# Herbicide Trials for Management of Flowering Rush in Detroit Lakes, Minnesota for 2012



A Report to the Pelican River Watershed District and Minnesota Department of Natural Resources

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## **Executive Summary**

#### **Conclusions**

- Field evaluations of 2012 treatments with the contact herbicide diquat have not only resulted in a decrease in aboveground biomass, but also decreases in both belowground biomass and rhizome bud density.
- Diquat treatments not only significantly reduced the nuisance problem, but reduced the potential for plants to regrow and spread.
- While some species declined with diquat treatments, notably elodea, leafy pondweed, clasping-leaf pondweed, sago pondweed and bladderwort; most species either did not change or increased, or otherwise followed a pattern similar to populations in reference plots.
- Additional research may indicate how native plant impacts may be mitigated.
- Imazapyr treatments did not statistically decrease aboveground biomass, belowground biomass, or rhizome bud density. However, insufficient time may have elapsed between treatment and posttreatment data collection to allow herbicidal activity to be fully expressed.

#### Recommendations

- Field evaluations and monitoring of diquat or other herbicides should be continued to determine if reduction in belowground biomass and rhizome bud density is repeatable.
- Mesocosm studies should also be performed to ensure that diquat treatments reducing belowground biomass and rhizome bud density can be reproduced under more controlled conditions.
- We recommend that other herbicide active ingredients and use patterns be evaluated under controlled conditions to determine if there are alternatives to diquat treatments, which may be field demonstrated in the future.
- The following mesocosm studies are recommended:
  - Repeated diquat treatments to determine how many submersed treatments per year are needed to reduce rhizome bud densities.
  - Screening of additional contact or systemic herbicides for control of submersed plants. Control should be defined as reduction in rhizome bud density.
  - Foliar treatments with imazapyr or other foliar herbicides to determine if they can reduce belowground biomass and rhizome bud density. These studies should include hardstem bulrush to evaluate potential selectivity.

o Granular formulations of systemic herbicides should be evaluated to determine if they will function on plants in shallow water in a similar way to the bareground applications performed for drawdown zones.

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#### Introduction

Flowering rush (*Butomus umbellatus* L.) is an emergent invasive plant that has invaded the Detroit Lakes area, in particular, Detroit Lake (Big Detroit, Little Detroit, and Curfman Lakes), Lake Sallie, Lake Melissa and Mill Pond (Becker County) since the 1960s. It is native to Europe and Asia and first entered the United States in 1928. Flowering rush has continued to be a problem in the lake for at least three decades.

Although flowering rush has been in North America for over forty years, very little useful information is known about its biology, ecology, and management. Bellaud (2009) reports that it was first observed in North America in St. Lawrence River (Quebec) in 1897. Flowering rush is currently found in all of the southern Canadian provinces except Alberta, and all of the states bordering Canada and the Great Lakes (NRCS 2013). Bellaud (2009) echoes our current state of affairs with flowering rush: "...there is not a wealth of information regarding the management of flowering rush infestations in North America." Bellaud (2009) cites Minnesota Department of Natural Resources research to support the recommendation to use imazapyr on the exposed foliage of flowering rush. Parkinson and others (2010) are also limited in their management recommendations, citing either imazapyr or imazamox foliar applications for management of flowering rush.

The US Army Engineer Research and Development Center (USAERDC) studied the available aquatic herbicides for control of submersed flowering rush plants from Minnesota and Idaho (Poovey et al. 2012). As part of their study, they determined that populations in both Idaho and Minnesota were triploid, as confirmed by ploidy and AFLP (Poovey et al. 2012). Their studies of Minnesota-derived plants used diquat, endothall and flumioxazin at relatively short exposure times. Flumioxazin did not reduce shoot biomass in either treatment. Diquat at the full label rate (0.37 ppm) and at 6 and 12 hours contact time significantly reduced shoot biomass relative to the reference. Endothall treatments at 1.5 and 3 ppm at both 12 and 24 hours exposure time also reduced shoot biomass. No treatments reduced belowground biomass. In contrast, their studies with Idaho-derived plants found flumioxazin at 400ppb and 24 hours exposure time controlled shoot biomass, and endothall at 3 ppm and 24 hour exposure time controlled both aboveground and belowground biomass (Poovey et al. 2012). They also note that repeated treatments with contact herbicides, or integration with systemic herbicides, would be needed to achieve long-term control.

The findings of the above research was then moved to outdoor mesocosm tank conditions, and USAERDC tested both submersed and emergent aquatic herbicides on Minnesota-derived flowering rush in outdoor mesocosm tanks at Mississippi State University. Submersed applications of 2,4-D, triclopyr, a tank mix of 2,4-D and triclopyr, imazamox, and a tank mix of 2,4-D and a surfactant did not decrease either shoot or belowground biomass (Wersal et al. 2013). In contrast, foliar treatments (including a surfactant) of 2,4-D,, triclopyr, a tank mix of 2,4-D and triclopyr, aminopyralid (not labeled for aquatic use), imazapyr, glyphosate, and a tank mix of imazapyr and glyphosate reduced both shoot and belowground biomass (Wersal et al. 2013). Foliar applications (including a surfactant) of imazamox or a tank mix of imazamox and glyphosate did not control belowground biomass, but did control shoot biomass (Wersal et al. 2013). All of these applications would require repeated treatments for successful control.

Studies also evaluated the use of herbicides to treat exposed soils, for managing flowering rush in areas in which drawdown could expose soil early in the growing season (Woolf et al. 2011). This project had two parts: a mesocosm study and a field study. While field studies are more widely accepted as realistic, it is difficult to get an adequate number of replicates and control for unexpected events. In addition, field trials require regulatory approval for many operations which, without previous data, might not be granted approval. Flowering rush from Pend Oreille Lake in Idaho was used in mesocosm tanks, as well as examining some field plots, to determine if the drawdown period might be exploited to better control flowering rush. In the mesocosm study at Mississippi State University, of acetic acid, aminopyralid (not labeled for aquatic use), flumioxazin, imazamox, fluridone, imazapyr, penoxsulam, and triclopyr were tested at the full broadcast label rate, and one-half that rate (Woolf et al. 2011). By 24 weeks after treatment (WAT), fluridone and triclopyr significantly reduced belowground biomass. The report speculated that granular herbicides may have sufficient transmission into soils to simulate this effect, as well. Field trials on bare soil found no significant differences between treatments with acetic acid, fluridone, imazamox, imazapyr, and triclopyr and an untreated reference, but this may have been due to insufficient replication. This study also found that benthic barrier, digging, and handpulling did not result in a significant reduction in flowering rush (Woolf et al. 2011).

The summation of this existing research on management indicated to us that the best herbicides for initial evaluation in a management program were endothall and diquat for submersed applications, and imazapyr for emergent plant foliar applications.

#### Previous findings.

We initiated a study of the biology, ecology, and management of flowering rush in the Detroit Lakes in 2010. The four foci of the project were 1) the phenology and life history of flowering rush, including carbohydrate storage; 2) analysis of plant biomass allocation across a depth gradient, including leaf height and exposure above the surface; 3) a whole-lake plant survey to examine the overlap of flowering rush with native plants and patterns of habitat selection by flowering rush, and 4) a field assessment of management activities. These studies extended throughout 2010 and 2011, with management field assessments continued in 2012 (this report).

*Phenology*. We found that flowering rush initiates growth later than hardstem bulrush, and begins to senesce before hardstem bulrush (Madsen et al. 2011). Aboveground biomass peaked in August, ranging between 400 to 650 gDW/m². Belowground biomass of reference sites varied little over the season, and was typically around 1,000 gDW/m². The density of rhizome buds in 2010 was relatively constant, around 350 N/m². Extrapolating to larger areas, this suggests that there are 1.5 million rhizome buds in every acre of flowering rush-infested littoral zone. New ramets are formed throughout the growing season, reaching a peak in mid-summer (Marko et al. 2012). The vast majority of biomass, as noted above, is belowground (Marko et al. 2012). Rhizome buds are continually formed throughout the growing season, but many sprout to form new ramets (Marko et al. 2012). Ramet density at the beginning of the growing season was 150 N/m², and peaked between 250 and 400 N/m² (Marko et al. 2012). Converting to acres, there are

as many as 1.7 M ramets (rosettes of leaves) per acre of a dense infestation of flowering rush. Aboveground biomass tends to contain from 2 to 8% starch dry weight, while belowground biomass can be as high as 18% dry weight of starch in early fall (Marko et al. 2012). No discernible low point in starch storage was observed.

Depth Allocation of Biomass. Plant height increases to a maximum of 5' in 4' deep water, then declines precipitously. Plants tend to have about 1.5' height above the water surface from 1 to 3 feet deep, and plants at 5' water depth or more are completely submersed. Submersed plants are found throughout those depth ranges, though (Madsen et al. 2012). The significance of this for management is that herbicide uptake from foliar applications would not be sufficient in plants growing in 3' water depth or more. Effective management of dense flowering rush would require some submersed treatments to manage the bulk of biomass. Above- and belowground biomass are strongly (but negatively) correlated to water depth; the shallowest plants have both the highest aboveground and belowground biomass. Ramet density is also strongly related to water depth, but the pattern was more of a stage shift in which habitats from 1' to 4' water depth had similar ramet densities, and habitats from 6 to 10' had a similar, but much lower, density. In shallow water, flowering rush will over time form a dense mat or turf of ramets, which then will accumulate sediment, organic matter, and other material to rapidly accrete, and build a false bottom. Flowering rush, then, is an ecosystem engineer which will fill in the littoral zone (Madsen et al. 2012).

Distribution Patterns. Whole-lake plant surveys of Big Detroit, Little Detroit, Curfman, Melissa, and Sallie Lakes found little change in plant community composition between 2010 and 2011 (Madsen et al. 2012). While flowering rush was established in a wide range of habitats and depths, hardstem bulrush (*Schoenoplectus acutus*) was typically found associated with flowering rush (Madsen et al. 2012). Flowering rush was found to be associated with a diverse range of native plants. This creates a management dilemma; namely, managing flowering rush without an excess adverse impact on native plant communities. While flowering rush was found out to depths of 16', it was most common in habitats from 1 to 4' deep. Its relative rarity in water from 0 to 1' deep may be attributed to a combination of ice scour and human intervention. The flowering rush was found in 70% of points in the 1' depth interval (Madsen et al. 2011).

Management. In 2010, we attempted a field demonstration of submersed herbicide applications in Detroit Lake (Madsen et al. 2011). Field demonstrations in lakes, while appearing logical to pursue, are actually difficult to perform. Most field demonstrations cannot achieve the level of replication found in terrestrial trials. Our colleagues in row crop weed science have the luxury of laying out plots with numerous treatments, and possibly several replicates per treatment, with little anxiety for cross-contamination (drift aside). In aquatic submersed applications, with herbicide applied to the water, the herbicide will dissipate into adjoining plots, thus contaminating the trial. After consultation with Minnesota DNR, we attempted to lay out a replicated plot trial with endothall treatments. We attempted to look at endothall applied either once, twice, or three times to the plot overs the season. With three replicates per treatment, and the necessity of three reference plots, the need for 12 trial plots spaced at least 200 yards apart resulted in plots of only 1 acre. Endothall was treated at a target concentration of 3 ppm. Endothall treatments did not result in a significant reduction of biomass or reduction in flowering rush percent frequency. Analysis of residue data by John Skogerboe (USAERDC) found half-

lives of much less than 12 hours, which is typically the required exposure time for control with endothall. We speculated that the plots were way too small.

