Early Season 2,4-D Herbicide and Deep Harvesting Treatment Effects on Eurasian Watermilfoil (*Myriophyllum spicatum*) and Native Macrophytes in Turville Bay, Lake Monona, Dane County, Wisconsin

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Summary: Eurasian watermilfoil (*Myriophyllum spicatum*) occurs throughout Dane County's Yahara Chain of Lakes (Mendota, Monona, Wingra, Waubesa, and Kegonsa). In 2006, the Dane County Board of Supervisors formed an Aquatic Plant Management (APM) Committee to assess currently available options for aquatic plant management in the Yahara Chain, including mechanical, chemical, and biological approaches. At the request of the APM Committee, a Turville Bay APM research project was designed to evaluate the efficacy, selectivity, and longevity of early-season 2,4-D herbicide treatments and mechanical harvesting on the control of invasive aquatic plants, as well as the effects on restoration of native aquatic plant communities. The overall project goal was to determine if either or both of the evaluated management approaches would be viable methods for controlling nuisance exotic plants while benefiting and protecting a healthy native plant community. This report describes the work undertaken and presents the study results.

This research project was supported by a Wisconsin Department of Natural Resources Aquatic Invasive Species grant, with matching funds from the Dane County Department of Land and Water Resources. The U.S. Army Corps of Engineers' Engineer Research and Development Center provided technical support. Mention of trade names and commercial products does not constitute endorsement of their use.

Cover Photo: Aerial photograph of Turville Bay, Lake Monona, Dane County, Wisconsin. Photograph from U.S. Department of Agriculture, 2010 National Agricultural Imagery Program, 1:30,000 resolution.

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Contents

List of Figures

List of Tables

Introduction

Eurasian Watermilfoil in the Yahara Chain of Lakes

Dane County waters have supported populations of the exotic, invasive, aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) since at least the 1960s (Couch and Nelson 1985), and the species now occurs throughout the Yahara Chain of Lakes (Mendota, Monona, Wingra, Waubesa, and Kegonsa). The macrophyte (aquatic plant) community in the Yahara Chain has been altered from the effects of years of urbanization, as well as the introduction of aquatic invasive species such as EWM (Nichols and Lathrop 1994). In late July through early August 2008, Lake Monona (excluding the Monona Bay area) had an estimated littoral zone of 24% (757 acres) of the total lake area (3180 acres). EWM estimated frequency in the littoral zone was 64%, and in 2008 was the most dominant aquatic plant species in the lake (Marshall et al. 2011). With nuisance levels of EWM in some areas of Lake Monona, management actions are deemed necessary to maintain the aesthetically, recreationally, and ecologically valuable areas within the lake.

Formation of an Aquatic Plant Management Committee

In January 2006, Dane County's Board of Supervisors formed an Aquatic Plant Management (APM) Committee to assess currently available options for aquatic plant management in the Yahara Chain, including mechanical, chemical, and biological approaches. Committee selection covered a broad range of backgrounds to ensure diversity of representation and involvement. The committee held a series of fact-gathering meetings that involved county staff, representatives of the aquatic herbicide industry, Wisconsin Department of Natural Resources (Wisconsin DNR) staff representing fisheries, lake management, and research, U.S. Army Corps of Engineers staff, and many other stakeholders. The meetings were open to the public for both information and participation. The committee identified a collaborative, experimental approach to investigate early season herbicide use and mechanical harvesting as a high priority.

The Turville Bay APM research project was designed at the request of the APM Committee to evaluate the efficacy, selectivity, and longevity of early-season 2,4-D herbicide treatments and mechanical harvesting on the control of invasive aquatic plants as well as restoration of native aquatic plant communities. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a semi-selective herbicide approved for aquatic use, primarily targeting dicots such as EWM and coontail (*Ceratophyllum demersum*), but is also known to affect monocots under certain operational applications and conditions (Nault et al. 2012, Nault et al. *In review*). Mechanical harvesting is generally non-selective, and in current operational practices is usually conducted later in the summer to provide short-term, seasonal nuisance relief. Early-season treatments of either management approach could potentially increase selectivity of target exotic species which are actively growing, while minimizing non-target impacts on many native plant species which are not actively growing (Nichols and Shaw 1986). We sought to evaluate treatment impacts and efficacy of both methods conducted as an early-season treatment.

Project Goal

The overall goal of this study was to determine if either or both of the evaluated management approaches (early season 2,4-D treatment and early season deep harvesting) are viable methods to control nuisance exotic plants and aid in restoration of native plant communities. The project was not designed to eliminate aquatic plants from the Yahara Chain; rather, it was undertaken to further identify and refine management methods that can control exotic plants, while benefiting and protecting a healthy native plant community. Whereas historically permitted management had been intended primarily to provide short-term nuisance relief, it was hypothesized that strategic application early in the growing season would present heretofore unrealized opportunities for selectivity of exotics and potentially for native plant restoration.

Project Objectives

The project had two principle objectives:

- Use a scientific approach to measure impacts and results to determine if either control method is successful in facilitating an increase in distribution and density of native plants and a decrease in distribution and density of EWM.

- Prepare a final report including recommendations, based on study results, to suggest how the county will work with Wisconsin DNR, recreational users, and riparian property owners to implement possible management methods to seek native plant restoration and control of aquatic exotics.

Project Cooperators and Roles

The project involved multiple cooperators. Each undertook various roles:

- Dane County: project coordination, grant and permit application and administration, public information and coordination of public input, staff participation in mechanical harvesting aspects of the project, and collection of water quality data.

- Wisconsin DNR Bureau of Science Services: baseline plant frequency and biomass collection and analysis, staff participation in research design and implementation, and water quality analysis; Wisconsin DNR management programs also contributed staff to planning meetings and annual check-ins.

- U.S. Army Corps of Engineers Research and Development Center: staff participation in research design and implementation, supervision of herbicide application, and collection and analysis of herbicide concentration data.

Methods

Study Site Selection and Design

Lake Monona is a 3,359-acre, eutrophic drainage lake on the Yahara River with a maximum depth of 74 feet and an average depth of 27 feet. Turville Bay is located along the southern shore of Lake Monona (Figure 1), and is approximately 80 acres in size with an average depth for all plots of 7 feet.

Turville Bay was chosen as the study site due to its reasonable isolation from prevailing winds, which helped minimize any potential “drift” of the applied herbicide. This area has a long history of mechanical harvesting management along the eastern side of the bay and an unmanaged area along the undeveloped western shore. In the past, Turville Bay has experienced heavy EWM growth, and the presence of a relatively diverse community of natives in the shallow bay made it a good candidate to determine their response following the two treatment strategies. The initial pre-management baseline survey results from 2007 indicated that all of the plots were largely vegetated and had similar characteristics of depth, substrate, plant diversity, biomass, and frequencies of occurrence (Table 1, Figure 2). Seven 5-acre study plots were delineated in the Turville Bay project, divided into two herbicide treated plots, two mechanically harvested plots, and three untreated control plots (Figure 1).

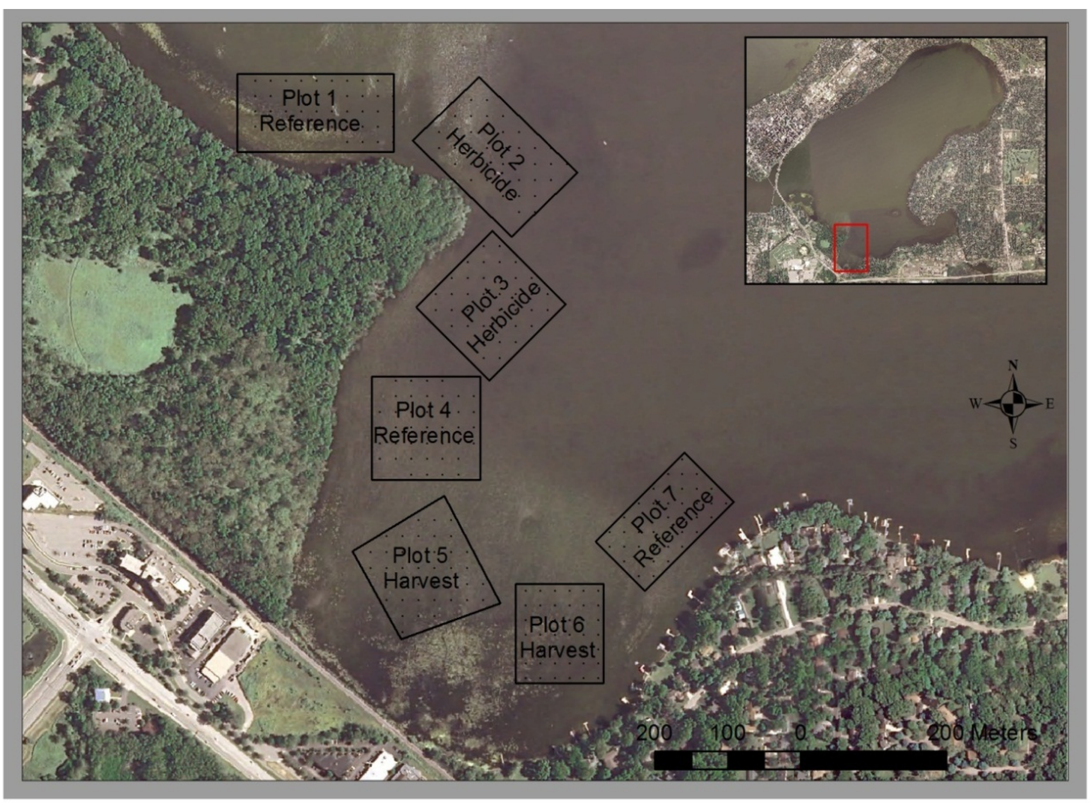


Figure 1. *Turville Bay study plot locations. Plots 1, 4, and 7 are untreated reference plots, plots 2 and 3 are herbicide treatments, and plots 5 and 6 are deep harvesting treatments. Inset map indicates location of Turville Bay on the southern shore of Lake Monona, Dane County, Wisconsin.*

Table 1. *Turville Bay study plot characteristics, August 2007.*

		August Plots 2007						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Summary	% vegetated	84.0	100.0	93.0	100.0	95.0	95.0	98.0
	Species richness	4.0	6.0	7.0	6.0	4.0	6.0	2.0
	Simpson diversity index	0.7	0.7	0.7	0.6	0.5	0.6	0.5
	Avg # species / vegetated site	2.2	2.5	2.4	2.1	1.8	2.1	2.0
	Avg # native species / vegetated site	1.3	1.5	1.6	1.2	1.0	1.2	1.0
	Average rake fullness	2.4	2.3	1.8	2.0	1.6	2.1	2.6
	Mean biomass/plot (g)	26.0	43.0	15.5	22.3	9.3	30.0	40.4
	Average Depth (ft)	7.5	5.7	6.4	6.8	6.7	6.9	8.2
Frequency of Occurrence (vegetated)	Eurasian watermilfoil	92.0	100.0	84.0	93.0	87.0	95.0	95.0
	Chara sp.			3.0				
	Clasping-leaf pondweed	19.0	18.0	30.0			8.0	
	Coontail	83.0	87.0	92.0	100.0	87.0	95.0	100.0
	Curly-leaf pondweed					3.0		
	Sago Pondweed	22.0	21.0	16.0	7.0	3.0	10.0	
	White water crowfoot				2.0			
	Water star-grass			8.0			3.0	
	Wild celery		3.0	5.0	2.0		3.0	
Avg. Rake Fullness (vegetated)	Eurasian watermilfoil	1.7	2.0	1.5	1.7	1.4	1.7	1.8
	Chara sp.			1.0				
	Clasping-leaf pondweed	1.0	1.0	1.5			1.3	
	Coontail	1.9	1.7	1.6	1.6	1.4	1.7	2.3
	Curly-leaf pondweed					1.0		
	Sago Pondweed	1.0	1.0	1.3	1.0	1.0	1.5	
	White water crowfoot				1.0			
	Water star-grass			1.3			1.0	
	Wild celery		1.0	1.5	1.0		1.0	

