INTRODUCTION

In February 2008, the North and South Twin Lakes Riparian Association (NSTLRA) successfully applied for a Wisconsin Department of Natural Resources (WDNR) Aquatic Invasive Species (AIS) Control Grant to conduct a four-year project aimed at reducing the Eurasian water milfoil (EWM, *Myriophyllum spicatum*) population within the lakes. While 2011 was originally slated to be the fourth and final year of treatments and monitoring under this grant-funded program, the successful control of EWM on South Twin Lake in 2010 resulted in a budget surplus within the grant. This surplus was used to fund additional EWM treatment and monitoring in 2012. This report discusses the fifth year of treatment on North and South Twin Lakes. Information regarding treatments completed from 2008-2011 can be found in their respective reports.

Following the 2011 EWM peak-biomass survey, a conditional treatment permit map was created proposing approximately 4.8 acres of treatment in South Twin Lake and 6.8 acres in North Twin Lake (Map 1). The treatment sites within South Twin Lake were proposed to be applied with liquid 2,4-D at a rate of 4.0 ppm ae (acid equivalent). All of the treatment areas in North Twin Lake except for site G-12 were proposed to be treated with Renovate Max G at 4.0 ppm ae, a granular combination of 2,4-D and triclopyr. Treatment site G-12 was proposed to be treated with Renovate LZR Max at 3.0 ppm, also a granular combination of 2,4-D and triclopyr, but this product is supposed to have a slower herbicide release rate which is theorized to increase exposure time. The herbicide doses were increased from 2011 because ongoing research indicates that rapid dilution of the herbicide occurs within these small treatment sites, which likely played an important role in why the 2011 treatments on North Twin Lake did not meet expectations. These treatment scenarios rely on a short exposure time (often hours) to cause mortality and therefore require a high herbicide concentration to compensate for the short exposure time. If sufficient herbicide concentration and exposure times are not met, the EWM that is targeted may not be killed by the treatment, simply injured and will rebound at a later date.

On May 2, 2012 Onterra staff visited North and South Twin Lakes to survey the proposed treatment areas and refine their boundaries as appropriate. Unfortunately, as a result of the spring pre-treatment survey, the treatment acreage was increased to 14.0 acres in South Twin Lake and 9.8 acres in North Twin Lake; encompassing areas of EWM that were not located in the summer of 2011 (Map 1). During this survey, a temperature, dissolved oxygen, and pH profile was collected in approximately 33 feet of water in South Twin Lake. Figure 1 illustrates that the lake was not strongly stratified at the time of the survey, with water temperatures were 11.7°C (53°F) near the surface and 9.3°C (49°F) near the bottom. Dissolved oxygen was near 11 mg/L throughout most of the water column, and pH was 8.1 at 15 feet.

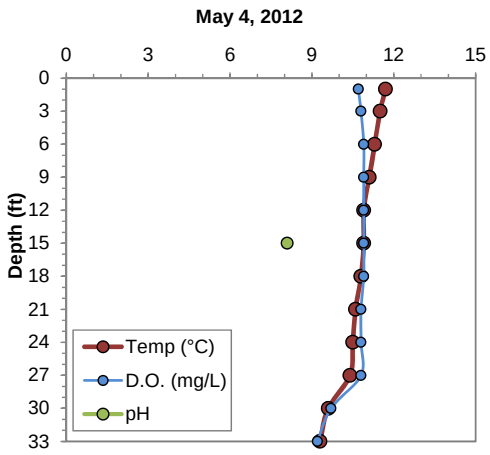


Figure 1. Temperature, dissolved oxygen, and pH profile collected from South Twin Lake. May 4, 2012.

The 2012 final treatment areas on North Twin Lake were treated on May 23, 2012 by Stantec, Inc. They reported a water temperature of approximately 60°F, an ambient air temperature of 68°F, and south winds at 5-10 mph increasing to 10-20 mph at the time of application. The final treatment areas in South Twin Lake were treated by Stantec, Inc.

on May 31, 2012. They reported a water temperature of 62°F, an ambient air temperature of 62°F, and variable and light winds at the time of application.

