INTRODUCTION

Following the discovery of Eurasian water milfoil (EWM) in North and South Twin Lakes, Vilas County, in 2001, the North and South Twin Lakes, Riparian Association (NSTLRA) has successfully applied for several grants through the Wisconsin Department of Natural Resources (WDNR) to help fund a campaign to control this invasive plant.

After the 2010 peak-biomass survey, a conditional treatment permit map was created proposing the treatment of 13.4 acres of EWM in North Twin Lake and 161 acres in South Twin Lake (Map 1). It was proposed that the treatment sites in North Twin Lake be treated using granular 2,4-D (Navigate) at 200 lbs per acre. However, the association's applicator suggested that Renovate Max G be used as it may be more effective. This herbicide is a combination of granular 2,4-D and triclopyr theorized to have synergistic effects compared to the respective herbicide components alone. Since the whole-lake liquid 2,4-D treatment on South Twin Lake was moderately successful in 2009, it was proposed that this strategy be used again in 2010 but with a higher application rate.

On May 25, 2010, Onterra ecologists visited North and South Twin Lakes to survey the proposed treatment areas and determined that their boundaries did not require any refining. During this survey, surface water temperatures were around 58°F. Renovate Max G was applied to treatment areas in North Twin Lake at a rate of 2.0 ppm on May 26, 2010 by Bonestroo. Post treatment surveys were completed by Onterra on August 24, 2010. Liquid 2,4-D was applied to the treatment areas on South Twin Lake on the afternoons of May 25 and 26, 2010 by Bonestroo at a rate of 2.5 ppm over the EWM treatment areas with a goal that the calculated lake-wide concentration would be 0.240 ppm.

2010 TREATMENT MONITORING

The goal of herbicide treatments is to maximize target species (EWM) mortality while minimizing impacts to valuable native aquatic plant species. Monitoring herbicide treatments and defining their success incorporates both quantitative and qualitative methods. As the name suggests, quantitative monitoring involves comparing number data (or quantities) such as plant frequency of occurrence before and after the control strategy is implemented. Qualitative monitoring is completed by comparing observational data such as EWM colony density ratings before and after the treatments.

Quantitative evaluation methodologies follow WDNR protocols in which point-intercept data is collected within treatment areas both the summer before and the summer immediately following the treatments take place. Since the intent on South Twin Lake was to have the herbicide disperse throughout the entire lake, quantitative evaluation was made using a whole-lake point-intercept survey which included data collection at 621 sub-sample locations. Although 621 sampling points are distributed over the lake, only those points that are located at or below the maximum depth of plant growth (littoral zone) are used in the analysis. In North Twin Lake, 41 point-intercept sub-sample locations, EWM and native aquatic plant species presence and rake-fullness were documented along with water depth and substrate type. Specifically, these surveys aim to determine if statistical differences in occurrence of EWM and native species occur following the herbicide application.

Quantitatively, a specific treatment site is deemed to be successful if the EWM frequency following the treatments is statistically reduced by at least 50%. Evaluation of treatment-wide effectiveness follows the same criteria based upon pooled sub-sample data from all of the treatment sites. Further, a noticeable decrease in rake fullness ratings within the fullness categories of 2 and 3 should be observed and preferable, there would be no rake tows exhibiting a fullness of 2 or 3 during the post treatment surveys.

Spatial data reflecting EWM locations were collected using a sub-meter Global Positioning System (GPS) during the late summers of 2009 and 2010, when this plant is assumed to be at its peak biomass or growth stage. Comparisons of these surveys are used to qualitatively evaluate the 2010 herbicide treatments on North and South Twin Lakes. Qualitatively, a successful treatment on a particular site would include a reduction of EWM density as demonstrated by a decrease in density rating (e.g. highly dominant to dominant). In terms of a treatment as a whole, at least 75% of the acreage treated that year would decrease by one level of density as described above for an individual site.

Although it is never the intent of the treatments to impact native species, it is important to remember that in spot treatment scenarios like on North Twin Lake, these non-target impacts can only be considered in the context of the areas treated and not on a *lake-wide* basis. In other words, the impact of the treatments on a non-target species in the treatment areas cannot be extrapolated to the entire population of that plant within the lake, unless the plant species is only found in locations where the herbicide applications took place. However, on South Twin Lake where the herbicide was expected to dissipate throughout the entire lake and a whole-lake point-intercept survey was conducted, the non-target impacts can be considered on a lake-wide basis. While 2,4-D is thought to be selective towards broad-leaf (dicot) species at the concentration and exposure times observed during the 2010 treatments on North and South Twin Lakes, emerging data from the WDNR and US Army Corps of Engineers suggests that some narrow-leaf (monocot) species may also be impacted by this herbicide.