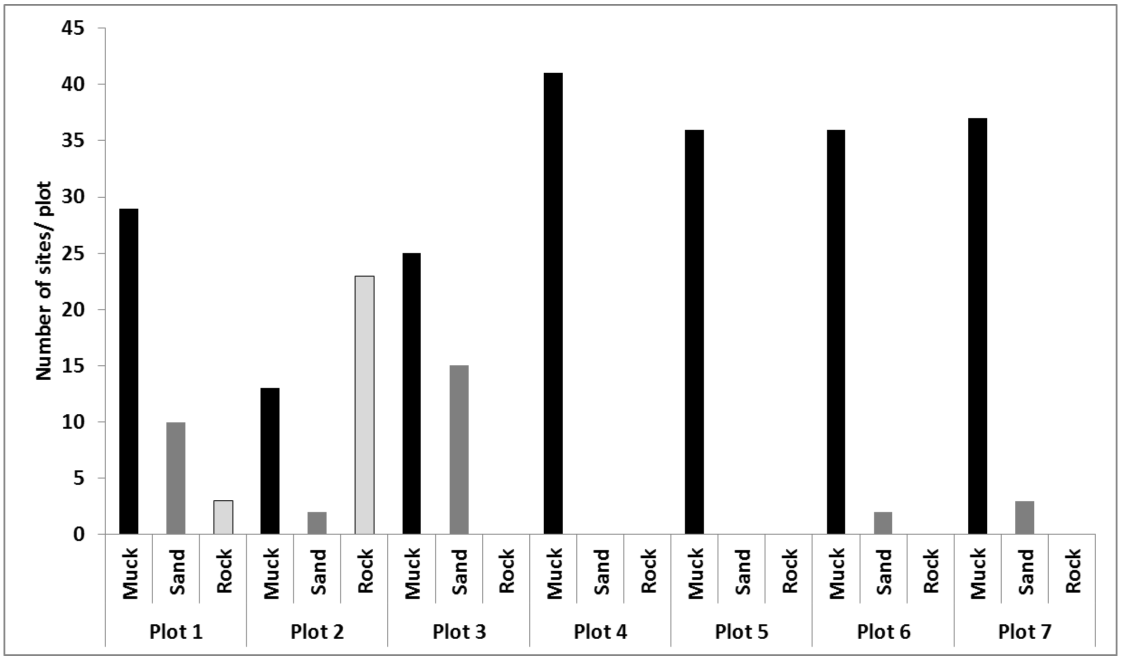


Figure 2. *Number of sites where each sediment type (muck, sand, or rock) was found in each study plot in Turville Bay, August 2007.*

Herbicide and Deep Harvesting Treatments

We treated two of the managed plots (P2 and P3) chemically with a granular formulation of 2,4-D (Navigate®) applied in spring (late April to early May) for three consecutive years (2008-2010) (Table 2). We timed the herbicide treatments based on the presence of actively growing EWM in conjunction with water temperatures of 10° C (50° F). In 2008, an application rate of 100 lbs/acre was used in plot areas shallower than 5 feet and 150 lbs/acre in areas deeper than 5 feet. In both 2009 and 2010, an application rate of 150 lbs/acre was used uniformly throughout the chemical treatment plots regardless of depth. In 2011 and 2012, we did not treat these two plots in order to monitor plant responses following repeated chemical treatments.

Table 2. *Total granular 2,4-D herbicide applied to treatment plots, 2008-2010, calculated as both active ingredient (ai) and acid equivalent (ae), and target concentrations per plot.*

Year	Total Product Applied (pounds)	2,4-D Active Ingredient (ai) (pounds)	2,4-D Acid Equivalent (ae) (pounds)	Target Concentration per Plot (µg/L)
2008	1160	320	220	1200-1300
2009	1500	414	285	1570-1750
2010	1500	414	285	1570-1750

Two of the other managed plots (P5 and P6) underwent mechanical "deep" harvesting for four consecutive years (2008-2011). This deep harvesting technique employed cutters that extended down 4.5-5.0 feet from the water's surface, and was conducted a single time each year when EWM was within 1.0-1.5 feet of the water's surface. The exact timing of harvesting varied from year to year depending on the environmental conditions within each particular year, such as unexpectedly high water levels or prolonged cold spring water temperatures. In 2009 and 2010, plots were harvested in late May and early June, and in 2008 and 2011, harvesting occurred in early July. In 2012, no further harvesting occurred and the managed plots were monitored to measure the aquatic plant community responses after the cessation of repeated harvesting efforts. We used the remaining three plots (P1, P4, and P7) as untreated controls throughout the entire study in order to better understand the degree to which natural interannual variation was affecting the plant community.

Herbicide Concentration Monitoring

We collected water samples from all plots and analyzed them for 2,4-D concentrations over time. Three sample sites were located in each of the two herbicide treated plots (P2 and P3), one sample site was located in each of the other plots, and an additional sample site was located outside of all the plots (Figure 3). In 2008 and 2009, each sample site located in the herbicide treated plots was sampled both near the surface and bottom (¼ and ¾ of the total water column depth). All other sites were sampled at mid-depth (½ of the total water column depth). In 2010, all samples were taken 1 foot above the sediment surface. Samples were collected pre-treatment to determine background levels and again within the first few hours after chemical treatment. Additional sampling occurred at approximately one, two, four, seven, 14, and 21 days after treatment (DAT). In 2008, sample analysis was provided by Applied Biochemists using traditional methods, which did not include the addition of a fixative agent such as muriatic acid at the time of collection. In 2009 and 2010, following collection of each sample, 3 to 4 drops of muriatic acid were added and samples were then stored in a refrigerator until shipped for analysis. 2,4-D concentration analyses of these samples were conducted at the University of Florida Center for Aquatic and Invasive Plants via an enzyme-linked immunosorbant assay (ELISA). All herbicide concentrations were reported as 2,4-D acid equivalent (ae).

Aquatic Plant Monitoring

A modified grid-based, point-intercept approach was used in both June and August from 2007 to 2012 in order to compare frequencies of occurrence of plant species over time. Point-intercept grids were created and geo-referenced for each of the seven experimental plots. Each plot contained approximately 40 evenly spaced sample points, a sample size chosen to limit the amount of error acceptable for statistical analyses. At each sample point, a double-sided collection rake was lowered straight through the water column to rest lightly on the sediment bottom, twisted twice around to collect plants, and pulled straight out of the water following methods outlined in Hauxwell et al. (2010). We recorded depth and substrate at each point, and each species on the rake was identified to species following Crow and Hellquist (2000a, b). Plants on the rake were then collected and processed for total biomass weights (Johnson and Newman 2011), and field crews visually estimated the relative percent dry weight of each species present. Samples were dried in an oven for at least 48 hours at 65º C (149ºF), and were then removed and weighed. A random selection of 10% of points from each plot was selected and sorted by species prior to drying in order to provide a quality control check on dry weight field estimates for individual species. Comparison of in-field proportional percent estimations of dry weights to actual lab-weighed and sorted samples were very similar and fell within the 95% confidence interval (Figure 4).

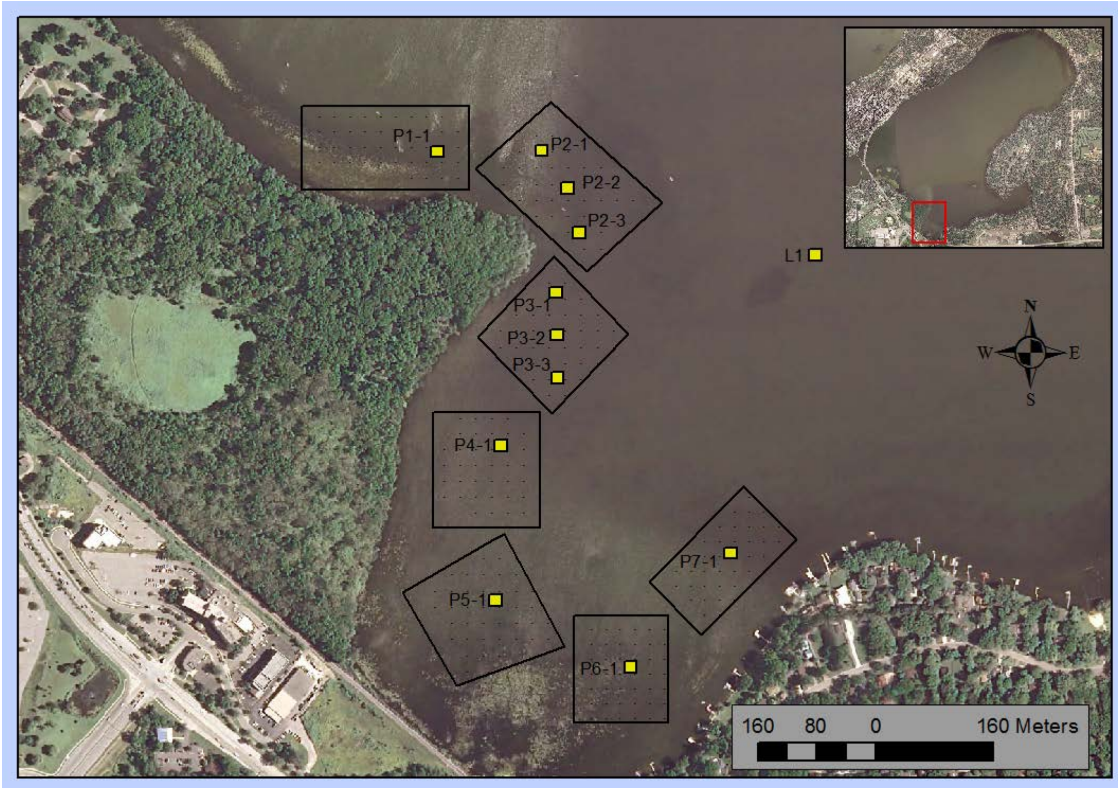


Figure 3. *Herbicide concentration sampling locations, 2008-2010. In 2010, only sites in plots P2, P3, and P4 were sampled.*

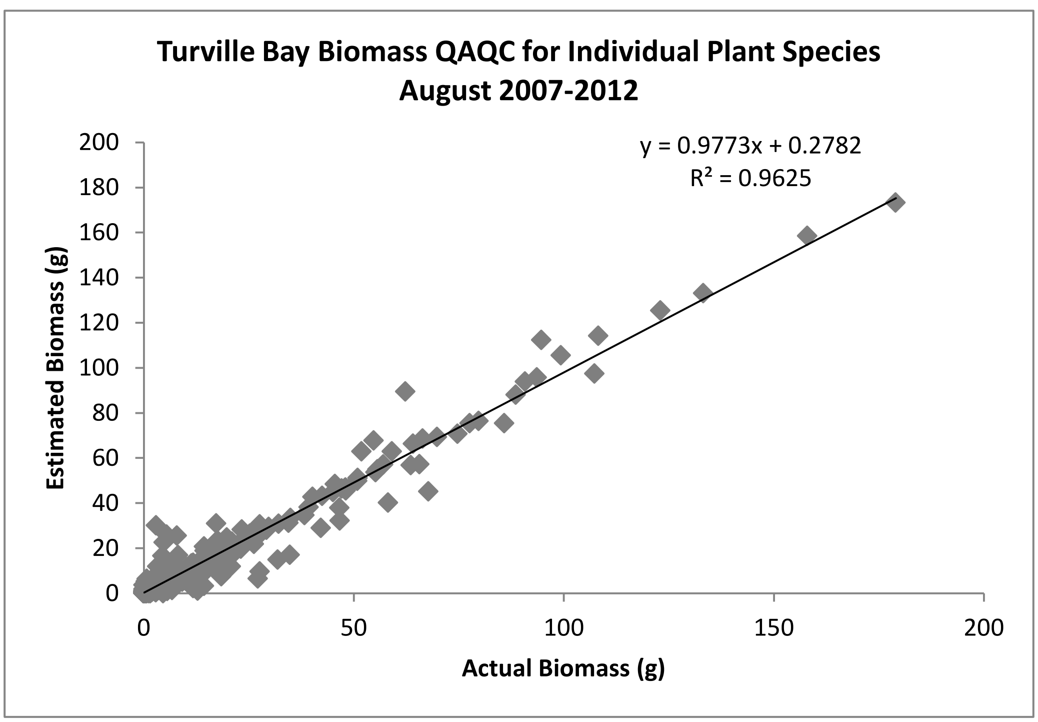


Figure 4. *August 2007-2012 comparison of in-field proportional percent estimations of dry weights to actual lab-weighed and sorted samples (R^2 = 0.9625).*

Statistical Methods

We used generalized linear mixed models to analyze changes in August frequency of occurrence and biomass. This approach allowed us to account for random error introduced by natural plot differences and sampling over multiple years. We included year and plot in the model as random effects to account for this. In this way, our analysis approach is conservative, and significance applied to the models is more robust than if data were analyzed as independent observations. To determine if treatments differed overall from the untreated controls, we combined treatment by type (chemical, harvested, and reference plots) and used these treatment types as the predictor of species occurrence. We only analyzed species that occurred with a frequency of at least 5% at any one sampling event and did not analyze less frequent species. We tested biomass data for outliers using boxplots and deleted extreme outliers which were more than 3 times above or below the third or first interquartile range, respectively. We did not analyze the June data due to differing harvesting times, which included two years where harvesting was completed after the June plant data were collected and made inferences to treatment effects unreliable. However, we did use the June data to investigate potential changes in curly-leaf pondweed (*Potamogeton crispus*) occurrence due to its differing early-season growth pattern from other aquatic species. We used $\alpha < 0.05$ to determine significant results, and all statistical analysis was completed using the R statistical environment (R Development Core Team 2011). Asterisks displayed on the following frequency and biomass graphs indicate a statistically significant difference from reference conditions.