In 2011, we therefore designed a management demonstration plot with larger plots. We had two 10-acre plots for endothall treatments, located on the flats, and we added two 1-acre plots for diquat (Madsen et al. 2012). Our rational in adding the diquat plots was that diquat is commonly used to treat small plots, and requires a much shorter exposure time than even endothall. Working with John Skogerboe, we added a dye dissipation study component for both endothall and diquat plots, as well as residue collection for endothall. An inexpensive ELISA analytical method is available for endothall residues, but not for diquat. We initially planned for two treatments with both endothall and diquat, but we canceled the second endothall treatment after its poor performance with the first treatment. Biomass data indicated that endothall treatments did not reduce above- or belowground biomass with the first treatment, but diquat treatments significantly reduced flowering rush aboveground biomass (but not belowground biomass). Assessment of the species composition of the plots found no reduction in native species diversity after the second treatment of diquat. Dye studies found that the herbicide moved quickly in a mass out of the large plots, following shoreline currents. Half-life of dye in endothall plots ranged from 3 to 6 hours at best. While 3 to 6 hours is sufficient for control with diquat, it is not sufficient to control plants using endothall. We also observed that wind direction had a significant effect on water residence time. Therefore, we recommended that submersed plant treatments be done with diquat, until such time as alternatives can be identified.

## Herbicide Background

Diquat. Diquat is the common name for the chemical 6,7-dihyropyrido[1,2-a:2',1'-c]pyrazinediium (Senseman 2007). It is commonly formulated as a dibromide salt. Highly soluble in water, it is widely used in spot and broadcast treatments to control ornamental, turf, noncrop, and aquatic weeds. In aquatics, it is used both as a foliar treatment and as a submersed aquatic treatment (Senseman 2007). Diquat is also commonly used in tank mixtures with other herbicides, such as 2,4-D, to increase the spectrum and longevity of control (Senseman 2007). The mode of action for diquat is to inhibit photosynthesis in photosystem I. The resulting free radicals create superoxides that oxidize organic molecules, lipids, and fatty acids, destroying cell membranes (Senseman 2007). Plants rapidly absorb the molecule, and symptoms appear within hours in plants exposed to light. Diquat is photodegraded, particularly by ultraviolet light, and is not metabolized by plants. In distilled water, the typical half-life is 1000 days; but under normal conditions it binds quickly with any soil particle and becomes immobilized (Senseman 2007).

For aquatic use, it is a standard treatment for many emergent and floating plants, such as American frogbit, duckweed, watermeal, and waterlettuce (Madsen et al. 1998, Wersal and Madsen 2009). For use on duckweed and watermeal, it can be used either as a submersed application or a surface broadcast application (Wersal and Madsen 2009). For some submersed plants, diquat has been tank-mixed with chelated copper herbicides (Pennington et al. 2001). For some species and systemic herbicides, tank mixing with diquat might not be beneficial; diquat may be antagonistic with some systemic herbicides (Wersal and Madsen 2010). Although light is required for symptoms to appear, the timing of application (light or dark, morning or afternoon) does not affect efficacy (Wersal et al. 2010).

The main drawback to use of diquat is that it is rapidly adsorbed by soil particles suspended in the water, so turbidity (suspended particles) significantly affects the efficacy of diquat (Hofstra et al. 2001, Poovey and Getsinger 2002).

While species do vary in their susceptibility to diquat, some research indicates that diquat can control some submersed species with as short a contact time as 2.5 hours (Skogerboe et al. 2006). Of all herbicides labeled for aquatic use, diquat is recognized for having the shortest exposure time requirements (Netherland 2009, Madsen 2000).

Diquat is relatively unaffected by water temperature, as long as the plants are actively growing. While the common recommendation for early season control of curlyleaf pondweed has generally been to use endothall, early research found that diquat was just as effective at low temperatures as endothall (Netherland et al. 2000, Poovey et al. 2002).

Diquat, as mentioned earlier, is slow to degrade in the environment, but will rapidly be adsorbed by soil particles (WHO 2004). In pond studies, diquat rapidly dissipated from the water, and was below detection levels in 8 to 38 days (Langeland and Warner 1986). Parsons and others (2007) likewise found a rapid decline in diquat concentrations in a study of a clear Washington State lake, but did persist in the water and dissipate to off-treatment areas. Given rapid adsorption, expensive analytical costs, and the speed with which diquat is taken up by plants, residue analyses for the purpose of ensuring efficacy are rare.

*Imazapyr*. Imazapyr inhibits the plant-specific enzyme acetolactate synthase, so is classed as an ALS inhibitor (Netherland 2009, Senseman 2007). It is used as a foliar applied herbicide to emergent and floating leaved plants, but does not have activity in water (Netherland 2009). So long as no standing water is above the sediment, it can have a soil residual effect (Netherland 2009). Imazapyr is used as a spot or broadcast herbicide applied to the foliage of living plants, and is widely used in forestry, noncrop areas, rights of way, and aquatic sites. It is a broadspectrum herbicide, and generally is used with a nonionic surfactant (Senseman 2007). It is photodegraded, and the rate of photodegradation is greatly accelerated in water. The half-life in soil is 25-142 days, while that in water is 2-3 days (Senseman 2007). Weed control is often effective for multiple years. Imazapyr is rapidly translocated from both roots and leaves, and uptake is often less than 24 hours (Senseman 2007).

When applied to foliage, imazapyr has been shown effective against such hard-to-control species as alligatorweed (Hofstra and Champion 2010), parrotfeather (Wersal and Madsen 2007), common reed (Kay 1995, True et al. 2010), giant reed (Bell 2011, Spencer et al. 2009), smooth cordgrass (Patten 2002) and even woody vegetation such as melaleuca (Stocker and Sanders 1997, Serbesoff-King 2003) and Carolina willow (Hutchinson and Langeland 2010).

In considering potential nontarget effects associated with use of imazapyr in the Willapa Bay estuary, Patten (2003) concluded that submersed plants would be unaffected, and only plants exposed above the water line would potentially be affected. Therefore, submersed species occurring under stands of emerged flowering rush would not be adversely affected by imazapyr treatments. Bulrush, however, is listed in the imazapyr label [Habitat] for control (BASF 2004).

#### Goals

We therefore selected diquat for treatment of submersed flowering rush stands and imazapyr to treat emergent stands of flowering rush in less than 2' water depth. Our initial concept was that diquat would control the submersed plants, and imazapyr would have to be implements in shallow shoreline areas where diquat was not able to be applied or failed to control robust emergent plants.

For diquat treatments, our goal was to measure efficacy of control and reduction of reproductive ability through reduction of belowground biomass and rhizome bud density through biomass sampling, and to evaluate the impact of diquat treatments on native plant communities using a point intercept survey method.

For imazapyr treatments, our goal was to measure efficacy of control and reduction of reproductive ability through reduction of belowground biomass and rhizome bud density through biomass sampling. Given the lack of native plant growth and difficulty of navigation in shallow stands of dense flowering rush, we decided not to evaluate the impact of these relatively limited applications on native plant diversity.

#### **Materials and Methods**

#### **Herbicide Treatments**

Two types of herbicide treatments were made to manage flowering rush populations at designated treatment areas: 1) treatments of submersed or mostly submersed plants with the contact herbicide diquat using drop hoses from a boat, in 4 feet and less of water; and 2) treatments of emergent plants with the systemic herbicide imazapyr, in water depths of less than 2 feet with leaves that are at least 1 foot above the water surface.

Diquat treatments. From two feet to four feet deep, a rate of two gallons per surface acre were used, and in water depths from shoreline to two feet deep, a rate of one gallon per surface acre were applied; as per the US EPA label. The target water column concentration was 0.37 ppm of diquat. No dissipation studies were performed using either dye or residue sampling. No inexpensive ELISA kit for diquat analysis is yet available, and residues in the water rapidly bind to suspended sediment particles. These repeated treatments of the same areas were done twice during the growing season, the first in June, and the second in July (Table 2). Treatments occurred in Big Detroit, Curfman (Figure 1), Sallie (Figure 2), and Melissa Lakes (Figure 3, Table 1, 2). Diquat formulation used was a 2 lbs. per gallon diquat cation formulation (Tribune, Syngenta Crop Protection, LLC, Greensboro, NC).

*Imazapyr treatments*. Imazapyr treatments were done on emergent plants in less than two feet water depth on August 2, 2012 (Table 2, 3, Figure 4). Given the scarcity of emergent flowering rush in July, we selected one plot in Little Detroit (Imz-Trt-2), and the selected a previously untreated plot in Big Detroit Lake (Imz-Trt-1) for treatment (Figure 4), with one reference plot

(Imz-Ref) the same as for diquat evaluations, but with samples taken in shallower water (Figure 4). Submersed treatments had not been expected to control plants in water less than one foot deep, but control with submersed applications exceeded our expectations in most areas.

## **Assessment Approach**

We assessed the response of flowering rush to both submersed and emergent herbicide applications using biomass estimates, and assess the impact of submersed applications on aquatic plant communities using a point intercept method.

Biomass estimates. Assessment of both submersed and emergent treatments in this system were done by sampling biomass collected with a 6" diameter biomass coring device to collect both shoots and rhizomes (Madsen et al. 2007, Figure 5). Twenty cores per plot were collected before each proposed treatment, and at the end of the growing season in September (Table 3). After washing to remove sediment, cores were either shipped to Mississippi State University for processing, or held on ice until returned to campus. Cores were separated into aboveground and belowground biomass. Ramets, rhizomes and rhizome buds (Figure 6A,B) were counted, but not separated from the remainder of belowground biomass. Plants were dried for 48 hours at 50C or greater, and weighed for biomass. Successful applications should reduce rhizome weight and rhizome bud number. For the submersed diquat treatments, four treatment plots (Detroit 1 and 3, Sallie 2, and Melissa 7) were sampled for biomass, and four reference plots (Detroit 1 and 2, Sallie Ref, and Melissa Ref); for a total 160 biomass samples per time (Table 3). Treatments were not randomly assigned to plots, but were assigned based on the relative abundance of flowering rush to minimize the variability of biomass samples. In some instances, reference plots were selected to avoid treating stands of hardstem bulrush, even though submersed applications of diquat should not affect hardstem bulrush. For the submersed treatment study, biomass samples were taken at predetermined points randomly selected from the point intercept points (below) of those plots. For the emergent treatments with imazapyr, we selected two treatment plots and one reference plot. Twenty biomass samples were collected from each plot before treatment, and twenty biomass samples from the reference plot, in waters less than two feet deep. For posttreatment samples, twenty biomass samples were taken from each plot. Posttreatment samples were taken about six weeks after treatment. Biomass sample points were not predetermined, but selected by a random walk method along the shoreline at the appropriate depth. Statistical analysis of biomass data was performed using a two-way analysis of variance (ANOVA), with the two factors being treatment (diquat-treated vs. untreated reference) and time of sampling, and the interaction factor being treatment\*time. Analysis was done using Statistix (Analytical Software, Tallahassee, FL).