2010 TREATMENT RESULTS

South Twin Lake

Incredibly, during the 2010 post treatment survey, Onterra ecologists could not locate any EWM growing in South Twin Lake, even in areas that were classified as having 'dominant' and 'highly dominant' EWM in 2009 (Map 1 and 2). This obviously resulted in all of the treatment areas being reduced by at least one density rating, exceeding the qualitative success criteria (75% reduction) for the 2010 treatment.

This year was the second year of whole-lake scale treatments on South Twin Lake, and Figure 1 displays the decline in EWM beginning in 2009 following the first whole-lake treatment. In 2008, approximately 21% of the littoral point-intercept sampling locations contained EWM, followed by 10% in 2009, and 0% in 2010. The decline of EWM in 2010 represents a statistically valid reduction in occurrence of 100%, exceeding the quantitative success criteria (50% reduction in occurrence). A rake fullness rating of 1-3 was used to determine the abundance of EWM at each point-intercept location. Figure 2 displays the proportions of EWM

rake-fullness ratings from 2008 to 2010, and illustrates the reduction in occurrence and density of EWM within South Twin Lake.



Figure 1. South Twin Lake EWM littoral occurrence prior to and following a 2009 and 2010 whole-lake liquid 2,4-D application. Created using data from 2008 pre-treatment survey and 2009/2010 post treatment surveys.



Figure 2. South Twin Lake proportions of EWM rake-fullness ratings prior to and following a 2009 and 2010 whole-lake liquid 2,4-D application. Created using data from 2008 pre-treatment survey and 2009/2010 post treatment surveys.

Ten native aquatic plant species were found to have statistically declined in 2010 following the treatment (Table 1). Like EWM, northern water milfoil, water marigold, and alternate-flowered water milfoil are dicots and are particularly susceptible to the herbicide application. Northern water milfoil was located at approximately 28% of the point-intercept locations in 2008, but was not detected at all during the 2010 survey. While their populations were reduced, water marigold and alternate-flowered water milfoil were still located in the 2010 survey.

The remaining seven native species that showed declines in occurrence are non-dicots, and are not thought to be particularly sensitive to dicot-selective herbicides. However, emerging data gathered this year from South Twin Lake and other lakes with similar large-scale liquid treatments in the northern region suggests that some of these plants may be prone to decline after treatment. It is believed that these native species will begin to re-colonize from healthy populations in North Twin Lake.

Table 1. Statistical analysis of EWM and native aquatic plant occurrence prior to and
following a 2009 and 2010 whole-lake liquid 2,4-D application. Created using data from
2008 pre-treatment survey and 2009/2010 post treatment surveys.

