AIRR-097-11
Final Report



## INTRODUCTION

**Photo 1. Forest Lake, Fond du Lac County.**

In 2010, the Forest Lake Improvement Association, Inc. (FLIA) contracted with Onterra to initiate a three-year aquatic invasive species (AIS) control and monitoring project for Forest Lake, Fond du Lac County (Photo 1, Map 1). The non-native, invasive plant Eurasian water milfoil (EWM, *Myriophyllum spicatum*) was first documented in Forest Lake in 1992. Later in 2010, it was confirmed via DNA analyses that the EWM in Forest Lake was actually a hybrid between EWM and the indigenous northern water milfoil (*Myriophyllum sibiricum*). As will be discussed further in this report, hybrid EWM (HWM) presents some complications for management as research is indicating that some hybrid strains may have a higher tolerance to aquatic herbicides than pure strains of EWM. Members of the FLIA indicated that the HWM had spread and occupied the majority of Forest Lake's littoral areas, matting on the surface and interfering with recreational activities and reducing the lake's aesthetic appeal.

The goal of this three-year project (2011-2013) is to reduce the HWM population within Forest Lake to more manageable levels while at the same time minimizing impacts to valuable native aquatic plant species. Reducing the HWM population will alleviate recreational interference and improve the lake's ecological integrity. At the end of the three year project, the FLIA is hopeful that the AIS populations in the lake have been reduced to levels that on an annual basis require minimal or no use of herbicides. This report discusses the final year of HWM treatment monitoring under this project. Specific details pertaining to the 2011 and 2012 treatments on Forest Lake can be found in their respective annual treatment reports.

## 2013 HWM CONTROL STRATEGY DEVELOPMENT

Within the 2012 treatment report, Onterra presented three HWM management options to the FLIA for consideration, along with elaboration of the pros and cons of each strategy:

- *Conduct another whole-lake2,4-D treatment in 2013*
- *No Treatment in 2013, continue monitoring HWM Population*
- *Conduct a 2,4-D spot treatment targeting the colonized areas of EWM in 2013*

After deliberation, the FLIA chose to move forward with a limited 2,4-D spot treatment in 2013. Following this strategy, approximately 3.3 acres were targeted for treatment on Forest Lake during the early-spring of 2013 (Map 1). The FLIA had expressed concerns regarding the existence of HWM in additional areas of the lake that are not within the proposed treatment strategy, especially in shallow near-shore areas that may interfere with recreational use of the lake. While these areas wouldn't be a part of the herbicide control program, they were proposed to be included within the 2013 control program as areas targeted by strategic hand-removal by volunteers.

Only the colonized areas of HWM were considered for treatment in 2013. Because all of the proposed treatment sites are quite small (0.5-2.1 acres), they were are proposed to be treated with granular 2,4-D at the maximum application rate (4.0 ppm ae). Ongoing research clearly indicates

that conducting spot treatments on small areas can be challenging due to extremely high herbicide dissipation rates. The only way to influence herbicide dissipation rates on these small treatment sites is to ensure that the herbicide treatment occurs when water movement caused by wind is minimal.

Unlike whole-lake treatments where the intent is to dilute the herbicide to a specified lake-wide (epilimnion-wide) concentration, spot-treatments are intended to only affect the target plant within the specified application areas. Although herbicide degradation may occur before the herbicide reaches equilibrium with the entire volume of Forest Lake or its epilimnion, these concentrations were calculated to understand if there would be a potential for unintended lake-wide herbicide impacts from the proposed 2013 treatment. The calculations indicate that the lake-wide 2,4-D concentrations would be approximately 0.171 ppm ae if the herbicide from the 3.3 acres of treatment dissipated evenly throughout the entire volume of the lake prior to degradation. It is believed that these concentrations are below levels that would significantly impact aquatic plants on a lake-wide basis. Ongoing research suggests that when using granular products, the calculated whole-lake concentrations are overstated compared to what would actually be measured due to factors such as the availability of 2,4-D granules that sunk into or bound with the sediment.. However, these calculations help to understand the potential herbicide concentrations in non-targeted areas.

## PRETREATMENT CONFIRMATION AND REFINEMENT SURVEY

On May 14, 2013, Onterra ecologists conducted the HWM Pretreatment Confirmation and Refinement Survey on Forest Lake. Based upon a temperature profile collected during the survey, the lake was determined to be weekly stratified with water temperatures in the upper 50s°F a few feet down and not dropping below 50°F until greater than 20 feet deep. Because this was a spot treatment, it was not necessary to wait for stratification to occur as was necessary in 2011 and 2012 when whole-lake treatment strategies were implemented.

Based upon on the pretreatment survey, only a slight expansion of C-13 was recommended, otherwise all other areas were recommended to be targeted as originally proposed (Map 1). As discussed within the 2012 treatment report, low density occurrences of HWM were located in areas outside of the areas targeted for control. However, these occurrences did not meet the density threshold that warrant treatment at this time (i.e. colonized areas mapped with area-based methods [polygons]). Within an upcoming management planning effort, the FLIA will be able to use the knowledge gained over the past few years to develop new thresholds (triggers) that would guide future management actions.

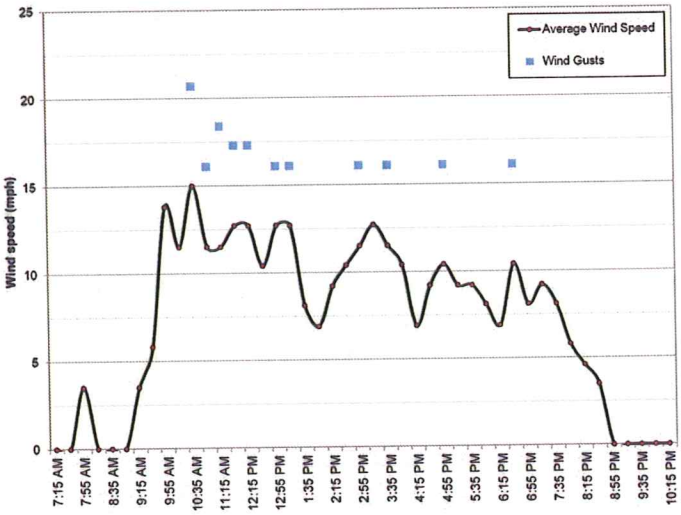



**Figure 1. West Bend wind speeds on May 29, 2013.** Created using data from the National Weather Service.

The 2013 final treatment areas in Forest Lake were treated by Aquatic Biologists, Inc. on May 29, 2013. Treatment sites were targeted with granular 2,4-D (Navigate®) at a rate of 4.00 ppm ae (Map 1). The treatment was conducted between 7:00 am and 10:00 am when wind speeds were approximately calm (Figure 1). However, wind speeds did increase as the day progressed.

## MONITORING METHODOLOGIES

The objective of an herbicide treatment strategy is to maximize target species (HWM) 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 AIS colony density ratings before and after the treatments.

### Quantitative Aquatic Plant Monitoring

The goal of an herbicide treatment strategy is to maximize target species (HWM) 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 HWM colony density ratings before and after the treatments.

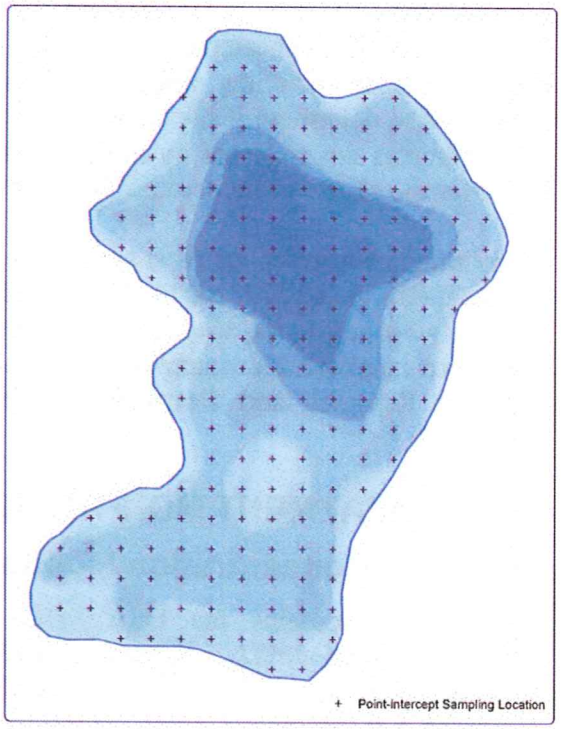



**Figure 2. Whole-lake point-intercept survey sampling locations on Forest Lake.**

Because a whole-lake treatment was conducted on Forest Lake in 2011 and 2012, the whole-lake point-intercept method as described by the WDNR Bureau of Science Services (PUB-SS-1068 2010) was used to complete a quantitative evaluation of the occurrences of HWM and native aquatic plant species (Figure 2). Point-intercept surveys have been conducted annually on Forest Lake since 2011 by Onterra, while a point-intercept survey was conducted by the WDNR in 2008 prior to the discovery of HWM. Comparing data collected before and after the treatment allows for a statistical comparison of aquatic plant occurrences and a quantitative determination of treatment efficacy on a lake-wide scale.

## Qualitative HWM Monitoring

Using sub-meter GPS technology, HWM locations were mapped the year prior to treatment (2012) in late-summer when HWM is at or near its peak growth, and in the late summer immediately following the treatment (2013). The HWM population was mapped by using either 1) point-based or 2) area-based methodologies. Large colonies >40 feet in diameter are mapped using polygons (areas) and were qualitatively attributed a density rating based upon a five-tiered scale from *Highly Scattered* to *Surface Matting*. Point-based techniques were applied to HWM locations that were considered as *Small Plant Colonies* (<40 feet in diameter), *Clumps of Plants*, or *Single or Few Plants*.

Qualitative monitoring of herbicide treatments includes comparing spatial data reflecting HWM locations and densities during the peak-growth stages the summer before the treatment the summer immediately following the treatment. Based upon a pre-determined success criterion, an effective treatment would include a 75% reduction of HWM as demonstrated by a decrease in density rating (e.g. *Highly Dominant* to *Dominant*).

## Herbicide Concentration Monitoring

In-lake herbicide concentrations are also monitored as a part of some treatment strategies, especially those including whole-lake treatments. In Forest Lake, 2,4-D concentrations were monitored to determine if the target concentrations had been met. With this type of monitoring, water samples are collected by trained volunteers from multiple locations and depths within the lake over the course of numerous days following treatment.

Water samples were collected at six sites at time intervals approximately 1, 3, 6, and 24 hours after treatment (HAT) and 5, 7, 10, 14, 21, 28, and 35 days after treatment (DAT). Site FO1, located over the lake's deepest point, water was sampled from the surface (3 feet), 15, 20, and 25 feet using a Van Dorn sampler. The samples were fixed (preserved) with acid and shipped to the U.S. Army Engineer Research and Development Center (USAERDC) where the herbicide analysis is completed.

# POST TREATMENT MONITORING RESULTS

## Herbicide Concentration Monitoring Results

Appendix A contains the USAERDC draft *Forest Lake, Manitowoc County, Herbicide Concentration Monitoring Summary, 2013* (October 29, 2013) with more detail regarding the herbicide concentration monitoring sampling study on Forest Lake. The information within Appendix A is referenced within the following section.

Herbicide application rates were formulated volumetrically, targeting 2,4-D at 4.0 ppm ae. This means that sufficient 2,4-D was applied within the *Application Area* such that if it mixed evenly with the *Treatment Volume*, it would equal 4.0 ppm ae. This standard method for determining spot treatment use rates is not without flaw, as no physical barrier keeps the herbicide within the *Treatment Volume* and herbicide dissipates horizontally out of the area before reaching equilibrium (Figure 3). Particularly with granular herbicides it is theorized that some of the 2,4-D granules sink into or bind with the sediment, not allowing a portion of the product to be included within herbicide measurements within the water column. Granular herbicides are also thought to release the herbicide more slowly in certain situations (e.g. lower pH); however more research is needed to quantify these statements.

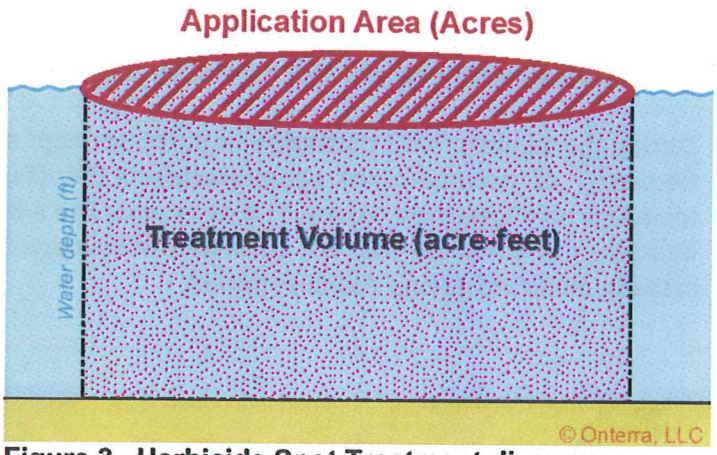


**Figure 3. Herbicide Spot Treatment diagram.**

Initial herbicide concentrations within two of the three treatment sites was slightly greater than sampling locations in other parts of the lake, however never exceeded 0.156 ppm ae even though the herbicide was applied at 4.0 ppm ae. Some of the factors discussed above likely contributed to this low concentration measured within the treatment areas. As shown on Figure 4 of Appendix A, herbicide concentrations within the treatment sites was only marginally higher that within non-treated parts of the lake for the duration of this sampling period. Only one herbicide sample exceeded the irrigation standard (0.1 ppm ae). The low concentrations observed through 336 HAT (14 DAT) were only marginally above the detection limit and therefore the USAERDC laboratory ceased analyzing additional samples.

While calculations indicate that the lake-wide 2,4-D concentrations would be approximately 0.171 ppm ae if the herbicide dissipated evenly throughout the entire volume of the lake, average 0-7 DAT concentrations were 0.038 ppm ae.

## Aquatic plant Monitoring Results

On August 27, 2013, Onterra ecologists visited Forest Lake to complete the post-treatment assessments of the 2013 treatment. This included conducting the whole-lake point-intercept survey, as well as mapping the HWM occurrences within the lake.

### *Efficacy*

The summer 2012 HWM Peak-Biomass Survey indicated approximately 1.4 acres of the lake contained colonized areas of HWM, with additional HWM occurrences being mapped throughout the lake using point-based methodologies (Map 2). The post treatment mapping survey conducted during the late-summer of 2013 indicated that the HWM population showed lake-wide expansion during this time period, albeit still at relatively low occurrences compared with before the 3 year control program was initiated. Within the 2013 treatment sites where the herbicide was directly

applied, HWM colony size and density reduction was moderately observed. However, it is suspected that these lower populations and densities may be a result of the HWM plants within these areas being injured and will recover by 2014.

During the summer 2012 pretreatment point-intercept survey, HWM in Forest Lake had a littoral frequency of occurrence of approximately 4.2% (Figure 4). During the August 2013 post treatment point-intercept survey, HWM increased to a littoral frequency of 10.8% (Figure 4). This indicates that quantitatively, the 2013 control strategy did not meet the success criteria and HWM actually increased during this time period.

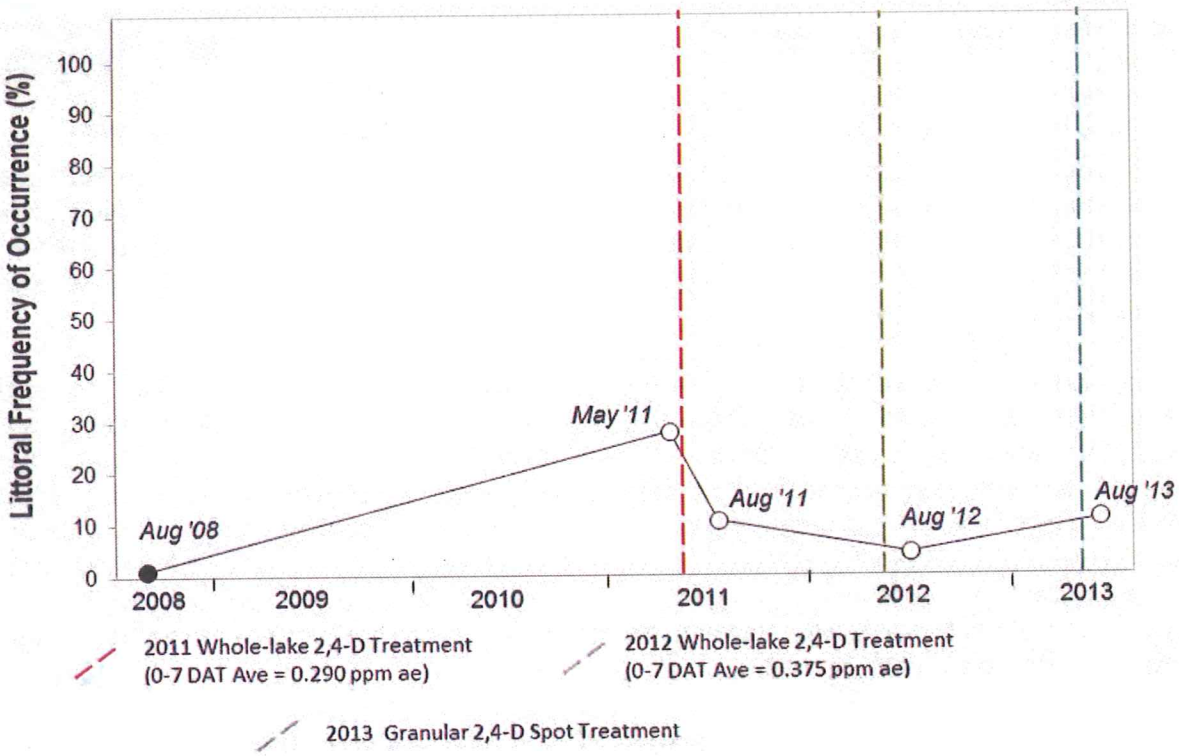



**Figure 4. HWM littoral frequency of occurrence in Forest Lake from 2008 and 2010-2013.** Created using data from WDNR 2006 and Onterra 2010-2013 point-intercept surveys. Open circle represents statistically valid change from previous survey.

## *Selectivity*

The littoral frequencies of native aquatic plant species available from the four (2008, 2011, 2012, 2013) summer point-intercept survey are shown in Figure 5. As illustrated, the native plant community was largely unaffected by the 2013 treatment. Three native species displayed statistically valid increases (stiff pondweed, slender naiad, and flat-stemmed pondweed). It is believed that differences in field identification between Illinois pondweed, variable-leaf pondweed, and a hybrid pondweed (variable pondweed x Illinois pondweed) explain the slight increases and decreases of these species.