*Point Intercept.* To assess the community impact of submersed diquat treatments, point intercept sampling (Madsen 1999) was done on all nine treated plots, and four reference plots (Table 4). The grid interval was no less than 25 m. There were not an equal number of points per plot. Statistical analysis was performed using a McNemar's test, testing for a statistically-significant change in frequency between the current time and the previous time interval. Analysis was performed using SAS (SAS Institute, Inc., Cary, NC)

#### **Results and Discussion**

## **Submersed Diquat Treatments**

Biomass. The measurement of abundance, such as biomass, is the best method to evaluate the effectiveness of control (Madsen 1993a, Madsen and Bloomfield 1993). Since the aboveground biomass often causes the nuisance problem, reduction in biomass may measure the reduction in nuisance potential. While reduction of the nuisance potential is important to resource user perception, it is also important to contribute to the long-term management of the invasive plant species. For flowering rush, the two best indicators of reduction in long-term growth potential are rhizome abundance, which may be measured by belowground biomass since rhizomes are the dominant constituent of belowground biomass; and rhizome bud density, since buds appear to be the perennating and regrowth propagule (Marko et al. 2012, Madsen et al. 2012). Rhizomes are the main location to store carbohydrates, essential for overwintering and for regrowth from management. Rhizome buds are the individual growing points from which new ramets or leaves regrow. Reductions in these two constituents indicate long-term control. A two-way analysis of variance (ANOVA) found a significant reduction in aboveground biomass from diquat treatments in the four treated plots, from 72 gDW/m<sup>2</sup> in May to 0.83 gDW/m<sup>2</sup> in September (Figure 7, Table 6). In contrast, reference plots had a significant increase in aboveground biomass, from 33 gDW/m<sup>2</sup> in May to 120 gDW/m<sup>2</sup> in September (Figure 7, Table 6). The significant interaction (Treatment\*Month) term is the result of treated plots decreasing in abundance and untreated reference plots increasing in abundance.

An examination of plot-by-plot means for each month confirm this statistical trend, that treated plots had reduced aboveground biomass and reference plots had increased biomass across the season (Figure 8). Diquat was very effective in controlling aboveground biomass of flowering rush, and comments from lake users and shoreline owners (granted, an unscientific and nonstatistical survey) generally indicated satisfaction with the reduction in the nuisance growth. Management of plots with diquat in 2011 also were successful in reducing aboveground biomass, but only in two one-acre plots (Madsen et al. 2012).

In 2011, we did not observe a reduction in belowground biomass with two diquat treatments, albeit with a much smaller sample size (Madsen et al. 2012). In contrast, a two-way ANOVA found a significant treatment effect from diquat treatments on belowground biomass (Figure 9, Table 7). Reference plot biomass was constant from May and July and significantly higher in September. Diquat-treated plots, on the other hand, were highest in May (350 gDW/m²), and declined to 64 gDW/m² in September (Figure 9, Table 7). Belowground biomass samples are notoriously variable, in part due to the difficulty of consistently cleaning sediment and debris from the sample. The significant interaction (Treatment\*Month) term is the result of treated plots decreasing in abundance and untreated reference plots increasing in abundance. Other studies have likewise not detected a reduction in belowground biomass from most herbicide treatment of flowering rush, including field treatments in Idaho and mesocosm studies in Mississippi (Woolf et al. 2011). An examination of plot-by-plot means by month do show significant reductions in belowground biomass of some treated plots, such as the Flats of Big Detroit Lake (DL-DIQ-1), they also show potential outliers in the data for the Sallie Lake reference (Figure 10). Repeating this finding would ensure confidence that treatments are, in

fact, reducing belowground biomass, as would controlled experiments under mesocosm conditions.

Rhizome bud density should be a more conservative measure of reduction in the potential for plants to regrow. A two-way ANOVA of rhizome bud density found no statistically-different change in bud density across the season for untreated reference plots, but did find a significant decrease in bud density between pretreatment values in May and posttreatment values in July and September of diquat-treated plots (Figure 11, Table 8). In May, bud density averaged 170 N/m<sup>2</sup>, while July averaged 20 N/m<sup>2</sup> and September averaged 29 N/m<sup>2</sup>; a decrease of 80-90%. The significant interaction (Treatment\*Month) term is the result of treated plots decreasing in density and untreated reference plots not changing in density. An examination of plot-by-plot means reiterates that treated plots had significantly fewer rhizome buds posttreatment, while rhizome buds tended to increase or remain the same in untreated reference plots (Figure 12). This finding supports the concept that repeated treatments with diquat not only reduces the nuisance of topped-out flowering rush, but actually contributes to long-term control. Future monitoring of rhizome bud density will, hopefully, substantiate this result. Continued monitoring may be done without reference plots, with the objective of tracking rhizome buds as the significant propagule, as has been done for curlyleaf pondweed (Potamogeton crispus L.) turions (Johnson et al. 2012), hydrilla (Hydrilla verticillata (L.f.) Royle) subterranean turions (Richardson 2012), and waterchestnut (Trapa natans L.) seeds (Madsen 1993b, Methe et al. 1993). A controlled study in mesocosm tanks would also strengthen the case that this management program has long-term benefit to controlling flowering rush.

Point Intercept. While decreasing the nuisance growth and reducing the long-term potential to spread and regrow is important for managing invasive plants, this benefit must be weighed against possible damage to the native plant community. A point intercept study was performed to evaluate the impact on native plant species and the overall community. This sampling did not detect a decrease in the abundance of native plants, but rather if plants survived and continued at the same frequency. Thousands of point intercept observations were collected at thirteen plots (nine treated plots, four reference plots) in the five basins (Table 4). For the diquat treated plots, seven species were found to increase, eight to decrease, and seventeen did not change (Tables 9, 11). This compares favorably with the reference plots, in which seven increased, five decreased, and twenty remained the same (Tables 10, 11). The species that decreased in the diquat plots that did not decrease in the reference plots were elodea, leafy pondweed, clasping-leaf pondweed, sago pondweed and bladderwort. Clasping-leaf had lower numbers in July, but increased again in September. While some species did appear to be significantly reduced by diquat treatments, most species were not affected in frequency by the treatments.

## **Imazapyr Emergent Treatments**

Imazapyr has been the herbicide of choice for treatment of emergent flowering rush in the past. In this study, we found no significant reduction in aboveground biomass (Figure 13), rhizome bud density (Figure 14), or belowground biomass (Figure 15). However, insufficient time may have elapsed between treatment and collection of posttreatment biomass. Typically, imazapyr may take several months to completely kill the plant and, since it is readily translocated, it often kills both the aboveground and belowground biomass. A visual inspection of the Big Detroit

Lake treatment site did show plants that were healthy before treatment in July (Figure 16), and obviously in the process of dying by September (Figure 17). Field trials of imazapyr typically test treated areas one-year posttreatment to fully evaluate herbicidal effects. A controlled mesocosm experiment would better evaluate both the potential for imazapyr treatments to reduce belowground biomass and rhizome bud density, and determine the length of time required for successful control.

## **Summary**

In summary, submersed contact herbicide treatments on large blocks using diquat herbicide exceeded our expectations in three ways. First, the treatments were much more effective at controlling aboveground biomass and nuisance growth than expected. Localized dissipation allowed control of aboveground biomass, including emergent leaves, even in very shallow water. The effectiveness of diquat in controlling flowering rush all but obviated the need for emergent plant treatments, which were only needed in areas in which diquat was not applied. Second, diquat treatments reduced belowground biomass and rhizome bud density, contributing to long-term control. While a single year's result is premature to base a new treatment program, this result is a hopeful indication that populations of flowering rush could in fact be reduced with this herbicide usage pattern. Third, the adverse effect of diquat treatments on native plant communities was much less than expected. Diquat is often considered the ultimate in broad-spectrum herbicides, yet a number of submersed species were not apparently reduced by diquat applications. On the other hand, we did not measure abundance, and we did note that many species appeared to have some herbicide damage. Further documentation of treatments may indicate if long-term applications will reduce populations.

The imazapyr treatments on emergent plants did not reduce biomass, but this may have been due to the short interval between application and evaluation.

#### **Conclusions and Recommendations**

#### **Conclusions**

- Field evaluations of 2012 treatments with the contact herbicide diquat have not only resulted in a decrease in aboveground biomass, but also decreases in both belowground biomass and rhizome bud density.
- Diquat treatments not only significantly reduced the nuisance problem, but reduced the potential for plants to regrow and spread.
- While some species declined with diquat treatments, notably elodea, leafy pondweed, clasping-leaf pondweed, sago pondweed and bladderwort; most species either did not change or increased, or otherwise followed a pattern similar to populations in reference plots.
- Additional research may indicate how native plant impacts may be mitigated.
- Imazapyr treatments did not statistically decrease aboveground biomass, belowground biomass, or rhizome bud density. However, insufficient time may have elapsed between treatment and posttreatment data collection to allow herbicidal activity to be fully expressed.

#### **Recommendations**

- Field evaluations and monitoring of diquat or other herbicides should be continued to determine if reduction in belowground biomass and rhizome bud density is repeatable.
- Mesocosm studies should also be performed to ensure that diquat treatments reducing belowground biomass and rhizome bud density can be reproduced under more controlled conditions.
- We recommend that other herbicide active ingredients and use patterns be evaluated under controlled conditions to determine if there are alternatives to diquat treatments, which may be field demonstrated in the future.
- The following mesocosm studies are recommended:
  - Repeated diquat treatments to determine how many submersed treatments per year are needed to reduce rhizome bud densities.
  - Screening of additional contact or systemic herbicides for control of submersed plants. Control should be defined as reduction in rhizome bud density.
  - Foliar treatments with imazapyr or other foliar herbicides to determine if they can reduce belowground biomass and rhizome bud density. These studies should include hardstem bulrush to evaluate potential selectivity.
  - Granular formulations of systemic herbicides should be evaluated to determine if they will function on plants in shallow water in a similar way to the bareground applications performed for drawdown zones.

## Acknowledgements

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#### **Literature Cited**

Bellaud, M. 2009. Chapter 13.10: Flowering Rush. pp. 141-144. In: L.A. Gettys, W.T. Haller, and M. Bellaud, eds. Biology and Control of Aquatic Plants: A Best Management Practices Handbook. Aquatic Ecosystem Restoration Foundation, Marietta, GA. 200pp.

BASF Corporation. 2004. Habitat Herbicide Specimen Label. BASF Corporation, Research Triangle Park, NC

Bell, C.F. 2011. Giant reed (*Arundo donax* L.) response to glyphosate and imazapyr. Journal of Aquatic Plant Management 49:111-113.

Hofstra, D.E. and P.D. Champion. 2010. Herbicide trials for the control of alligatorweed. Journal of Aquatic Plant Management 48:79-83.