						2008-2009		2009-2010		2008-2010	
	Scientific Name	Com m on Nam e	2008 FOO	2009 FOO	2010 FOO	% Change	Direction	% Change	Direction	% Change	Direction
Dicots	Myriophyllum spicatum	Eurasian w ater milfoil	20.7	10.2	0.0	-50.6	▼	-100.0	▼	-100.0	V
	Myriophyllum sibiricum	Northern w ater milfoil	28.3	12.2	0.0	-56.8	•	-100.0	•	-100.0	V
	Megalodonta beckii	Water marigold	14.1	5.9	0.6	-58.0	V	-89.1	V	-95.4	V
	Myriophyllum alterniflorum	Alternate-flow ered water milfoil	3.6	4.6	0.6	27.7		-86.0	▼	-82.2	•
	Ceratophyllum demersum	Coontail	23.4	20.8	21.0	-11.0		0.8	A	-10.2	
	Myriophyllum tenellum	Dw arf w ater milfoil	1.0	0.0	0.0	-100.0		0.0	-	-100.0	V
	Ranunculus aquatilis	White water-crow foot	0.7	0.3	0.0	-49.8		-100.0		-100.0	
	Potamogeton gramineus	Variable pondw eed	46.7	46.5	37.4	-0.4		-19.6	•	-19.9	•
	Najas flexilis	Slender naiad	33.2	31.4	17.1	-5.6		-45.5	V	-48.5	V
	Potamogeton zosteriformis	Flat-stem pondw eed	31.3	26.1	5.2	-16.6	V	-80.2	V	-83.5	V
	Elodea canadensis	Common w aterw eed	24.7	26.4	15.5	7.0		-41.4	V	-37.2	V
	Heteranthera dubia	Water stargrass	22.4	8.9	4.2	-60.2	•	-52.9	•	-81.3	•
	Potamogeton pusillus	Small pondw eed	18.4	12.5	2.6	-31.9	V	-79.4	V	-86.0	V
	Potamogeton richardsonii	Clasping-leaf pondw eed	11.5	17.5	16.8	51.9	▲	-4.1	V	45.7	▲
	Potamogeton friesii	Fries' pondw eed	9.2	3.0	0.6	-67.8	•	-78.3	•	-93.0	•
	Potamogeton amplifolius	Large-leaf pondw eed	3.6	3.6	0.6	0.3	A	-82.2	▼	-82.2	V
ots	Vallisneria americana	Wild celery	60.9	43.2	53.5	-29.0	▼	23.9	▲	-12.0	\square
ŝ	Potamogeton robbinsii	Fern pondw eed	31.3	32.7	33.5	4.6	A	2.7		7.4	A
Ę	Chara sp.	Muskgrasses	29.3	17.2	22.3	-41.4	▼	29.7		-24.0	\square
ž	Potamogeton praelongus	White-stem pondw eed	10.5	9.9	10.3	-5.9		4.3		-1.9	
	Eleocharis acicularis	Needle spikerush	5.9	4.0	5.5	-33.1		38.5	A	-7.4	\square
	Isoetes sp.	Quillw ort species	3.9	2.6	5.5	-33.1		107.7		38.9	
	Nitella sp.	Stonew orts	2.3	2.6	0.6	14.7	A	-75.6	V	-72.0	\square
	Schoenoplectus acutus	Hardstem bulrush	1.0	3.0	2.9	201.0		-2.3		194.2	
	P. richarsonii hybrid	Clasping-leaf hybrid	0.7	0.3	1.0	-49.8		193.2	A	47.1	A
	Juncus pelocarpus	Brown-fruited rush	0.0	0.0	0.3	0.0	-	100.0		100.0	
	Potamogeton strictifolius	Stiff pondw eed	0.0	0.3	0.3	100.0		-2.3		100.0	A
	Stuckenia pectinata	Sago pondw eed	0.0	0.0	0.3	0.0	-	100.0		100.0	
	Elatine minima	Waterw ort	0.0	0.3	0.0	100.0		-100.0	V	0.0	-

2008 N = 303, 2009 N = 304, 2010 N = 310

Note: Point-intercept sub-sampling locations used were located in littoral areas determined by the maximum depth of plant growth for each year

FOO = Frequency of Occurrence

▲ or ▼ = Statistically Different (Chi-square; $\alpha = 0.05$)

▲ or ∇ = Not Statistically Different (Chi-square; α = 0.05)

Due to the large scale of the treatment on South Twin Lake, it was one of a number of lakes selected for herbicide residual monitoring. Water sampling was coordinated by the Engineer Research and Development Center, a division of the USACE and conducted by NSTLRA volunteers (Map 1). The data indicate that herbicide did not dissipate into North Twin Lake. The mean lake-wide herbicide concentration from application to 7 days after treatment was 0.575 ppm acid equivalent (a.e.); almost 2.5 times higher than the target concentration of 0.240 ppm.

The herbicide concentration did not drop below the irrigation restriction limit of 0.100 ppm a.e. until approximately 24 days following the application. Appendix A contains the USACE residual monitoring report.

While emerging results appear clear that liquid 2,4-D mixes horizontally within the lake, little information exists regarding if 2,4-D vertically mixes into deep areas of the lake during stratification. After discussions between Onterra and John Skogerboe from the US Army Corps of Engineers (USACE), it was hypothesized that if the lake was thermally stratified, the 2,4-D would dissipate throughout the warmer, upper zone of the lake (epilimnion), but not into the cooler, deeper water zones of the lake (metalimnion and hypolimnion). This hypothesis was tested in 2010 on a lake in Manitowoc County and the results indicate that the herbicide residuals did not vertically mix throughout the entire water column. While this was not specifically tested on South Twin Lake, it is possible that the lake was thermally stratified at the time of the treatment and the 2,4-D only mixed within the upper zone of the lake contributing to the higher than expected 2,4-D concentrations.