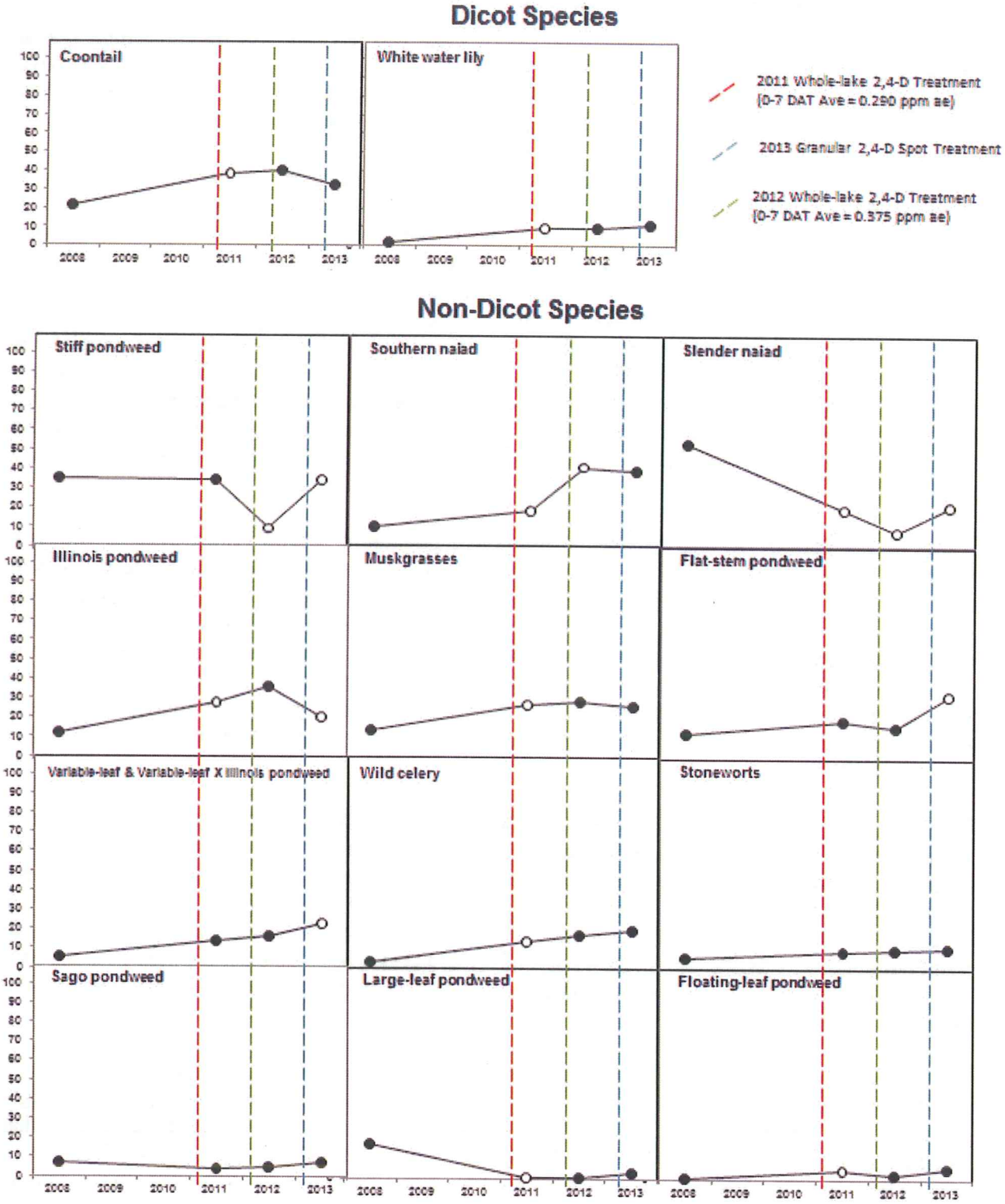



**Figure 5. Pre- and post-treatment littoral occurrence of native aquatic plant species in Forest Lake.** Created using data from WDNR 2008 and Onterra 2011-2013 point-intercept surveys. Open circle represents statistically valid change from previous survey.

## CONCLUSIONS AND DISCUSSION

The 2013 granular 2,4-D spot treatment on Forest Lake was not effective on controlling HWM either within the areas targeted for treatment, nor lake-wide. As discussed within the 2012 treatment report, this is likely due to the fact that these small treatment sites did not allow sufficient herbicide concentration and exposure times for HWM mortality to occur.

The concept of heterosis, or hybrid vigor, is the concept that hybrid individuals typically have improved function compared to their pure-strain parents. HWM typically has thicker stems, is a prolific flowerer, and grows much faster than pure-strain EWM (LaRue et al. 2012). In response to field observations of unexplained reduced success in controlling populations of HWM, researchers conducted scientific case studies to better understand the efficacy of early-season whole lake low-dose treatments of 2,4-D on pure-stain EWM and HWM populations. As has been the case for Forest Lake, preliminary results show that several study lakes with HWM populations demonstrated reduced susceptibility to 2,4-D treatments in comparison with other pure-strain EWM treatments. This is corroborated by recent laboratory and growth chamber research which indicates that some HWM populations are less susceptible to certain auxin herbicides (2,4-D and triclopyr) than pure-strains of EWM (Glomski and Netherland 2010, Poovey et al. 2007, SePRO unpublished data).

Albeit higher than in the last two years, the HWM population within Forest Lake is still at a relatively low level. The HWM is widely dispersed and is currently not forming dense colonies in any locations; likely not having an acute impact on the ecosystem or causing recreational interference to lake users. Due to Forest Lake's small water volume, almost any spot-treatment that would be conducted would have whole-lake implications. Therefore, Onterra recommends that a whole-lake treatment would be the most appropriate way to target the HWM within Forest Lake if future active management is to occur. However, there likely does not contain a sufficient HWM population within Forest Lake at this time to warrant such and all-encompassing treatment strategy in 2014.

While the whole-lake 2,4-D treatments conducted in 2011 and 2012 effectively reduced the HWM population within Forest Lake, Onterra is not confident that a subsequent whole-lake 2,4-D treatment conducted at the same dose utilized in 2012 (approximately 0.4 ppm ae) will produce the desired HWM control results. While a higher dose of 2,4-D would almost certainly equate to increased HWM control, it may also lead to additional collateral impacts to the native aquatic plant community. But the native plant community of Forest Lake has been resilient over the past two whole-lake 2,4-D treatments and targeting a slightly higher whole-lake 2,4-D dose in the future may be appropriate.

HWM control projects conducted on English Lake (Manitowoc County) and Frog Lake (Florence County) are similar to the activities taken place on Forest Lake. Successively increased target concentrations of whole-lake 2,4-D treatments were conducted with control being less that anticipated for pure-strain EWM control projects. Both of these lake groups opted to forego conducting an even higher concentration whole-lake 2,4-D treatment in 2013, choosing to initiate alternative treatment strategies that are less commonly used in Wisconsin.

English Lake conducted a combination whole-lake 2,4-D and endothall treatment in 2013 (0.25 ppm ae/0.75 ppm ai) resulting in 100% HWM control and some measureable, but small, native

plant impacts. The use of this strategy is significantly more costly than using 2,4-D alone. Also, this lake did not contain the high value native plant community that is present if Forest Lake and the selectivity observed on English Lake may not be directly transferable to Forest Lake. Frog Lake conducted a whole-lake fluridone treatment in 2013 (7.5 ppb) which also was shown to be almost 100% effective at controlling the HWM population within the lake. While much more cost-effective than using a combination of 2,4-D and endothall, increased native plant impacts were observed in association with this treatment. Additional monitoring on both lakes in 2014 and beyond will allow an understanding of the long-term efficacy and selectivity of these herbicide treatment strategies.

In summary, Onterra proposes that no herbicide treatment is conducted on Forest Lake in 2014. The spot treatment strategy conducted in 2013 was largely ineffective and Onterra does not feel that the level of HWM within Forest Lake currently warrants a whole-lake treatment strategy, particularly with the uncertainty of what whole-lake treatment strategy would be best suited for Forest Lake and the FLIA. The FLIA (and other managing entities) will need to realize that in order to effectively control the HWM population in Forest Lake, aggressive herbicide strategies needed to be implemented, which could have increased collateral effects on the native aquatic plant community compared to more-typical use rates employed for pure-strain EWM control projects. Discussions within the FLIA during future management planning efforts will determine whether the increased financial and potentially ecological costs of conducting future treatment strategies are warranted.

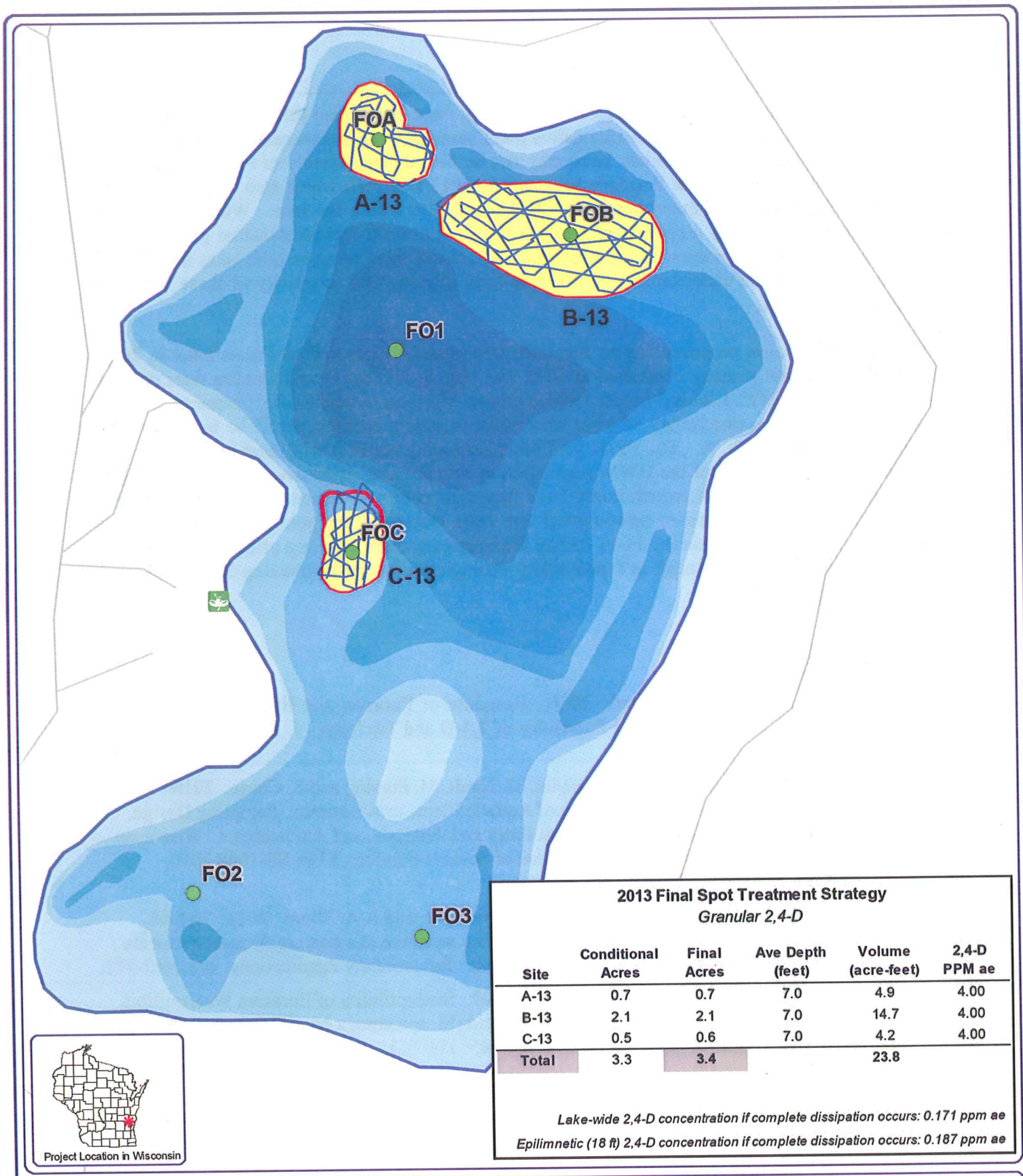
## LITERATURE CITED

Glomski, L.M. and M.D. Netherland. 2010. Response of Eurasian and Hybrid Watermilfoil to Low Use Rates and Extended Exposures of 2,4-D and Triclopyr. J. Aquat. Plant Manage. 48: 12-14.

Hauxwell, J., S. Knight, A. Wagner, Mikulyuk, M. Nault, M. Porzky, and S. Chase. 2010. Recommended Baseline Monitoring of Aquatic Plants in Wisconsin: Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications. Wisconsin Department of Natural Resources Bureau of Science Services, PUB-SS-1068 2010. Madison, Wisconsin, USA.

LaRue, E.A, M.P. Zuellig, M.D. Netherland, M.A. Heilman, and R.A. Thum. 2012. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). Evolutionary Applications. 6(3):462-471.

Poovey, A.G., J.G. Slade, and M.D. Netherland. 2007. Susceptibility of Eurasian Watermilfoil (Myriophyllum spicatum) and a Milfoil Hybrid (M. spicatum x M. sibiricum) to Triclopyr and 2,4-D Amine. J. Aquat. Plant Manage. 45: 111-115.




**2013 Final Spot Treatment Strategy**

*Granular 2,4-D*

| Site | Conditional Acres | Final Acres | Ave Depth (feet) | Volume (acre-feet) | 2,4-D PPM ae |
|---|---|---|---|---|---|
| A-13 | 0.7 | 0.7 | 7.0 | 4.9 | 4.00 |
| B-13 | 2.1 | 2.1 | 7.0 | 14.7 | 4.00 |
| C-13 | 0.5 | 0.6 | 7.0 | 4.2 | 4.00 |
| Total | 3.3 | 3.4 | | 23.8 | |

*Lake-wide 2,4-D concentration if complete dissipation occurs: 0.171 ppm ae*

*Epilimnetic (18 ft) 2,4-D concentration if complete dissipation occurs: 0.187 ppm ae*




N W E S

290

Feet

## Legend

- 2013 Conditional Spot-Treatment Area
- 2013 Final Spot-Treatment Area
- Herbicide Concentration Monitoring Location
- Applicator Treatment Track

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

Sources:
Roads and Hydro: WDNR
Aquatic Plants: Onterra, 2012-13
Map Date: May 15, 2013
Filename: ForestFDL_HWM_Z013Final.mxd

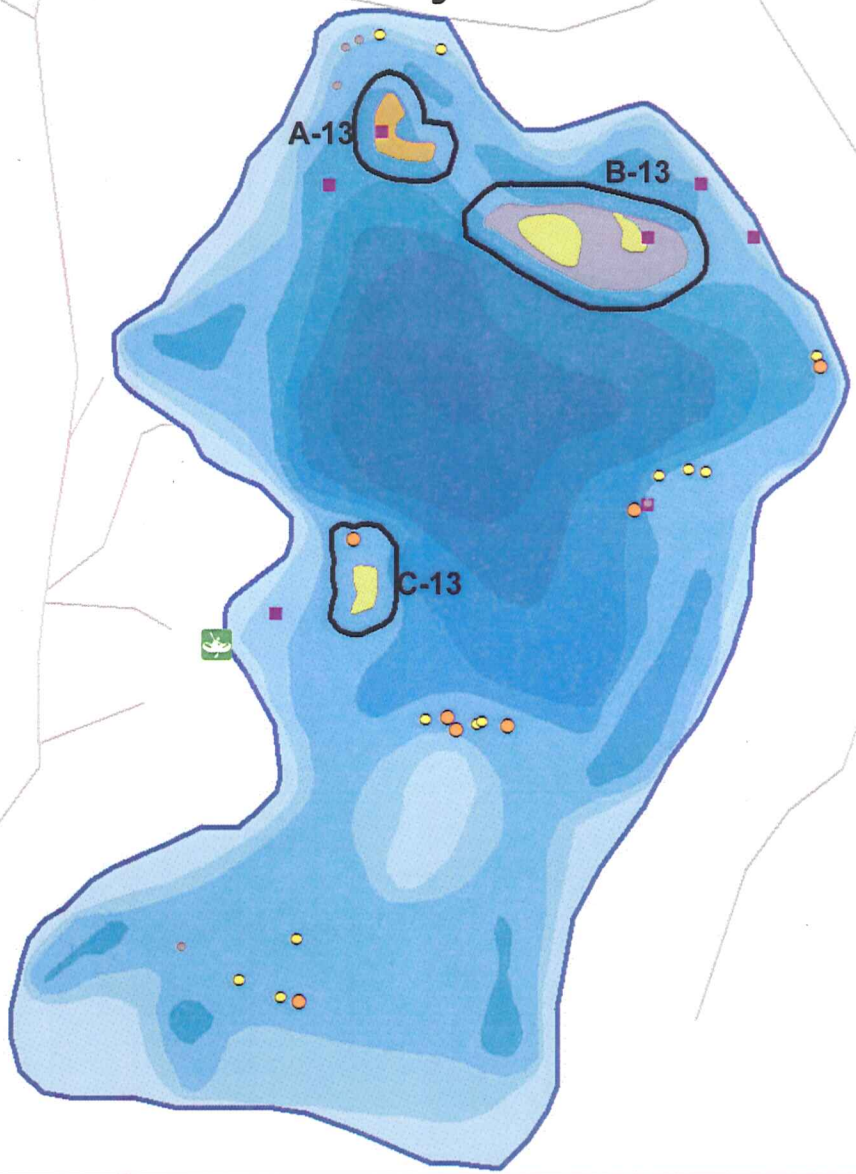
Map 1

# Forest Lake

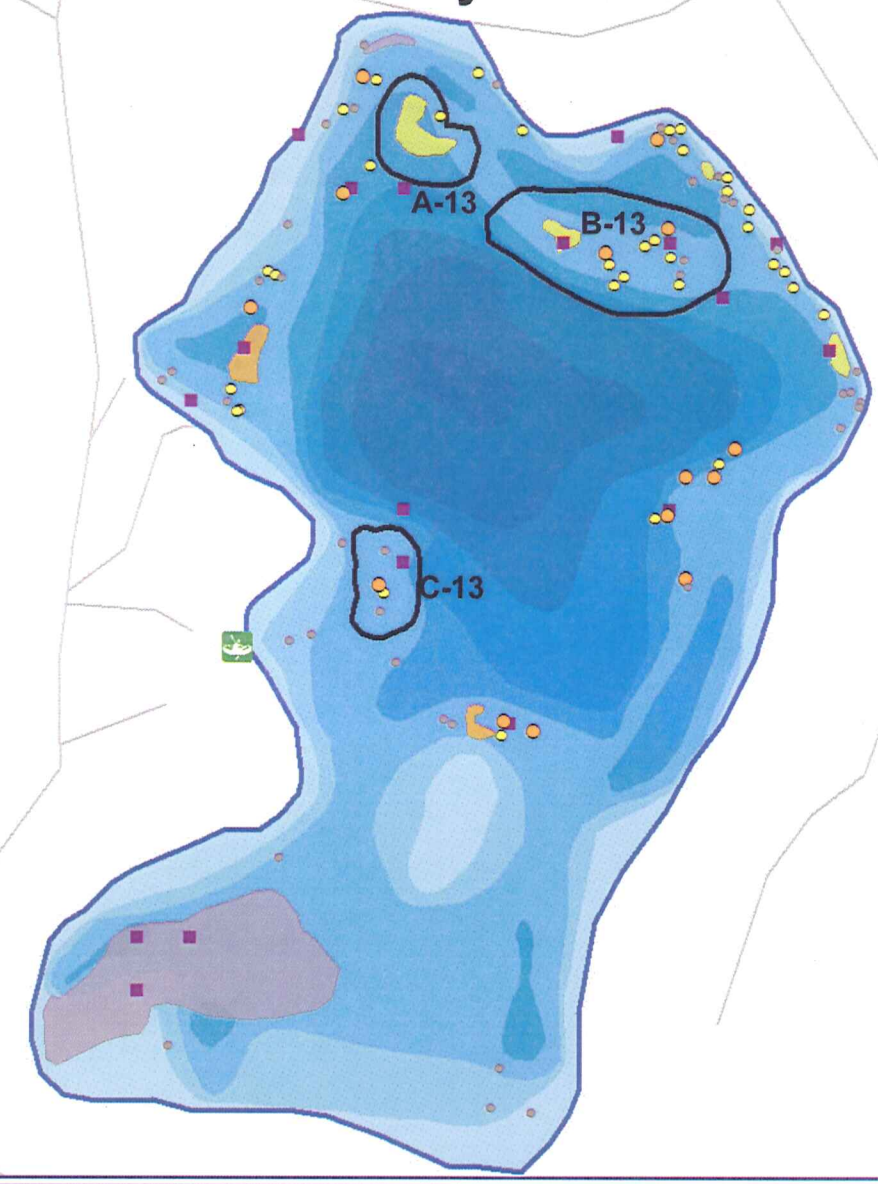
Fond du Lac County, Wisconsin

## 2013 Final HWM Treatment Areas

## 2012 Summer Survey Results




## 2013 Summer Survey Results




N
W E
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375
Feet

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Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR, digitized by Onterra
Aquatic Plants: Onterra, 2012-13
Map Date: February 18, 2014
Filename: Map2_Forest_EWM_2012&2013PB.mxd