Results

Herbicide Concentration Monitoring

***2008* –** The target herbicide concentration rate per plot was 1,200-1,300 µg/L ae. Pre-treatment background readings were below detectable limits for all samples taken. The maximum 2,4-D concentration recorded in the herbicide treated plots was 21 µg/L ae observed at the first post-treatment sampling event conducted one DAT (Figure 5). The maximum 2,4-D concentration recorded two DAT was 12 µg/L ae, with a mean concentration in the treatment plots of 5 µg/L ae. These very low measured concentrations indicate that the herbicide was very quickly dissipated off the treatment sites within the first few hours after treatment. Concentrations of 2,4-D measured in the untreated areas and harvesting plots were negligible and near detection limits in all samples (<10 µg/L ae). By comparison, the water use restrictions listed on the 2,4-D label are 100 µg/L ae for irrigation and 70 µg/L ae for human drinking.

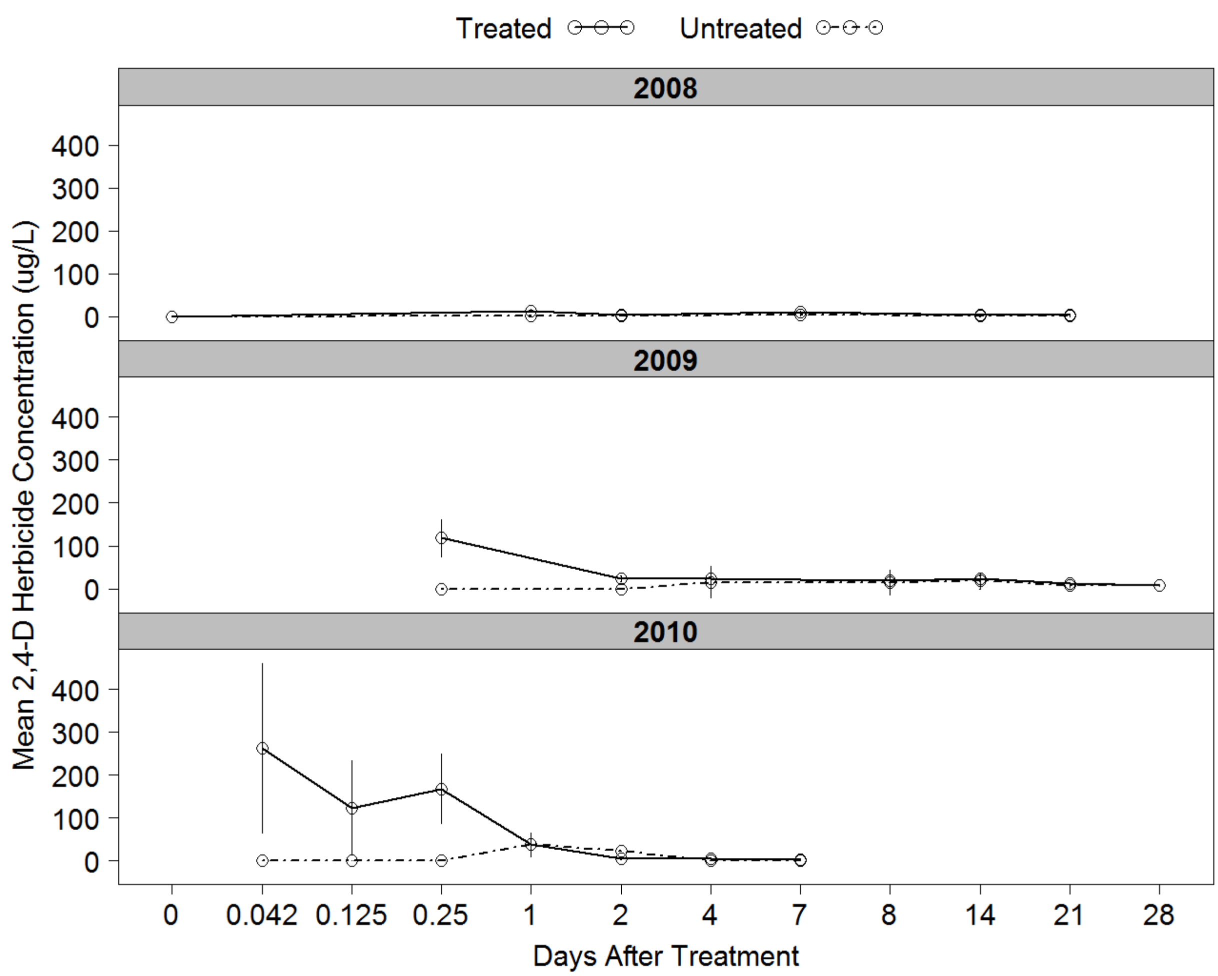


Figure 5. *Mean herbicide concentration (± 95% confidence interval) in Turville Bay at treated (solid lines) and untreated (dashed lines) plots, 2008-2010.*

2009 – Initial 2,4-D concentrations within the treatment plots collected at six hours after treatment (HAT) ranged from less than 5 µg/L ae to greater than 250 µg/L ae, compared with the target application concentrations of 1,570 to 1,750 µg/L ae. Weather prevented collection of herbicide concentration samples at one DAT, however all herbicide concentration samples within the treated areas were less than 50 µg/L ae by two DAT, with a mean concentration of 23 µg/L ae. Concentrations of 2,4-D in the untreated areas and harvesting plots were mostly near detection limits (< 10 µg/L ae), but varied from 0 to 45 µg/L ae throughout different plots and individual sampling events. These results were similar to results observed in 2008, which also suggested very rapid dissipation of the herbicide off the treatment sites.

2010 – Previous herbicide concentration monitoring in 2008 and 2009 showed that concentrations of 2,4-D in the untreated areas and harvesting plots were near or below detection limits (10 µg/L ae), so herbicide concentration sampling was conducted only within the treatment plots (P2 and P3) and in adjacent plot P4 during 2010. Initial 2,4-D concentrations in the treatments plots at one HAT ranged from 63 to 125 µg/L ae in plot P2 and 149 to 587 µg/L ae in plot P3, compared to the target application concentrations of 1,570 and 1,750 µg/L ae, respectively. Mean concentrations at one HAT were 94 µg/L ae in plot P2 and 431 µg/L ae in plot P3. The mean concentration for 0 to 24 HAT was 98 µg/L ae in plot P2 compared to 198 µg/L ae in plot P3. Concentrations in both plots were less than 100 µg/L ae (irrigation restriction level) by approximately 18 HAT, indicating rapid dissipation from target areas. Concentrations in the adjacent untreated plot (P4) were < 40 µg/L ae at one and two DAT indicating that minimal 2,4-D drifted into this area. These results were slightly higher, but similar to results seen in 2008 and 2009.

Aquatic Plant Monitoring

Invasive Plant Species – Two non-native aquatic plant species are found in Turville Bay, EWM and curly-leaf pondweed. During the pre-treatment year (2007), there were no differences between frequencies of occurrence of EWM in the reference or treatment plots (Figure 6). EWM decreased significantly in comparison to the reference plots for all years of herbicide treatments, and one year of control was maintained following the cessation of treatments ($p < 0.001$). In the harvested plots, EWM frequency of occurrence decreased significantly following the third and fourth year of treatment ($p < 0.001$), but did not exhibit decreases in the first two years of harvesting. During the last year of data collection (2012), when no herbicides or harvesting occurred, all managed plots returned to pre-treatment reference levels indicating that control of this aquatic invasive plant requires continued active management. The mean biomass of EWM followed a similar overall trend as the frequency data, however, the only significant decline in mean biomass observed was in both herbicide and harvested plots during the first year of treatment ($p < 0.001$).

Curly-leaf pondweed is typically treated using the herbicide endothall, but has also been shown to be susceptible to 2,4-D at higher exposure concentrations (Belgers et al. 2007), or effectively controlled using a combination of endothall and 2,4-D (Skogerboe and Getsinger 2006). Curly-leaf pondweed has a natural tendency to emerge early in the growing season and senesce during the summer, and for this reason we analyzed the results of the June survey data instead of August. From 2009 to 2011, curly-leaf pondweed frequency of occurrence was significantly lower in the herbicide treatment plots compared to the reference plots (Figure 7, $p < 0.001$). Curly-leaf pondweed in the harvested plots was not significantly different from reference plots. It is important to note that the frequency of occurrence and biomass in the harvested treatments for June 2008 and 2011 may be overestimated as the cutting had not occurred prior to our survey.

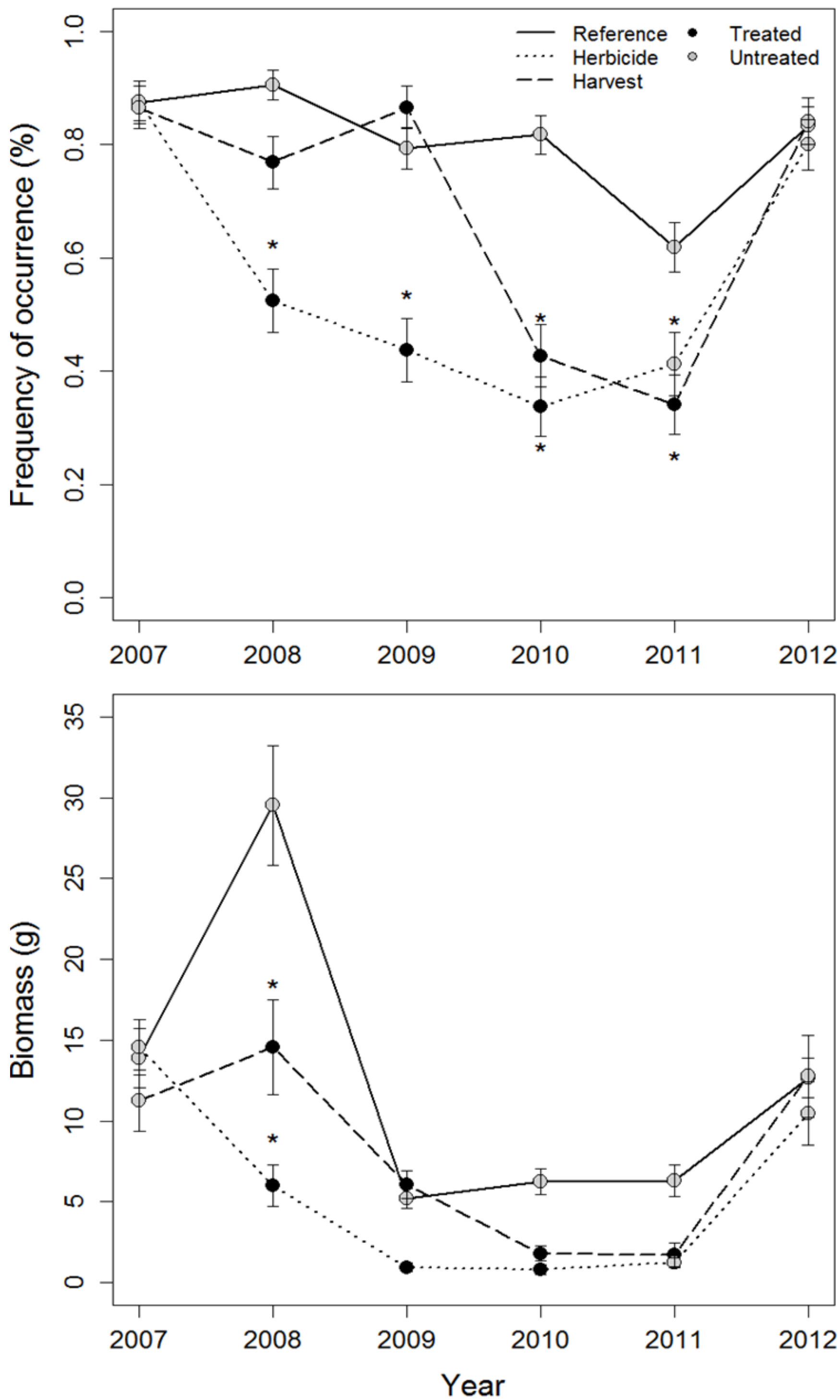


Figure 6. *August Eurasian watermilfoil frequency of occurrence (%) ± standard error (top) and mean biomass (g) per treatment ± standard error (bottom) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

