred on 28 June 2003 in a 6-ha portion of the lake. This area was not treated again in future applications.

Table 7. Mean (±1 SE) biomass (g dry weight) of aquatic plants in two herbicide-treated lakes located in Minnesota, June and August 2003 to 2005. Numbers followed by the same letter are not significantly different for a given plant species and lake (LSD, p≤0.05).

Bush Lake	Jun 03	Aug 03	Apr 04	Jun 04	Aug 04	Apr 05	Jun 05	Aug 05
Eurasian watermilfoil	10.4 ± 2.8 a	3.3 ± 1.6 ab	1.2 ± 0.4 bc	0.0 ± 0.0 d	0.2 ± 0.2 c	0.9 ± 0.3 bc	0.0 ± 0.0 d	0.2 ± 0.1 c
Curlyleaf pondweed	1.0 ± 0.3 a	0.0 ± 0.0 b	0.7 ± 0.3 a	0.0 ± 0.0 b	0.4 ± 0.3 ab	0.8 ± 0.4 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Coontail	11.6 ± 3.8 b	43.4 ± 15.5 a	9.3 ± 2.4 b	7.1 ± 2.2 b	25.7 ± 6.3 ab	—[2]	9.0 ± 1.9 b	32.2 ± 7.9 a
Other native species[1]	10.0 ± 3.2 c	10.5 ± 4.6 c	6.6 ± 4.7 c	3.8 ± 2.1 c	12.5 ± 4.6 c	—	33.1 ± 9.1 b	50.6 ± 4.5 a
Zumbra Lake	**Jun 03**	**Aug 03**	**Apr 04**	**Jun 04**	**Aug 04**	**Apr 05**	**Jun 05**	**Aug 05**
Eurasian watermilfoil	26.8 ± 8.3 a	16.1 ± 4.4 a	2.4 ± 0.7 b	0.0 ± 0.0 c	0.1 ± 0.0 c	0.8 ± 0.3 bc	0.2 ± 0.1 c	1.8 ± 0.5 b
Curlyleaf pondweed	0.6 ± 0.3 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Coontail	45.6 ± 14.3 c	106 ± 29.7 a	38.9 ± 12.0 c	40.1 ± 8.2 c	54.8 ± 13.4 bc	—	55.0 ± 9.3 bc	97.8 ± 20.4 ab
Other native species[1]	1.7 ± 0.7 b	1.7 ± 0.8 b	0.0 ± 0.0 b	0.9 ± 0.5 b	9.8 ± 3.1 a	—	1.0 ± 0.1 b	7.1 ± 2.2 a

[1] Native species other than coontail
[2] Data not collected

Eurasian watermilfoil biomass was reduced by > 99 percent in June 2004 compared to June 2003 and April 2004 (Table 7). Some plant recovery was observed in August 2004 but was mostly in areas treated with 2,4-D in June 2003. The lake was again treated in April 2005 to control curlyleaf pondweed returning from turions sprouting from the sediment, and recovering Eurasian watermilfoil in the areas not treated in 2004. The herbicide applications in 2005 continued to suppress Eurasian watermilfoil biomass at very low levels, ≤ 1 percent of total plant biomass. Occurrence of Eurasian watermilfoil was significantly reduced in June and August, 2004 and 2005 (< 10 percent) compared to the same months in 2003 (36 percent), as shown in Table 8.

Observations indicated that most curlyleaf pondweed was dead by mid-May, approximately 3 weeks after herbicide application, and therefore production of vegetative reproductive propagules (turions) was probably eliminated. Percent occurrence of curlyleaf pondweed was significantly reduced in June 2004 and 2005 compared to June 2003, following the herbicide treatments in April (Table 8). Some scattered plants from newly sprouted turions were observed in June 2004, August 2004, and August 2005. These plants were most likely from turions deposited on the sediment in previous years, and sprouted well after the 2004 herbicide applications. Though still present at posttreatment sampling events, curlyleaf pondweed accounted for only ≤ 1 percent of the total biomass in the lake (Table 7).

The mean number of plant species per sample point in Bush Lake ranged from 3.3 in August 2003 to 3.8 in August 2005, and the mean number of native plant species per sample point ranged from 3.0 in 2003 to 3.7 in 2005 (Table 4). The mean number of total plant species in June 2003 was 3.7 while the

Table 8. Percent occurrence of aquatic plants in two herbicide-treated lakes located in Minnesota, April, June, and August 2003 to 2005. Asterisks denote significant changes in percent occurrence compared to the corresponding pretreatment month (Jun 03 or Aug 03, Chi square, p≤0.05).