Utilizing data from Figure 3 of Appendix A, it appears that 2,4-D concentrations between day 3 and day 7 range from 500 to 450 ppm a.e. Using that data and back-calculating a mixing zone that does not include the entire water column, the measured concentrations can be achieved if herbicides were dispersed within the upper 12 to 15 feet of the lake. Unfortunately, the closest temperature profile was taken approximately 28 days after the treatment (June 21, 2010). These data show that the lake was indeed stratified between 12-15 feet.

North Twin Lake

Following the 2010 treatment on North Twin Lake, approximately 87% of the 13.4 acres treated were reduced by at least one density rating, exceeding the qualitative success criteria (75% reduction). In 2009, there were approximately 6.7 acres of EWM that were classified as either 'dominant' or 'highly dominant', and in 2010 this was reduced to approximately 1.5 acres (Map 1 and 2). Large reductions in EWM were most apparent in sites A-10, B-10, and D-10 where EWM could not be observed from the surface (Map 2). Numerous random rake tows yielded a few occurrences of EWM within these areas, but the EWM appeared impacted by the treatments. Control on site E-10 was not as effective where dense EWM could easily be observed and the colony was found to extend approximately 20 feet outwards in all directions (Map 2).

During the summer of 2009, approximately 36% of the point-intercept locations contained EWM compared to 14% following the 2010 treatment (Figure 3), demonstrating a statistically valid 60% reduction in EWM occurrence within the 2010 treatment areas and exceeding the quantitative success criteria (50% reduction in occurrence). The occurrence of EWM in 2010 within treatment sites A-10 and D-10 was not shown to be statistically different from its occurrence in 2009 (Figure 3), and this is likely due to the small number of point-intercept sampling locations within these sites not fully representing what occurred within the treatment site... Due to its small size and other factors, no point-intercept sampling locations were placed within treatment site E-10. Figure 4 displays the proportions of EWM rake-fullness ratings from the pre- and post treatment surveys, and illustrates the reduction in EWM occurrence and density.



Figure 3. North Twin Lake EWM occurrence in point-intercept locations displayed by treatment site comparing summer 2009 to summer 2010. Created using data from 2009 pre-treatment survey and 2010 post treatment survey.



Figure 4. North Twin Lake proportions of EWM rake-fullness ratings from 42 pointintercept sampling sites located in 2010 treatment areas. Created using data from 2009 pre-treatment survey and 2010 post treatment survey.

One native species, large-leaf pondweed, was found to have statistically declined within application areas in North Twin Lake in 2010 following the treatment (Table 2). Large-leaf pondweed, unlike EWM, is a monocot and is not thought to be particularly susceptible to dicot-selective herbicides, so it is not clear if this species' decline was a direct result of the treatment. No native dicot (broad-leaf) species declined following the 2010 treatment, and two native monocot species, wild celery and fern pondweed, exhibited statistically valid increases (Table 2), indicating that native species may be colonizing areas previously occupied by EWM.

Table 2.	Statistical	comparison	of EWM ar	d native	aquatic	plant	occurrence	data	from
2009 pre-	and 2010 p	oost treatmen	t surveys.						

							Chi-square Analysis	
			2009	2010	Percent		Statistically	
	Scientific Name	Common Name	FOO	FOO	Change	Direction	Different	p-value
cots	Myriophyllum spicatum	Eurasian water milfoil	35.7	14.3	-60.0	V	Yes	0.023
	Ceratophyllum demersum	Coontail	47.6	54.8	15.0		No	0.513
	Megalodonta beckii	Water marigold	2.4	4.8	100.0		No	0.557
	Ranunculus aquatilis	White water-crowfoot	2.4	0.0	-100.0		No	0.314
	Myriophyllum sibiricum	Northern water milfoil	0.0	2.4	100.0		No	0.314
	Vallisneria americana	Wild celery	26.2	47.6	81.8	A	Yes	0.042
	Potamogeton amplifolius	Large-leaf pondweed	9.5	0.0	-100.0	▼	Yes	0.040
	Potamogeton robbinsii	Fern pondweed	4.8	19.0	300.0	A	Yes	0.043
	Elodea canadensis	Common waterweed	73.8	76.2	3.2		No	0.801
Ś	Potamogeton zosteriformis	Flat-stem pondweed	23.8	23.8	0.0	-	No	1.000
ö	Najas flexilis	Slender naiad	16.7	16.7	0.0	-	No	1.000
ġ	Potamogeton pusillus	Small pondweed	9.5	9.5	0.0	-	No	1.000
uo	Chara sp.	Muskgrasses	7.1	11.9	66.7		No	0.457
z	Potamogeton friesii	Fries' pondweed	4.8	0.0	-100.0		No	0.152
	Potamogeton gramineus	Variable pondweed	4.8	4.8	0.0	-	No	1.000
	Nitella sp.	Stoneworts	2.4	0.0	-100.0	V	No	0.314
	Potamogeton richardsonii	Clasping-leaf pondweed	2.4	4.8	100.0		No	0.557
	Potamogeton praelongus	White-stem pondweed	0.0	2.4	100.0		No	0.314