Hofstra, D.E., J.S. Clayton, and K.D. Getsinger. 2001. Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: II. The effects of turbidity on diquat and endothall efficacy. Journal of Aquatic Plant Management 39:25-27.

Hutchinson, J.T. and K.A. Langeland. 2010. Evaluation of aerial herbicide application for reduction of woody vegetation in a floodplain marsh. Journal of Aquatic Plant Management 48:40-46.

Johnson, J.A., A.R. Jones, and R.M. Newman. 2012. Evaluation of lakewide, early season herbicide treatments for controlling invasive curlyleaf pondweed (*Potamogeton crispus*) in Minnesota lakes. Lake and Reservoir Management 28:346-363.

Kay, S.H. 1995. Efficacy of wipe-on applications of glyphosate and imazapyr on common reed in aquatic sites. Journal of Aquatic Plant Management 33:25-26.

Langeland, K.A. and J.P. Warner. 1986. Persistence of diquat, endothall, and fluridone in ponds. Journal of Aquatic Plant Management 24:43-46.

Madsen, J.D. 1993a. Biomass techniques for monitoring and assessing control of aquatic vegetation. Lake and Reservoir Management 7:141-154.

Madsen, J.D. 1993b. Waterchestnut seed production and management in Watervliet Reservoir, New York. Journal of Aquatic Plant Management 31:271-272.

Madsen, J.D. 1999. Point and line intercept methods for aquatic plant management. APCRP Technical Notes Collection (TN APCRP-M1-02), U.S. Army Engineer Research and Development Center, Vicksburg, MS. 16 p.

Madsen, J.D. 2000. Advantages and Disadvantages of Aquatic Plant Management Techniques. US Army Engineer Research and Development Center Miscellaneous Report ERDC/EL MP-00-1, Vicksburg, MS. September 2000.

Madsen, J.D., and J.A. Bloomfield. 1993. Aquatic vegetation quantification symposium: An overview. Lake and Reservoir Management 7:137-140.

Madsen, J.D., M. Marko, J. Cheshier, and C. Olson. 2011. Flowering Rush in the Detroit Lakes Chain, Becker County, Minnesota. Letter report to Pelican River Watershed District, March, 2011. 23pp.

Madsen, J.D., C.S. Owens, and K.D. Getsinger. 1998. Evaluation of four herbicides for management of American frogbit (*Limnobium spongia*). Journal of Aquatic Plant Management 36:148-150.

Madsen, J.D., R.M. Wersal, M.D. Marko, and J.G. Skogerboe. 2012. Ecology and Management of Flowering Rush (*Butomus umbellatus*) in the Detroit Lakes, Minnesota. Geosystems Research Institute Report 5054, July 27, 2012.

Madsen, J. D., R. M. Wersal, and T. E. Woolf. 2007. A new core sampler for estimating biomass of submersed aquatic macrophytes. Journal of Aquatic Plant Management 45:31-34.

Marko, Michelle, John Madsen, Casey Olson and Ryan Smith. 2012. Ecology of Flowering Rush (*Butomus umbellatus*) in Detroit Lakes, Becker County, Minnesota. Letter report to Pelican River Watershed District, Concordia College, Moorhead, MN. August 2012.

Methe, B.A., R.J. Soracco, J.D. Madsen, and C.W. Boylen. 1993. Seed production and growth of waterchestnut as influenced by cutting. Journal of Aquatic Plant Management 31:154-157.

Netherland, M.D. 2009. Chapter 11. Chemical Control of Aquatic Weeds. pp. 65-78. In: L.A. Gettys, W.T. Haller, and M. Bellaud, eds. Biology and Control of Aquatic Plants: A Best Management Practices Handbook. Aquatic Ecosystem Restoration Foundation, Marietta, GA. 200pp.

Netherland, M.D., J.D. Skogerboe, C.S. Owens, and J.D. Madsen. 2000. Influence of water temperature on the efficacy of diquat and endothall versus curlyleaf pondweed. Journal of Aquatic Plant Management 38:25-32.

Parkinson, H., J. Mangold, V. Dupuis, and P. Rice. 2010. Biology, Ecology and Management of Flowering Rush (*Butomus umbellatus*). Montana State University Extension Service EB0201, December 2010.

Parsons, J.K., K.S. Hamel, and R. Wierenga. 2007. The impact of diquat on macrophytes and water quality in Battle Ground Lake, Washington. Journal of Aquatic Plant Management 45:35-39.

Patten, K. 2002. Smooth cordgrass (*Spartina alterniflora*) control with imazapyr. Weed Technology 16:826-932.

Patten, K. 2003. Persistence and non-target impact of imazapyr associated with smooth cordgrass control in an estuary. Journal of Aquatic Plant Management 41:1-6.

Pennington, T.G., J.G. Skogerboe, and K.D. Getsinger. 2001. Herbicide/copper combinations for improved control of *Hydrilla verticillata*. Journal of Aquatic Plant Management 39:56-58.

Poovey, A.G. and K.D. Getsinger. 2002. Impacts of inorganic turbidity on diquat efficacy against *Egeria densa*. Journal of Aquatic Plant Management 40:6-10.

Poovey, A.G., C.R. Mudge, R.A. Thum, C. James, and K.D. Getsinger. 2012. Evaluations of contact herbicides for controlling two populations of submersed flowering rush. Journal of Aquatic Plant Management 50:48-54.

Poovey, A.G., J.G. Skogerboe, and C.S. Owens. 2002. Spring treatments of diquat and endothall for curlyleaf pondweed control. Journal of Aquatic Plant Management 40:63-67.

Richardson, R.J. 2012. Considering monoecious hydrilla subterranean turion biology in management programs. Abstract, 52<sup>nd</sup> Annual Meeting, Aquatic Plant Management Society, July 22-25, 2012, Salt Lake City, UT.

Senseman, S.A. (ed.) 2007. Herbicide Handbook, Ninth Edition. Weed Science Society of America, Lawrence, KS. 458p.

Serbesoff-King, K. 2003. Melaleuca in Florida: A literature review on the taxonomy, distribution, biology, ecology, economic importance and control measures. Journal of Aquatic Plant Management 41:98-112.

Skogerboe, J.G., K.D. Getsinger, and L.M. Glomski. 2006. Efficacy of diquat on submersed plants treated under simulated flowing water conditions. Journal of Aquatic Plant Management 44:122-125.

Spencer, D.F., W. Tan, P.-S. Liow, G.G. Ksander, and L.C. Whitehand. 2009. Evaluation of a late summer imazapyr treatment for managing giant reed (*Arundo donax*). Journal of Aquatic Plant Management 47:40-43.

Stocker, R.K. and D.D. Sanders, Jr. 1997. Control of melaleuca seedlings and trees by herbicides. Journal of Aquatic Plant Management 35:55-59.

True, S.L., R.J. Richardson, P.L. Hipkins, and A.P. Gardner. 2010. Efficacy of selected aquatic herbicides on common reed. Journal of Aquatic Plant Management 48:121-123.

United States Department of Agriculture, Natural Resources Conservation Service [NRCS]. 2013. Plants Database, profile for *Butomus umbellatus* L., accessed April 20, 2013. http://plants.usda.gov.

Wersal, R.M. and J.D. Madsen. 2007. Comparison of imazapyr and imazamox for control of parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.). Journal of Aquatic Plant Management 45:132-136.

Wersal, R.M. and J.D. Madsen. 2009. Combinations of diquat and methylated seed oil surfactant for control of common duckweed and watermeal. Journal of Aquatic Plant Management 47:59-62.

Wersal, R.M. and J.D. Madsen. 2010. Combinations of penoxsulam and diquat as foliar applications for control of waterhyacinth and common salvinia: Evidence of herbicide antagonism. Journal of Aquatic Plant Management 48:21-25.

Wersal, R.M., J.D. Madsen, J.H. Massey, W. Robles, and J.C. Cheshier. 2010. Comparison of daytime and night-time applications of diquat and carfentrazone-ethyl for control of parrotfeather and Eurasian watermilfoil. Journal of Aquatic Plant Management 48:56-58.

Wersal, R.M., A.G. Poovey, J.D. Madsen, K.D. Getsinger, and C.R. Mudge. 2013. Herbicide applications for control of emergent flowering rush under mesocosm conditions. In review for submission to Journal of Aquatic Plant Management.

World Health Organization [WHO] 2004. Diquat in Drinking-water. World Health Organization, Geneva.

Woolf, T., J. Madsen, and R. Wersal. 2011. Flowering Rush Control Project for Lake Pend Oreille, Idaho: Preliminary Summary on Mesocosm and Field Evaluations. Geosystems Research Institute Report #5048, Geosystems Research Institute, Mississippi State University, September 2011.

Table 1. Treatment areas for diquat submersed treatments by basin, plot, average depth, and amount of herbicide to be used. Amount of herbicide calculated at two gallons per acre.

		Planned Area	
Lake Basin	Plot number	(Acres)	
Big Detroit	1	82.4	
Big Detroit	2	19.8	
Big Detroit	3	14.7	
BIG DETROIT	<b>SUBTOTAL</b>	116.9	
Curfman	1	2.2	
Curfman	2	10.4	
CURFMAN	SUBTOTAL	12.6	
Sallie	1	12.6	
Sallie	2	4.7	
	~		
SALLIE	SUBTOTAL	17.3	
Melissa	4	4.9	
Melissa	7	8.7	
MELISSA	SUBTOTAL	13.6	
TOTALS		160.4	

Table 2. Diquat<sup>1</sup> and imazapyr<sup>2</sup> treatment dates, areas, and volumes along with application conditions. Weather data from application records (PLM Lake and Land Management

Corporation, unpubl. records)

Basins	Area (acres)	Volume of Formulated	Rate (gal./acre)	Wind Direction	Wind Speed (mph)
		Herbicide	(gui./ dere)	(cardinal)	(mpn)
		(gallons)		(**************************************	
First diquat appl	lication, June, 201	,	1	1	1
Detroit	117	212.2	1.81	ESE	3
Curfman	12.7	20.9	1.65	ESE	3-7
Melissa	13.6	27.2	2.0	ESE	3-5
Sallie	17.3	32.2	1.86	ESE	3-7
Second diquat a	pplication, July 1	9, 2012			
Detroit	117.1	212	1.81	SSE	3-7
Curfman	7	14	2.0	SSE	3-7
Melissa	13.6	29	2.0	SSE	3-7
Sallie	17.3	30.5	1.76	SSE	3-7
Imazapyr applic	ation, August 2, 2	2012			
Big Detroit	9.71	2.5	0.26	NE	3-7
and Little					
Detroit (both					
plots)					

<sup>&</sup>lt;sup>1</sup>Tribune, Syngenta Crop Protection, Greensboro, NC

<sup>&</sup>lt;sup>2</sup>Habitat, BASF Corporation, Research Triangle Park, NC

Table 3. Treatment areas for imazapyr emergent treatments by basin, plot, average depth, and amount of herbicide to be used. Imazapyr rate was calculated at three pints per acre of the two lbs. per gallon formulation.