Bush Lake	Jun 03	Aug 03	Apr 04	Jun 04	Aug 04	Apr 05	Jun 05	Aug 05
Eurasian watermilfoil	36	37	21	4*	18*	26	13*	18*
Curlyleaf pondweed	24	0	15	3*	8*	26	19	8*
Native species	91	90	73	88	93	68	97	100
All species	93	91	80	88	93	76	97	100
Zumbra Lake	**Jun 03**	**Aug 03**	**Apr 04**	**Jun 04**	**Aug 04**	**Apr 05**	**Jun 05**	**Aug 05**
Eurasian watermilfoil	80	64	62	6*	8 *	23	22*	26*
Curlyleaf pondweed	37	0	2	0*	1	3	0*	0
Native species	90	92	65	85	92	80	92	89
All species	90	92	79	85	92	80	92	89

mean number of native plant species per sample point was only 2.6. By June 2005 these numbers were 3.8 and 3.7, respectively, indicating that the invasive species no longer dominated the plant community. Total numbers of native plants recorded in Bush Lake during the August plant evaluations were 19 in 2003, 20 in 2004, and 18 in 2005.

Some reduction in coontail biomass occurred between 2003 and 2004 (Table 7), similar to the untreated reference lakes Auburn and Pierson. No change occurred in the biomass of other native plant species between 2003 and 2004; however, a significant increase in the biomass of other native plant species did occur in 2005. Much of the change was caused by an 18-percent increase in elodea from August 2003 to 2005 (Table 9), but other species contributed to the community shift including northern milfoil (*Myriophyllum sibiricum* Komarov), slender naiad (*Najas flexilis* Willd), fragrant water lily (*Nymphaea odorata* Aiton), big leaf pondweed (*Potamogeton amplifolius* Tuckerman), sago pondweed, wild celery, water stargrass (*Zosterella dubia* Jacq), and musk grass (*Chara* spp.). Native species other than coontail, elodea, and musk grass accounted for 3 percent of total biomass in August of 2003 and 24 percent in August 2005.

Occurrence of coontail increased significantly from 2003 to June 2005, and August 2005, primarily by moving into 4.6-m deeper water (Table 9). Occurrence of other native species was variable, with numerous significant differences. Large increases occurred in elodea, Illinois pondweed, wild celery, and water star grass. The largest declines occurred in large leaf pondweed and flat-stem pondweed.

Zumbra Lake - herbicide treated. Zumbra Lake is 65.6 ha with a littoral zone of 37.1 ha, or 57 percent of the total surface area (Table 1). It is classified as eutrophic based on Carlson's Trophic Status Index (Moore and Thornton 1988). In June 2003, Eurasian watermilfoil was measured in 80 percent of sample points and 37 percent of sample points contained curlyleaf pondweed. Native plants were found at 90 percent of sample points. Secchi disk readings averaged 2.3 m from 1999 to 2003, 3.2 m in 2004, and 2.6 m in 2005.

Between June and August 2003, Eurasian watermilfoil exhibited leaf necrosis and declining biomass similar to conditions on Auburn Lake (above), but differences in biomass were not significant. Eurasian watermilfoil biomass was reduced by > 99 percent in June 2004 compared to June 2003

Table 9. Bush Lake percent occurrence data.