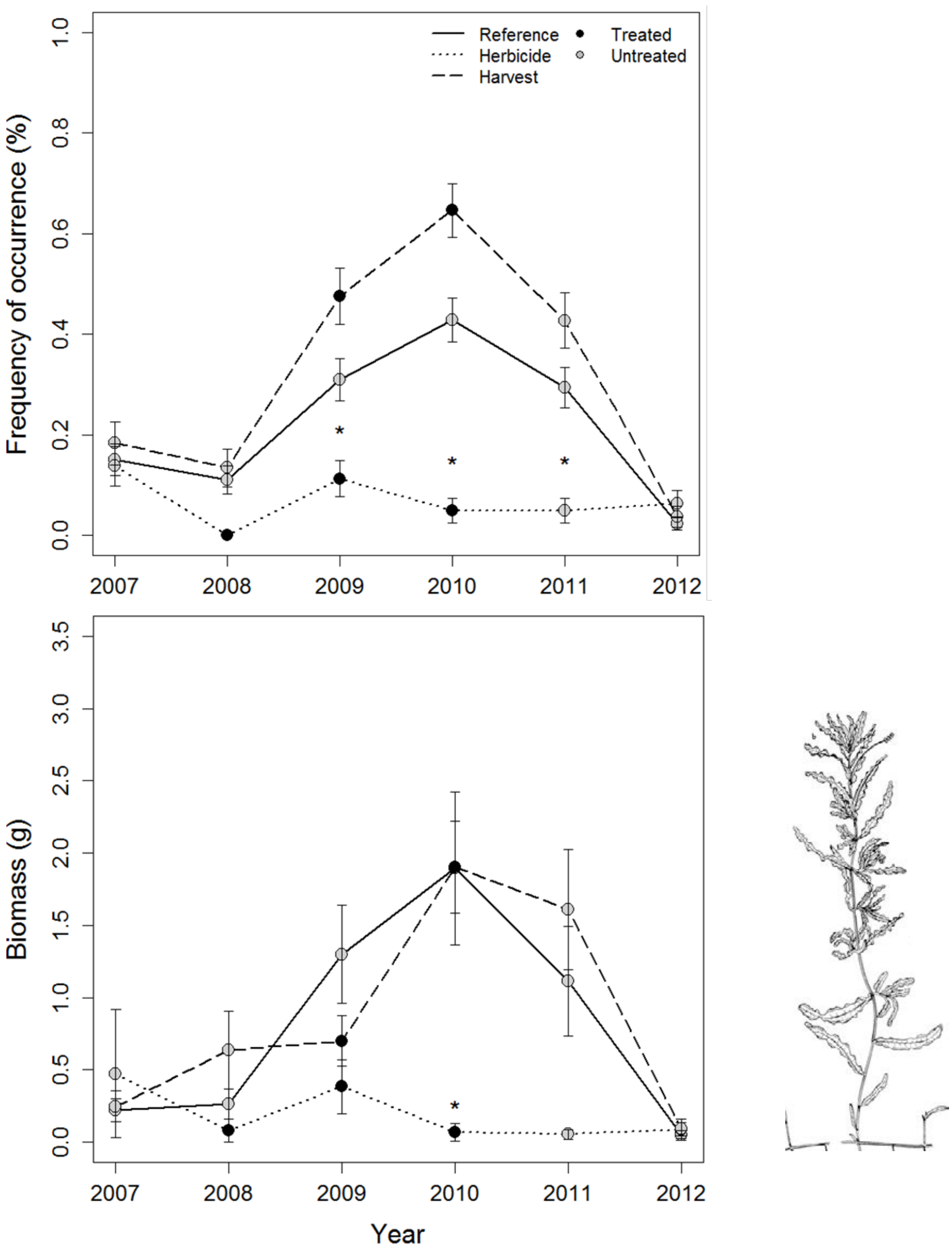


Figure 7. *June survey results of curly-leaf pondweed frequency of occurrence (%) ± standard error (top) and mean biomass (g) per treatment ± standard error (bottom) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012. In 2008 and 2011, harvesting occurred post survey.*

Native Plant Species – We collected 13 native plant species during the course of the study. Seven of these species occurred with a frequency of occurrence greater than 5% (Tables 3-4 and 6) and are discussed here in greater detail based on results from surveys conducted in August. Data for the remaining six native species with frequencies of occurrence less than 5% are shown in Appendix B.

Table 3. *Summary of the aquatic plant species found with frequencies greater than 5% during the Turville Bay study. Arrows indicate statistically significant ($p < 0.05$) increases or decreases in frequency of occurrence (%) in either the herbicide or harvested plots in relation to the reference plots. n.s. = no significant difference, - = no analysis, ND = species not found during survey.*

Species	Treatment	2007	2008	▲▼	2009	▲▼	2010	▲▼	2011	▲▼	2012	▲▼
*Myriophyllum spicatum**	Reference	87.3	90.5		79.4		81.7		61.9		83.3	
	Herbicide	87.5	52.5	▼	43.8	▼	33.8	▼	41.3	▼	80.0	n.s.
	Harvest	86.6	76.8	n.s.	86.6	n.s.	42.7	▼	34.1	▼	84.1	n.s.
*Potamogeton crispus**	Reference	ND	0.8		3.9		0.8		1.5		ND	
	Herbicide	ND	2.5	-	ND	▼	1.3	▼	1.3	▼	ND	n.s.
	Harvest	1.2	ND	n.s.	6.1	n.s.	ND	n.s.	7.3	n.s.	ND	n.s.
Ceratophyllum demersum	Reference	88.9	82.5		79.4		90.5		84.1		81.7	
	Herbicide	85.0	67.5	▼	67.5	▼	71.2	▼	60.0	▼	75.0	n.s.
	Harvest	86.6	95.1	▲	96.3	▲	82.9	▼	59.8	▼	84.1	n.s.
Stuckenia pectinata	Reference	8.7	2.4		9.5		22.2		20.6		19.8	
	Herbicide	17.5	20.0	n.s.	22.5	▲	11.3	▼	31.3	n.s.	38.8	n.s.
	Harvest	6.1	4.9	n.s.	12.2	n.s.	12.2	n.s.	14.6	n.s.	17.1	n.s.
Potamogeton foliosus	Reference	ND	ND		0.8		ND		0.8		ND	
	Herbicide	ND	ND	-	3.8	▲	ND	-	8.8	▲	12.5	▲
	Harvest	ND	ND	-	6.1	▲	4.9	▲	6.1	▲	1.2	n.s.
Valisneria americana	Reference	0.8	0.8		ND		0.8		0.8		0.8	
	Herbicide	3.8	3.8	▲	5.0	▲	6.3	▲	10.0	▲	10.0	▲
	Harvest	1.2	2.4	n.s.	ND	-	ND	-	ND	-	ND	-
Heteranthera dubia	Reference	ND	0.8		ND		ND		0.8		ND	
	Herbicide	3.8	3.8	n.s.	1.3	n.s.	5.0	n.s.	6.3	n.s.	17.5	▲
	Harvest	1.2	1.2	n.s.	ND	-	2.4	n.s.	1.2	n.s.	ND	-
Elodea canadensis	Reference	ND	ND		4.0		26.2		13.5		4.0	
	Herbicide	ND	ND	-	2.5	n.s.	21.3	n.s.	2.5	n.s.	11.3	n.s.
	Harvest	ND	1.2	n.s.	34.1	▲	36.6	n.s.	19.5	n.s.	25.6	▲
Potamogeton richardsonii	Reference	5.6	2.4		1.6		7.1		11.1		11.1	
	Herbicide	22.5	23.8	n.s.	21.3	n.s.	12.5	n.s.	18.8	n.s.	22.5	n.s.
	Harvest	3.7	4.9	n.s.	7.3	n.s.	3.7	n.s.	7.3	n.s.	3.7	n.s.
*indicates invasive species; ND = not detected; n.s. = not significant												

Table 4. *Summary of total number of individual native and invasive species which significantly increased or decreased from reference levels, 2008-2012.*

	2008		2009		2010		2011		2012	
	Herbicide	Harvest	Herbicide	Harvest	Herbicide	Harvest	Herbicide	Harvest	Herbicide	Harvest
Total Native Increases	1	1	3	2	1	1	2	1	3	1
Total Native Decreases	1	0	1	0	2	1	1	1	0	0
Total Invasive Increases	0	0	0	0	0	0	0	0	0	0
Total Invasive Decreases	1	0	2	0	2	1	2	1	0	0

The dominant native species in regard to biomass in all the plots was coontail (*Ceratophyllum demersum*) as indicated by the similarity in the frequency and mean total native biomass (Figure 8). Overall mean native biomass per point was 21.0 g and 73.1% of this was coontail.

Coontail in the herbicide plots followed the same pattern of decrease as that of EWM, with significant declines observed from 2008 to 2011 ($p < 0.001$). The decline persisted one year post-treatment before returning to pre-treatment reference levels in 2012. Coontail frequency in the early-season harvesting plots increased significantly during the first two years of treatment ($p < 0.01$) before declining significantly the fourth year of treatment ($p < 0.001$). The harvested plots returned to reference levels the first year after cessation of treatment. The mean biomass of coontail increased across the study period in the reference plots.

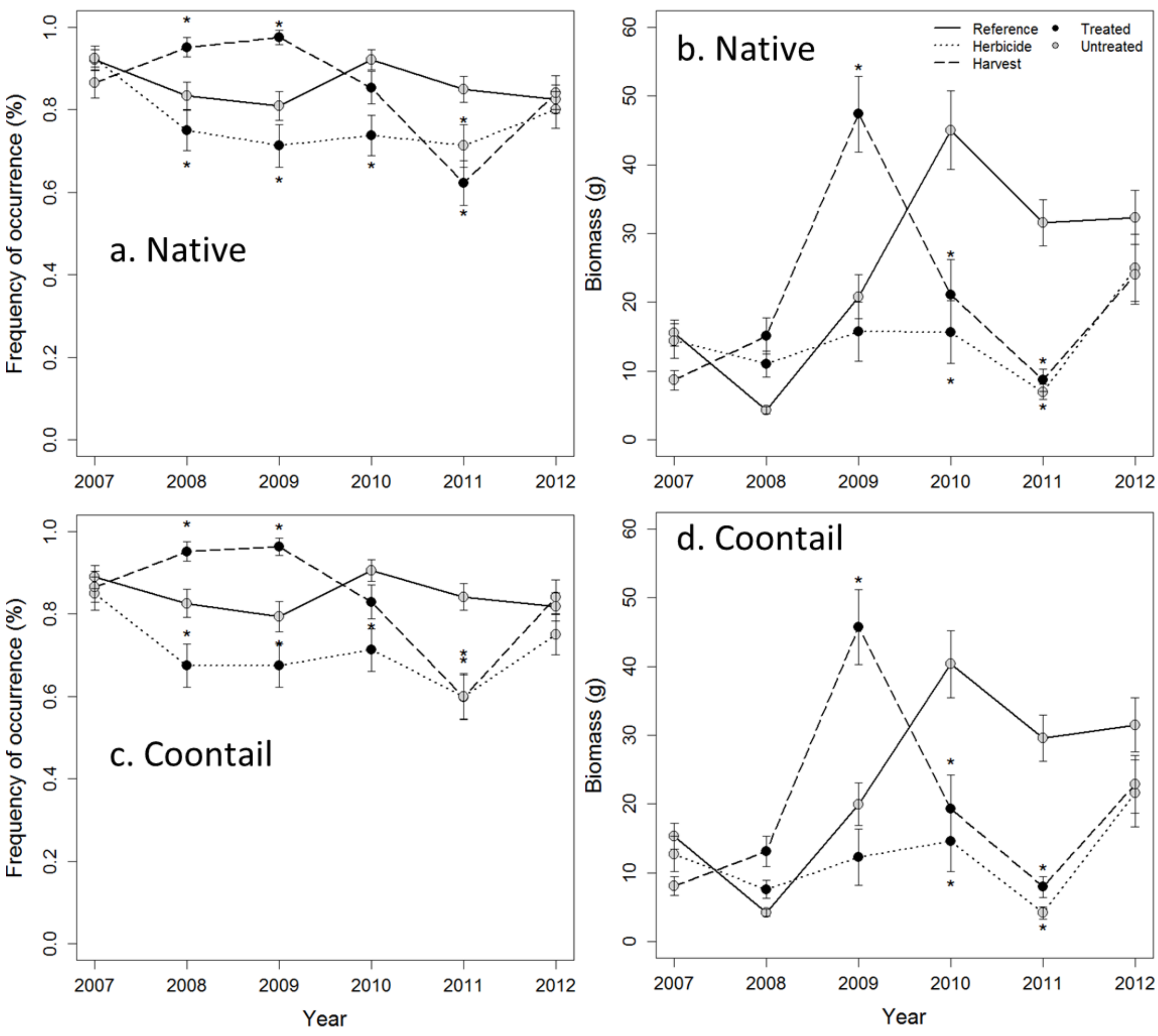
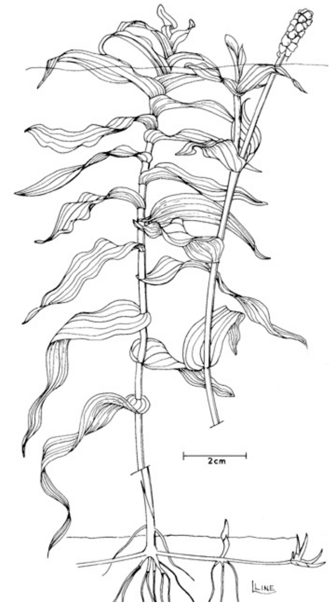