Lake Basin	Plot Number	Area (Acres)	Notes
Big Detroit	Imz-Trt-1	5	A total of only 9.7
Little Detroit	Imz-Trt-2	7.9	Acres was treated
Little Detroit	Imz-Ref	5	

Table 4. Submersed diquat treatment and reference sites at which twenty biomass samples will be collected in May, July, and September of 2012, in the Detroit Lakes basins.

Lake Basin	Type (Treatment or	Plot Number
	Reference)	
Big Detroit	Treatment	DL-DIQ-1
Big Detroit	Treatment	DL-DIQ-3
Little Detroit	Reference	DL-REF-1
Little Detroit	Reference	DL-REF-2
Sallie	Treatment	S-DIQ-1
Sallie	Reference	S-REF-1
Melissa	Treatment	M-DIQ-7
Melissa	Reference	M-REF-1

Table 5. A list of all diquat treatment and reference plots at which point intercept samples will be taken. The point interval is relatively constant, so the number of points will vary by plot.

Lake Basin	Type (Treatment or	Plot Number
	Reference)	
Big Detroit	Treatment	DL-DIQ-1
Big Detroit	Treatment	DL-DIQ-2
Big Detroit	Treatment	DL-DIQ-3
Little Detroit	Reference	DL-REF-1
Little Detroit	Reference	DL-REF-2
Curfman	Treatment	CF-DIQ-1
Curfman	Treatment	CF-DIQ-2
Sallie	Treatment	S-DIQ-1
Sallie	Treatment	S-DIQ-2
Sallie	Reference	S-REF-1
Melissa	Treatment	M-DIQ-4
Melissa	Treatment	M-DIQ-7
Melissa	Reference	M-REF-1

Table 6. Two-way analysis of variance of aboveground biomass (gDW/m<sup>2</sup>) from four diquat treatment and four reference plots across three basins of Detroit Lakes. N=479. Treatment\*Month is the interaction term.

Source	Degrees of Freedom	F-score	p-value
Treatment	1	26.77	0.0000
Month	2	4.56	0.0109
Treatment*Month	2	29.18	0.0000

Table 7. Two-way analysis of variance of belowground biomass (gDW/m<sup>2</sup>) from four diquat treatment and four reference plots across three basins of Detroit Lakes. N=479. Treatment\*Month is the interaction term.

Source	Degrees of Freedom	F-score	p-value
Treatment	1	9.65	0.0020
Month	2	4.61	0.0104
Treatment*Month	2	11.21	0.0000

Table 8. Two-way analysis of variance of rhizome bud density (N/m<sup>2</sup>) from four diquat treatment and four reference plots across three basins of Detroit Lakes. N=479. Treatment\*Month is the interaction term.

Source	Degrees of Freedom	F-score	p-value
Treatment	1	6.52	0.0110
Month	2	5.21	0.0058
Treatment*Month	2	6.46	0.0017

Table 9. Summary of species percent frequency of occurrence by month for all diquat-treated plots in all lakes for 2012. May is pretreatment data, July and September are four weeks after the first and second diquat treatment, respectively. An asterisk indicates a significant difference from the previous month, as indicated by a McNemar's test.

Common Name	SPECIES	MAY %	JULY %	SEPT %
Water marigold	Bidens beckii	0.6	0.3	0
Flowering rush	Butomus umbellatus	77.9	33.1*	31.3
Coontail	Ceratophyllum demersum	5.5	20*	27.1*
Chara	Chara	45.8	73.4*	68.6*
Moss	Drepanocladus	6.5	15.1*	8.5*
Elodea	Elodea canadensis	13.1	3.1*	1.1
Water stargrass	Heteranthera dubia	0.3	1.3	0
Common duckweed	Lemna minor	0	0	0
Forked duckweed	Lemna trisulca	8.6	10.6	12.4
Northern water milfoil	Myriophyllum sibiricum	7.9	10.3	15.8
Slender naiad	Najas flexilis	0	4.1*	3.1
Yellow pond lily	Nuphar lutea	3.1	9.3*	5.4
White water lily	Nymphaea odorata	0.3	0	1.5
Curly leaf pondweed	Potamogeton crispus	12.7	0.6*	0.7
Leafy pondweed	Potamogeton foliosus	2.4	4.1	0*
Variable pondweed	Potamogeton gramineus	0.3	0.6	0
Illinois pondweed	Potamogeton illinoensis	21.7	17.9	25.8
Floating pondweed	Potamogeton natans	0.3	0	0
White stem pondweed	Potamogeton praelongus	9.3	11	9.3
small pondweed	Potamogeton pusillus	0	1.7	0
American pondweed	Potamogeton nodosus	0	0	0
Clasping leaf-pondweed	Potamogeton richardsonii	35.5	18.6*	28.2*
Fern-leaf pondweed	Potamogeton robbinsii	0	0	0.3
Flat-stem pondweed	Potamogeton zosteriformis	11.3	20.3*	25.5
Water crowfoot	Ranunculus sp.	0	0	0
Spiral ditch-grass	Ruppia cirrhosa	1.7	0.6	0.3
White water crowfoot	Ranunculus longirostris	14.1	0*	0
Hardstem bulrush	Schoenoplectus acutus	2.7	3.1	3.1
Sago pondweed	Stuckenia pectinata	21.7	5.8*	8.9
Bladderwort	Utricularia macrorhiza	20.6	8.6*	6.9
Cattail	Typha sp.	0	1.7	0
Water celery	Vallisneria americana	0	43.7*	70.1*

Table 10. Summary of species percent frequency of occurrence by month for all submersed application reference plots in all lakes for 2012. May is pretreatment data, July and September are four weeks after the first and second diquat treatment, respectively. An asterisk indicates a significant difference from the previous month, as indicated by a McNemar's test.

Common Name	SPECIES	MAY %	JULY %	SEPT%
Water marigold	Bidens beckii	0.0	1.3	1.7
Flowering rush	Butomus umbellatus	65.6	39.62*	43.4
Coontail	Ceratophyllum demersum	1.9	10.06*	23.0
Chara	Chara	54.8	71.7*	62.8
Moss	Drepanocladus	1.9	7.55*	0.9
Elodea	Elodea canadensis	15.3	14.7	10.6
Water stargrass	Heteranthera dubia	0.0	1.3	2.7
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	1.9	0.6	8.0
Northern water milfoil	Myriophyllum sibiricum	28.1	25.2	52.2
Slender naiad	Najas flexilis	0.0	11.32*	9.7
Yellow pond lily	Nuphar lutea	6.4	11.3	8.0
White water lily	Nymphaea odorata	0.0	11.32*	7.1
Curly leaf pondweed	Potamogeton crispus	7.0	1.26*	0.0
Leafy pondweed	Potamogeton foliosus	0.6	1.9	0.0
Variable pondweed	Potamogeton gramineus	0.0	1.9	3.5
Illinois pondweed	Potamogeton illinoensis	24.2	27.7	48.7
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	7.6	0*	8.0
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.9
Clasping leaf-pondweed	Potamogeton richardsonii	25.5	23.9	37.2
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	7.0	22.01*	15.0
Water crowfoot	Ranunculus sp.	0.0	3.1	0.0
Spiral ditch-grass	Ruppia cirrhosa	5.7	0.63*	6.2
White water crowfoot	Ranunculus longirostris	15.9	4.4*	0.0
Hardstem bulrush	Schoenoplectus acutus	22.3	21.4	16.8
Sago pondweed	Stuckenia pectinata	10.8	17.0	24.8
Bladderwort	Utricularia macrorhiza	15.9	18.9	1.8
Cattail	Typha sp.	0.0	0.6	0.0
Water celery	Vallisneria americana	0.0	21.38*	47.8

Table 11. Summary of the change in species percent frequency of occurrence by month for all reference and treatment plots. A plus ("+") indicates a statistical increase in the species for the reference or treatment plot, a minus ("-") indicates a decrease for the reference or treatment plots.

Common Name	SPECIES	Reference	Diquat
Water marigold	Bidens beckii		
Flowering rush	Butomus umbellatus	-	-
Coontail	Ceratophyllum demersum	+	+
Chara	Chara	+	+
Moss	Drepanocladus	+	+
Elodea	Elodea canadensis		-
Water stargrass	Heteranthera dubia		
Common duckweed	Lemna minor		
Forked duckweed	Lemna trisulca		
Northern water milfoil	Myriophyllum sibiricum		
Slender naiad	Najas flexilis	+	+
Yellow pond lily	Nuphar lutea		+
White water lily	Nymphaea odorata	+	
Curly leaf pondweed	Potamogeton crispus	-	-
Leafy pondweed	Potamogeton foliosus		-
Variable pondweed	Potamogeton gramineus		
Illinois pondweed	Potamogeton illinoensis		
Floating pondweed	Potamogeton natans		
White stem pondweed	Potamogeton praelongus	-	
small pondweed	Potamogeton pusillus		
American pondweed	Potamogeton nodosus		
Clasping leaf-pondweed	Potamogeton richardsonii		-
Fern-leaf pondweed	Potamogeton robbinsii		
Flat-stem pondweed	Potamogeton zosteriformis	+	+
Water crowfoot	Ranunculus sp.		
Spiral ditch-grass	Ruppia cirrhosa	-	
White water crowfoot	Ranunculus longirostris	-	-
Hardstem bulrush	Schoenoplectus acutus		
Sago pondweed	Stuckenia pectinata		-
Bladderwort	Utricularia macrorhiza		-
Cattail	Typha sp.		
Water celery	Vallisneria americana	+	+
Increasers		7	7
Decreasers		5	8
No change		20	17

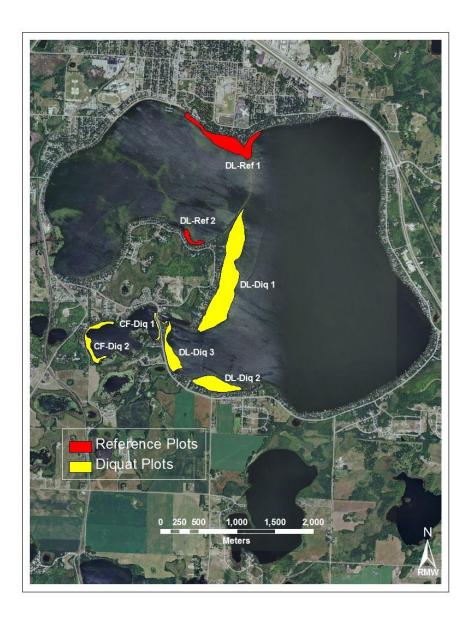


Figure 1. Diquat treatment areas of delineated flowering rush beds (yellow) for Big Detroit, Little Detroit, and Curfman Lakes. Reference areas are in red.