Project Location in Wisconsin

### Legend

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clump of Plants
- Small Plant Colony
- PI Location with EWM
- 2013 Final Treatment Area

Map 2

Forest Lake
Fond du Lac County, Wisconsin

**2012 & 2013 HWM Survey Results**

**Draft: Forest Lake, Fond du Lac County,**
**2,4-D Concentration Monitoring Summary, 2013**
**29 October 2013**

**John Skogerboe**

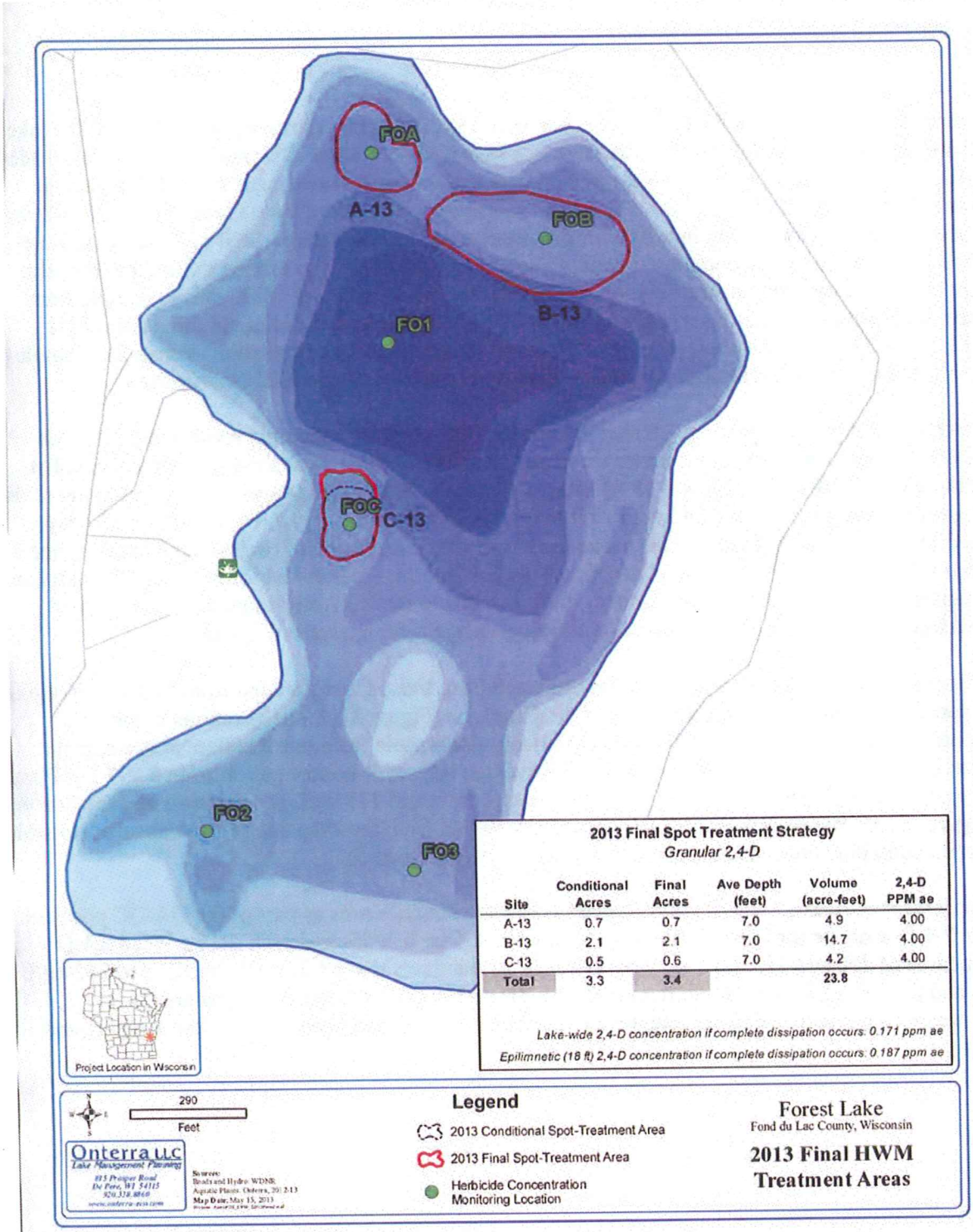
Forest Lake has an area of 51 acres, maximum depth of 32 ft, and a mean depth of 11 feet. The lake is listed on WI DNR Lakes Finder web page as a seepage lake with a 99% much bottom. On 29 May 2013, three areas on Forest Lake were treated with a granular formulation of 2,4-D (Navigate) to control hybrid milfoil (*Myriophyllum spicatum x Myriophyllum sibiricum*) (Figure 1). The 2,4-D was applied to areas infested with hybrid milfoil at a target application rate of 4000 ug/L ae (4 mg/L ae). The lake wide 2,4-D concentration, assuming complete dissipation and mixing throughout the lake was 171 ug/L assuming no stratification and 187 if the lake stratified at 18 ft. Water sample sites were established at 3 three sites (FOA, FOB, and FOC) within treated areas and three sites (FO1, FO2, and FO3) in untreated areas to quantify 2,4-D dissipation and degradation (Figure 2). Sample site FO1 was sampled at multiple depths to quantify the effects of potential stratification.

Water samples were collected at all sites except for FO1 using an integrated water sampler which collects a water sample from the entire water column. Water samples at site FO1were collected at multiple depths (0-10, 15, 20, and 25 ft) using a van dorn. Water samples were collected at intervals of approximately 1, 3, 6, and 24 hours after treatment (HAT) and 5, 7, 10, 14, 21, 28, and 35 days after treatment (DAT). Samples were taken to shore after completion of each sample interval, and 3 drops of muriatic acid were added to each sample bottle to fix the herbicide and prevent degradation. Samples were then stored in a refrigerator, until shipped to the US Army Engineer Research and Development Center (ERDC) laboratory in Gainesville, FL for analysis of 2,4-D.

Concentrations of 2,4-D in water samples collected from treated areas ranged from 12.6 to 156 ug/L ae compared to the target concentration of 4000 ug/L ae (Figure 3). Concentrations in samples exceed the irrigation standard (100 ug/L ae) in only one sample from one location, at one sample interval (156 ug/L ae, site FOC, 1 HAT). The average lake wide concentration from 0 to 7 DAT was 38 ug/L ae compared to a potential lake wide concentration of 171 ug/L ae assuming no stratification (Figure 4). Herbicide concentration in samples collected from the deep site (FO1) indicated possible stratification but concentrations were to low to quantify (Figure 5).

Herbicide standards for 2,4-D are included in all batch analysis runs as part of the QA/QC protocol. The 2,4-D analysis for Forest Lake were conducted in four batches and each run included 2,4-D standards of 250 and 125 ug/L ae. The 250 ug/L ae standards ranged from 231 ug/L ae to 262 ug/L ae, and the 125 ug/L ae standard ranged from 120 to 136 ug/L ae (Figure 6). The analytical laboratory rechecked the water samples to verify that the acid had been added to the samples, and it was.

# Figure 1. Forest Lake 2,4-D Treatment Areas 2013




**2013 Final Spot Treatment Strategy**
*Granular 2,4-D*

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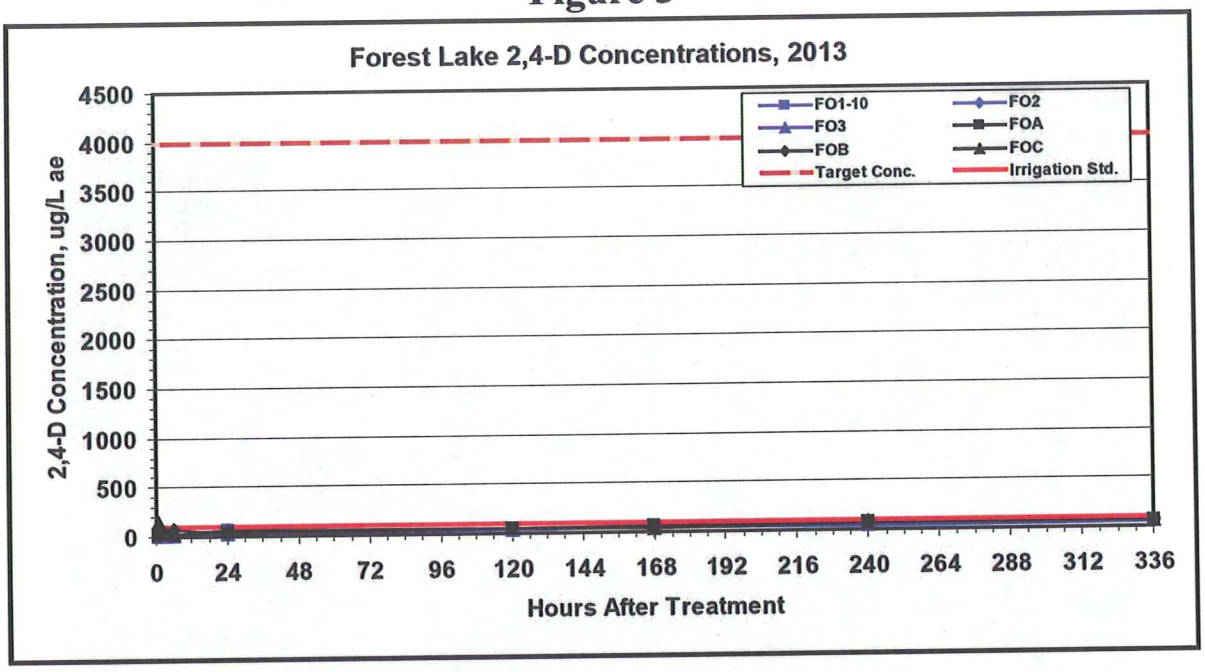
*Lake-wide 2,4-D concentration if complete dissipation occurs: 0.171 ppm ae*

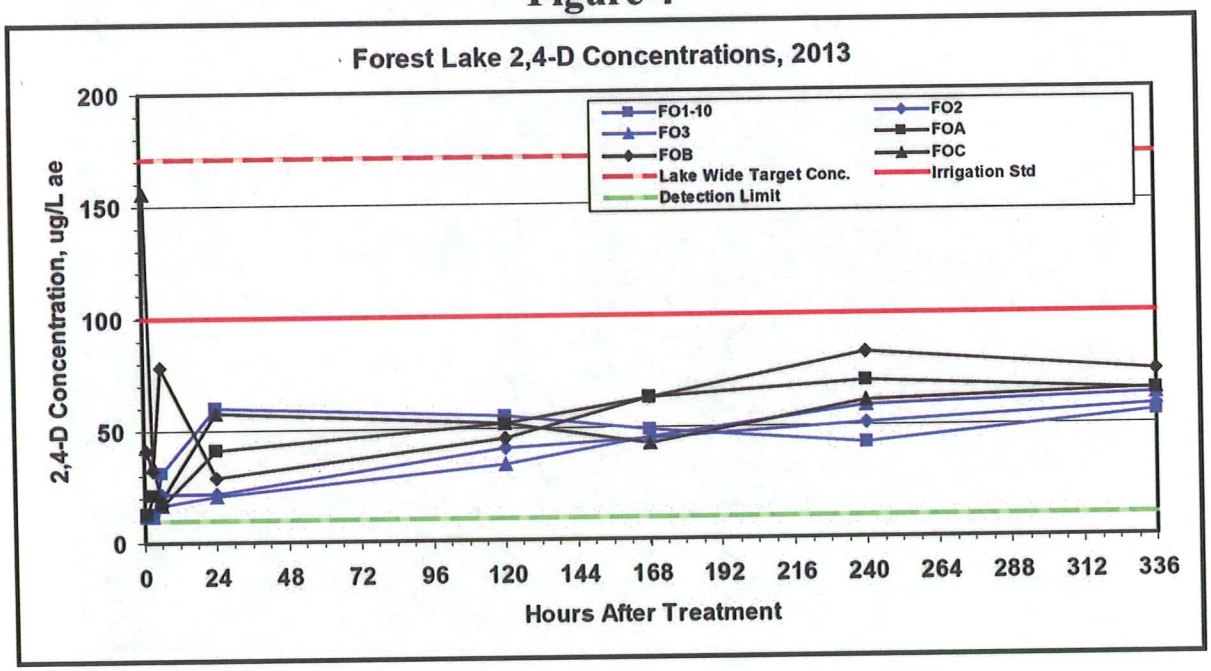
*Epilimnetic (18 ft) 2,4-D concentration if complete dissipation occurs: 0.187 ppm ae*

# Figure 2. Forest Lake 2,4-D Sample Locations 2013

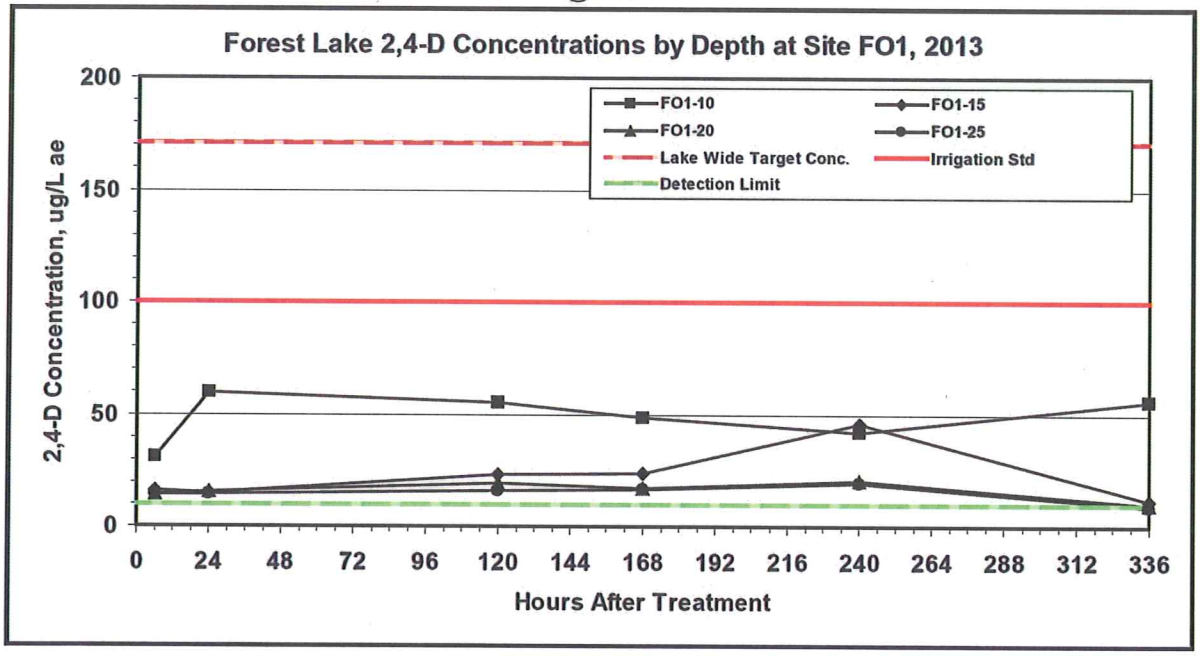
# Figure 3



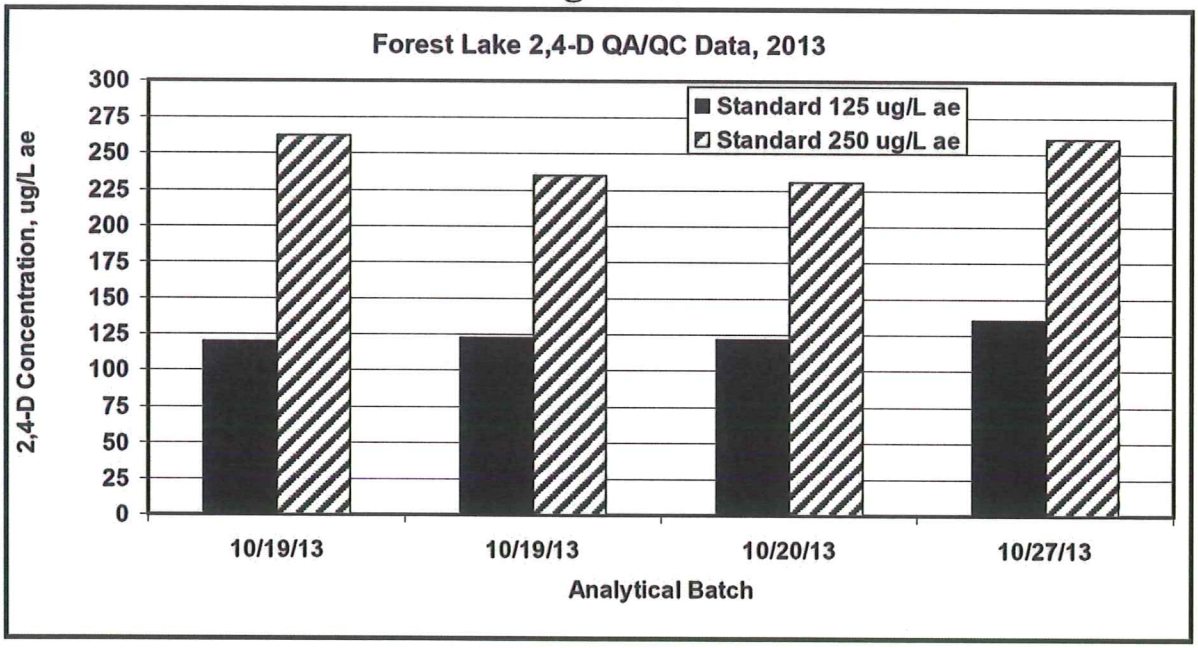

# Figure 4



# Figure 5




# Figure 6



## INTRODUCTION

**Photo 1. Forest Lake, Fond du Lac County.**

In 2010, the Forest Lake Improvement Association, Inc. (FLIA) contracted with Onterra to initiate a three-year aquatic invasive species (AIS) control and monitoring project for Forest Lake, Fond du Lac County (Photo 1, Map 1). The non-native, invasive plant Eurasian water milfoil (EWM, *Myriophyllum spicatum*) was first documented in Forest Lake in 1992. Later in 2010, it was confirmed via DNA analyses that the EWM in Forest Lake was actually a hybrid between EWM and the indigenous northern water milfoil (*Myriophyllum sibiricum*). As will be discussed further in this report, hybrid EWM presents some complications for management as research is indicating that some hybrid strains may have a higher tolerance to aquatic herbicides than pure strains of EWM. Members of the FLIA indicated that the hybrid EWM had spread and occupied the majority of Forest Lake's littoral areas, matting on the surface and interfering with recreational activities and reducing the lake's aesthetic appeal.