Figure 8. *Mean August combined native plant and coontail frequency of occurrence (%) ± standard error (a and c) and combined native plant and coontail mean biomass (g) per treatment ± standard error (b and d) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*



Sago pondweed (*Stuckenia pectinata*) frequency of occurrence increased over the course of the study across all plots (Figure 9). In 2009, there was significantly more sago pondweed in the herbicide plots than the reference plots ($p = 0.013$). In 2010, sago pondweed exhibited a significant decline in the herbicide plots ($p = 0.028$), but it rebounded in 2011 and continued its overall increasing trend over time. There was no difference between reference and harvested plots during the study. Sago biomass decreased significantly in the herbicide plot in 2010 ($p < 0.001$) and was significantly lower in the harvested plots compared to the reference plots in both 2010 and 2011.

We found no significant difference in the frequency of occurrence in clasping-leaf pondweed (*Potamogeton richardsonii*) between the reference and managed plots. The mean biomass in the first year of herbicide treatment was significantly higher in herbicide treatment plots ($p = 0.009$), and except for 2010, was higher overall in the herbicide treatment plots than observed in the other plots (Figure 10).

Leafy pondweed (*Potamogeton foliosus*) increased significantly in both the herbicide and harvesting plots beginning in 2009 ($p < 0.01$, Figure 11), possibly indicating that the reduction of previously highly dominant species such as EWM and coontail opened up habitat for this native species.

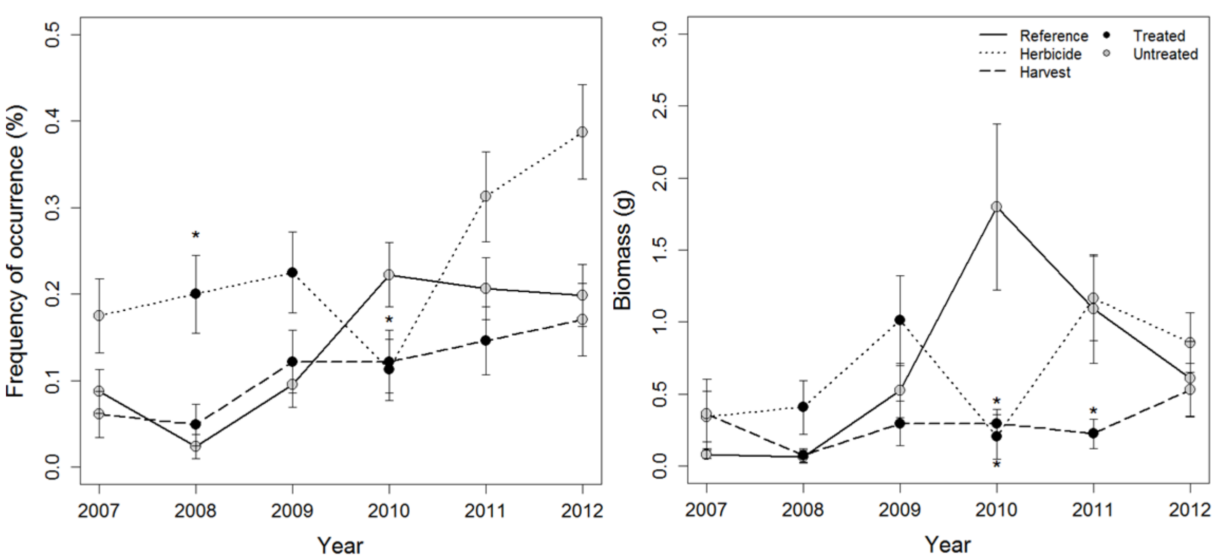


Figure 9. *August sago pondweed frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

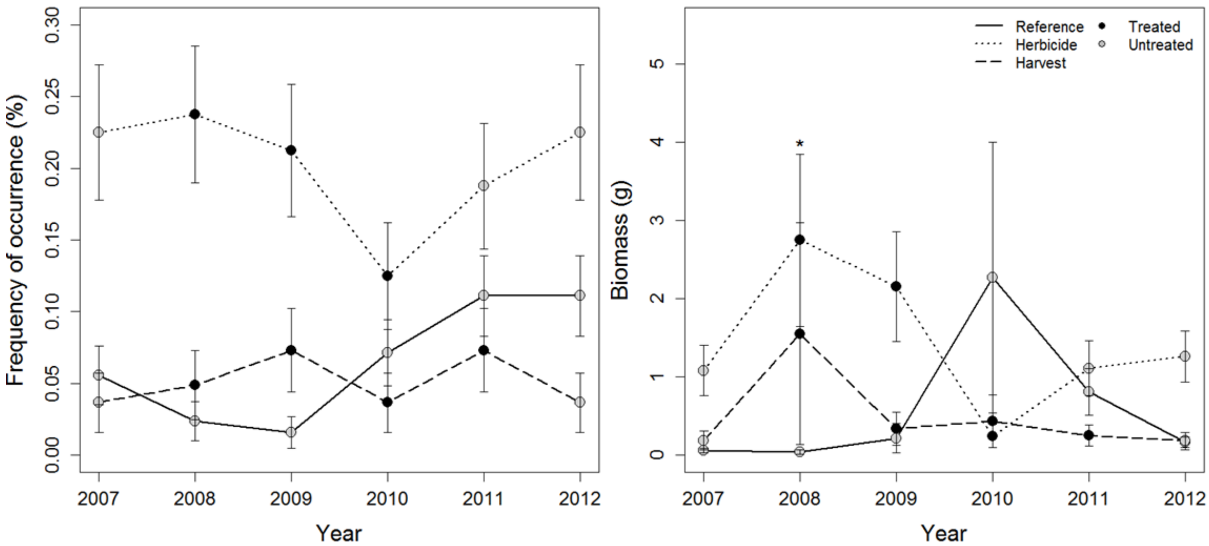


Figure 10. *August clasping-leaf pondweed frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

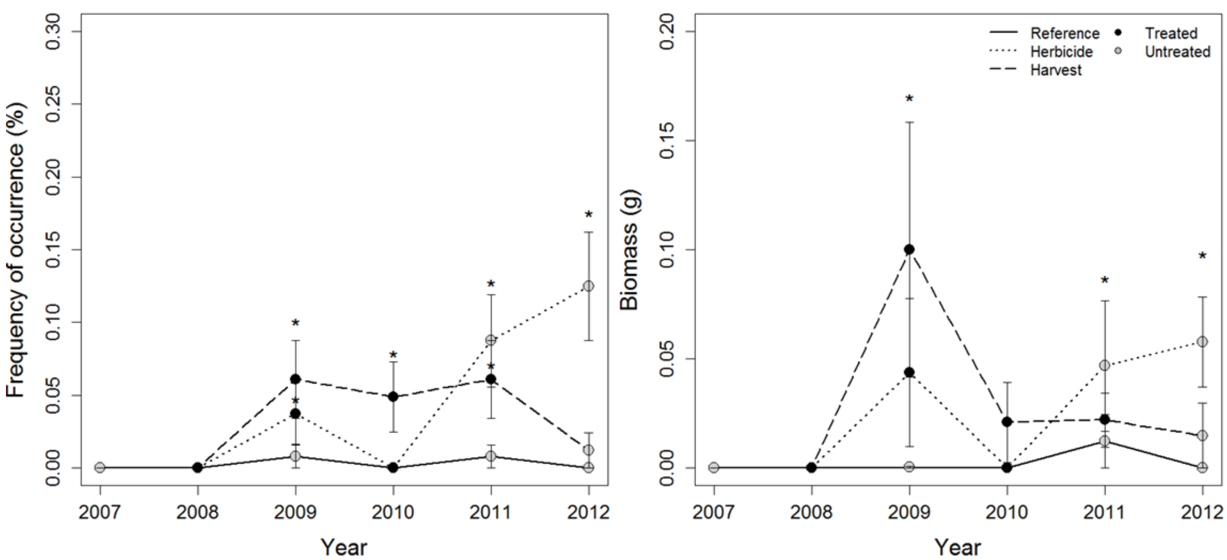


Figure 11. *August leafy pondweed frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

Wild celery (*Vallisneria americana*) frequency of occurrence and biomass increased significantly across the study period in the herbicide plots ($p < 0.01$; Figure 12). This also may possibly indicate that creation of niche habitat by reducing highly dominant species may be increasing native presence and diversity. The frequency of occurrence within the harvested plots did not differ from the reference plots across the study period and was below 5% frequency of occurrence in both areas.

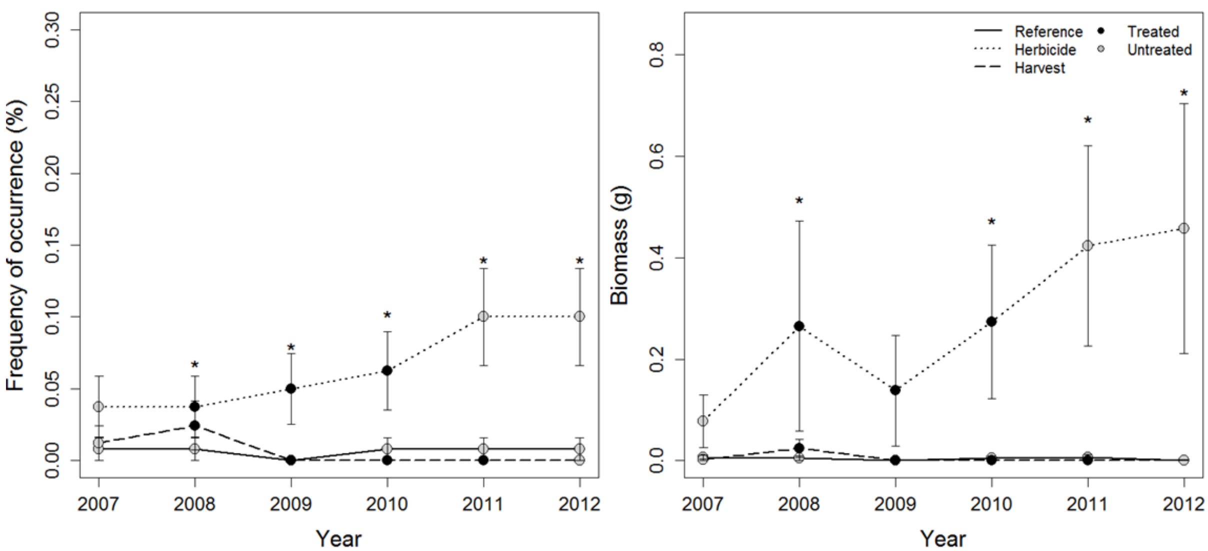


Figure 12. *August wild celery frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

Water star-grass (*Heteranthera dubia*) underwent an increasing trend in the herbicide plots beginning in 2010 but its frequency of occurrence and biomass only became significantly higher the final year of the study ($p < 0.001$; Figure 13). The frequency of occurrence in the harvested plots did not differ from the reference plots during the study period. The mean biomass in 2008 was significantly higher in the harvested plot compared to the reference ($p = 0.016$). These results again may indicate a species' ability to increase in distribution with increased habitat availability.