Survey Points	June Percent Occurrence					August Percent Occurrence				
	June 2003#	June 2004	Significance p =	June 2005	Significance p =	August 2003	August 2004	Significance p =	August 2005	Significance p =
Total survey points	190	190		187		190	189		186	
All native species	91	88	0.76	97	0.44	90	93	0.72	100	0.21
Myriophyllum spicatum	36	4**	0.01	13**	0.01	37	18**	0.01	18**	0.01
Potamogeton crispus	24	3**	0.01	19	0.45	0	8*	0.01	8*	0.01
Brasenia schreberi	1	1	1.00	1	0.99	1	1	0.10	0	0.32
Ceratophyllum demersum	56	61	0.58	75*	0.03	67	81	0.13	89*	0.01
Elodea canadensis	1	4	0.18	46*	0.01	2	12*	0.01	38*	0.01
Myriophyllum sibiricum	4	1	0.18	4	0.98	4	9	0.15	9	0.15
Najas flexilis	3	2	0.65	16*	0.01	17	31*	0.03	22	0.36
Nelumbo lutea	2	3	0.65	2	0.99	2	3	0.65	2	0.98
Nymphaea odorata	31	31	1.0	44	0.08	33	42	0.24	43	0.16
Potamogeton amplifolius	15	2**	0.01	4**	0.01	16	9	0.15	10	0.24
Potamogeton foliosus	13	2**	0.01	2**	0.01	0	2	0.15	1	0.31
Potamogeton illinoensis	6	5	0.76	22*	0.01	8	19*	0.03	18*	0.04
Potamogeton richardsonii	3	0	0.08	1	0.32	4	1	0.18	2	0.42
Potamogeton robbinsii	21	13	0.15	4**	0.01	13	8	0.27	8	0.28
Potamogeton zosteriformis	49	6**	0.01	0**	0.01	53	1**	0.01	0**	0.01
Ranunculus longirostris	18	5**	0.01	5**	0.01	10	1**	0.01	0**	0.01
Stukenia pectinata	6	4	0.52	9	0.41	9	14	0.28	10	0.78
Sagittaria graminea	6	4	0.52	23*	0.01	6	18*	0.01	25*	0.01
Vallisneria americana	8	13	0.26	34*	0.01	25	39*	0.05	43*	0.01211
Utricularia vulgaris	10	0**	0.01	0**	0.01	0	0		0	
Zannichellia palustris	1	0	0.32	1	0.99	11	0**	0.01	1**	0.00377
Zosterella dubia	3	6	0.31	39*	0.01	11	23*	0.03	28*	0.00322
Chara sp	5	9	0.28	32*	0.01	6	12	0.14	20*	0.00371
Aquatic moss	1	1	1.00	1	0.99	0	1	0.31	2	0.15181

** or red number indicate a statistically significant decrease of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
* or blue number indicate a statistically significant increase of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
# 2003 data are pretreatment and 2004-2005 data are posttreatment.

(Table 7). No plant recovery was observed in August 2004, but some recovery did occur by April 2005, mostly in areas treated with granular endothall. The total area treated with the endothall, 2,4-D combination was reduced from 33.8 ha in May 2004 to 11.5 ha (mostly in one block of 5.1 ha) in April 2005. Some slight plant recovery occurred by August 2005 in areas not treated in April 2005 or spot-treated with granular endothall. Occurrence of Eurasian watermilfoil (Table 8) was significantly reduced from 80 to 6 percent in June 2004 following the April herbicide treatment, and no significant recovery occurred by August 2004. Occurrence in both June and August remained significantly less than in comparable months in 2003. Eurasian watermilfoil was generally scattered or found in small clumps.

Curlyleaf pondweed biomass was very low in June 2003 and was nearly absent in April 2004 and 2005 (Table 7). Occurrence of curlyleaf pondweed was 37 percent in June 2003, but < 5 percent in April 2004 and 2005 (Table 8). All of the curlyleaf pondweed occurred in a 2.4-ha area that was treated with liquid endothall alone. No curlyleaf pondweed was found in June or August 2005.

The mean number of plant species per sample point ranged from 2.9 in June 2003 to 2.4 in August 2005, and the mean number of native plant species per sample point ranged from 1.8 in June 2003 to 2.1 in August 2005 (Table 4). Total numbers of native plants recorded in Zumbra Lake during the August evaluations were 10 in 2003, 15 in 2004, and 12 in 2005.

Coontail biomass was relatively stable through 2003 and 2005 (Table 7). Biomass of other native plant species increased significantly from 2003 to 2005, mostly from an increase in elodea, slender naiad, and sago pondweed. Occurrence of all species and all native species did not change significantly from year to year (Table 8). The only native plant species that declined on Lake Zumbra was big leaf pondweed in June; however, healthy, robust stands of big leaf pondweed were observed in herbicide-treated areas in August 2004 and 2005.

Several native species showed significant increases in occurrence in 2004 and 2005 (Table 10). No native plant species increased > 10 percent; however, most species showed some increase in occurrence, particularly elodea, Illinois pondweed, smartweed (*Polygonum amphibium* L), and bulrush (*Scirpus validus* Vahl).

SUMMARY: Early spring, low-dose applications of endothall combined with 2,4-D in 2004, followed by selective spot-treatments with the same herbicides in 2005, provided > 99 percent reduction of Eurasian watermilfoil biomass in Bush and Zumbra Lakes through August of the treatment years. This treatment regime also reduced occurrence of Eurasian watermilfoil by 75 to 85 percent through 2005 in both lakes. Curlyleaf pondweed biomass and occurrence were reduced in both lakes in June following treatment. In contrast, untreated reference lakes, Auburn and Pierce, continued to be dominated by nuisance levels of Eurasian watermilfoil and potentially problematic levels of curlyleaf pondweed. In addition, water clarity in the treated lakes remained at pretreatment levels through the 2005 growing season.