The goal of this three-year project (2011-2013) is to reduce the hybrid EWM population within Forest Lake to more manageable levels while at the same time minimizing impacts to valuable native aquatic plant species. Reducing the hybrid EWM population will alleviate recreational interference and improve the lake's ecological integrity. At the end of the three year project, the FLIA is hopeful that the AIS populations in the lake have been reduced to levels that on an annual basis require minimal or no use of herbicides. This report discusses the second year of hybrid EWM treatment monitoring under this project. Specific details pertaining to the 2011 treatment on Forest Lake can be found in the 2011 treatment report.

## 2012 HYBRID EWM CONTROL STRATEGY DEVELOPMENT

On August 19, 2011, Onterra ecologists visited Forest Lake to conduct a meander-based hybrid EWM survey. This survey is designed to visually inspect as much lake-area as possible with the intent of locating and mapping hybrid EWM. Density-based qualitative assessments are best done at this time of year because hybrid EWM should be at or near its peak growth. Three colonies comprised of dominant hybrid EWM were mapped in the west-central and northern portions of the lake, while numerous single plants, clumps of plants, and small plant colonies were located in the southern, central, and northern areas of the lake (Map 1). Results of this survey were used in determining the treatment strategy developed for the spring of 2012.

Following discussions between Onterra, the FLIA, WDNR, and US Army Corps of Engineers (USACE) over the winter of 2011/2012, an initial strategy for treatment of hybrid EWM in the spring of 2012 was developed. Onterra ecologists visited Forest Lake on March 28, 2012 to conduct the spring pre-treatment and refinement survey. During this survey, treatment areas determined in the previous months within the office are visited on the lake to ensure that colonial expansion or retraction have not occurred, or other aspects of the EWM colony have not changed. This survey is conducted only days prior to the anticipated application of herbicides in the early spring. EWM often overwinters as above-ground biomass and begins growing earlier than native

plants in the spring. While it may be difficult to see from the surface, supplemental use of a submersible video camera allowed for this refinement survey to be accomplished. Onterra ecologists located hybrid EWM growing throughout most of the predetermined treatment areas by completing numerous transects and using a submersible video camera as necessary.

## Background on Herbicide Application Strategy

Herbicides that target submersed plant species are directly applied to the water, either as a liquid or an encapsulated granular formulation. Factors such as water depth, water flow, treatment area size, and plant density work to dilute herbicide concentration within aquatic systems. Understanding concentration-exposure times are important considerations for aquatic herbicides. Successful control of the target plant is achieved when it is exposed to a lethal concentration of the herbicide for a specific duration of time. Much information has been gathered in recent years, largely as a result of a joint research project between the WDNR and USACE. Based on their preliminary findings, lake managers have adopted two main treatment strategies; 1) whole-lake treatments, and 2) spot treatments.

Spot treatments are a type of control strategy where the herbicide is applied to a specific area (treatment site) such that when it dilutes from that area, its concentrations are insufficient to cause significant effects outside of that area. Spot treatments typically rely on a short exposure time (often hours) to cause mortality and therefore are applied at a much higher herbicide concentration than whole-lake treatments.

Whole-lake treatments are those where the herbicide is applied to specific sites, but when the herbicide reaches equilibrium within the entire volume of water (of the lake, lake basin, or within the epilimnion of the lake or lake basin); it is at a concentration that is sufficient to cause mortality to the target plant within that entire lake or basin. The application rate of whole-lake treatments is dictated by the volume of water in which the herbicide will reach equilibrium with. Because exposure time is so much greater, target herbicide levels for whole-lake treatments are significantly less than for spot treatments. This was the strategy that was utilized on Forest Lake in 2011. Whole-lake treatments are typically conducted when the target plant is spread throughout much of the lake. Therefore, a low-dose, whole-lake liquid 2,4-D treatment was proposed again for Forest Lake in 2012. Internal discussions within the FLIA took place with the association deciding to move forward with the proposed strategy as outlined by Onterra. The association also assembled volunteers to aid in the monitoring of the treatment.

Emerging data, including herbicide concentration data collected from Forest Lake in 2011, appears clear that liquid 2,4-D mixes horizontally within the lake, but does not vertically mix into deep areas of the lake during stratification. In other words, 2,4-D would dissipate throughout the upper, warmer water zone of the lake (epilimnion), but not into the deeper, colder water zones of the lake (metalimnion and hypolimnion).

Current research indicates that whole-lake liquid 2,4-D treatments are effective on EWM when the target whole-lake herbicide concentration is between 0.250 ppm acid equivalent (ae) and 0.350 ppm a.e. and is maintained for approximately 14 days. The epilimnetic target herbicide concentration for Forest Lake in 2011 was 0.300 ppm ae, and post-treatment monitoring showed that the average concentration from 0-7 days after the treatment was close at 0.290 ppm ae. While hybrid EWM occurrence was reduced by over 60% following the 2011 treatment, a greater level

of control was expected given the herbicide concentration. Emerging research on hybrid EWM is indicating that there is some level of tolerance to aquatic herbicides. In 2011, Onterra ecologists collected hybrid EWM specimens from two lakes in Wisconsin and sent them to SePRO and the USACE for herbicide resistance testing. Cultures of these plants were grown, and then experimental groups were exposed to varying concentrations of 2,4-D. While the results are still preliminary, the overall conclusion of the study is that these two strains of hybrid EWM appear to be less responsive to 2,4-D than a pure EWM reference strain.

While hybrid EWM density was reduced following the 2011 treatment, it was still spread throughout much of the lake's littoral area, making spot-treatments in 2012 an unrealistic option. Because of this, it was recommended that a whole-lake (epilimnion) approach be utilized again in 2012, but with a higher epilimnion-wide herbicide concentration to gain better control of the hybrid EWM. The liquid 2,4-D would be applied at a rate of 1.00 ppm ae to areas of hybrid EWM that would achieve a calculated epilimnetic concentration of 0.365 ppm ae (Map 1).

Devising a strategy to attain the target epilimnetic concentration is a process that involves two primary objectives: 1) determine the water volume for the herbicide application areas and the entire lake, and 2) properly identify the depth at which stratification is occurring at the time of herbicide application so the volume of the epilimnion can be calculated. Bathymetry from the 1965 WDNR Lake Survey Map was brought into Geographic Information Systems (GIS) software and digitized. This produced a spatially-rectified map of Forest Lake's contours with which area-based calculations (acres), and finally total lake volume could be made (Map 1).

One day prior to treatment on April 11, 2012, Onterra ecologists collected a final temperature/dissolved oxygen profile on Forest Lake. Based on this information, it appeared that the epilimnion extended to a depth of 18 feet, an increase of approximately four feet from the late-March pre-treatment survey (Figure 1). Onterra created a final dosing plan for the treatment based upon this information and provided the plan to the herbicide applicator for treatment the following day.

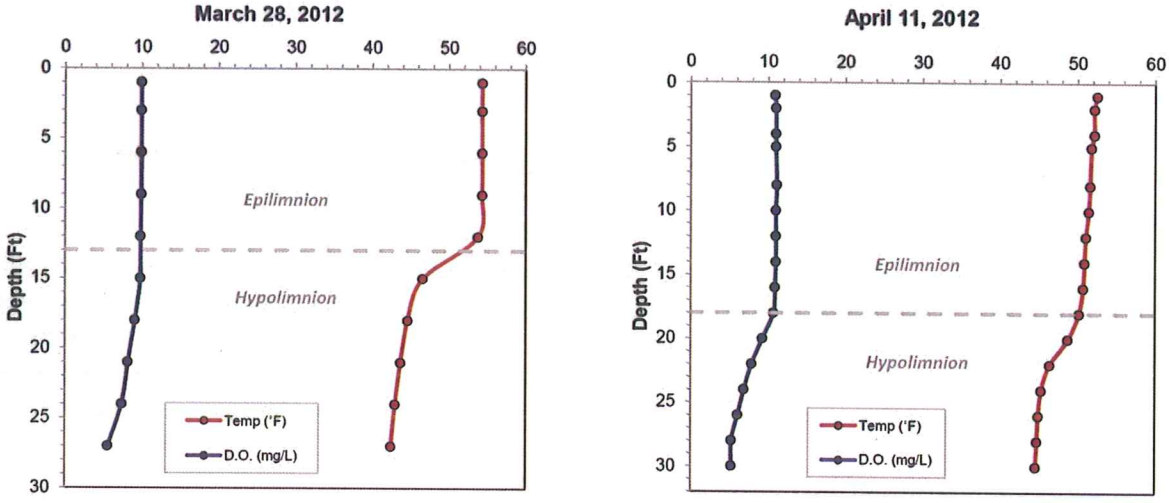



**Figure 1. Temperature and dissolved oxygen profiles collected on Forest Lake in March and April, 2012.**

The 2012 final treatment areas in Forest Lake were treated by Aquatic Biologists, Inc. on April 12, 2012. Treatment sites A-12, B-12, C-12, and D-12 were applied with liquid 2,4-D at a rate of 1.00 ppm ae to achieve an epilimnetic concentration of 0.365 ppm ae. The applicator reported an ambient air temperature of 42°F and northeast winds at approximately 5-10 miles per hour at the time of application.

## 2012 TREATMENT MONITORING

The goal of an herbicide treatment strategy is to maximize target species (hybrid 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 hybrid EWM colony density ratings before and after the treatments.

Because a whole-lake treatment was conducted on Forest Lake, the whole-lake point-intercept method as described by the WDNR Bureau of Science Services (PUB-SS-1068 2010) was used to complete a quantitative evaluation of the occurrences of hybrid EWM and native aquatic plant species. These surveys were completed by Onterra in the summer of 2012 following the treatment and are compared to data collected from the same surveys that were conducted in 2011. Hybrid EWM occurrence in the summers of 2011 and 2012 will also be compared to data gathered during the spring 2011 pre-treatment point-intercept survey. In addition, the response of Forests Lake's native aquatic plant community over the course of the 2011 and 2012 treatments will be examined by comparing data gathered during a 2008 point-intercept survey by the WDNR.

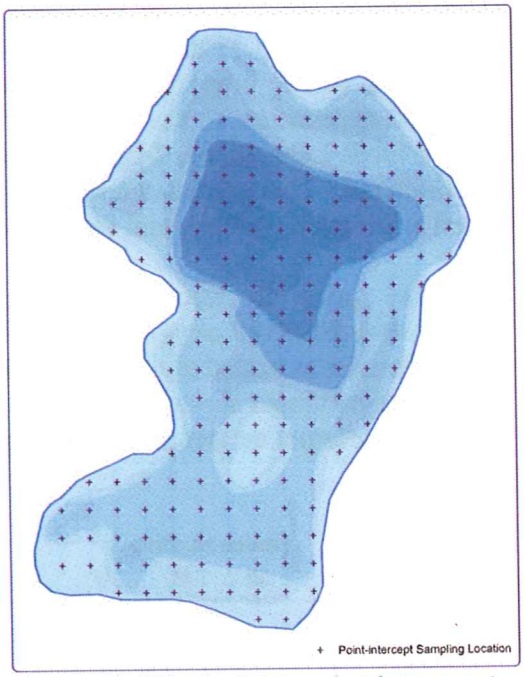



**Figure 2. Whole-lake point-intercept survey sampling locations on Forest Lake.**

Comparing data collected before and after the treatment allows for a statistical comparison of aquatic plant occurrences and a quantitative determination of treatment efficacy on a lake-wide level. Based upon guidance from the WDNR, a point spacing of 33 meters was used resulting 184 points evenly distributed across Forest Lake (Figure 2). Quantitatively, a treatment is deemed successful if the point-intercept data show that the hybrid EWM frequency of occurrence following the treatment is reduced by at least a statistically valid 50% ($\alpha = 0.05$). Only those points that fell within the maximum depth of plant growth (littoral zone) are used in the analyses.

Qualitative monitoring of herbicide treatments includes comparing spatial data reflecting hybrid EWM locations and densities in late-summer prior to and immediately following the treatment when this plant is assumed to be at or near its peak growth. Comparisons of the survey results are used to qualitatively evaluate the 2012 herbicide treatments on Forest Lake. Qualitatively, a

successful treatment on a particular site would include a reduction of hybrid 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 be one level of density as described above for an individual site.

The 2012 treatment on Forest Lake was again selected to participate in an herbicide concentration monitoring project being conducted by the WDNR and USACE. Water samples were collected a five sites, four of which were located within herbicide application areas (Map 1). The fifth was located over Forest's Lake's deepest location and was sampled at multiple depths to measure herbicide concentrations vertically throughout the water column. Water samples were collected by a FLIA volunteer at intervals of 1, 2, 4, 7, 10, 14, 18, 21, 29, and 36 days after treatment (DAT).

## 2012 TREATMENT RESULTS

On August 10, 2012, Onterra ecologists visited Forest Lake to complete the post-treatment assessment of the 2012 whole-lake treatment. During the 2011 point-intercept survey, aquatic plants were found growing to a maximum depth of 22 feet, while they were found growing to a similar maximum depth of 23 feet in 2012. Of the 184 sampling locations within the lake, 163 fell at or below the maximum depth of plant growth (littoral zone) in 2011, and 166 fell at or below the maximum depth of plant growth in 2012. Similarly, the WDNR found aquatic plants growing to a maximum depth of 20.5 feet in 2008 resulting in 163 littoral sampling points. The data collected from these littoral sampling locations are used in the following analyses.

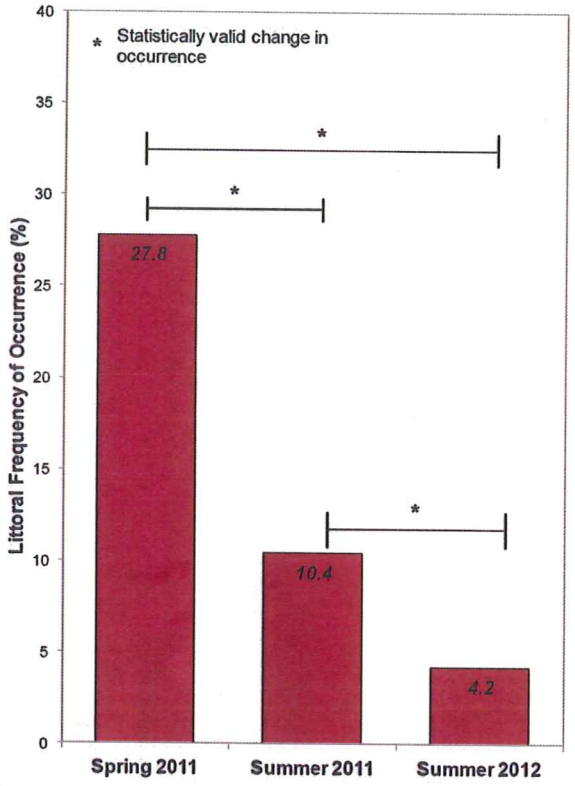



**Figure 3. Hybrid EWM littoral occurrence in Forest Lake from 2011-2012.** Created using data from Onterra point-intercept surveys.

In the spring of 2011, prior to implementation of the first whole-lake treatment of this multi-year control project, almost 30% of point-intercept locations contained hybrid EWM (Figure 3). Comparing the spring 2011 and the summer 2011 point-intercept surveys, 62.5% reduction in hybrid EWM occurrence was documented in association with the 2011 whole-lake treatment. Only 7 (4.2%) of the 166 littoral sampling locations contained hybrid EWM during the summer 2012 survey, representing a statistically valid 59.6% reduction in occurrence compared to the summer 2011 survey, exceeding the quantitative success criterion (50% reduction) for the 2012 whole-lake treatment. Figure 3 illustrates that following two consecutive whole-lake treatments in 2011 and 2012, hybrid EWM has been reduced by a statistically valid 85% since the spring of 2011.

With low-density hybrid EWM populations such as currently exist on Forest Lake; meeting the qualitative density-based mapping success criteria is not as straight forward as the quantitative point-intercept based methodologies. Comparing Map 1 to Map 2, clearly the hybrid EWM population has been reduced over this time period. Almost no hybrid EWM remains within the southern part of the lake following the 2012 treatment. However, areas that contained colonized hybrid EWM during the late-summer of 2011 continue to contain hybrid EWM in 2012.

Data concerning native aquatic plant species were also collected at the same littoral sampling locations during the summers of 2011 and 2012. Statistical analyses are only performed on those species that had a littoral occurrence of greater than 5% in a least one of the two surveys. Figure 4 displays the littoral occurrence of these aquatic plants and illustrates that two native species (stiff pondweed, slender naiad) exhibited statistically valid declines following the 2012 treatment. Unlike hybrid EWM, stiff pondweed and slender naiad are monocots and were not historically thought to be susceptible to dicot-selective herbicides like 2,4-D. However, data collected from Forest Lake and other lakes in Wisconsin indicating that some of these plants may be prone to decline following these types of treatments. Another native naiad species, southern naiad, exhibited a statistically valid increase of 123% from 2011 to 2012 (Figure 4). Two frequently-occurring native dicot species, coontail and white water lily did not see statistically valid changes in their occurrence following the 2012 treatment.

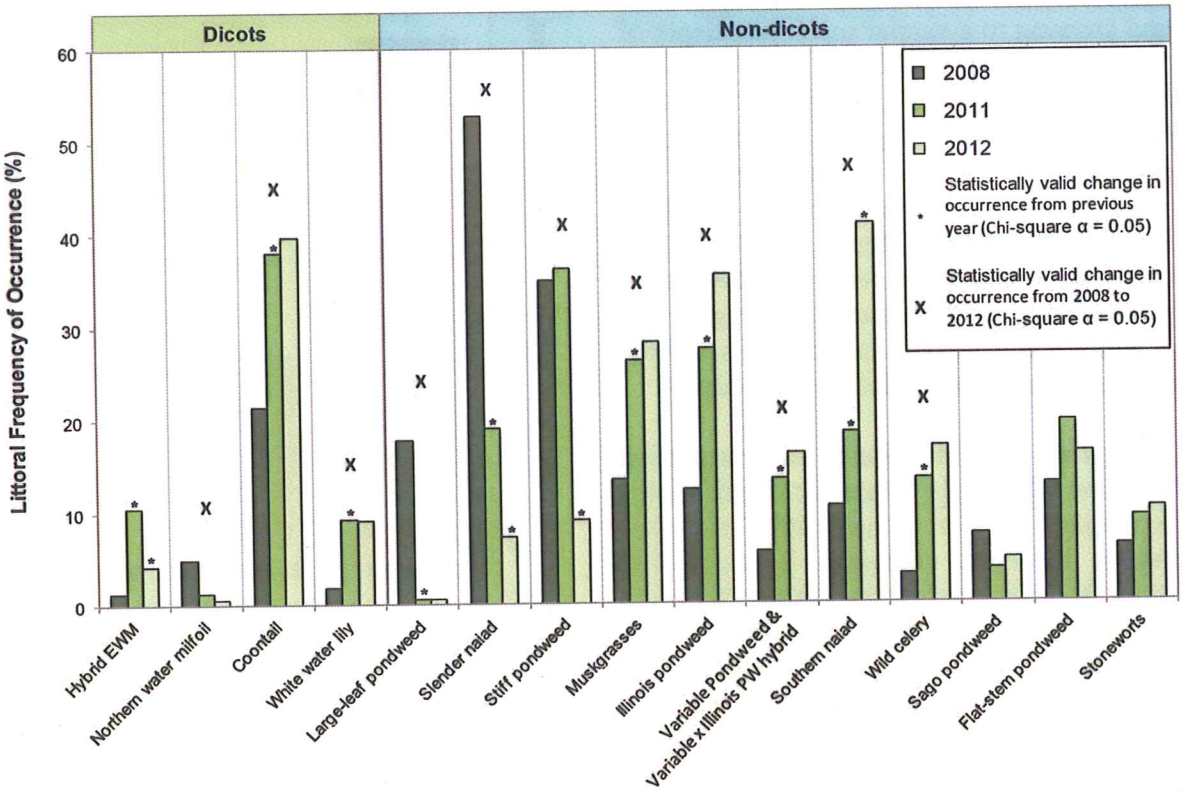



**Figure 4. Littoral occurrence of aquatic plants in Forest Lake from 2008, 2011, and 2012 summer point-intercept surveys.** Only those species with >5% littoral occurrence in either year are displayed. Created using data from WDNR (2008) and Onterra (2011, 2012) point-intercept surveys.