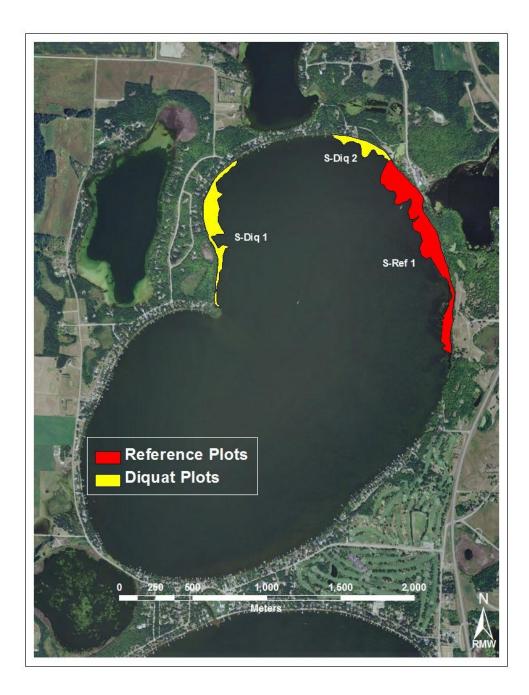


Figure 2. Treatment areas of delineated flowering rush beds (yellow); with reference areas indicated in red for Sallie Lake.

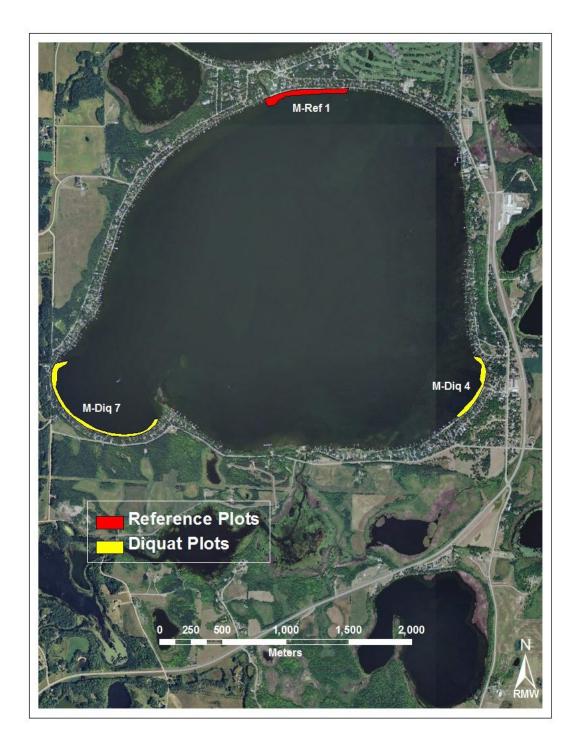


Figure 3. Treatment areas of delineated flowering rush beds (yellow) for Melissa Lake. Reference areas are in red.



Figure 4. Imazapyr treatment areas (yellow) in Big Detroit and Little Detroit Lakes. The reference plot (red) is in Little Detroit Lake.



Figure 5. The 6" diameter core biomass sampler in use, operated by Kris Madsen of Mississippi State University and assisted by Sydney Redmond of Concordia College. The sampler is inserted into the bottom, suction applied by a temporary cap or valve, and brought to the surface where the core is transferred to a sorting bucket. Photo by J. Madsen, GRI.



Figure 6A. The rhizome of flowering rush with two rhizome buds, indicated by the yellow arrows. Rhizome buds initiate new shoots and are the main form of vegetative propagation in flowering rush. Photo by J. Madsen, GRI.



Figure 6B. Rhizome bud of flowering rush from Detroit Lake, detached. Rhizome buds may break off, and become independent propagules. Photo courtesy of C. Welling, MN DNR.

## **Aboveground Biomass**

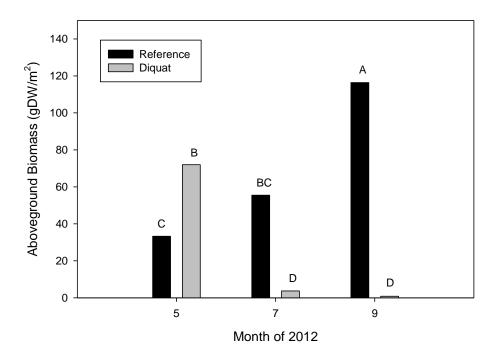


Figure 7. Grand means of aboveground biomass  $(gDW/m^2)$  from ANOVA of untreated reference vs. diquat treated plots for four treated and four reference plots in Detroit Lakes basins in 2012. Means with the same letter are not significantly different at the p=0.05 level.

# **Aboveground Biomass**

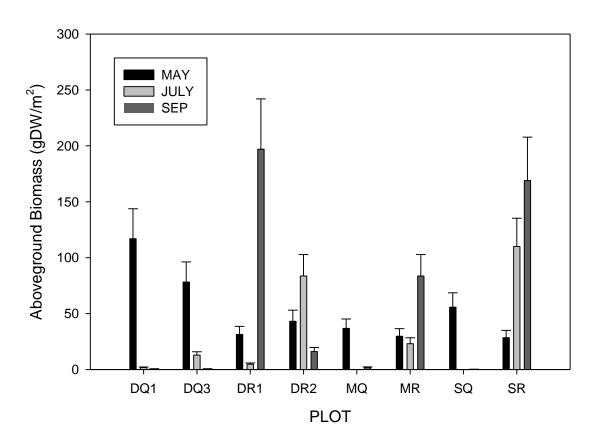


Figure 8. Aboveground biomass of submersed diquat application plots for May, July and September of 2012 in the Detroit Lakes. DQ1, Big Detroit Treated Plot 1; DQ3, Big Detroit Treated Plot 3; DR1, Big Detroit Reference Plot 1; DR2, Big Detroit Reference Plot 2; MQ, Melissa Treated Plot 7; MR, Melissa Reference Plot; SQ, Sallie Lake Treated Plot 1; SR, Sallie Lake Reference Plot.

#### **Belowground Biomass**

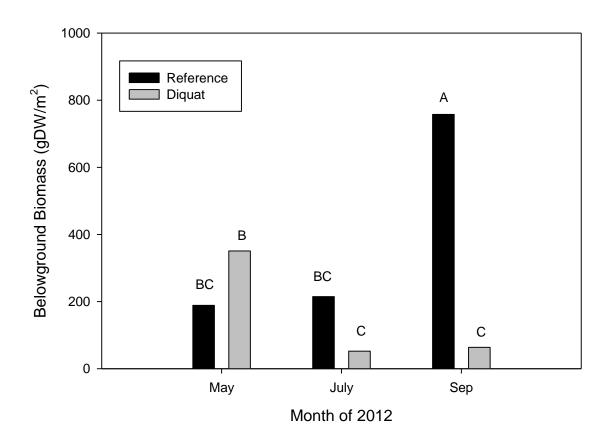


Figure 9. Grand means of belowground biomass ( $gDW/m^2$ ) from ANOVA of untreated reference vs. diquat treated plots for four treated and four reference plots in Detroit Lakes basins in 2012. Means with the same letter are not significantly different at the p=0.05 level.

### **Belowground Biomass**

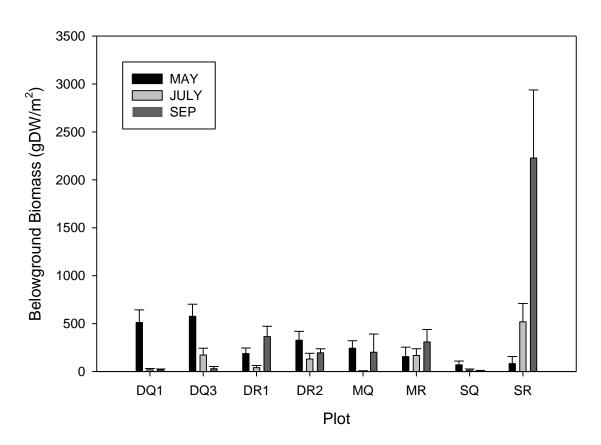


Figure 10. Belowground biomass (gDW/m²) of flowering rush in submersed diquat treatment study, Detroit Lakes, in May, July and September of 2012. DQ1, Big Detroit Treated Plot 1; DQ3, Big Detroit Treated Plot 3; DR1, Big Detroit Reference Plot 1; DR2, Big Detroit Reference Plot 2; MQ, Melissa Treated Plot 7; MR, Melissa Reference Plot; SQ, Sallie Lake Treated Plot 1; SR, Sallie Lake Reference Plot.

#### Rhizome Bud Density

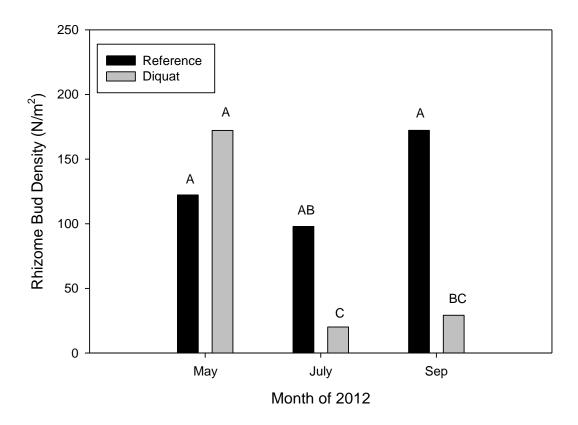


Figure 11. Grand means of rhizome bud density  $(N/m^2)$  from ANOVA of untreated reference vs. diquat treated plots for four treated and four reference plots in Detroit Lakes basins in 2012. Means with the same letter are not significantly different at the p=0.05 level.

# Bud Density (N/m<sup>2</sup>)

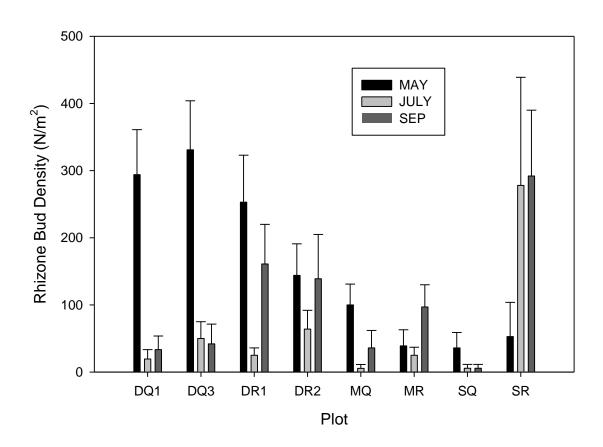


Figure 12. Rhizome bud density (N/m²) of flowering rush in submersed diquat treatment study, Detroit Lakes, in May, July and September of 2012. DQ1, Big Detroit Treated Plot 1; DQ3, Big Detroit Treated Plot 3; DR1, Big Detroit Reference Plot 1; DR2, Big Detroit Reference Plot 2; MQ, Melissa Treated Plot 7; MR, Melissa Reference Plot; SQ, Sallie Lake Treated Plot 1; SR, Sallie Lake Reference Plot.