2012 TREATMENT MONITORING

The goal of any herbicide treatment strategy 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 visual data such as EWM colony density ratings before and after the treatments.

EWM treatment quantitative evaluation methodologies follow WDNR protocols in which point-intercept data are collected within treatment areas before and after the treatment. On North Twin Lake, data of this type was collected at 28 point-intercept sub-sample locations that were sampled during the summers of 2011 and/or 2012 (Figure 2). Quantitative monitoring was not made within the treatment areas on South Twin Lake because the proposed treatment areas were originally too small and no data were collected in the summer of 2011. At these 28 sampling locations, EWM and native aquatic plant species presence and rake-fullness were documented. Specifically, these surveys aim to determine if statistically valid differences in frequencies of occurrence of EWM and native species occur following the herbicide application.

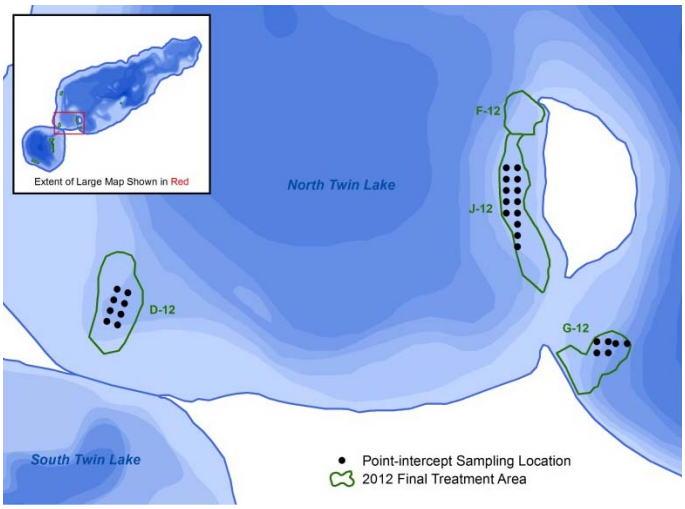


Figure 2. 2012 Quantitative monitoring plan for North Twin Lake.

Quantitatively, a specific treatment site is deemed to be successful if the EWM frequency following the treatments exhibit a statistically valid reduction 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 preferably, 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 2011 and 2012, when this plant is assumed to be at its peak biomass or growth stage. Comparisons of the survey results are used to qualitatively evaluate the 2012 herbicide treatment 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 (lake-wide), 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, 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. While 2,4-D is thought to be selective towards broad-leaf (dicot) species at the concentration and exposure times observed during the 2012 treatment 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. However, the majority of declines in non-dicot native species occurred when whole-lake treatment strategies were used. This strategy is much different from the spot treatment strategies employed in 2012 on North and South Twin Lakes,

2012 TREATMENT RESULTS

Post-treatment surveys were completed by Onterra on September 12 and 13, 2012. Prior to treatment, six (21.4%) of the 28 sampling locations within the treatment areas on North Twin Lake contained EWM. Following the 2012 treatment, 11 (39.3%) of the 28 sampling locations were found to contain EWM, representing an increase in occurrence of 83%. This failed to meet the quantitative success criterion of a 50% reduction in occurrence. Data concerning native aquatic plants were also collected at these same 28 sampling locations. As Figure 3 illustrates, some native species saw reductions in their occurrence; however, these reductions were not statistically valid (Chi-square $\alpha = 0.05$) and cannot be attributed to the 2012 herbicide treatments. Figure 4 displays the rake-fullness ratings for EWM within the treatment areas on North Twin Lake and shows that while its occurrence increased from 2011 to 2012, its density did not increase.