Comparing the 2011 and 2012 point-intercept data to data collected during the WDNR 2008 point-intercept survey allows for an examination of species' responses to two consecutive years of whole-lake treatments on Forest Lake. As Figure 4 illustrates, four native aquatic plant species have shown statistically valid reductions in occurrence from 2008 to 2012. These include one dicot (northern water milfoil) and three monocots (large-leaf pondweed, slender naiad, and stiff pondweed). It remains unknown if the observed increase of southern naiad and corresponding decrease of slender naiad is a real phenomenon or if these changes simply reflect incorrect identification in the field of these extremely similar looking species.

Seven native species saw statistically valid increases in their occurrences from 2008 to 2012, and include two dicots (coontail and white water lily), four monocots (Illinois pondweed, variable pondweed and variable pondweed x Illinois pondweed hybrid, southern naiad, and wild celery), and one macroalgae (muskgrasses) (Figure 4 and Table 1).

**Table 1. Aquatic plant species located in Forest Lake during 2008, 2011, and 2012 summer point-intercept surveys.** Created using data from WDNR (2008) and Onterra (2011, 2012) point-intercept surveys.

| Life Form | Scientifc Name | Common Name | Coefficient of Conservatism (C) | 2008 LFOO | 2011 LFOO | 2012 LFOO |
|---|---|---|---|---|---|---|
| E | *Eleocharis palustris* | Creeping spikerush | 6 | | 0.6 | |
| FL | *Nymphaea odorata* | White water lily | 6 | 1.8 | 9.2 | 9.0 |
| | *Nuphar variegata* | Spatterdock | 6 | 0.6 | 3.1 | 4.2 |
| Submergent | *Ceratophyllum demersum* | Coontail | 3 | 21.1 | 38.0 | 39.8 |
| | *Chara spp.* | Muskgrasses | 7 | 13.3 | 26.4 | 28.3 |
| | *Myriophyllum sibiricum* | Northern water milfoil | 7 | 4.8 | 1.2 | 0.6 |
| | *Myriophyllum spicatum (hybrid)* | Eurasian water milfoil (hybrid) | Exotic | 1.2 | 10.4 | 4.2 |
| | *Najas flexilis* | Slender naiad | 6 | 51.8 | 19.0 | 7.2 |
| | *Najas guadalupensis* | Southern naiad | 7 | 10.2 | 18.4 | 41.0 |
| | *Nitella spp.* | Stoneworts | 7 | 6.0 | 9.2 | 10.2 |
| | *Potamogeton amplifolius* | Large-leaf pondweed | 7 | 17.5 | 0.6 | 0.6 |
| | *Potamogeton friesii* | Fries' pondweed | 8 | 4.2 | | |
| | *Potamogeton gramineus* | Variable pondweed | 7 | | 12.9 | 16.3 |
| | *Potamogeton gramineus x illinoensis* | Variable x illinois pondweed | N/A | 5.4 | 0.6 | |
| | *Potamogeton illinoensis* | Illinois pondweed | 6 | 12.0 | 27.6 | 35.5 |
| | *Potamogeton natans* | Floating-leaf pondweed | 5 | 0.6 | 4.3 | 2.4 |
| | *Potamogeton pusillus* | Small pondweed | 7 | | | 1.8 |
| | *Potamogeton strictifolius* | Stiff pondweed | 8 | 34.3 | 36.2 | 9.0 |
| | *Potamogeton zosteriformis* | Flat-stem pondweed | 6 | 12.7 | 19.6 | 16.3 |
| | *Sagitaria sp. (rosette)* | Arrowhead rosette | N/A | 2.4 | 2.5 | 3.0 |
| | *Stuckenia pectinata* | Sago pondweed | 3 | 7.2 | 3.7 | 4.8 |
| | *Vallisneria americana* | Wild celery | 6 | 3.0 | 13.5 | 16.9 |
| S/E | *Eleocharis acicularis* | Needle spikerush | 5 | 0.6 | | |

*E = Emergent; FL = Floating Leaf; S/E = Submergent and Emergent*
*LFOO = Littoral Frequency of Occurrence*
*2008 N = 166; 2011 N = 163; 2012 N = 166*

The native species richness along with the plant community's average coefficient of conservatism was used to calculate the Floristic Quality Index for Forest Lake's aquatic plant community in 2008, 2011, and 2012. Floristic Quality Assessment (FQA) is used to evaluate the closeness of a lake's aquatic plant community to that of an undisturbed lake. The higher the floristic quality, the closer a lake is to an undisturbed system. FQA is an excellent tool for comparing individual lakes and the same lake over time.

Average species conservatism utilizes the coefficient of conservatism values for each of those species in its calculation (Table 1). A species coefficient of conservatism value indicates that species likelihood of being found in an undisturbed system. The values range from one to ten. Species that are normally found in disturbed systems have lower coefficients, while species frequently found in less-disturbed systems have higher values. For example, cattail is a disturbance-tolerant species and has value of 1, while Oakes pondweed is a sensitive and rare species that has a value of 10. On their own, the species richness and average conservatism values for a lake are useful in assessing a lake's plant community; however, the best assessment of the lake's plant community health is determined when the two values are used to calculate the lake's floristic quality (equation shown below).

$$FQI = \text{Average Coefficient of Conservatism} * \sqrt{\text{Number of Native Species}}$$

Figure 5 compares the FQI components of Forest Lake from 2008, 2011, and 2012. In addition, the FQI components of Forest Lake are compared to median values of other lakes within the Southeastern Till Plains (SETP) Ecoregion and the entire State of Wisconsin. Native plant species richness, or the number of native plant species, was similar in all three years: 19, 19, and 18 in 2008, 2011, and 2012, respectively, falling above the mean, but below the 75$^{th}$ percentile( (upper quartile) for lakes within the SETP Ecoregion and the state. Average conservatism values for all three years remained the same at 6.1, and also fall above the mean for lakes within the ecoregion and the state. Using the native species richness and conservatism values to calculate Forest Lake's FQI shows that it remained relatively the same over all three years, exceeding the 75$^{th}$ percentile for lakes in the ecoregion and falling just slightly below the 75$^{th}$ percentile for lakes in the state.

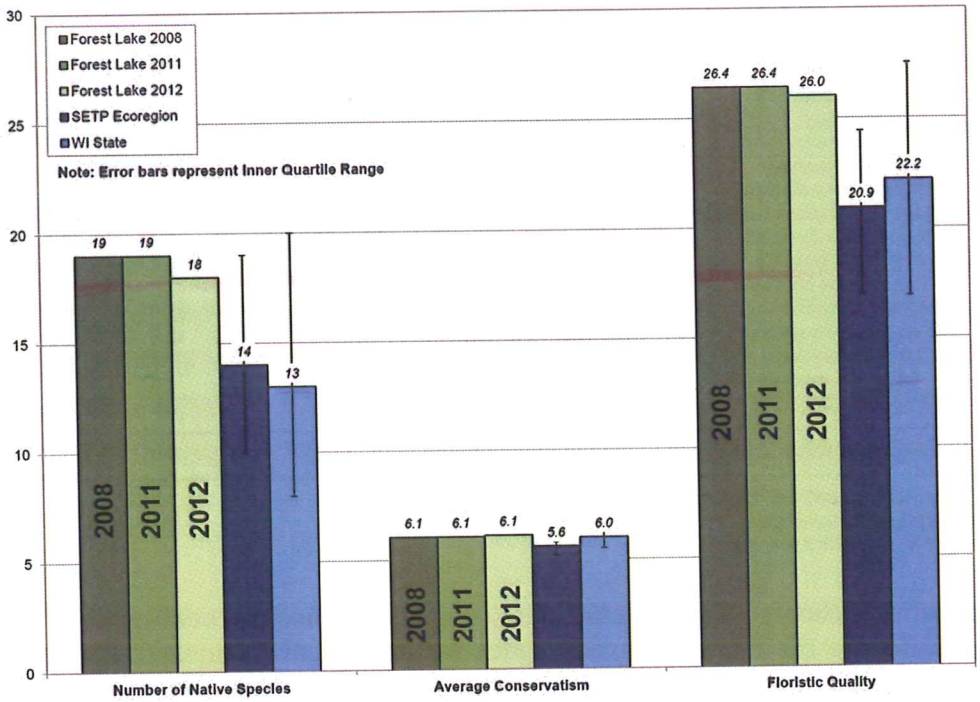



**Figure 5. Forest Lake Floristic Quality Analysis.** Created using data from 2008, 2011, and 2012 point-intercept surveys. Analysis follows Nichols (1999).

Diverse aquatic plant communities are an important component of lake ecology as they have higher resilience to environmental disturbances. A plant community with a mosaic of species with differing morphological attributes provides zooplankton, macroinvertebrates, fish and other wildlife with diverse structural habitat and various sources of food. While species richness is solely the total number of native plant species found within the lake, diversity takes into account how evenly those plant species are distributed within the community. An ecological tool called Simpson's Diversity Index is commonly used to determine a plant community's diversity. Using data collected from the 2008, 2011, and 2012 point-intercept surveys, the diversity of Forest Lake's aquatic plant community increased from 0.88 in 2008 to 0.91 and 0.90 in 2011 and 2012 (Figure 6). Diversity index values for Forest Lake in 2012 fall in the 75th percentile for lakes in both the SETP Ecoregion and with other lakes in the state.

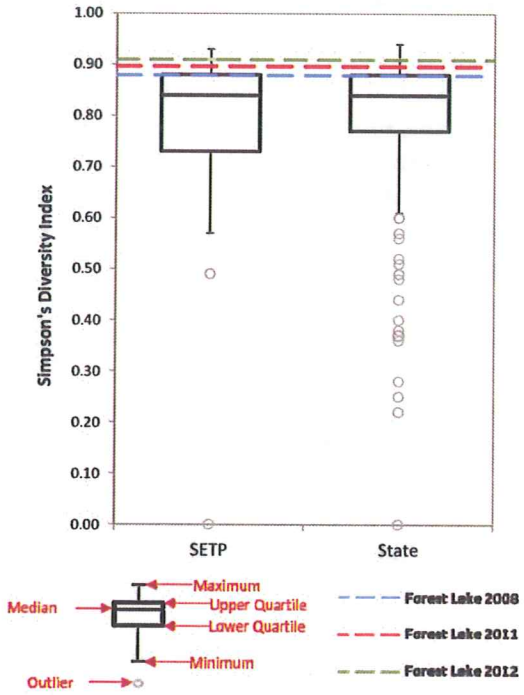



**Figure 6. Forest Lake Simpson's Diversity Index.** Created using data from 2008, 2011, and 2012 point-intercept surveys.

### *2012 Herbicide Concentration Monitoring on Forest Lake*

As indicated in Figure 1, Forest Lake was stratified at the time of herbicide application, with the upper, warmer layer of water (epilimnion) extending to a depth of around 18 feet. The herbicide was applied to the application areas with the intent that it would dilute throughout the epilimnion to a concentration of 0.365 ppm ae. Five locations were sampled by a FLIA volunteer (John Bardenwerper) to measure herbicide concentrations following the 2012 treatment. Four locations (FO1, FO3, FO4, FO5) were located within areas were the herbicide was directly applied, while one site (FO2) was located over the deepest location of Forest Lake (Map 1).

The mean herbicide concentration from all five sampling locations from 0-7 days after treatment was 0.351 ppm ae, just below the target level of 0.365 ppm ae. Herbicide concentrations remained above 0.300 ppm ae to at least 21 days after treatment, and dropped to near 0.200 ppm aw at 29 days after treatment. Herbicide concentrations were still above the irrigation restriction limit of 0.100 ppm ae at the 36 days after treatment, the final sampling period. Herbicide concentrations collected at the lake's deep hole indicate that the lake was stratified. Appendix A contains the USACE draft report with more detail regarding the herbicide concentration monitoring sampling study on Forest Lake.

## 2013 CONTROL STRATEGY

Overall, the 2012 hybrid EWM treatment on Forest Lake was successful. The quantitative success criterion of at least a 50% reduction in hybrid EWM occurrence was met, while the qualitative monitoring methodologies shown a decrease in hybrid EWM occurrence, its continued existence in a few areas are troubling. Based upon the measured concentration-exposure time following the treatment, the level of hybrid EWM control was to be expected. While some native species declined in their occurrence, the Floristic Quality Analysis showed that Forest Lake's aquatic plant community is of high quality and remained relatively unchanged from 2008 to 2012.

In early February, the FLIA was provided three possible control options for 2013:

- **Conduct another whole-lake treatment in 2013** – Hybrid EWM control has been demonstrate on Forest Lake utilizing this technique, but there likely isn't sufficient hybrid EWM on the lake to justify such an all-encompassing treatment strategy. As discussed above, a few native plant species had population declines during this period of whole-lake treatment strategies. It is suspected that the populations of northern water milfoil, large-leaf pondweed, and stiff pondweed will rebound once the scale of the control program decreases. It is believed that if another whole-lake treatment were to occur on Forest Lake again in 2013, the impacts to the native aquatic plant community may outweigh the benefits of hybrid EWM control.

- **No Treatment in 2013, continue monitoring hybrid EWM Population** – The EWM population on Forest Lake is significantly reduced over the course of the 3-year project. There probably isn't enough EWM to warrant another whole-lake treatment (bullet point above). And there are some concerns that the small-scale spot treatment may be ineffective due to rapid dilution (bullet point below). For these reasons, it may be appropriate to not conduct a treatment in 2013. Obviously there are concerns that this option might allow the EWM to regain a foothold in the lake – but the EWM population may stay the same or spread slowly. Perhaps the initiation of a whole-lake treatment strategy in a few years when the population gets high again may be a viable management strategy at that time.

- **Conduct a spot treatment targeting the colonized areas of EWM in 2013** – Unlike the whole-lake treatment strategy that relies on a low-dose liquid 2,4-D application to be sustained for a long period of time, this spot-treatment strategy would target specific hybrid EWM colonies with a high dose of granular 2,4-D. Because the lake is relatively small, there will be some whole-lake implications, but at less than half the levels if a whole-lake strategy is planned. Some concerns exist regarding the small size of the treatment sites and that the herbicide may move off site too quickly to provide hybrid EWM control. But if these areas experienced 4-8 hours of high concentration, coupled with 7-14 days of a very low 2,4-D concentration, it is anticipated that the treatment would be effective. Because the whole-lake herbicide concentrations would be less, the impacts on the native plant community may also be lessened and/or confined to the areas where the herbicide was directly applied.

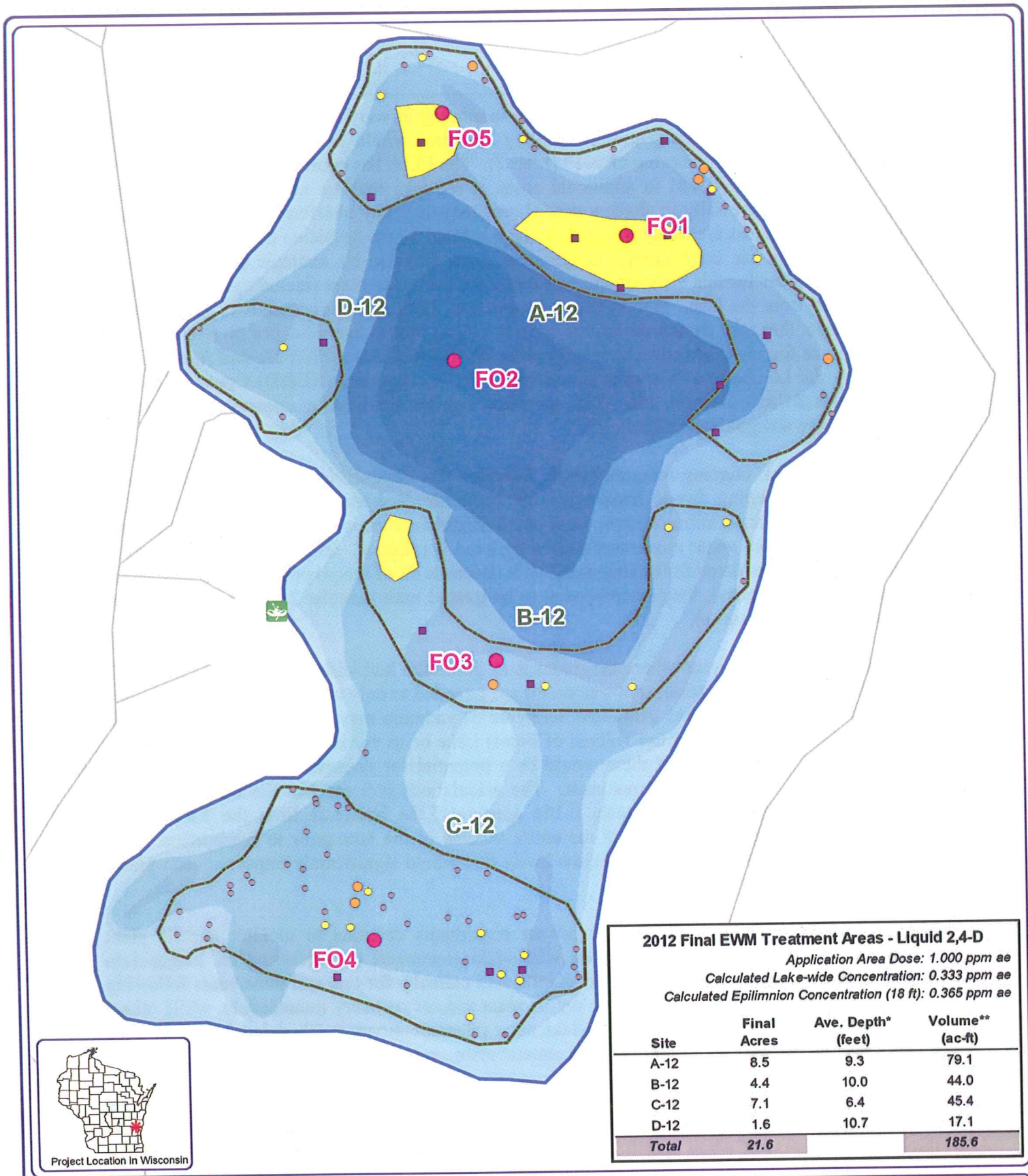
After deliberation, the FLIA has slight preference in moving forward with a limited spot treatment in 2013. Following this strategy, approximately 3.3 acres would be targeted for treatment on Forest Lake during the early-spring of 2013 (Map 2). The FLIA has expressed concerns regarding the existence of hybrid EWM in additional areas of the lake that are not within the proposed treatment strategy, especially in shallow near-shore areas that may interfere with recreational use of the lake. These areas are likely not going to be impacted by the herbicide treatment, but should be included within the 2013 control program as areas targeted by strategic hand-removal by volunteers. 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 needs to be paid to all plant fragments that may detach during the control effort. Additional guidance on hand-removal methods can be found within educational pamphlet, *Eurasian Water Milfoil Manual Removal,* co-authored by the Lumberjack Resource Conservation & Development (RC&D) Council, Inc. and Golden Sands RC&D Council, Inc. This pamphlet can be obtained by contacting the Golden Sands RC&D (www.goldensandsrcd.org).