## **Aboveground Biomass**

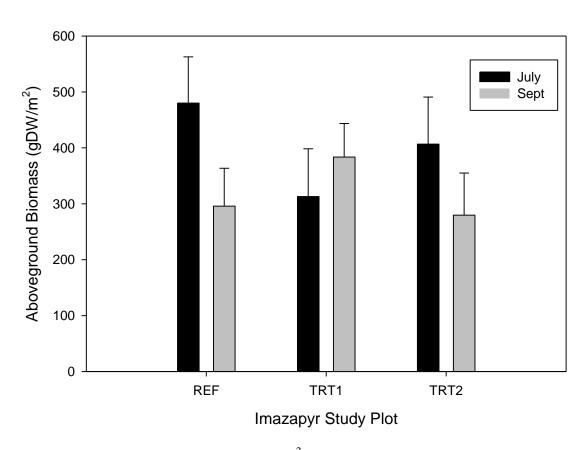


Figure 13. Aboveground biomass (gDW/m²) of flowering rush in imazapyr treatment study in 2012 of Detroit Lakes. REF, Reference; TRT1, treatment plot 1 (Big DL); TRT2, treatment plot 2 (Little DL).

### **Belowground Biomass**

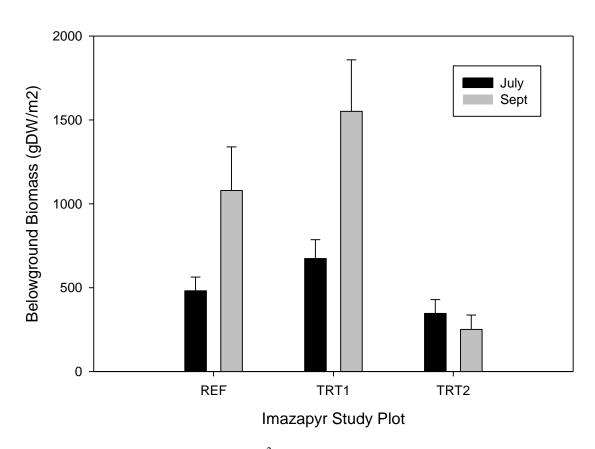


Figure 14. Rhizome bud density (N/m²) of flowering rush in imazapyr treatment study in 2012 of Detroit Lakes. REF, Reference; TRT1, treatment plot 1 (Big DL); TRT2, treatment plot 2 (Little DL).

# Rhizome Bud Density

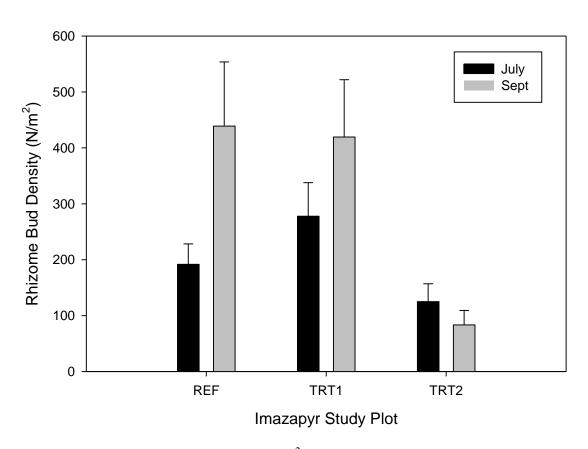


Figure 15. Belowground biomass (gDW/m²) of flowering rush in imazapyr treatment study in 2012 of Detroit Lakes. REF, Reference; TRT1, treatment plot 1 (Big DL); TRT2, treatment plot 2 (Little DL).



Figure 16. Big Detroit Lake imazapyr treatment site before treatment, in July 2012.



Figure 17. Flowering rush is chlorotic in the Big Detroit Lake imazapyr treatment site in September 2012, about six weeks after treatment, but not sufficiently advanced to count as dead.

Appendices. Species percent frequency of occurrence for all treatment and reference plots, by plot, for May, July, and September 2012.				

Appendix 1. Species percent frequency of occurrence for plot DL-DIQ-1 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	81.4	27.1	37.3
Coontail	Ceratophyllum demersum	0.0	3.4	13.7
Chara	Chara	74.6	98.3	100.0
Moss	Drepanocladus	6.8	16.9	2.0
Elodea	Elodea canadensis	13.6	1.7	0.0
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	0.0	1.7	3.9
Northern water milfoil	Myriophyllum sibiricum	0.0	5.1	15.7
Slender naiad	Najas flexilis	0.0	5.1	7.8
Yellow pond lily	Nuphar lutea	0.0	0.0	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	1.7	0.0	0.0
Leafy pondweed	Potamogeton foliosus	0.0	3.4	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	16.9	16.9	37.3
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	6.8	8.5	13.7
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	47.5	13.6	43.1
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	0.0	3.4	15.7
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water crowfoot	Ranunculus longirostris	0.0	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	18.6	16.9	15.7
Bladderwort	Utricularia macrorhiza	33.9	0.0	17.6
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	71.2	98.0

Appendix 2. Species percent frequency of occurrence for plot DL-DIQ-2 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	90.9	34.3	36.4
Coontail	Ceratophyllum demersum	3.0	20.0	18.2
Chara	Chara	84.8	97.1	100.0
Moss	Drepanocladus	3.0	8.6	13.6
Elodea	Elodea canadensis	3.0	0.0	0.0
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	6.1	2.9	0.0
Northern water milfoil	Myriophyllum sibiricum	3.0	5.7	9.1
Slender naiad	Najas flexilis	0.0	0.0	4.5
Yellow pond lily	Nuphar lutea	6.1	5.7	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	15.2	0.0	0.0
Leafy pondweed	Potamogeton foliosus	0.0	5.7	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	18.2	40.0	40.9
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	0.0	0.0	4.5
small pondweed	Potamogeton pusillus	0.0	14.3	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	54.5	45.7	40.9
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	0.0	17.1	22.7
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water crowfoot	Ranunculus longirostris	3.0	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	48.5	8.6	18.2
Bladderwort	Utricularia macrorhiza	18.2	5.7	0.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	71.4	90.9

Appendix 3. Species percent frequency of occurrence s for plot DL-DIQ-3 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	84.2	38.5	36.8
Coontail	Ceratophyllum demersum	5.3	20.5	21.1
Chara	Chara	36.8	79.5	78.9
Moss	Drepanocladus	21.1	53.8	18.4
Elodea	Elodea canadensis	31.6	5.1	2.6
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	10.5	28.2	18.4
Northern water milfoil	Myriophyllum sibiricum	0.0	7.7	10.5
Slender naiad	Najas flexilis	0.0	10.3	2.6
Yellow pond lily	Nuphar lutea	5.3	30.8	26.3
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	7.9	0.0	2.6
Leafy pondweed	Potamogeton foliosus	0.0	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	42.1	23.1	50.0
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	10.5	28.2	21.1
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf- pondweed	Potamogeton richardsonii	50.0	17.9	36.8
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	7.9	20.5	15.8
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	7.9	0.0	0.0
White water crowfoot	Ranunculus longirostris	10.5	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	23.7	5.1	7.9
Bladderwort	Utricularia macrorhiza	26.3	10.3	2.6
Cattail	Typha sp.	0.0	7.7	0.0
Water celery	Vallisneria americana	0.0	76.9	94.7

Appendix 4. Species percent frequency of occurrence for plot CF-DIQ-1 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	79.2	70.8	55.0
Coontail	Ceratophyllum demersum	8.3	37.5	50.0
Chara	Chara	33.3	75.0	55.0
Moss	Drepanocladus	0.0	0.0	0.0
Elodea	Elodea canadensis	37.5	0.0	0.0
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	16.7	33.3	25.0
Northern water milfoil	Myriophyllum sibiricum	20.8	16.7	15.0
Slender naiad	Najas flexilis	0.0	0.0	0.0
Yellow pond lily	Nuphar lutea	8.3	8.3	5.0
White water lily	Nymphaea odorata	4.2	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	12.5	0.0	0.0
Leafy pondweed	Potamogeton foliosus	0.0	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	4.2	0.0
Illinois pondweed	Potamogeton illinoensis	29.2	25.0	5.0
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem	Potamogeton praelongus	0.0	4.2	5.0
pondweed				
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf- pondweed	Potamogeton richardsonii	25.0	8.3	10.0
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	0.0	0.0	5.0
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water	Ranunculus longirostris	0.0	0.0	0.0
crowfoot				
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	37.5	4.2	10.0
Bladderwort	Utricularia macrorhiza	16.7	4.2	5.0
Cattail	Typha sp.	0.0	8.3	0.0
Water celery	Vallisneria americana	0.0	45.8	75.0

Appendix 5. Species percent frequency of occurrence for plot CF-DIQ-2 in 2012.

Common Name	SPECIES	May	July	Sept
Water manicald	Di I LLii	0.0	0.0	0.0
Water marigold	Bidens beckii			
Flowering rush	Butomus umbellatus	71.4	55.6	57.1
Coontail	Ceratophyllum demersum	14.3	44.4	78.6
Chara	Chara	52.4	72.2	71.4
Moss	Drepanocladus	0.0	0.0	0.0
Elodea	Elodea canadensis	4.8	0.0	0.0
Water stargrass	Heteranthera dubia	0.0	5.6	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	4.8	33.3	28.6
Northern water milfoil	Myriophyllum sibiricum	33.3	11.1	0.0
Slender naiad	Najas flexilis	0.0	0.0	7.1
Yellow pond lily	Nuphar lutea	14.3	38.9	21.4
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	9.5	0.0	7.1
Leafy pondweed	Potamogeton foliosus	4.8	5.6	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	14.3	11.1	21.4
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	14.3	11.1	14.3
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	23.8	0.0	0.0
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	0.0	0.0	0.0
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water crowfoot	Ranunculus longirostris	9.5	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	14.3	11.1	14.3
Sago pondweed	Stuckenia pectinata	71.4	5.6	14.3
Bladderwort	Utricularia macrorhiza	42.9	38.9	28.6
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	16.7	42.9

Appendix 6. Species percent frequency of occurrence for plot Sallie S-DIQ-1 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	86.1	30.5	22.2
Coontail	Ceratophyllum demersum	5.5	33.3	22.2
Chara	Chara	30.5	58.3	61.1
Moss	Drepanocladus	2.7	11.1	22.2
Elodea	Elodea canadensis	8.3	11.1	2.7
Water stargrass	Heteranthera dubia	2.7	2.7	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	2.7	5.5	16.6
Northern water milfoil	Myriophyllum sibiricum	8.3	2.7	11.1
Slender naiad	Najas flexilis	0.0	2.7	0.0
Yellow pond lily	Nuphar lutea	0.0	2.7	0.0
White water lily	Nymphaea odorata	0.0	0.0	11.1
Curly leaf pondweed	Potamogeton crispus	16.6	2.7	0.0
Leafy pondweed	Potamogeton foliosus	2.7	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	2.7	2.7	2.7
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	8.3	8.3	2.7
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf- pondweed	Potamogeton richardsonii	36.1	11.1	16.6
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	22.2	27.7	13.8
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water crowfoot	Ranunculus longirostris	25.0	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	13.8	19.4	16.6
Sago pondweed	Stuckenia pectinata	0.0	0.0	5.5
Bladderwort	Utricularia macrorhiza	8.3	2.7	2.7
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	19.4	47.2