Common waterweed (*Elodea canadensis*) did not differ significantly in the herbicide and reference plots, but it did increase significantly in the harvested plots in 2009 and 2012 ($p < 0.001$; Figure 14). A similar pattern is reflected in the mean biomass data with significantly higher values in 2009 and 2010 ($p < 0.001$).

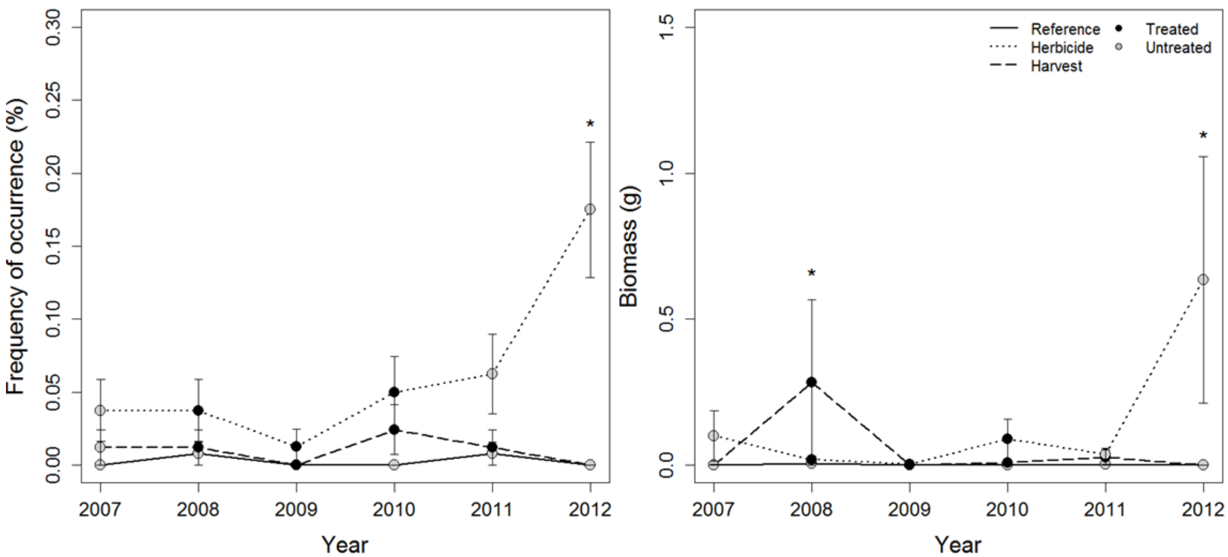


Figure 13. *August water star-grass frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

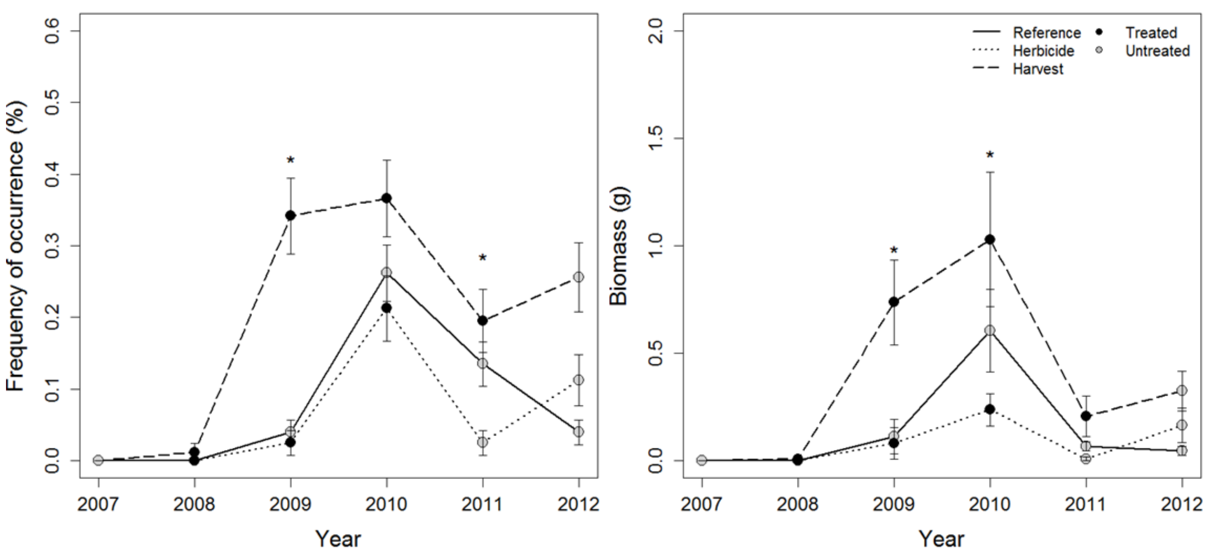


Figure 14. *August common waterweed frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

Discussion

Both chemically treated and mechanically harvested plots were effective at reducing EWM frequency of occurrence and biomass over the study period. The reduction in EWM was sustained during all years of chemical treatment as well as the initial year after treatment ceased. Curly-leaf pondweed was also suppressed in herbicide treatment plots the second and third treatment years, as well as the year after treatment ceased. The herbicide treatment had immediate effects on coontail that were observed for all treatments years and the year following treatment cessation. Sago pondweed was the only other native which exhibited a significant decline in the herbicide treatment plots during 2010, but rebounded in frequency of occurrence in 2011. Four native species with frequencies of occurrence greater than 5% increased within the herbicide plots during the study.

Herbicide treatment efficacy is dependent on both the concentration of herbicide used and exposure time within the treatment area (Green and Westerdahl 1990, Netherland et al. 1991). Based on previously conducted laboratory 2,4-D exposure time and concentration relationships, effective control of EWM requires maintained concentrations at 2000 µg/L ae (2.0 ppm) for 24 hours, with lower rates of herbicides requiring longer contact times to achieve similar results (i.e. 1000 µg/L ae for 36-48 hours and 500 µg/L ae for 72 hours) (Green and Westerdahl 1990). The rapid rate at which 2,4-D dissipated from the treatment areas in this study was not anticipated, but is supported by more recent research into small-scale, granular 2,4-D treatments (J. Skogerboe, pers. comm.). Therefore, the herbicide concentration sampling regime in 2008 and 2009 was likely not intensive enough to capture the effective concentrations within the first 24 hours. In 2010, sampling was further refined to try to capture this initial spike within the first few hours after application occurred, although even with the increase in concentration sampling intensity, herbicide concentrations were well below the intended target concentrations. In 2008, the lack of addition of muriatic acid at the time of sample collection may have allowed herbicide degradation to continue after sample collection, and this may explain the reduced herbicide concentrations in 2008 compared to those of 2009 and 2010. Despite the lower than anticipated herbicide concentrations observed in the water column, treatments were effective in decreasing EWM by approximately 50% the first year of treatment and continued to reduce EWM with the successive chemical treatments.

The harvested plots did not show initial control of EWM during the first two years of treatments, but showed declines in EWM following the third and fourth years of consecutive harvesting. EWM, however, returned to pre-treatment reference levels the first year after harvesting treatments stopped. Harvesting did not reduce curly-leaf pondweed frequency or biomass throughout the study. The effects of harvesting on coontail were varied, with increases observed in the first two years of treatments and decreases observed in the third and fourth years of treatment. Repeated yearly, early-season harvesting efforts did not negatively impact any other native species during this study. Two other native species with frequencies of occurrence greater than 5% also increased in harvested plots during the study. Our results of achieving within-season control are consistent with other EWM harvesting projects (Perkins and Sytsma 1987, Painter 1988).

EWM and curly-leaf pondweed are known to emerge earlier in the growing season than many native species (Nichols and Shaw 1986), and by reducing their biomass before other native plant species have fully emerged, managers could provide natives an opportunity to grow with less competition from these invasive species. To capitalize on this early season approach, the timing of harvesting was based on when plants neared the water's surface which made implementation variable from year to year. The harvesters have limited depths in which they can cut (4.5-5.0 feet), which became an operational issue the first two years of treatment due to unusually high water levels in those years. This may have reduced the overall effectiveness of harvesting and may help to explain the lack of control observed in those years. The original study design intended to achieve cutting down to 6.0-6.5 feet, but due to the physical limitations of the harvesters this was not possible. In the future, if harvesters can be modified to cut at deeper depths, it would achieve the goal of reaching closer to the sediment's surface and could potentially improve control. Also, implementing an early-season harvesting regime as opposed to a peak growth method may reduce the overall harvesting effort needed in a growing season, and could potentially save on staff hours and operational costs associated with running the equipment. In contrast, herbicide treatments by design are inherently easier to employ and less dependent on water levels, and are limited more by water temperatures and wind speeds.

Overall, the chemically treated plots observed more years of continuous control of EWM than the harvested treatment plots. Herbicide treatments incurred a greater overall impact on coontail, a dominant species in the Yahara system, in comparison to harvesting treatment plots. However, more native species increased in frequency of occurrence in the herbicide treatment plots than the harvested plots, and overall impacts on native species using either treatment technique was minimal. Although the drivers affecting aquatic plant composition of lakes have been studied (Mikulyuk et al. 2011), very few studies have examined the restoration of native species after habitat management efforts (Reid et al. 2009, Shafer and Bergstrom 2010). The rate and extent of native plant succession into managed areas depends heavily on the existing habitat conditions and their level of suitability for plant growth (Prach and Hobbs 2008) in addition to the continued suppression of the invader.

The use of either small-scale, early-season treatment minimized negative non-target impacts to the less dominant native plant species within Turville Bay. It is difficult to aggressively manage invasive species while minimizing impacts to natives in a system that is highly diverse. Controlling invasive species on a small scale where diversity is low, however, may be a better option (Rinella et al. 2009). In small shallow areas of a larger system, such as Turville Bay, treating with an herbicide or harvesting mechanically may be the best options for control, and maintaining control requires continued monitoring and adaptive management. There have been several short-term, small-scale projects that have examined the effects of herbicides on aquatic organisms such as insects, zooplankton, amphibians, and fish (Boone and James 2003, Relyea 2005, Cattaneo et al. 2008), however, the long-term effects of continued herbicide use on an ecosystem are understood poorly. There is also evidence that invasive plant species are less sensitive to herbicides or develop resistance to herbicides after repeated exposures, for example, with hydrilla (*Hydrilla verticillata*) and the herbicide fluridone (Puri et al. 2007, Richardson 2008, LaRue et al. 2013). Potential ecosystem impacts and developing resistance to herbicides should be considered carefully when repeatedly employing herbicides in a management plan. Another important consideration is the hybridization of Eurasian watermilfoil with the native northern watermilfoil (*Myriophyllum sibiricum*) within Wisconsin lakes (Moody and Les 2002). Several strains of these hybrids have been shown to grow faster and to be less affected by 2,4-D treatments, which may increase their invasiveness and affect how they are ultimately managed (LaRue et al. 2013). The long-term effects of repeated mechanical harvesting are also understood poorly as most studies are conducted over the short term and some only examined the target species (Peterson et al. 1974, Mikol 1985, Painter 1988, Engel 1990). Because long-term ecosystem changes caused by continued management are understood poorly (Nichols 1991), biological monitoring of treatment areas is necessary to determine the effectiveness of management and to detect any adverse changes that may occur throughout the management period and beyond.

Conclusions

1. The use of early-season 2,4-D treatments on small target areas of EWM may provide selective nuisance control.

2. The use of early-season harvesting may also provide nuisance control of EWM in small areas of larger lake systems. Successive years of treatment, however, may be necessary to begin to achieve good control.

3. The long-term ecosystem impacts of herbicide and harvesting treatments are not well understood and need further study.