As discussed earlier, ongoing research clearly indicates that conducting spot treatments on small areas can be challenging due to extremely high herbicide dissipation rates. The only way to influence herbicide dissipation rates on these small treatment sites is to ensure that the herbicide treatment occurs when water movement caused by wind is minimal. Only the colonized areas of hybrid EWM are considered for treatment in 2013. Because all of the proposed treatment sites are quite small (0.5-2.1 acres), they are proposed to be treated with granular 2,4-D at the maximum application rate (4.0 ppm ae).

Unlike whole-lake treatments where the intent is to dilute the herbicide to a specified lake-wide (epilimnion-wide) concentration, spot-treatments are intended to only affect the target plant within the specified application areas. Although herbicide degradation may occur before the herbicide reaches equilibrium with the entire volume of Forest Lake or its epilimnion, these concentrations were calculated to understand if there would be a potential for unintended lake-wide herbicide impacts from the proposed 2013 treatment. The calculations indicate that the lake-wide 2,4-D concentrations would be approximately 0.166 ppm ae if the herbicide from the 3.3 acres of treatment dissipated evenly throughout the entire volume of the lake prior to degradation. It is believed that these concentrations are below levels that would significantly impact aquatic plants on a lake-wide basis.

Members of the WDNR, USACE, and private consultants continue to monitor aquatic plant communities of lakes that have undergone whole-lake treatments to understand the longer-term effects of this treatment strategy. Their data collected annually for two and three years following whole-lake treatments indicate that some native plant species recovery immediately, while others remain slower to recover. Another whole-lake point-intercept survey is scheduled for Forest Lake in 2013 to continue monitoring of the lake's aquatic plant community.

Following the conclusion of the control project, the FLIA needs to consider updating its lake management plan to reflect the success and limitations learned. Along with establishing new thresholds (triggers) of when specific herbicide treatment strategies warrant implementation, the lake management planning process could include a holistic understanding of the Forest Lake ecosystem involving assessments of the water quality, watershed, shoreline condition, and stakeholder perceptions of Forest Lake.




**2012 Final EWM Treatment Areas - Liquid 2,4-D**

*Application Area Dose: 1.000 ppm ae*
*Calculated Lake-wide Concentration: 0.333 ppm ae*
*Calculated Epilimnion Concentration (18 ft): 0.365 ppm ae*

| Site | Final Acres | Ave. Depth* (feet) | Volume** (ac-ft) |
|---|---|---|---|
| A-12 | 8.5 | 9.3 | 79.1 |
| B-12 | 4.4 | 10.0 | 44.0 |
| C-12 | 7.1 | 6.4 | 45.4 |
| D-12 | 1.6 | 10.7 | 17.1 |
| *Total* | *21.6* | | *185.6* |




N
W E
S

280
Feet

**Legend**

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- PI Location with EWM (August 2011)
- 2012 Conditional Treatment Area
- 2012 Final Treatment Area
- Herbicide Concentration Sampling Location

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

Sources:
Roads and Hydro: WDNR
Aquatic Plants: Onterra, 2011
**Map Date:** January 16, 2013
Filename: Map1_Forest_EWM_2011PI_T2012.mxd

Map 1
Forest Lake
Fond du Lac County, Wisconsin
**2011 Hybrid EWM Locations & 2012 Final Treatment Areas**


This Map & Strategy are Not For Permit Use

**2013 Preliminary Spot Treatment Strategy**
*Granular 2,4-D*

| Site | Acres | Ave Depth (feet) | Volume (acre-feet) | 2,4-D PPM ae |
|---|---|---|---|---|
| A-13 | 0.7 | 7.0 | 4.9 | 4.00 |
| B-13 | 2.1 | 7.0 | 14.7 | 4.00 |
| C-13 | 0.5 | 7.0 | 3.5 | 4.00 |
| Total | 3.3 | | 23.1 | |

While not designed as a whole-lake treatment, will have the following lake-wide concentrations if complete dissipation occurs:

*Calculated Lake-wide Concentration: 0.166 ppm ae*
*Calculated Epilimnion Concentration (18 ft): 0.182 ppm ae*


N W E S

290
Feet

**Legend**

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- PI Location with EWM (August 2012)
- 2012 Final Treatment Area
- 2013 Preliminary Treatment Strategy

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Sources:
Roads and Hydro: WDNR
Aquatic Plants: Onterra, 2012
**Map Date:** January 16, 2013
Filename: Map2_Forest_FT013_Grad1.mxd

Map 2
# Forest Lake
Fond du Lac County, Wisconsin
## 2012 Hybrid EWM Locations & 2013 Preliminary Treatment Strategy v1

**Draft: Forest Lake, Fond du Lac County,**
**2,4-D Herbicide Monitoring Summary, 2012**

**15 December 2012**

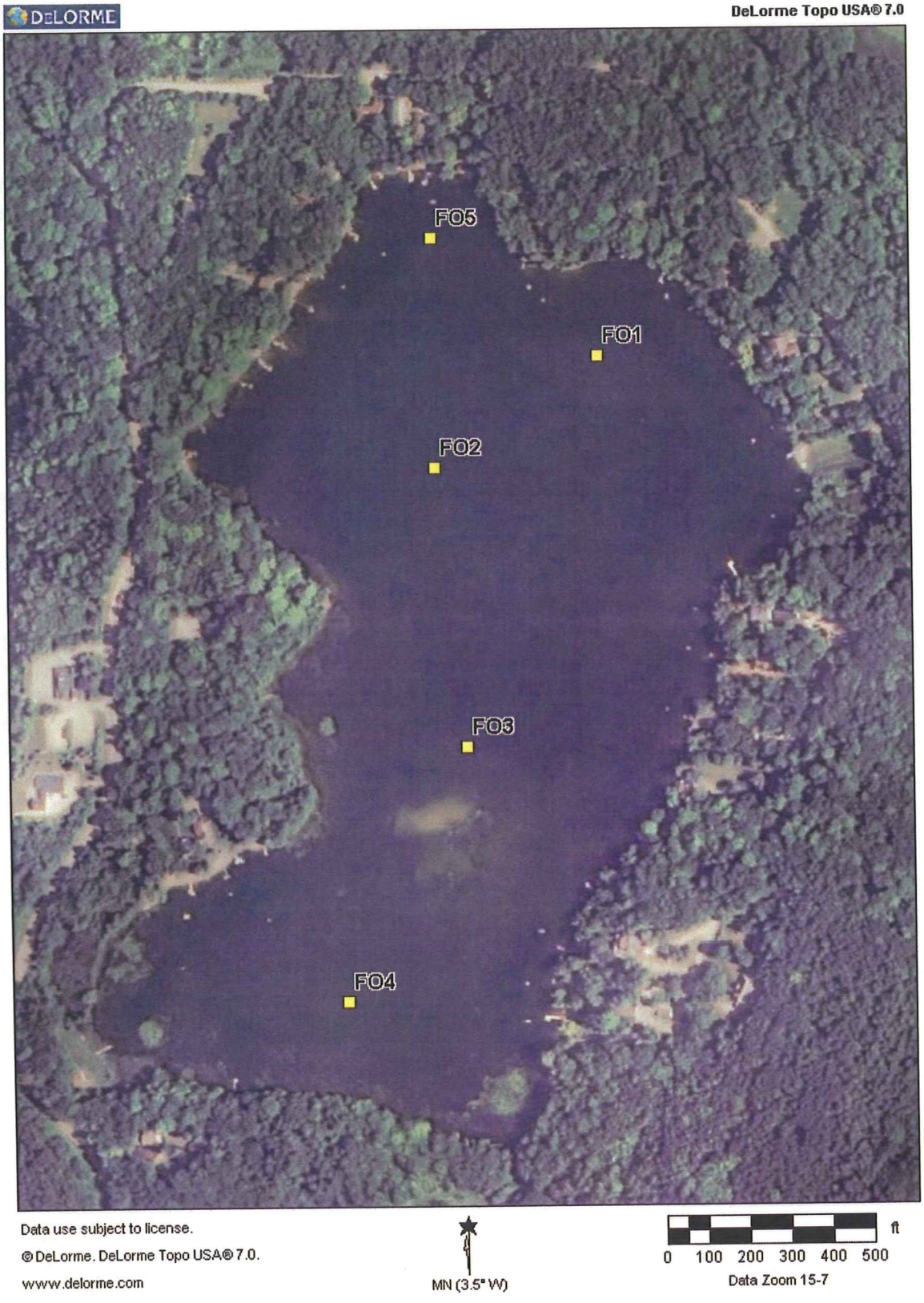
**John Skogerboe**
**US Army Engineer Research and Development Center (ERDC)**

On 1 May 2012 Forest Lake was treated with a liquid formulation of 2,4-D at a lake wide target application rate of 350 ug/L ae to control hybrid milfoil. The total quantity of herbicide was determined based on the belief that the lake would be stratified. The depth of the thermocline was measured shortly before herbicide application and the herbicide quantity was adjusted accordingly. Water sample locations were established at five locations to monitor herbicide concentrations and exposure times following the application (Figure 1).

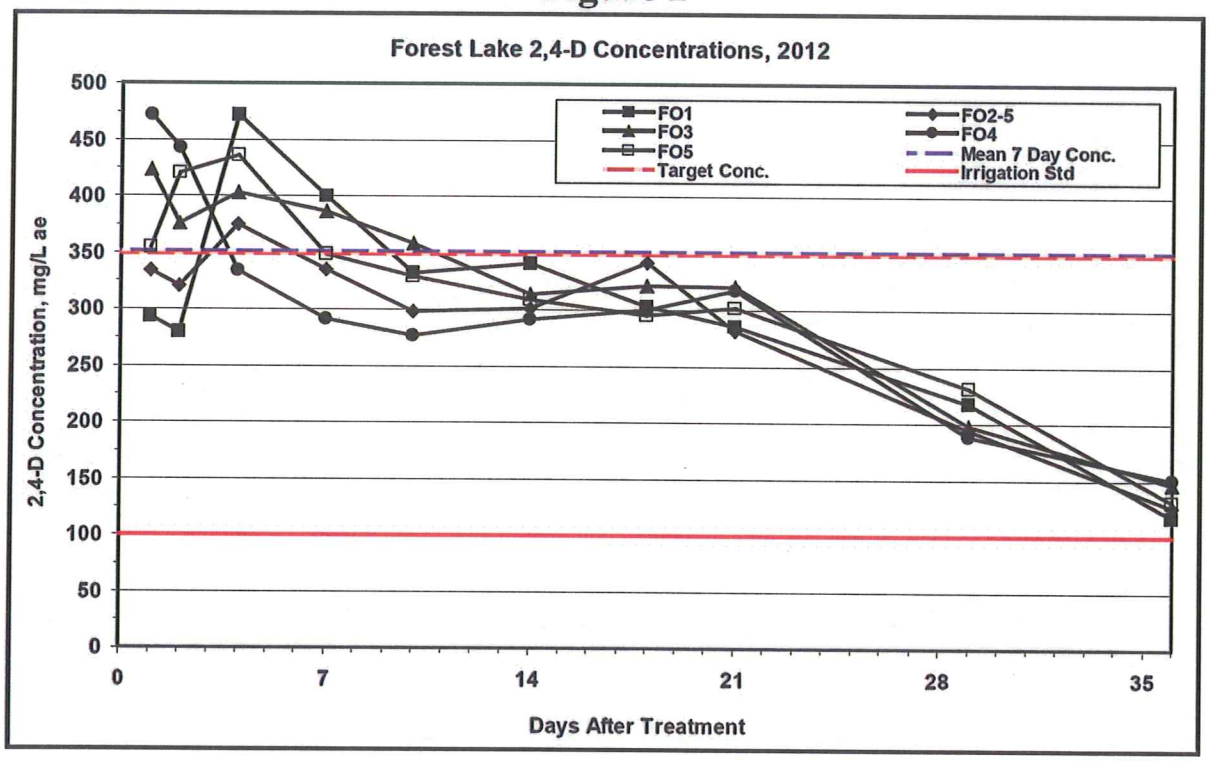
Water samples were collected using an integrated water sampler which collects a water sample from the entire water column. One sample site (FO2) was located in a deep section of the lake and sampled at multiple depths (5, 10, 15, 20, and 25 ft). Water samples were collected at intervals of 1, 2, 4, 7, 10, 14, 18, 21, 29 and 36 days after treatment (DAT). Samples were taken to shore after completion of each sample interval, and 3 drops of muriatic acid were added to each sample bottle to fix the herbicide and prevent degradation. Samples were then stored in a refrigerator, until shipped to the ERDC laboratory in Gainesville, FL for analysis of 2,4-D.

The peak mean 2,4-D concentration was 390 ug/L ae ($\leq$ 15 ft) and occurred at 4 DAT (Figure 2, Figure 3). The mean concentration from 0 to 7 DAT was 351 ug/L ae compared to the target concentration of 350 ug/L ae. The mean 2,4-D concentration was greater than the irrigation standard of 100 ug/L ae at 36 DAT. Herbicide concentrations in samples from the deep site, FO2, were greater from depths 5, 10, and 15 ft than from deeper depths 20 and 25 ft indicating that the lake was stratified (Figure 4).

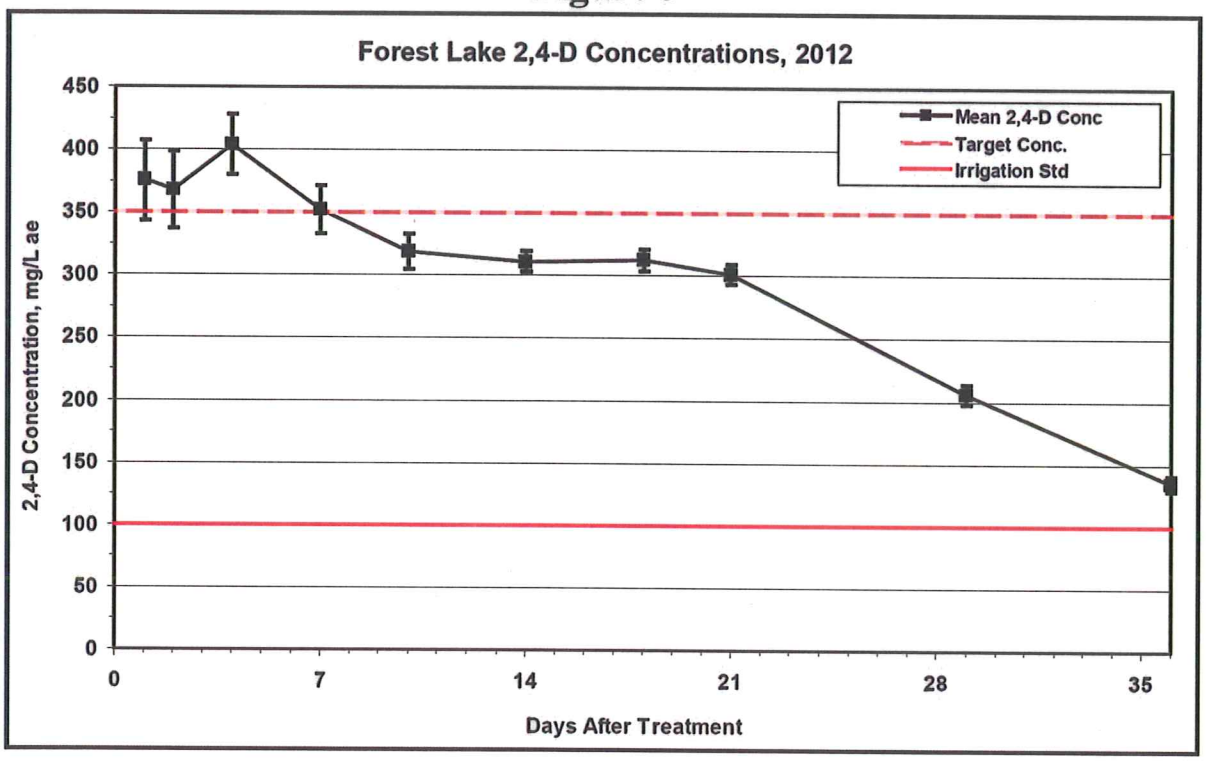
## Figure 1. Forest Lake 2,4-D Sample Locations, 2012



# Figure 2




# Figure 3



# Figure 4

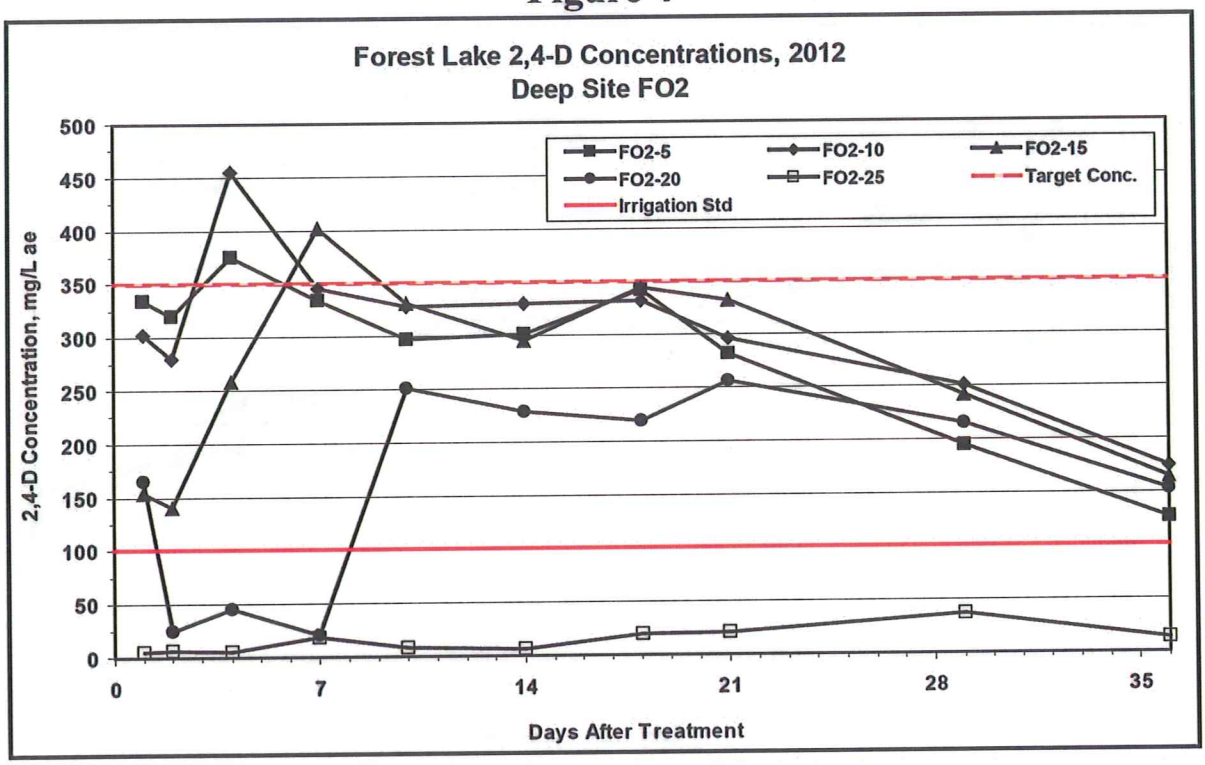
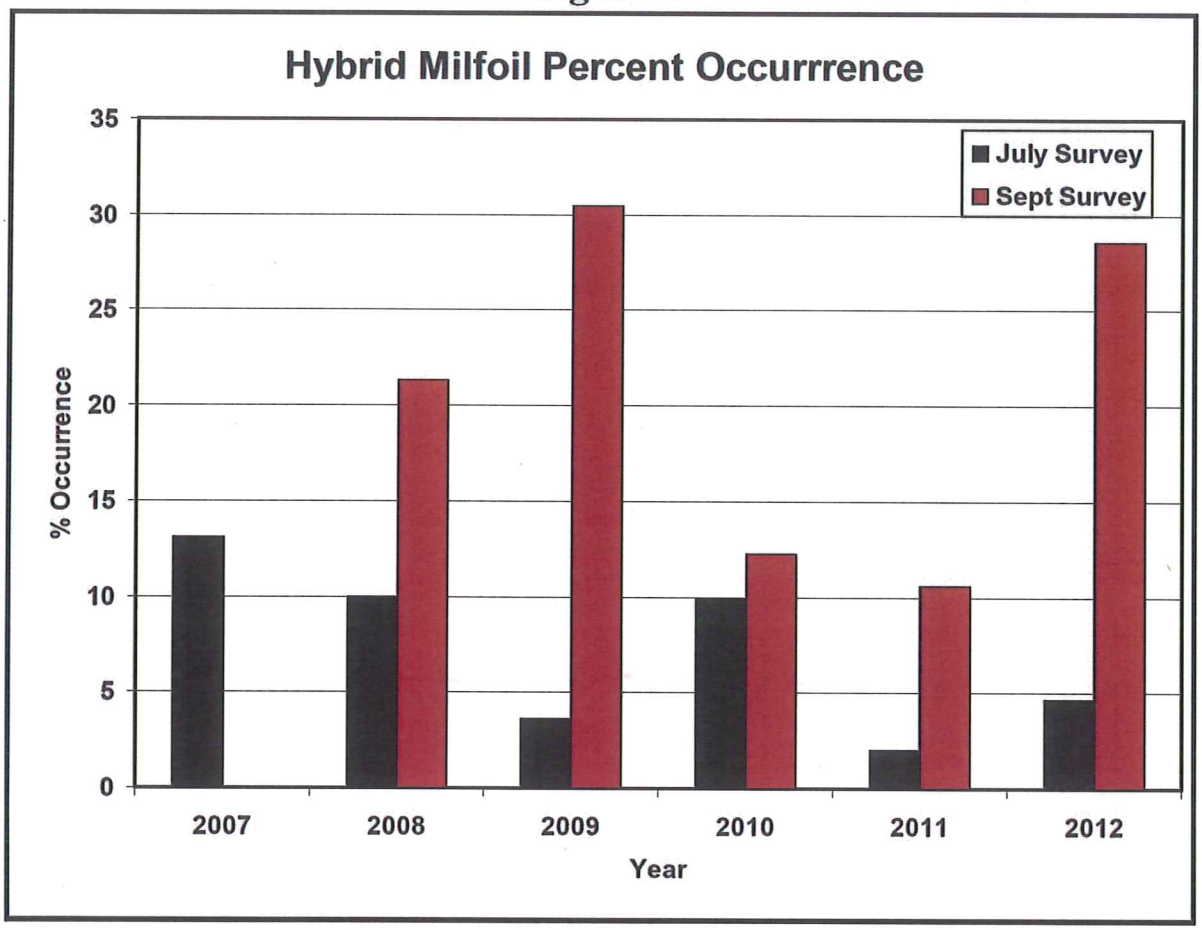