Of the 5.3 acres of colonized EWM treated in 2012 on North and South Twin Lakes, only 49% saw a reduction in density by at least one rating, falling short of the qualitative success criteria (75% reduction). These declines were observed within treatment sites E-12, F-12, and G-12 (Map 1 and Map 2). A dominant colony of EWM was located in 2012 just outside of treatment area G-12 (Map 2). The two treatment sites within South Twin Lake appeared to be ineffective as numerous single plants, clumps of plants, and small plant colonies were observed in these areas. In addition, numerous occurrences of EWM were located outside of the treatment areas in South Twin Lake, indicating that the population is beginning to rebound following the 2010 whole-lake treatment (Map 2).

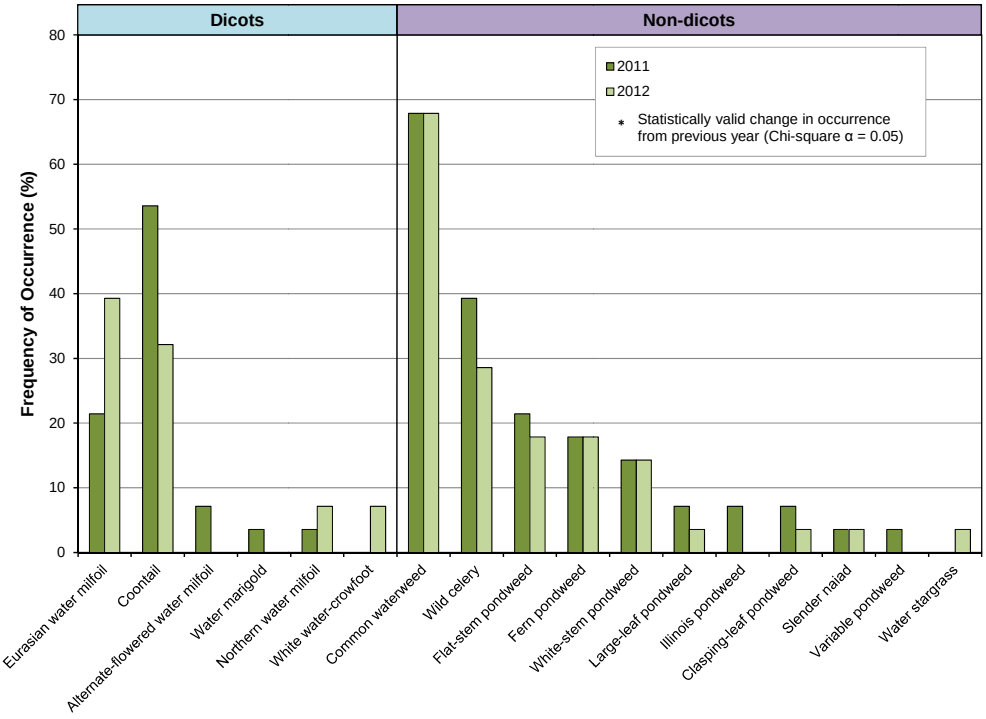


Figure 3. 2011 pre- and 2012 post-treatment occurrence of aquatic plant species within treatment areas on North Twin lake. Created using data collected from 28 sampling locations during the 2011 pre- and 2012 post-treatment surveys.