4. Deciding which control method to use should be based on the overall management goals and time scale to achieve those goals.

5. Small-scale management activities within large lakes can provide temporary, localized nuisance control of EWM with little impact to natives.

6. Long-term restoration of an aquatic plant community after a successful invader becomes established remains a challenge for managers.

Acknowledgments

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Line drawings of plants courtesy of the University of Florida Center for Aquatic and Invasive Plants. Available at http://plants.ifas.ufl.edu/linedrawings.

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Appendix A – Water Quality Monitoring Data

Water quality was monitored from April through September, 2008-2012, in order to track any changes that may have occurred as a result of these management activities. Water clarity was measured monthly using a Secchi disk, and total phosphorus and chlorophyll *a* were also sampled monthly (Figure 15). Samples were collected at one or two locations within the center of Turville Bay midway between the plots. One site outside of the bay also was monitored monthly. The collection method did not lend itself to statistical comparison of the research plots within this study. Trophic state indices (TSI, Table 5) were calculated using the statewide equations for Secchi depth, chlorophyll, and total phosphorus according to Lillie et al. (1993) based on the original TSI work by Carlson (1977). The monthly means for chlorophyll range from 11-23 µg/L. The monthly means for total phosphorus ranged from 45 to 80 µg/L. Chlorophyll and total phosphorus exhibited an inverse temporal trend from April through September. Water clarity was measured using a Secchi disk and the monthly mean ranged from 1.2-2.8 meters (4.0-9.2 feet) from April to September. As chlorophyll increased throughout the season, water clarity decreased. TSI values ranged from 42-63, exhibiting a gradual seasonal shift from mesotrophic to eutrophic conditions throughout the growing season (Table 5).

Table 5. *Monthly trophic state index (TSI) values in Turville Bay, 2008-2012.*

Chlorophyll a TSI						
Year	**April**	**May**	**June**	**July**	**August**	**September**
2008	ND	ND	49	48	59	57
2009	56	46	50	59	57	60
2010	ND	44	56	56	57	59
2011	57	46	54	56	59	ND
2012	ND	61	ND	55	57	ND
Total Phosphorus TSI						
Year	**April**	**May**	**June**	**July**	**August**	**September**
2008	ND	ND	63	59	59	57
2009	61	61	63	60	59	61
2010	62	62	62	58	58	57
2011	58	59	59	57	55	ND
2012	ND	62	ND	60	57	ND
Secchi Disc TSI						
Year	**April**	**May**	**June**	**July**	**August**	**September**
2008	ND	ND	49	51	59	57
2009	ND	ND	42	59	55	62
2010	56	45	56	55	55	54
2011	50	46	54	57	61	ND
2012	ND	45	ND	59	58	ND
ND = No data collected						
TSI Value Description:						
Oligotrophic = 0 - 39						
Mesotrophic = 40-49						
Eutrophic = 50 - 69						
Hypereutrophic = 70 - 100						

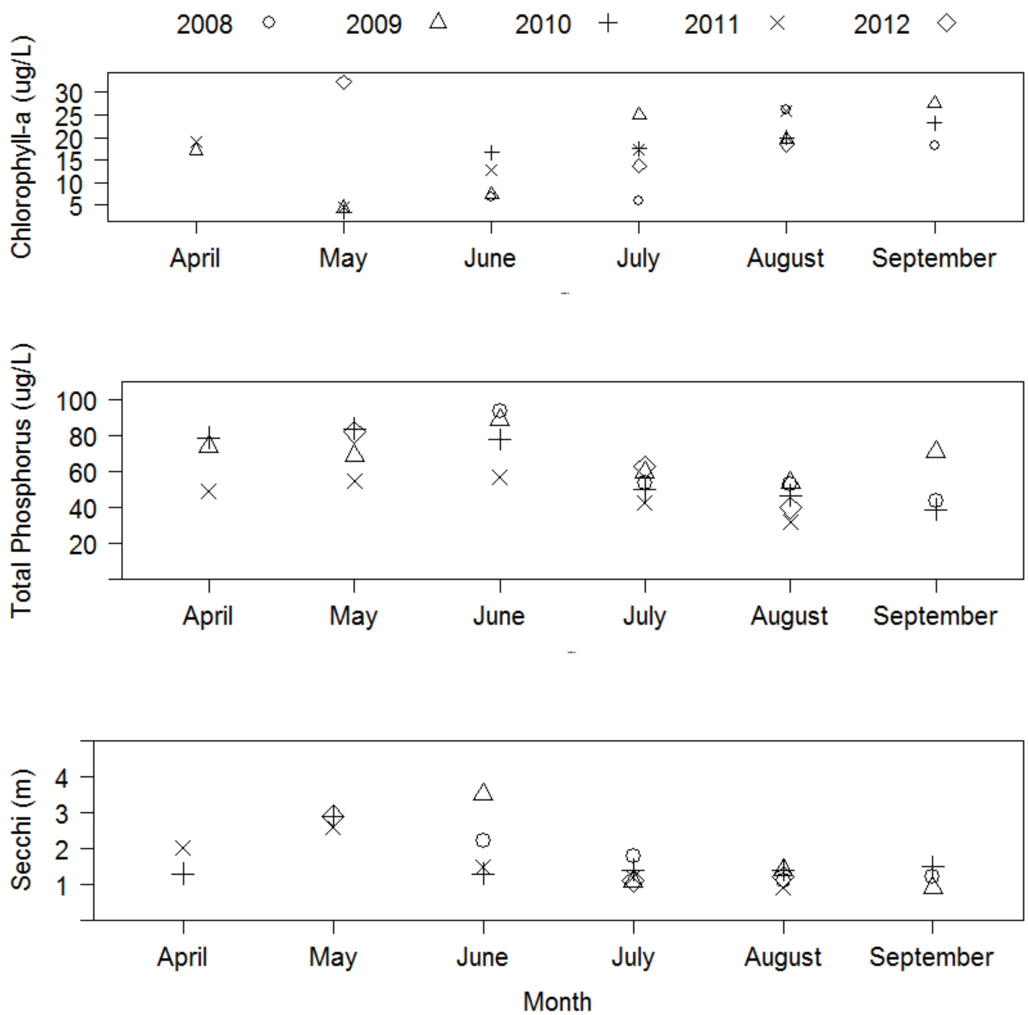

Figure 15. *Monthly chlorophyll* a *(µg/L), total phosphorus (µg/L), and Secchi depth (m) measurements in Turville Bay, 2008-2012.*

Appendix B – Data for Low Frequency Plant Species

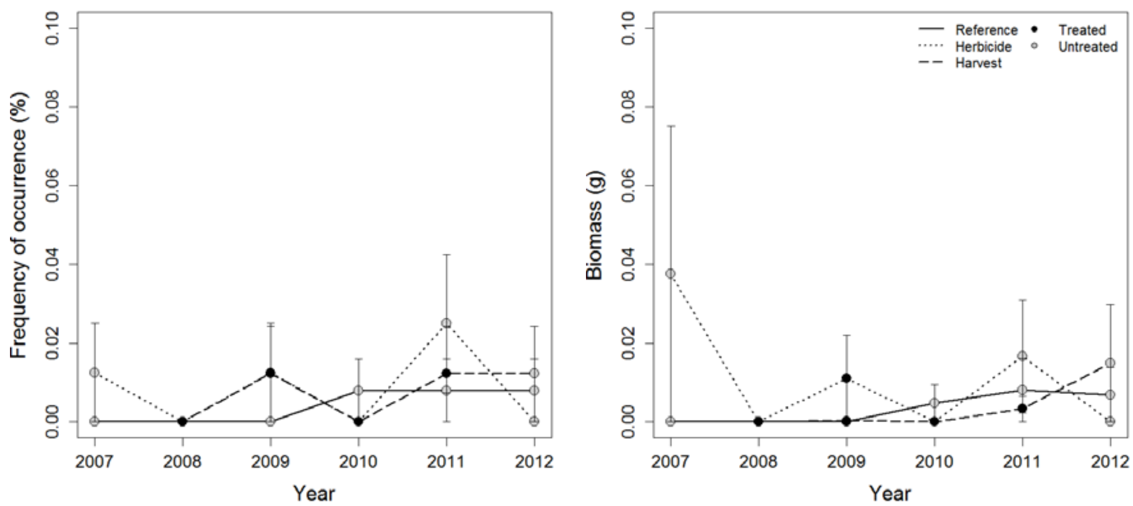

Figure 16. *August chara (*Chara *spp.) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

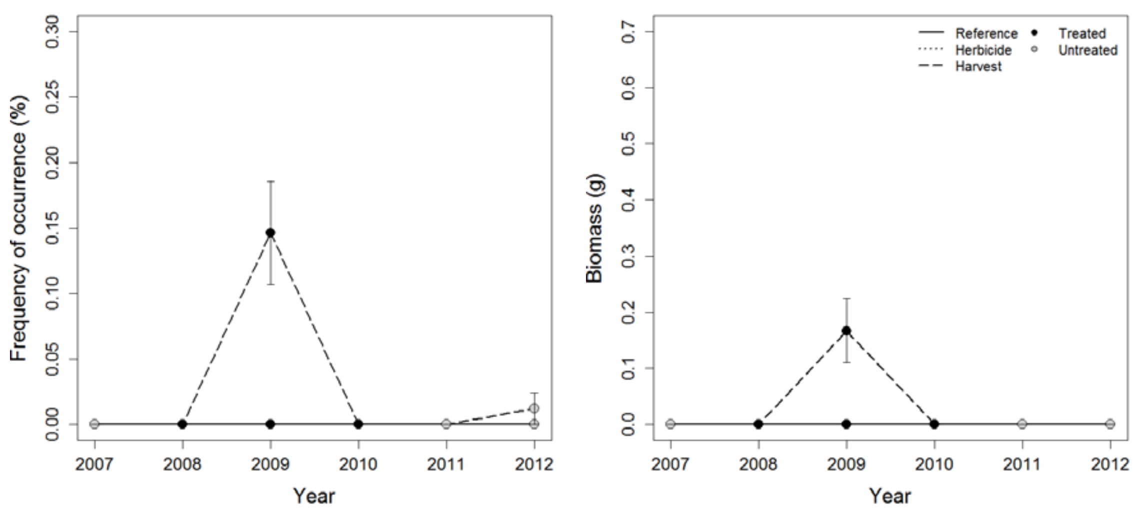

Figure 17. *August small duckweed (*Lemna minor*) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

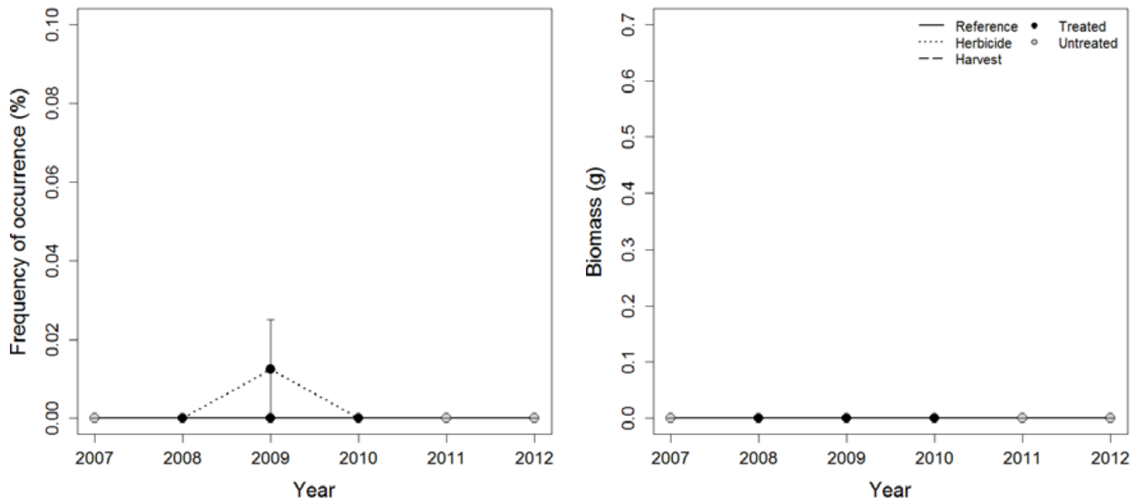

Figure 18. *August slender naiad (*Najas flexilis*) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