Forest Lake 2,4-D Concentrations, 2012
Deep Site FO2
2,4-D Concentration, mg/L ae
Days After Treatment
FO2-5
FO2-10
FO2-15
FO2-20
FO2-25
Target Conc.
Irrigation Std
500
450
400
350
300
250
200
150
100
50
0
0
7
14
21
28
35

**Figure 4**


Hybrid Milfoil Percent Occurrrence
35
30
25
20
15
10
5
0
% Occurrence
July Survey
Sept Survey
2007
2008
2009
2010
2011
2012
Year

# INTRODUCTION

The Forest Lake Improvement Association, Inc. (FLIA) contacted with Onterra in 2010 with the intent to conduct a three-year aquatic invasive species (AIS) monitoring project for Forest Lake, Fond du Lac County (Map 1). Eurasian water milfoil (EWM) (*Myriophyllum spicatum*) was first documented in Forest Lake in 1992, and later confirmed by DNA analysis in 2010 as a hybrid between EWM and the indigenous northern water milfoil (*Myriophyllum sibiricum*). Members of the FLIA indicated that EWM had spread and occupied the majority of the lake's littoral areas, matting on the surface and interfering with recreational activities and reducing the lake's aesthetic appeal. The goal of this three-year project (2011-2013) is to reduce the EWM population within Forest Lake to more manageable levels while at the same time minimizing impacts to valuable native aquatic plant species. Reducing the EWM population will alleviate recreational interference and initiate the recovery of the lake's ecological integrity. This report discusses the first year of EWM treatment monitoring under this project.

# 2011 CONTROL STRATEGY DEVELOPMENT

On May 4, 2011, Onterra ecologists visited Forest Lake to conduct an EWM pretreatment survey which consisted of two separate assessments: a whole-lake meander survey and a whole-lake aquatic plant point-intercept survey. The whole-lake meander survey is designed to visually survey as much lake-area as possible with the intent to locate and map EWM. Mapping of EWM in the early spring is not Onterra's standard protocol, and is usually conducted in mid- to late-summer when EWM is at or near its peak growth. However, EWM often overwinters as above-ground biomass and begins growing earlier than our native plants in the spring. While it may be difficult to see from the surface, supplemental use of a submersible video camera allowed for the preliminary mapping survey to be accomplished.

Onterra ecologists located EWM growing throughout most of the littoral areas of Forest Lake (Map 2) by completing numerous transects within littoral areas using a submersible video camera. Five contiguous areas of highly dominant EWM were located from Forest Lake, as well as additional EWM occurrences in almost all other parts of the lake. Based upon Onterra's experience, it was apparent that if these EWM colonies were left untreated, many of them would have reached the surface and formed dense mats by the end of the summer. Just prior to the pretreatment survey, FLIA members showed Onterra staff pictures from the previous year where EWM was flowering at the surface over large portions of the lake.

A whole-lake point-intercept survey was conducted in the spring prior to treatment to quantify the lake-wide occurrence of EWM. Comparing this data to a replicate whole-lake point-intercept survey conducted during the summer immediately following the treatment would allow for a quantitative evaluation of the treatment's efficacy. The whole-lake point-intercept survey methodology is discussed in more detail in the next section.

Herbicides that target submersed plant species are directly applied to the water, either as a liquid or an encapsulated granular formulation. Factors such as water depth, water flow, treatment area size, and plant density work to dilute herbicide concentration within aquatic systems. Understanding concentration-exposure times are important considerations for aquatic herbicides. Successful control of the target plant is achieved when it is exposed to a lethal concentration of the herbicide for a specific duration of time. Much information has been gathered in recent

years, largely as a result of a joint research project between the WDNR and US Army Corps of Engineers (USACE). Based on their preliminary findings, lake managers have adopted two main treatment strategies; 1) whole-lake treatments, and 2). spot treatments.

Spot treatments are a type of control strategy where the herbicide is applied to a specific area (treatment site) such that when it dilutes from that area, its concentrations are insufficient to cause significant affects outside of that area. Spot treatments typically rely on a short exposure time (often hours) to cause mortality and therefore are applied at a much higher herbicide concentration than whole-lake treatments. This has been the strategy historically used on Forest Lake.

Whole-lake treatments are those where the herbicide is applied to specific sites, but when the herbicide reaches equilibrium within the entire volume of water (of the lake, lake basin, or within the epilimnion of the lake or lake basin); it is at a concentration that is sufficient to cause mortality to the target plant within that entire lake or basin. The application rate of whole-lake treatments is dictated by the volume of water in which the herbicide will reach equilibrium with. Because exposure time is so much greater, target herbicide levels for whole-lake treatments are significantly less than for spot treatments.

Whole-lake treatments are typically conducted when the target plant is spread throughout much of the lake. Therefore, a low-dose, whole-lake liquid 2,4-D treatment was proposed for Forest Lake in 2011. This strategy was much different from what the association and herbicide applicator were anticipating. Internal discussions within the FLIA took place with the association deciding to move forward with the proposed strategy as outlined by Onterra. The association also assembled volunteers to aid in the monitoring of the treatment.

Emerging data appears clear that liquid 2,4-D mixes horizontally within the lake, but does not vertically mix into deep areas of the lake during stratification. In other words, 2,4-D would dissipate throughout the upper zone of the lake (epilimnion), but not into the deeper water zones of the lake (metalimnion and hypolimnion).

Current research indicates that whole-lake liquid 2,4-D treatments are effective on EWM when the target whole-lake herbicide concentration is between 0.250 ppm acid equivalent (a.e.) and 0.350 ppm a.e. and is maintained for approximately 14 days. The control strategy developed for Forest Lake included applying liquid 2,4-D over the highly dominant areas of EWM at a dose that would achieve a calculated epilimnion concentration of 0.300 ppm a.e. (Map 2). The target concentration was admittedly a conservative approach that would hopefully cause significant EWM mortality while minimizing impacts to native plant species. Beginning on the lower end of the dose gradient is standard procedure when treating invasives on a system-wide basis. As more is learned about the system, the dose can then be increased as appropriate.

Devising a strategy to attain the target epilimnion concentration is a process that involves two primary objectives: 1) determine the water volume for the herbicide application areas and the entire lake; and 2) properly identify the depth at which stratification is occurring at the time of herbicide application so the volume of the epilimnion can be calculated.

Bathymetry from the 1965 WDNR Lake Survey Map was brought into Geographic Information Systems (GIS) software and digitized. This produced a spatially-rectified map of the lake's

contours with which area-based calculations (acres) could be made (Map 1). Members of the FLIA indicated that the current water level of Forest Lake was lower than normal and likely lower than the 1965 map. Comparisons of the depth measurements collected at each of the spring point-intercept survey locations revealed that the depths in 1965 were a foot or more less than what was observed in 2011. It is believed that the level of water in Forest Lake was approximately 1-2 feet higher in 2011 than in 1965. Thus, the adjusted depths were used to calculate the volume of Forest Lake and the application areas.

On May 23, Onterra staff visited Forest Lake to train FLIA volunteers on collecting temperature/dissolved oxygen profiles and the water samples that would be sent on to the US Army Corps of Engineers (USACE) for 2,4-D residual analysis. During that visit, it was determined that the lake was beginning to stratify (Figure 1). On May 25, Onterra staff again visited Forest Lake to collect temperature/dissolved oxygen profiles. Based on this information, it appeared that the lake was stratifying around 10 feet. Onterra created an initial dosing plan for the treatment and provided the plan to the herbicide applicator for treatment the following day. Prior to the herbicide application on May 26, FLIA volunteers collected a temperature and dissolved oxygen profile. This information was conveyed to Onterra via telephone (Figure 1). These data indicate that the lake was mixing to approximately 12 feet. Onterra re-calculated the application rate based upon that epilimnetic depth (Map 2).

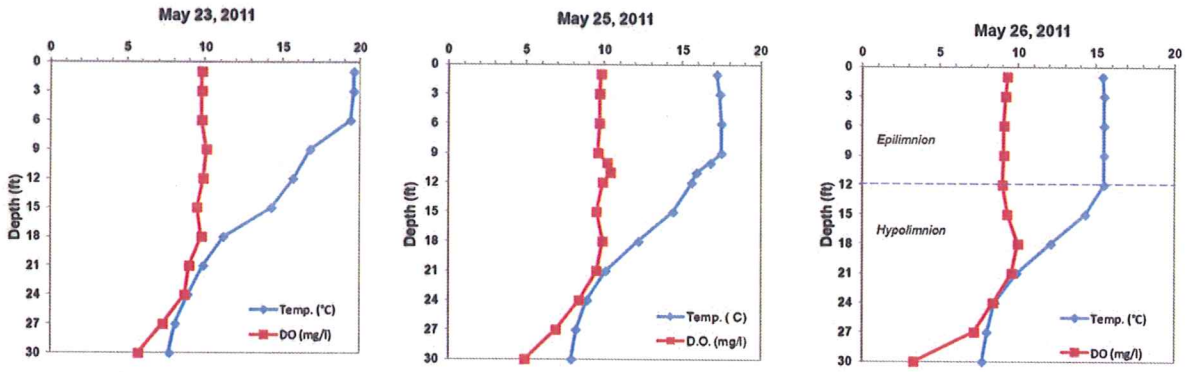



**Figure 1. Temperature/dissolved oxygen profile on Forest Lake.** May 25, 2011.

During the early afternoon of May 26, 2011, Aquatic Biologists Incorporated (ABI) applied liquid 2,4-D to the five treatment sites (19.3 acres) at the rate of 0.600 a.e. to achieve a concentration throughout the epilimnion of 0.300 ppm a.e. (Map 2). The applicator reported 15-25 mph winds out the north-northeast.

## 2011 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 visual data such as EWM colony density ratings before and after the treatments.

As mentioned previously, the whole-lake point-intercept method as described by the Wisconsin Department of Natural Resources (WNDR) Bureau of Science Services (PUB-SS-1068 2010) was used to complete a quantitative evaluation of EWM and native species' occurrences on Forest Lake in the spring prior and the summer immediately following the 2011 treatment. Conducting this survey before and after the treatment allows for a statistical comparison of aquatic plant occurrences and a quantitative determination of treatment efficacy. Based upon guidance from the WDNR, a point spacing of 33 meters was used resulting in 184 points evenly distributed across the lake (Map 1).

Quantitatively, a treatment is deemed successful if the point-intercept data show that the EWM frequency of occurrence following the treatment is reduced by at least a statistically valid 50% ($\alpha = 0.05$). Only those points that fell within the maximum depth of plant growth (littoral zone) are used in the analysis.

Spatial data reflecting EWM locations were collected using a sub-meter Global Positioning System (GPS) before and after the 2011 treatment. Comparisons of the survey results are used to qualitatively evaluate the 2011 herbicide treatment on Forest Lake.

It is never the intent of the treatments to impact valuable native aquatic plant species. While product labeling indicates that 2,4-D is selective towards broad-leaf (dicot) species at the concentration and exposure times used during the 2011 treatment on Forest Lake, emerging conclusions from the WDNR and US Army Corps of Engineers (USACE) suggests that some narrow-leaf (monocot) species may also be impacted by this herbicide. Although the point-intercept survey conducted prior to the treatment in the spring of 2011 did not record native species' occurrences, the same survey was conducted in the summer of 2008 and will allow for an assessment of the treatment's possible impacts to the native aquatic plant community.

The 2011 whole-lake treatment on Forest Lake was selected to participate in a residual herbicide monitoring project being conducted by the WDNR and USACE. Water samples were collected at five sites at various depths located around the lake both within and outside of herbicide application areas by a Forest Lake volunteer. Samples were collected over a time period from 0 to 34 days after the treatment and were properly fixed and sent to the USACE laboratory for analysis.

## 2011 TREATMENT RESULTS

On August 19, 2011, Onterra ecologists visited Forest Lake to complete the post-treatment assessment of the 2011 treatment. Map 3 displays the results of the mid-August 2011 peak-biomass survey. Comparisons of Map 2 and Map 3 show that the EWM control strategy was quite effective. It must be noted that these comparisons are from spring to summer; and had the EWM been mapped in the summer of 2010, when it was at its peak growth, these reductions in density following the treatment would likely have been more drastic.

Prior to the treatment in early May of 2011, 26.9% of the 163 littoral point-intercept sampling locations contained EWM compared to 10.4% following the 2011 treatment, representing a statistically valid reduction in EWM littoral occurrence of 61.3% and exceeding the quantitative success criteria (50% reduction in occurrence) (Figure 2). Interestingly, during the WDNR's point-intercept survey in 2008, EWM was found to have a littoral frequency of occurrence of only 1.2%.

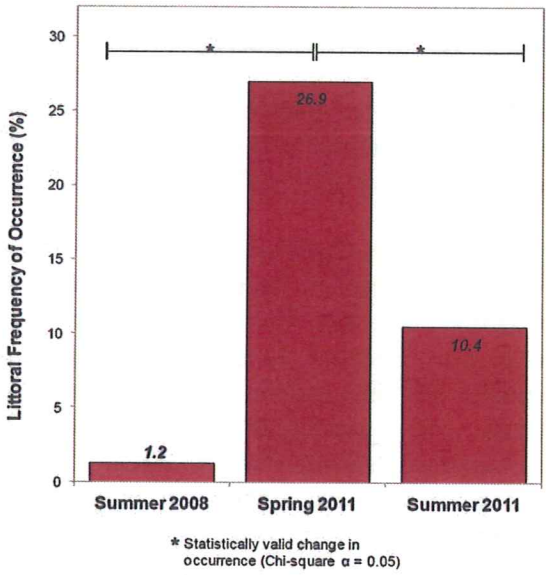



**Figure 2. EWM littoral frequency of occurrence from 2008 and 2011 surveys.** Created using data from 2008 WDNR and 2011 Onterra point-intercept surveys.

As discussed previously, a rake-fullness rating of 1-3 was used to determine the abundance of EWM at each point-intercept location. While EWM density is naturally lower in the spring, Figure 3 shows that not only did the occurrence of EWM decrease following the 2011 treatment, but so did the density as represented by the decline in the rake-fullness category of 2. Again, had the pretreatment point-intercept survey been conducted in the summer of 2010, when the EWM would be at peak-biomass, there would likely have been more rake-fullness ratings of 2 and 3.

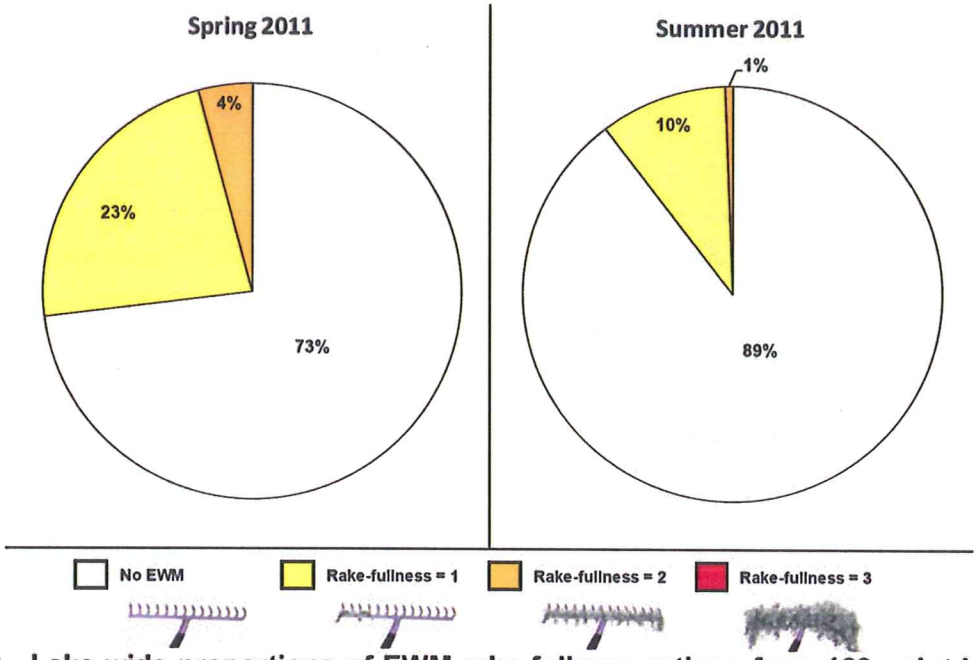



**Figure 3. Lake-wide proportions of EWM rake fullness ratings from 163 point-intercept sampling locations.** Created using data from 2011 pre- and post-treatment point-intercept surveys.