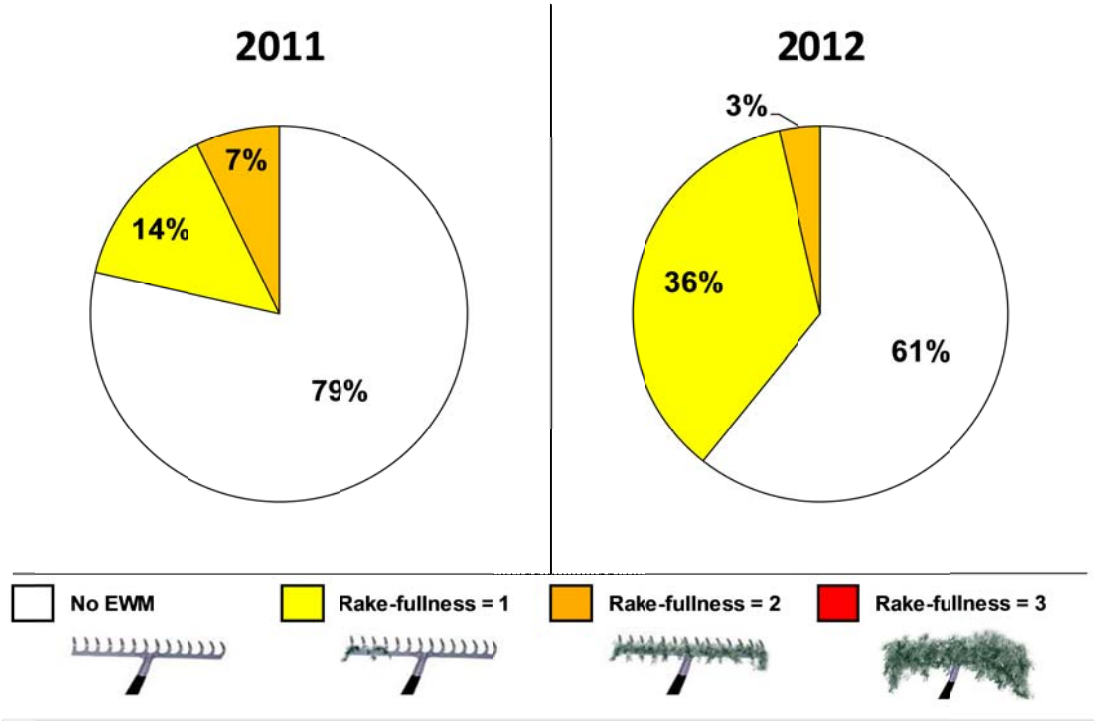


Figure 4. Proportion of EWM rake-fullness ratings from 28 sample locations within 2012 treatment areas in 2011 pre- and 2012 post-treatment on North Twin lake.

2013 CONTROL STRATEGY

As described previously, the 2012 treatments on North and South Twin Lakes were met with little success, with neither the quantitative nor qualitative success criteria being met. Following a series of whole-lake treatments in 2009 and 2010, the EWM has only begun to rebound within South Twin Lake; therefore, herbicide treatments are not proposed to occur in 2013. The NSTLRA would like to initiate coordinated hand-removal of EWM within South Twin Lake starting in 2013.

Spot treatment taking place on North Twin Lake have effectively reduced the density of EWM within the areas being targeted, but EWM continues to rebound within these areas after one or two summers following the treatment. For these reasons, the WDNR does not support a multi-year, grant-funded AIS control project on North and South Twin Lakes at this time; however, they do support a one-year trial control project to determine if a few experimental control measures may be applicable for the system. The NSTLRA applied for a one-year AIS Established Population Control Grant during the February 2013 grant cycle to fund the proposed treatment monitoring activities in 2013.

Map 2 displays the areas of colonized EWM in North Twin Lake that will be targeted for herbicide treatment. All of the treatment areas on this map include a 40-foot buffer area surrounding the outer extent of EWM growth which is included to assist in keeping sufficient herbicide concentrations within the treatment areas for a longer period of time. Two sites (F-13 and G-13) would be targeted for control using a combination of granular 2,4-D and triclopyr product at its maximum application rate (5.0 ppm ae).

An additional site (E-13) would be targeted for control using diquat at a standard rate (2 gallons per acre – 0.17ppm cation). This site has historically been difficult to treat successfully, likely due to its location which is in deep (nine feet) water, and has high water exchange. Diquat is a contact herbicide traditionally used to control nuisance levels of aquatic plants. This herbicide only requires a short exposure time and for that reason has been used in a few spot treatment scenarios targeting EWM. In these scenarios, the herbicide has been effective at removing the above ground biomass, but it is not clear whether or not it will result in multi-year control. This location endures much recreational boating traffic as watercraft move from North to South Twin Lake and removing the EWM biomass in this area may limit the spread of fragments to other parts of the lake.