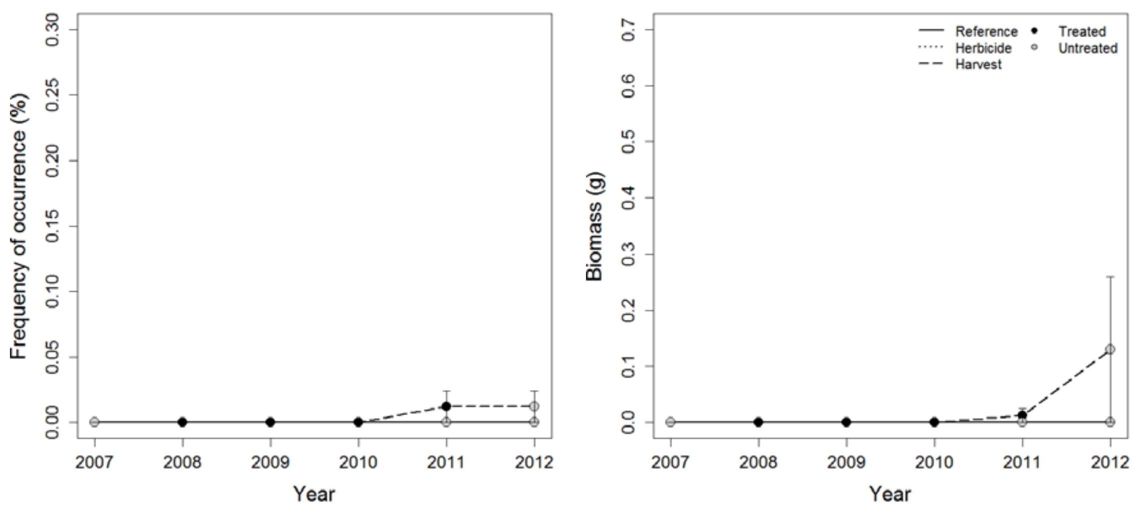

Figure 19. *August flat-stem pondweed (*Potamogeton zosteriformis*) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

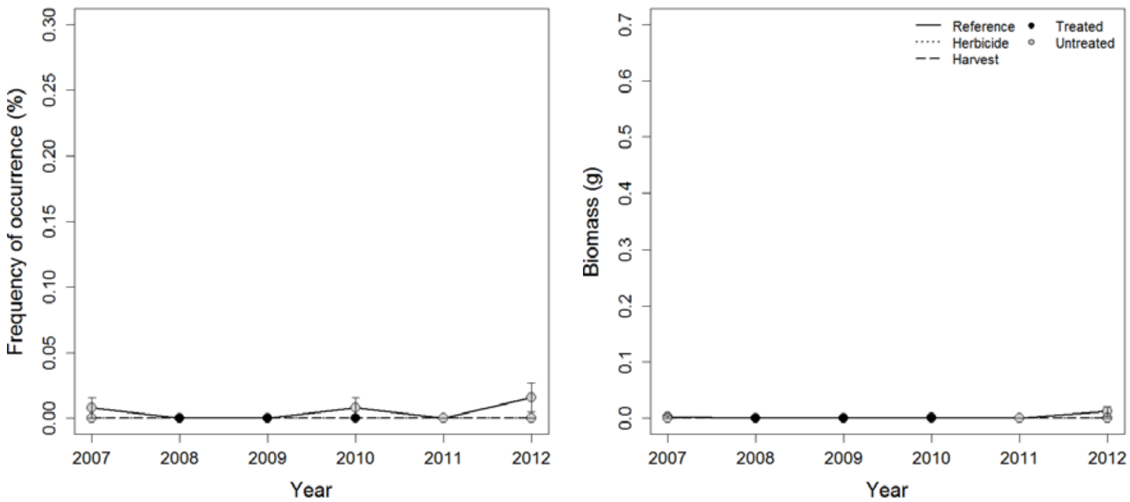

Figure 20. *August white water crowfoot (*Ranunculus aquatilis*) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

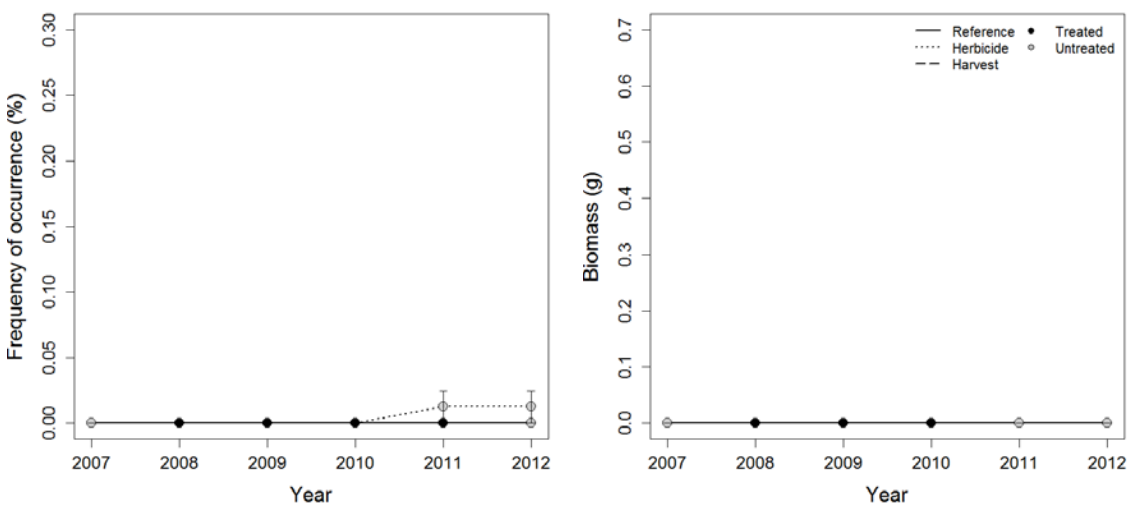

Figure 21. *August horned pondweed (*Zannichellia palustris*) frequency of occurrence (%) ± standard error (left) and mean biomass (g) per treatment ± standard error (right) for reference, herbicide, and mechanically harvested plots in Turville Bay, 2007-2012.*

Table 6. *Summary of mean August frequency of occurrence (%) and mean biomass (g) found in the reference, herbicide, and harvested plots, 2007-2012.*

		Reference						Herbicide						Harvest					
	Species	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Mean Frequency of Occurrence (%)	*Myriophyllum spicatum*	87.3	90.5	79.4	81.7	61.9	83.3	87.5	52.5***	43.7***	33.7***	41.2**	80.0	86.5	76.8	86.6	42.7***	34.1***	84.1
	Potamogeton crispus	ND	0.8	3.9	0.8	1.5	ND	ND	2.5	ND	1.2	1.2	ND	1.2	ND	6.1**	ND	7.3***	ND
	Ceratophyllum demersum	88.9	82.5	79.4	90.5	84.1	81.7	85.0	67.5**	67.5**	71.2*	60.0***	75.0	86.6	95.1*	96.3*	82.9	59.7***	84.1
	Stuckenia pectinata	8.7	2.4	9.5	22.2	20.6	19.8	17.5	20.0*	22.5	11.2*	31.2	38.7	6.1	4.9	12.2	12.2	14.6	17.1
	Potamogeton richardsonii	5.5	2.4	1.6	7.1	11.1	11.1	22.5	23.7	21.2	12.5	18.7	22.5	3.6	4.9	7.3	3.6	7.3	3.6
	Valisneria americana	0.8	0.8	ND	0.8	0.8	0.8	3.7	3.7*	5**	6.25***	10.0***	10.0***	1.2	2.4	ND	ND	ND	ND
	Elodea canadensis	ND	ND	4.0	26.2	13.5	4.0	ND	ND	2.5	21.2	2.5	11.3	ND	1.2	34.1***	36.6	19.5	25.6**
	Heteranthera dubia	ND	0.8	ND	ND	0.8	ND	3.7	3.7	1.2	5.0	6.2	17.5**	1.2	1.2	ND	2.4	1.2	ND
	Potamogeton foliosus	ND	ND	0.8	ND	0.8	ND	ND	ND	3.7*	ND	8.7***	12.5***	ND	ND	6.1**	4.9**	6.1**	1.2
	Chara sp.	ND	ND	ND	0.8	0.8	0.8	1.2	ND	1.3	ND	2.5	ND	ND	ND	1.2	ND	1.2	1.2
	Lemna minor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.4	ND	ND	1.2
	Najas flexilis	ND	ND	ND	ND	ND	ND	ND	ND	1.3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Potamogeton zosteriformis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.2	1.2
	Ranunculus aquatilus	0.8	ND	ND	0.8	ND	1.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Zannichelia palustris	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.3	1.3	ND	ND	ND	ND	ND	ND
Mean Biomass (g)	*Myriophyllum spicatum*	13.89	29.52	5.20	6.20	6.32	12.60	14.50	6.00***	0.952	0.794	1.23	10.45	11.25	14.56***	6.04	1.79	1.72	12.70
	Potamogeton crispus	ND	0.001	0.046	0.005	0.009	ND	ND	0.002	ND	0.013	0.014	ND	0.004	ND	0.455***	ND	0.067	ND
	Ceratophyllum demersum	15.37	4.22	19.90	40.33	29.50	31.50	12.70	7.60	12.20	14.6***	4.17***	21.57	8.12	13.10	45.7***	19.3**	7.94***	22.86
	Stuckenia pectinata	0.082	0.066	0.524	1.80	1.09	0.609	0.344	0.409	1.01	0.204***	1.17	0.857	0.361	0.075	0.296	0.292***	0.224**	0.530
	Potamogeton richardsonii	0.060	0.042	0.216	2.27	0.809	0.166	1.08	2.74***	2.16	0.246	1.11	1.27	0.189	1.55	0.341	0.439	0.252	0.184
	Valisneria americana	0.006	0.005	ND	0.005	0.006	0.001	0.077	0.265***	0.138	0.273***	0.424***	0.458***	0.002	0.025	ND	ND	ND	ND
	Elodea canadensis	ND	ND	0.112	0.605	0.067	0.047	ND	ND	0.082	0.237	0.009	0.166	ND	0.007	0.737***	1.029***	0.207	0.325
	Heteranthera dubia	ND	0.005	ND	ND	0.002	ND	0.100	0.019	0.004	0.089	0.038	0.634***	0.002	0.283**	ND	0.009	0.027	ND
	Potamogeton foliosus	ND	ND	0.000	ND	0.012	ND	ND	ND	0.044	ND	0.047*	0.058**	ND	ND	0.10***	0.021	0.219	0.148
	Chara sp.	ND	ND	ND	0.005	0.008	0.007	0.038	ND	0.011	ND	0.017	ND	ND	ND	0.000	ND	0.003	0.015
	Lemna minor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.167	ND	ND	ND
	Najas flexilis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Potamogeton zosteriformis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.012	0.130
	Ranunculus aquatilus	0.003	ND	ND	0.002	ND	0.012	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Zannichelia palustris	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	0.000	ND	ND	ND	ND	ND	ND
Summary	Mean Frequency of Occurrence of Natives (%)	20.9	17.8	19.1	21.2	16.6	17.1	22.3	13.7	8.3	12.9	10.4	29.8	19.7	2.9	5.5	27.5	7.5	15.7
	Mean Biomass/point (g)	29.5	37.5	27.9	52.2	39.2	45.0	29.3	19.7	17.4	17.3	9.1	35.4	19.7	31.2	57.0	23.8	10.5	36.8
	Mean Native Biomass/point (g)	15.6	4.3	20.8	45.0	31.6	32.3	14.3	11.0	15.7	15.6	7.0	25.0	8.7	15.1	47.3	21.1	8.7	24.1
	Avg # native spp/site	1.1	1.1	1.4	1.6	1.8	1.4	1.5	1.5	1.7	1.5	1.9	2.0	1.0	1.3	2.6	1.4	1.2	1.7

* = < 0.05, ** = <0.01, *** = <0.001

Notes

Science Services

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providing expertise for science-based decision-making

We develop and deliver science-based information, technologies, and applications to help others make well-informed decisions about natural resource management, conservation, and environmental protection.

Our Mission: The Bureau of Science Services supports the Wisconsin Department of Natural Resources and its partners by:

- conducting applied research and acquiring original knowledge.
- analyzing new information and emerging technologies.
- synthesizing information for policy and management decisions.
- applying the scientific method to the solution of environmental and natural resources problems.
- providing science-based support services for management programs department-wide.
- collaborating with local, state, regional, and federal agencies and academic institutions in Wisconsin and around the world.