These early season herbicide applications were designed to selectively control Eurasian watermilfoil and curlyleaf pondweed at a time of year when most native plants are not actively growing. Treatment of Eurasian watermilfoil and curlyleaf pondweed early in the growing season provided temporal selectivity by preventing exposure of many sensitive native plants to the herbicides during periods of active growth. Although some pondweed species declined in Bush Lake, many native

Table 10. Zumbra Lake percent occurrence data.

Survey Points	June Percent Occurrence					August Percent Occurrence				
	June 2003#	June 2004	Significance p =	June 2005	Significance p =	August 2003	August 2004	Significance p =	August 2005	Significance p =
Total Survey Points	206	208		206		207	206		206	
All native species	90	85	0.56	92	0.84272	92	92	0.96482	89	0.80
Myriophyllum spicatum	80	6**	0.01	22**	0.00000	64	8**	0.00000	26**	0.01
Potamogeton crispus	37	0**	0.01	0**	0.00000	0	1	0.31555	0	
Ceratophyllum demersum	76	73	0.70	85	0.36349	85	91	0.52254	87	0.81
Elodea canadensis	6	5	0.75	16*	0.02843	7	2	0.09339	11	0.33
Najas flexilis	1	0	0.31	8*	0.01831	1	5	0.09875	9*	0.01
Nymphaea odorata	42	37	0.50	60*	0.03991	47	58	0.20344	59	0.17
Polygonum amphibium	6	7	0.79	12	0.14813	3	13*	0.01047	11*	0.03
Potamogeton amplifolius	25	1**	0.01	3**	0.01	14	4**	0.02	4**	0.02
Potamogeton foliosus	1	0	0.31	2	0.56	0	4	0.04	1	0.32
Potamogeton illinoensis	1	5	0.10	11*	0.01	13	10	0.53	10	0.53
Potamogeton natans	0	0		0		1	0	0.32	1	0.10
Potamogeton praelongus	1	1	0.99	0	0.32	0	0		0	
Potamogeton pusillus	1	0	0.31	0	0.32	1	1	0.106	0	0.32
Potamogeton zosteriformis	1	0	0.31	0	0.32	0	1	0.32	0	
Ranunculus longirostris	2	0	0.15	0	0.16	0	0		0	
Scirpus validus	4	1	0.18	11	0.21	5	8	0.39	9	0.27
Stukenia pectinata	2	2	0.99	10*	0.02	0	5*	0.02	10*	0.01
Utricularia vulgaris	1	0	0.31	0	0.32	0	0		0	
Zannichellia palustris	1	0	0.31	2	0.56	0	1	0.32	0	
Zosterella dubia	0	0		2	0.16	0	2	0.15	1	0.32
Chara sp	6	3	0.31	2	0.41	0	4*	0.04	0	

** or red number indicate a statistically significant decrease of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
* or blue number indicate a statistically significant increase of post-treatment data compared to pre-treatment data (Chi-square, $p < 0.05$)
# 2003 data are pretreatment and 2004-2005 data are posttreatment

plant species expanded in frequency of occurrence in both Bush and Zumbra Lakes. In addition, mean number of native species per point increased in both lakes. Initial control of the invasive species in 2004, and aggressive maintenance control in 2005, enabled native plants to increase significantly in biomass in 2005. Total native plant biomass on Bush Lake increased by 80 percent following removal of most of the Eurasian watermilfoil and curlyleaf pondweed.

The use of endothall alone would have required higher concentrations (2 to 3 mg ai/L) to control Eurasian watermilfoil in the large treated blocks and would have risked damage to some of the native plants, particularly coontail. The use of 2,4-D alone at typical treatment rates of 2 to 4 mg ae/L to control Eurasian watermilfoil would not have controlled curlyleaf pondweed and would have risked damage to water lilies, which increased in frequency or remained unchanged. These plants are protected by MNDNR from general herbicide treatments.

Application of herbicide combinations allowed the simultaneous control of two invasive aquatic plant species. Control of one species may have allowed the other species to become dominant where two separate herbicide treatments would have been required to achieve desired control levels. In addition, the herbicide combination applied in early spring did not adversely impact the native plant population, including high value plants such as water lilies and bulrush. Overall native plant biomass and occurrence increased following the herbicide treatments.

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