Further, the NSTLRA and Onterra would identify specific areas for hand removal. Small isolated infestations of EWM can most appropriately be controlled using manual removal methods, likely through SCUBA or snorkeling efforts with scuba methodologies likely being more suitable for North and South Twin Lakes. In order for this technique to be successful, the entire plant (including the root) needs to be removed from the lake. During manual extraction, careful attention would need to be paid to all plant fragments that may detach during the control effort. The NSTLRA has identified a private firm that would be hired to carry out a large portion of the hand-removal strategy in 2013. During the subsequent EWM peak-biomass mapping survey, professional ecologists would visit all marked locations and assess if the plant was successfully removed similar to how an herbicide treatment is monitored

South Twin - 2012 Final EWM Treatment Areas

Liquid 2,4-D @ 4.0 ppm ae

Site	Proposed Acres	Final Acres	Ave. Depth (feet)	Volume (ac-f)
A-12	3.9	4.5	8.0	36.0
C_1-12	0.9	1.7	6.0	10.2
C_2-12		7.8	9.0	70.2
South Twin Total	4.8	**14.0**		116.4

North Twin - 2012 Final EWM Treatment Areas

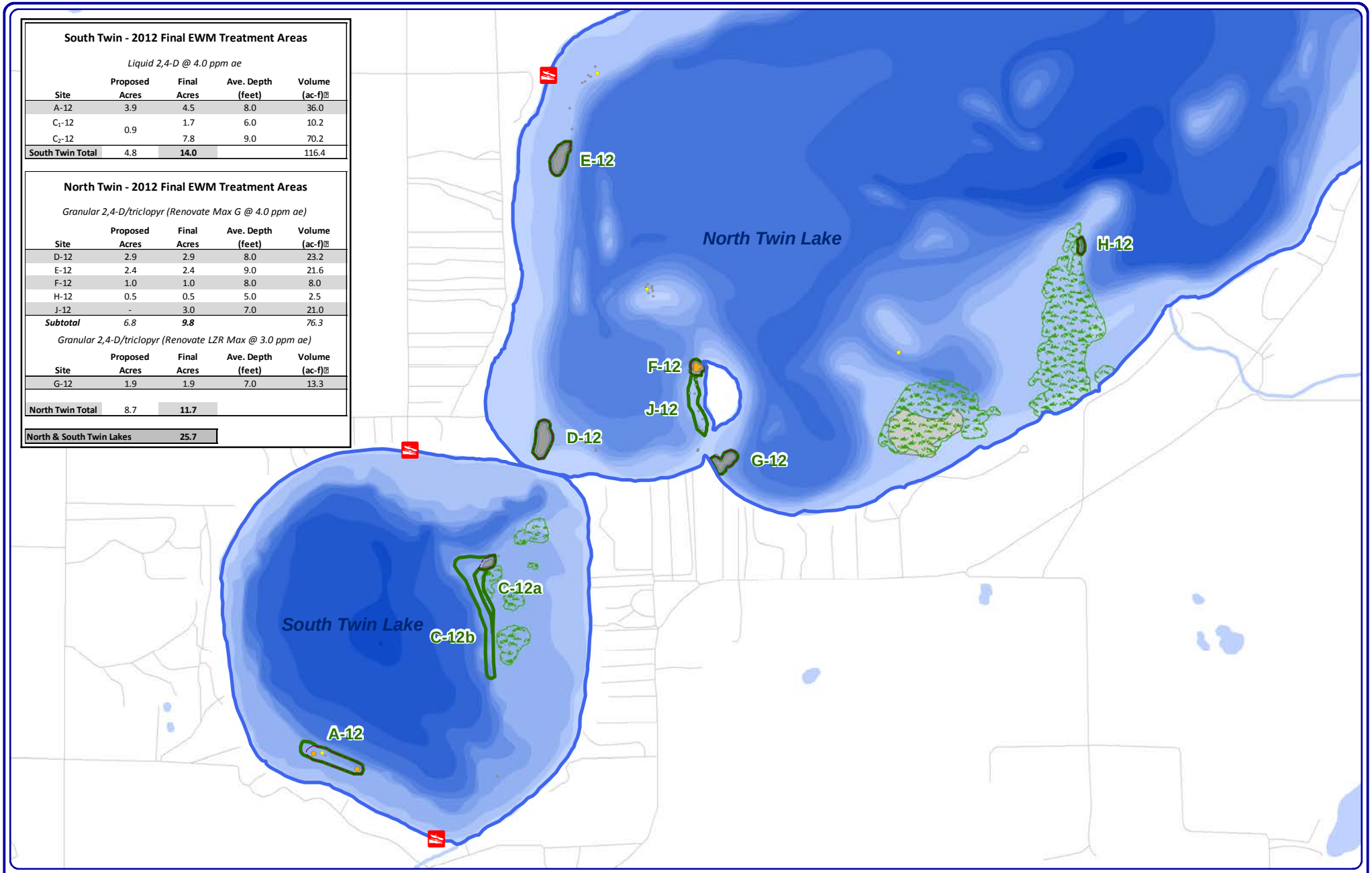
Granular 2,4-D/triclopyr (Renovate Max G @ 4.0 ppm ae)