Appendix 7. Species percent frequency of occurrence for plot S-DIQ-2 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	86.6	32.2	35.4
Coontail	Ceratophyllum demersum	0.0	3.2	6.4
Chara	Chara	3.3	64.5	32.2
Moss	Drepanocladus	6.6	9.6	3.2
Elodea	Elodea canadensis	0.0	0.0	0.0
Water stargrass	Heteranthera dubia	0.0	3.2	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	6.6	3.2	16.1
Northern water milfoil	Myriophyllum sibiricum	3.3	19.3	6.4
Slender naiad	Najas flexilis	0.0	6.4	0.0
Yellow pond lily	Nuphar lutea	0.0	3.2	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	23.3	0.0	0.0
Leafy pondweed	Potamogeton foliosus	6.6	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	13.3	3.2	0.0
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	6.6	3.2	6.4
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf- pondweed	Potamogeton richardsonii	26.6	16.1	32.2
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	33.3	35.4	45.1
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	3.3	6.4	0.0
White water crowfoot	Ranunculus longirostris	23.3	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	0.0	0.0	6.4
Bladderwort	Utricularia macrorhiza	0.0	6.4	0.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	16.1	48.3

Appendix 8. Species percent frequency of occurrence for plot M-DIQ-4 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	4.1	0.0	0.0
Flowering rush	Butomus umbellatus	29.1	5.2	0.0
Coontail	Ceratophyllum demersum	12.5	10.5	61.1
Chara	Chara	16.6	26.3	44.4
Moss	Drepanocladus	0.0	0.0	0.0
Elodea	Elodea canadensis	0.0	5.2	5.5
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	16.6	0.0	16.6
Northern water milfoil	Myriophyllum sibiricum	20.8	31.5	50.0
Slender naiad	Najas flexilis	0.0	10.5	0.0
Yellow pond lily	Nuphar lutea	0.0	10.5	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	4.2	0.0	0.0
Leafy pondweed	Potamogeton foliosus	8.3	21.0	0.0
Variable pondweed	Potamogeton gramineus	4.1	5.2	0.0
Illinois pondweed	Potamogeton illinoensis	12.5	26.3	22.2
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	33.3	21.0	5.5
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	16.6	36.8	38.8
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	33.3	78.9	61.1
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	4.1	0.0	0.0
White water crowfoot	Ranunculus longirostris	4.1	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	8.3	0.0	0.0
Bladderwort	Utricularia macrorhiza	4.1	10.5	0.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	5.2	50.0

Appendix 9. Species percent frequency of occurrence for plot M-DIQ-7 in 2012.

Common Name	SPECIES	May %	July %	Sept %
Water marigold	Bidens beckii	3.4	3.4	0.0
Flowering rush	Butomus umbellatus	75.8	13.7	7.1
Coontail	Ceratophyllum demersum	10.3	31.0	32.1
Chara	Chara	41.3	44.8	46.4
Moss	Drepanocladus	10.3	10.3	7.1
Elodea	Elodea canadensis	13.7	3.4	0.0
Water stargrass	Heteranthera dubia	0.0	3.4	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	24.1	3.4	0.0
Northern water milfoil	Myriophyllum sibiricum	3.4	10.3	32.1
Slender naiad	Najas flexilis	0.0	0.0	3.5
Yellow pond lily	Nuphar lutea	0.0	0.0	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	31.0	3.4	0.0
Leafy pondweed	Potamogeton foliosus	10.3	10.3	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	44.8	13.7	35.7
Floating pondweed	Potamogeton natans	3.4	0.0	0.0
White stem pondweed	Potamogeton praelongus	10.3	17.2	3.5
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	10.3	17.2	10.7
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	13.7	24.1	60.7
Water crowfoot	Ranunculus sp.	0.0	0.0	3.5
Spiral ditch-grass	Ruppia cirrhosa	0.0	0.0	0.0
White water crowfoot	Ranunculus longirostris	58.6	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	3.4	0.0	0.0
Bladderwort	Utricularia macrorhiza	24.1	20.6	7.1
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	10.3	46.4

Appendix 10. Species percent frequency of occurrence for plot DL-REF-1 in 2012.

Common Name	SPECIES	MAY	JULY	SEPT
		%	%	%
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	61.2	17.3	29.8
Coontail	Ceratophyllum demersum	0.0	11.5	10.6
Chara	Chara	85.7	98.1	93.6
Moss	Drepanocladus	2.0	21.2	212.0
Elodea	Elodea canadensis	18.3	3.8	0.0
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	0.0	0.0	0.0
Northern water milfoil	Myriophyllum sibiricum	63.3	13.5	38.3
Slender naiad	Najas flexilis	0.0	15.3	0.0
Yellow pond lily	Nuphar lutea	0.0	0.0	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	8.2	1.9	0.0
Leafy pondweed	Potamogeton foliosus	0.0	3.8	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	2.1
Illinois pondweed	Potamogeton illinoensis	18.4	17.3	51.1
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	16.3	0.0	12.8
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf- pondweed	Potamogeton richardsonii	34.7	28.8	31.9
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	4.1	9.6	6.4
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	6.1	0.0	0.0
White water crowfoot	Ranunculus longirostris	10.2	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	22.4	5.8	0.0
Bladderwort	Utricularia macrorhiza	18.4	1.9	2.1
Cattail	Typha sp.	0.0	1.9	0.0
Water celery	Vallisneria americana	0.0	34.6	63.8

Appendix 11. Species percent frequency of occurrence for plot DL-REF-2 in 2012.

Common Name	SPECIES	MAY %	JULY %	SEPT %
Water marigold	Bidens beckii	0.0	0.0	10.5
Flowering rush	Butomus umbellatus	63.0	61.5	42.1
Coontail	Ceratophyllum demersum	0.0	29.9	15.8
Chara	Chara	74.1	76.9	78.9
Moss	Drepanocladus	0.0	0.0	0.0
Elodea	Elodea canadensis	55.5	57.7	47.4
Water stargrass	Heteranthera dubia	0.0	0.0	10.5
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	0.0	3.8	5.3
Northern water milfoil	Myriophyllum sibiricum	22.2	57.7	89.5
Slender naiad	Najas flexilis	0.0	34.6	47.4
Yellow pond lily	Nuphar lutea	14.8	23.1	26.3
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	0.0	0.0	0.0
Leafy pondweed	Potamogeton foliosus	0.0	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	5.3
Illinois pondweed	Potamogeton illinoensis	51.9	69.2	73.7
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	11.1	0.0	5.3
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	5.3
Clasping leaf-pondweed	Potamogeton richardsonii	48.1	53.8	42.1
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	29.6	73.1	36.8
Water crowfoot	Ranunculus sp.	0.0	19.2	0.0
Spiral ditch-grass	Ruppia cirrhosa	11.1	0.0	0.0
White water crowfoot	Ranunculus longirostris	37.0	0.0	0.0
Hardstem bulrush	Schoenoplectus acutus	22.2	23.1	42.1
Sago pondweed	Stuckenia pectinata	14.8	53.8	47.4
Bladderwort	Utricularia macrorhiza	44.4	46.2	0.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	63.8	42.1

Appendix 12. Species percent frequency of occurrence for plot S-REF-1 in 2012.

Common Name	SPECIES	MAY %	JULY %	SEPT %
Water marigold	Bidens beckii	0.0	3.7	0.0
Flowering rush	Butomus umbellatus	88.2	57.4	61.9
Coontail	Ceratophyllum demersum	5.9	5.6	42.9
Chara	Chara	3.9	40.7	4.8
Moss	Drepanocladus	2.0	0.0	0.0
Elodea	Elodea canadensis	0.0	11.1	14.3
Water stargrass	Heteranthera dubia	0.0	3.7	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	5.9	0.0	38.1
Northern water milfoil	Myriophyllum sibiricum	13.7	33.3	81.0
Slender naiad	Najas flexilis	0.0	1.9	4.8
Yellow pond lily	Nuphar lutea	11.8	22.2	19.0
White water lily	Nymphaea odorata	0.0	33.3	38.1
Curly leaf pondweed	Potamogeton crispus	11.8	1.9	0.0
Leafy pondweed	Potamogeton foliosus	0.0	0.0	0.0
Variable pondweed	Potamogeton gramineus	0.0	0.0	0.0
Illinois pondweed	Potamogeton illinoensis	13.7	22.2	23.8
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	2.0	0.0	0.0
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	17.6	13.0	33.3
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	2.0	18.5	23.8
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	0.0	1.9	0.0
White water crowfoot	Ranunculus longirostris	17.6	9.3	0.0
Hardstem bulrush	Schoenoplectus acutus	56.9	51.9	52.4
Sago pondweed	Stuckenia pectinata	2.0	13.0	33.3
Bladderwort	Utricularia macrorhiza	5.9	31.5	0.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	11.1	33.3

Appendix 13. Species percent frequency of occurrence for plot M-REF-1 in 2012.

Common Name	SPECIES	MAY %	JULY %	SEPT %
Water marigold	Bidens beckii	0.0	0.0	0.0
Flowering rush	Butomus umbellatus	37.9	26.9	56.0
Coontail	Ceratophyllum demersum	0.0	0.0	36.0
Chara	Chara	75.9	80.8	44.0
Moss	Drepanocladus	3.4	3.8	0.0
Elodea	Elodea canadensis	0.0	0.0	0.0
Water stargrass	Heteranthera dubia	0.0	0.0	0.0
Common duckweed	Lemna minor	0.0	0.0	0.0
Forked duckweed	Lemna trisulca	0.0	0.0	0.0
Northern water milfoil	Myriophyllum sibiricum	0.0	0.0	28.0
Slender naiad	Najas flexilis	0.0	0.0	4.0
Yellow pond lily	Nuphar lutea	0.0	0.0	0.0
White water lily	Nymphaea odorata	0.0	0.0	0.0
Curly leaf pondweed	Potamogeton crispus	3.4	0.0	0.0
Leafy pondweed	Potamogeton foliosus	3.4	3.8	0.0
Variable pondweed	Potamogeton gramineus	0.0	11.5	8.0
Illinois pondweed	Potamogeton illinoensis	27.6	19.2	48.0
Floating pondweed	Potamogeton natans	0.0	0.0	0.0
White stem pondweed	Potamogeton praelongus	0.0	0.0	8.0
small pondweed	Potamogeton pusillus	0.0	0.0	0.0
American pondweed	Potamogeton nodosus	0.0	0.0	0.0
Clasping leaf-pondweed	Potamogeton richardsonii	3.4	7.7	48.0
Fern-leaf pondweed	Potamogeton robbinsii	0.0	0.0	0.0
Flat-stem pondweed	Potamogeton zosteriformis	0.0	3.8	8.0
Water crowfoot	Ranunculus sp.	0.0	0.0	0.0
Spiral ditch-grass	Ruppia cirrhosa	10.3	0.0	28.0
White water crowfoot	Ranunculus longirostris	3.4	7.7	0.0
Hardstem bulrush	Schoenoplectus acutus	0.0	0.0	0.0
Sago pondweed	Stuckenia pectinata	3.4	11.5	48.0
Bladderwort	Utricularia macrorhiza	3.4	0.0	4.0
Cattail	Typha sp.	0.0	0.0	0.0
Water celery	Vallisneria americana	0.0	3.8	36.0