2009 & 2010 N = 42

FOO = Frequency of Occurrence

▲ or ∇ = Statistically Different (Chi-square; α = 0.05)

▲ or ∇ = Not Statistically Different (Chi-square; α = 0.05)

Since Renovate Max G was first used in Wisconsin in the spring of 2010, SePRO conducted residual monitoring at sites within and near the 2010 treatment sites on North Twin Lake up to 30 hours following the application. There study found that a pulse of Renovate Max G herbicide occurred between 8-12 hours following application. Treatment sites that were observed to have good EWM control had relatively higher mean herbicide concentrations, while site E-10, which appeared to be unaffected by the treatment, had relatively lower mean herbicide concentrations. The lower herbicide concentrations measured in site E-10 were likely due to the treatment site's open-water location, making it more prone to water movement from wind/wave action and flow into South Twin Lake; causing the herbicide to dissipate at a more rapid rate. The concentrations and exposure times observed by SePRO are within a range that suggests that control of EWM should be good, but there may be some potential for recovery.

2011 TREATMENT STRATEGY

Overall, the 2010 EWM treatment on North and South Twin Lakes was extremely successful, with qualitative and quantitative success criteria being exceeded on both lakes. The whole-lake treatment on South Twin Lake was successful in controlling 100% of the EWM, with none being detected during the 2010 post treatment survey. North Twin Lake saw a 60% reduction in EWM occurrence within the 2010 treatment areas. While statistically valid reductions were observed in numerous native aquatic plant species in South Twin Lake, it is believed that with time these

species will begin to re-colonize from the healthy populations present in North Twin Lake. A whole-lake point-intercept survey is scheduled to be conducted on South Twin Lake in the summer of 2011 in order to continue assessing the long-term impacts of whole-lake treatments on native species.

Because no EWM was detected following the 2010 treatment, there are no treatments proposed for South Twin Lake in 2011. However, if EWM is shown to rebound in certain areas during the spring 2011 survey, a treatment may be proposed.



Figure 5. North Twin Lake common acreage comparison between 2010 treatment and proposed 2011 treatment.

Approximately 18.3 acres of EWM were located in 2010 and are proposed to be treated in North Twin Lake in 2011 (Map 2). As figure 5 illustrates, 82% (14.9) of the proposed 2011 treatment acres are new areas of EWM discovered in 2010. The remaining 18% (3.4) is comprised of areas that were treated in 2010 and require retreatment in 2011, and new areas that are adjacent to 2010 treatment areas and are a result of colonial expansion (Figure 5). The re-treatment of previously treated areas is not uncommon in EWM management as dense areas often require multiple years of treatment to significantly reduce a site's density and/or size.

The use of Renovate Max G was shown to be successful on North Twin Lake in 2010. However, great concerns were raised in 2010 by NSTRLA members due to an unforeseen 120-day irrigation restriction in association with this herbicide's use. The Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) recently ruled that the 120 day restriction for Renovate Max G is for agricultural purposes. Domestic or ornamental irrigation restrictions are set on a case by case basis by the manufacturer of the product (SePRO).

The 2010 treatment on North Twin Lake was the most effective to date. Due to the successes observed in 2010, Renovate Max G is proposed for treatment again in 2011. Map 2 shows this strategy using dosage recommendations from SePRO. Sites E-11, G-11, and I-11 were proposed to be treated at approximately 3.0 ppm a.e. because they are small sites with high water exchange. These factors reduce the exposure time and therefore require a higher concentration to be effective. A lower dose is recommended for Site H-11 where exposure times will likely be longer within this area due to the community of dense bulrushes. During the September 2010 survey, navigation within this area was not possible due to the dense bulrushes and therefore no quantitative treatment monitoring was conducted on this site. Based on the results of the spring 2011 pretreatment survey, it may be determined that H-11 is logistically unable to be treated.

Based on the areas proposed for treatment on Map 2, SePRO has indicated that there would be a 7 day domestic irrigation restriction put into effect after the treatment.