Site	Proposed Acres	Final Acres	Ave. Depth (feet)	Volume (ac-f)
D-12	2.9	2.9	8.0	23.2
E-12	2.4	2.4	9.0	21.6
F-12	1.0	1.0	8.0	8.0
H-12	0.5	0.5	5.0	2.5
J-12	-	3.0	7.0	21.0
Subtotal	*6.8*	***9.8***		*76.3*

Granular 2,4-D/triclopyr (Renovate LZR Max @ 3.0 ppm ae)

Site	Proposed Acres	Final Acres	Ave. Depth (feet)	Volume (ac-f)
G-12	1.9	1.9	7.0	13.3
North Twin Total	8.7	**11.7**		

North & South Twin Lakes	**25.7**



N W E S

2,000 Feet

Onterra LLC
Lake Management Planning
815 Prosper Road
De Pere, WI 54115
920.338.8860
www.onterra-eco.com

Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR, 1971; digitized by Onterra
Aquatic Plants: Onterra, 2011
Map Date: March 11, 2013
Filename:Map1_NSTwin_EWM_2011PB_T2012.mxd

Project Location in Wisconsin

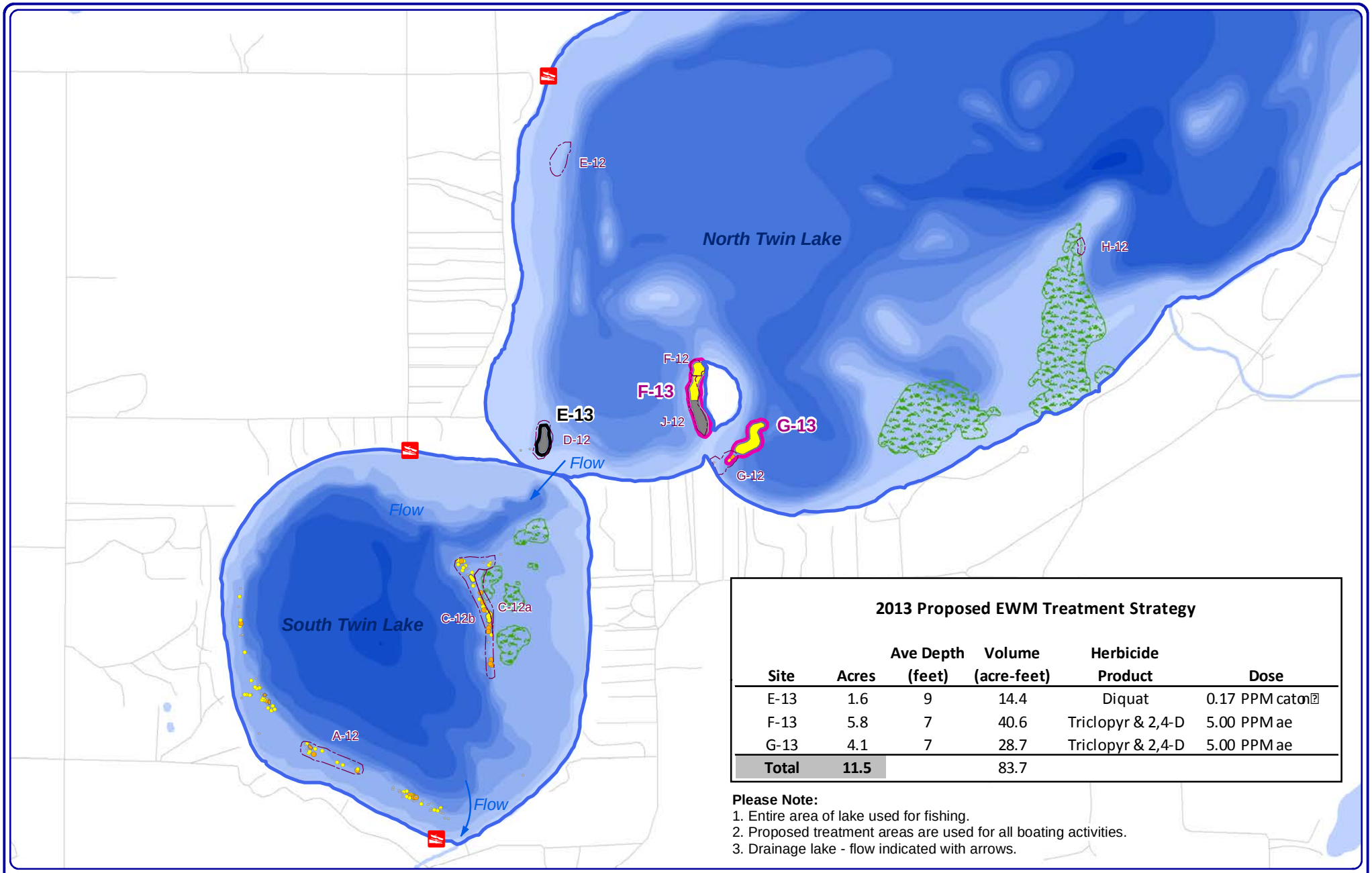
Legend

Eurasian water milfoil Locations (August 2011)

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2012 Conditional Treatment Area
- 2012 Final Treatment Area
- Hardstem Bulrush-dominated Community

Map 1
North & South Twin Lakes
Vilas County, Wisconsin

2011 EWM Locations & 2012 Treatment Areas



2013 Proposed EWM Treatment Strategy

Site	Acres	Ave Depth (feet)	Volume (acre-feet)	Herbicide Product	Dose
E-13	1.6	9	14.4	Diquat	0.17 PPM cation
F-13	5.8	7	40.6	Triclopyr & 2,4-D	5.00 PPM ae
G-13	4.1	7	28.7	Triclopyr & 2,4-D	5.00 PPM ae
Total	**11.5**		83.7		

Please Note:
1. Entire area of lake used for fishing.
2. Proposed treatment areas are used for all boating activities.
3. Drainage lake - flow indicated with arrows.

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Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR, 1971; digitized by Onterra
Aquatic Plants: Onterra, 2012
Map Date: March 11, 2013
Filename:Map2_NSTwin_EWM_T2013_Cond1.mxd


Project Location in Wisconsin

Legend

Eurasian water milfoil Locations (September 2012)
- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- Hardstem Bulrush Community
- 2012 Final Treatment Area

2013 Proposed Treatment Areas
- Diquat
- Granular 2,4-D/Triclopyr

Map 2
North & South Twin Lakes
Vilas County, Wisconsin
2012 EWM Locations & 2013 Proposed Treatment Areas v.1