It is important to note that comparing the 2008 and 2011 summer point-intercept datasets will allow for an understanding of the changes in the plant community during that time period; however, the changes may not be solely a result of the 2011 control action. As Figure 2 shows, EWM was at a significantly lower level during the summer of 2008 than it was during the 2011 spring pretreatment survey and it cannot be ruled out that changes in the native plant community may be a result of the incredible increase in occurrence of EWM during that timeframe.

Table 1 shows that two native species, slender naiad and large-leaf pondweed, saw statistically valid reductions in occurrence between the summer of 2008 and spring of 2011. Ongoing research indicates that reductions in these species following whole-lake treatments have occurred. Because slender naiad is an annual plant that reproduces primarily through seed, this species has been shown to rebound on many lakes after whole-lake herbicide treatment programs cease. Six native aquatic plant species including two dicots exhibited statistically valid increases in occurrence from 2008 to 2011 (Table 1). Despite the reductions in occurrence of slender naiad and large-leaf pondweed, it appears Forest Lake's native aquatic plant community was not adversely impacted by the 2011 treatment.

**Table 1. Statistical comparison of Forest Lake's native aquatic plant frequency data from the 2008 WDNR and 2011 Onterra post-treatment point-intercept survey.** Only species with greater than 5.0% frequency of occurrence in at least one of the two surveys are applicable for analysis.

| | Scientific Name | Common Name | 2008 LFOO | 2011 LFOO | Percent Change | Direction | Chi-square Analysis | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Statistically Valid | p-value |
| Dicots | *Ceratophyllum demersum* | Coontail | 21.5 | 38.0 | 77.1 | ▲ | Yes | 0.001 |
| | *Nymphaea odorata* | White water lily | 1.8 | 9.2 | 400.0 | ▲ | Yes | 0.004 |
| | *Myriophyllum sibiricum* | Northern water milfoil | 4.9 | 1.2 | -75.0 | ▽ | No | 0.054 |
| Non-dicots | *Najas flexilis* | Slender naiad | 52.8 | 19.0 | -64.0 | ▼ | Yes | 0.000 |
| | *Potamogeton amplifolius* | Large-leaf pondweed | 17.8 | 0.6 | -96.6 | ▼ | Yes | 0.000 |
| | *Potamogeton gramineus & illinoensis* | Variable and Illinois pondweed | 17.8 | 36.2 | 103.4 | ▲ | Yes | 0.000 |
| | *Chara spp.* | Muskgrasses | 13.5 | 26.4 | 95.5 | ▲ | Yes | 0.004 |
| | *Najas guadalupensis* | Southern naiad | 10.4 | 18.4 | 76.5 | ▲ | Yes | 0.040 |
| | *Vallisneria americana* | Wild celery | 3.1 | 13.5 | 340.0 | ▲ | Yes | 0.001 |
| | *Potamogeton strictifolius* | Stiff pondweed | 35.0 | 34.4 | -1.8 | | No | 0.907 |
| | *Potamogeton zosteriformis* | Flat-stem pondweed | 12.9 | 19.6 | 52.4 | △ | No | 0.099 |
| | *Stuckenia pectinata* | Sago pondweed | 7.4 | 3.7 | -50.0 | | No | 0.146 |
| | *Nitella spp.* | Stoneworts | 6.1 | 9.2 | 50.0 | △ | No | 0.298 |

2008 & 2011 N = 163
LFOO = Littoral Frequency of Occurrence
▲ or ▼ = Change Statistically Valid (Chi-square; α = 0.05)
△ or ▽ = Change Not Statistically Valid (Chi-square; α = 0.05)

## *2011 Residual monitoring*

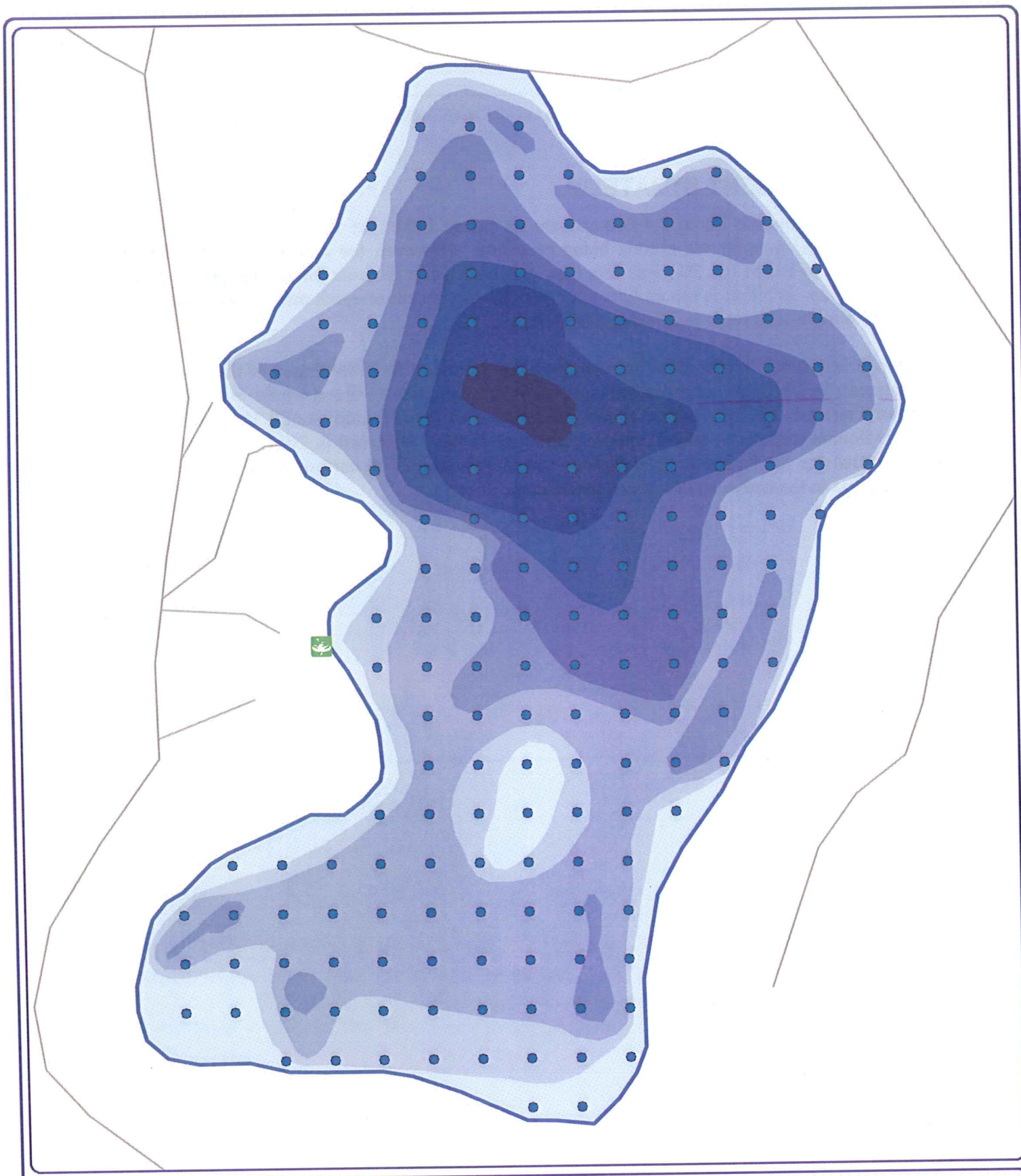
As indicated on Figure 1, the epilimnion of Forest Lake extended to 12 feet deep at the time of the treatment and the herbicide was applied to the lake such that this zone would have a target concentration of 0.300 ppm a.e. Herbicide residual analysis indicates that from 0 to 7 days after treatment, the average 2,4-D concentration was 0.290 ppm a.e. from samples collected in 10 feet of water or less. Average 2,4-D concentrations collected at 15 feet from 0 to 7 days after treatment were 0.155 ppm a.e. This may indicate that the lake continued to stratify deeper than 12 feet of water. Average 2,4-D concentrations at depths 20 feet and greater were significantly less than 0.050 ppm a.e. Appendix A contains the USACE draft report with more detail regarding the residual sampling study on Forest Lake. Please note that the draft report indicates

the incorrect treatment date. Also, the table on the map included as Figure 1 was revised and is properly listed on Map 2.

## 2012 TREATMENT STRATEGY

The 2011 large-scale liquid 2,4-D treatment on Forest Lake met with success, with both the qualitative and quantitative criteria being exceeded. However, the reduction in the lake-wide occurrence of EWM (61.3%) was less than expected given the concentration/exposure time of the herbicide. While it is too early to state definitively, recent research may indicate that hybrid milfoil is more tolerant to herbicides, particularly 2,4-D.

Though less dense, EWM is still spread throughout most of the littoral area of the lake, so spot treatments in 2012 are not a realistic option. It is recommended that a whole-lake approach be utilized again in 2012, but with a slightly higher lake-wide concentration of 0.350 ppm a.e. (Map 3). Minimal impacts to native aquatic plant species were observed in 2011, and it is believed that increasing the herbicide dose slightly will yield better EWM control while still minimizing its impacts to the native aquatic plant community.



N
W E
S

275
Feet

Onterra LLC
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920.338.8860
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Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR, digitized by Onterra
Map Date: January 25, 2012
Filename: *Map1_ForestFDL_Location.mxd*

Project Location in Wisconsin

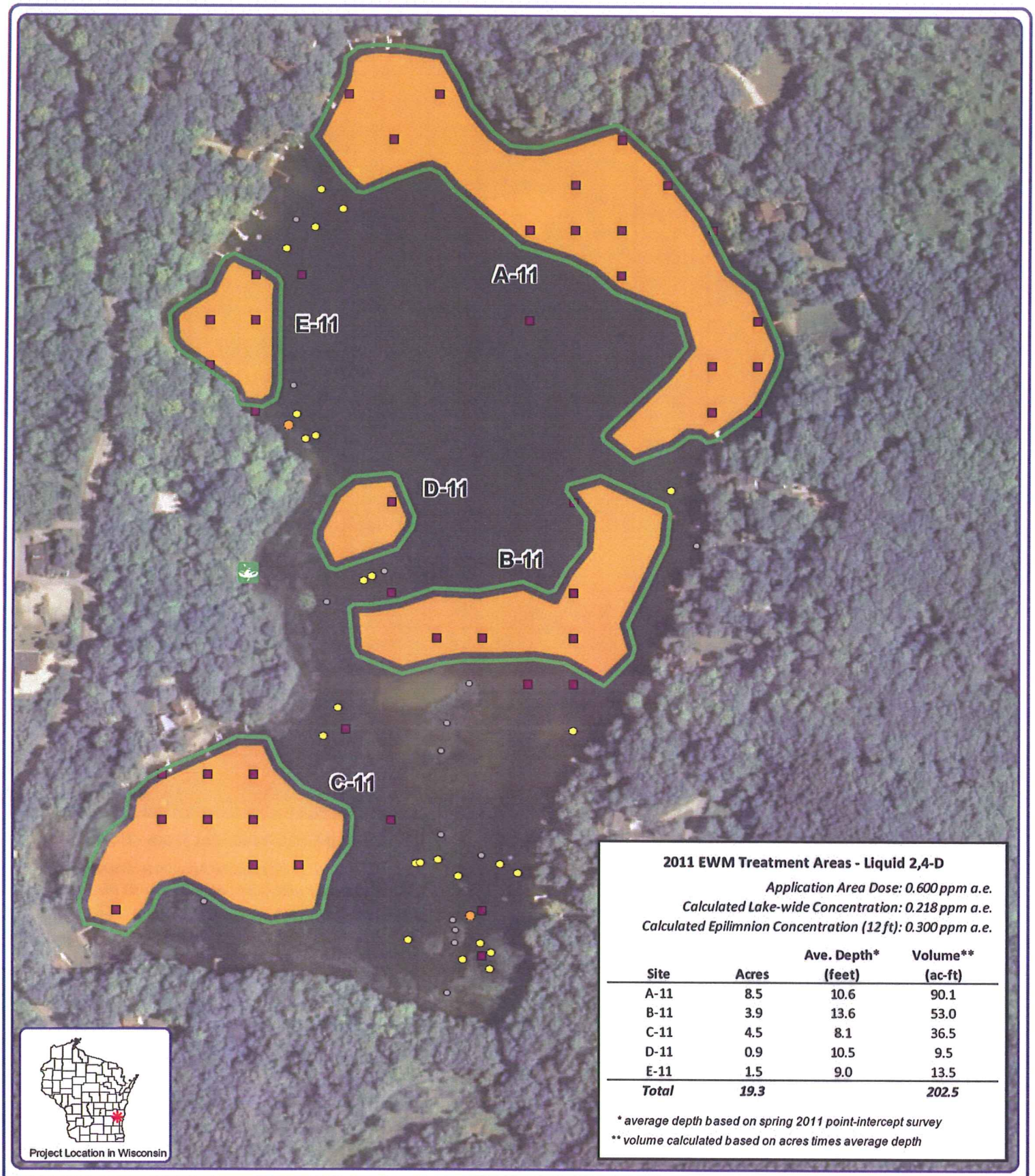
**Legend**

- Forest Lake ~51 acres
- Point-intercept Survey Location
  *33-meter spacing - 184 total points*
- Carry-in Access

Map 1

Forest Lake
Fond du Lac County, Wisconsin

**Project Location & Lake Boundaries**




**2011 EWM Treatment Areas - Liquid 2,4-D**

*Application Area Dose: 0.600 ppm a.e.*
*Calculated Lake-wide Concentration: 0.218 ppm a.e.*
*Calculated Epilimnion Concentration (12 ft): 0.300 ppm a.e.*

| Site | Acres | Ave. Depth* (feet) | Volume** (ac-ft) |
|---|---|---|---|
| A-11 | 8.5 | 10.6 | 90.1 |
| B-11 | 3.9 | 13.6 | 53.0 |
| C-11 | 4.5 | 8.1 | 36.5 |
| D-11 | 0.9 | 10.5 | 9.5 |
| E-11 | 1.5 | 9.0 | 13.5 |
| ***Total*** | ***19.3*** | | ***202.5*** |

** average depth based on spring 2011 point-intercept survey*
*** volume calculated based on acres times average depth*

Project Location in Wisconsin

290
Feet

**Legend**

- Highly Scattered *(None)*
- Scattered *(None)*
- Dominant *(None)*
- Highly Dominant
- Surface Matting *(None)*
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- PI Location with EWM *(May 2011)*
- 2011 Final Treatment Area

Onterra LLC
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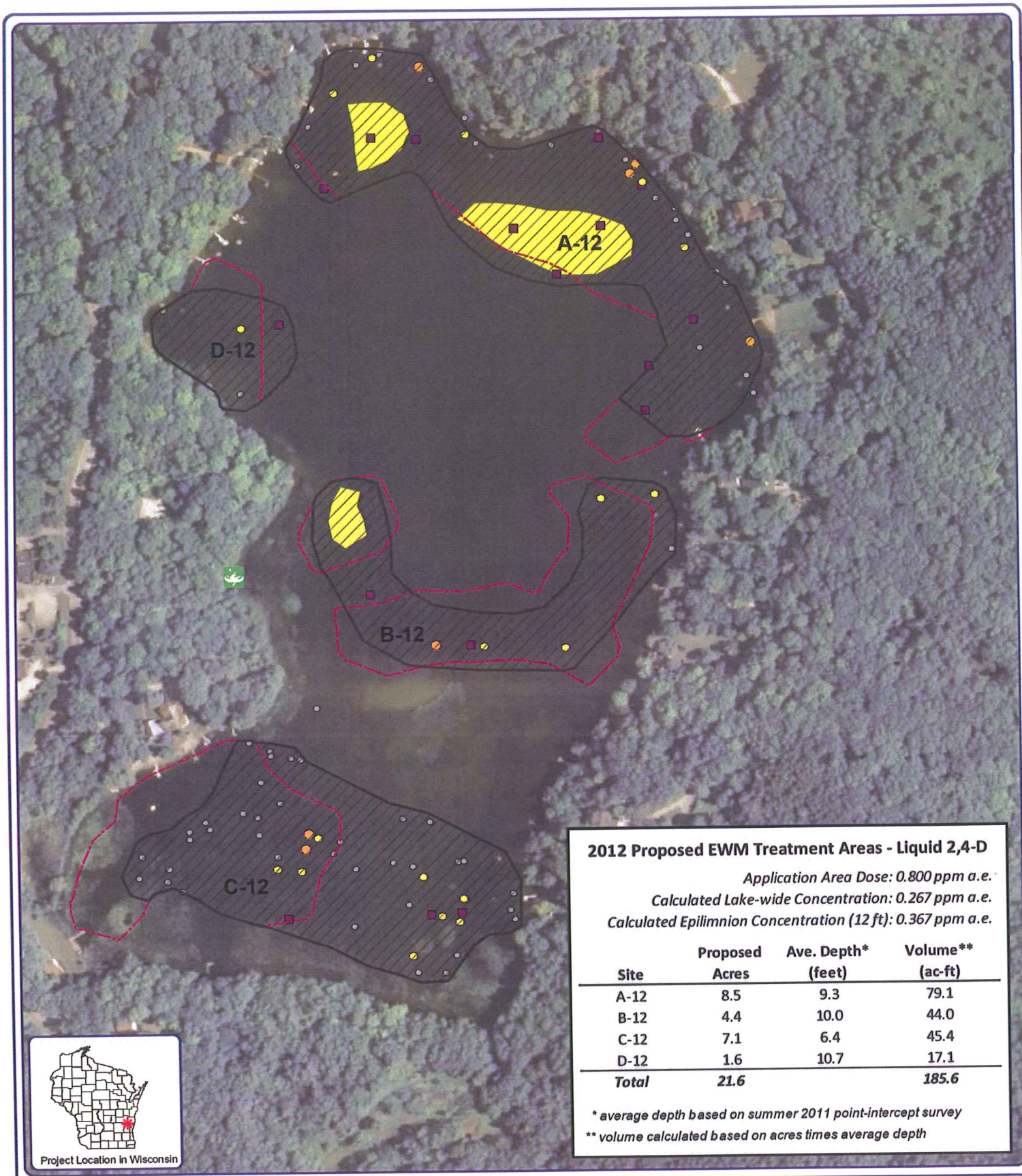
**Sources:**
Hydro: WDNR
Orthophotography: NAIP, 2010
Aquatic Plants: Onterra, 2011
**Map Date:** January 25, 2012
**Filename:** *Map2_ForestFDL_EWM_Spring11FB_T2011.mxd*

# Map 2
## Forest Lake
Fond du Lac County, Wisconsin
## Spring 2011 EWM Locations & 2011 Treatment Areas




## 2012 Proposed EWM Treatment Areas - Liquid 2,4-D

*Application Area Dose: 0.800 ppm a.e.*
*Calculated Lake-wide Concentration: 0.267 ppm a.e.*
*Calculated Epilimnion Concentration (12 ft): 0.367 ppm a.e.*

| Site | Proposed Acres | Ave. Depth* (feet) | Volume** (ac-ft) |
|---|---|---|---|
| A-12 | 8.5 | 9.3 | 79.1 |
| B-12 | 4.4 | 10.0 | 44.0 |
| C-12 | 7.1 | 6.4 | 45.4 |
| D-12 | 1.6 | 10.7 | 17.1 |
| ***Total*** | ***21.6*** | | ***185.6*** |

** average depth based on summer 2011 point-intercept survey*
*** volume calculated based on acres times average depth*




N
W E
S

300
Feet

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920.338.8860
www.onterra-eco.com

Sources:
Hydro: WDNR
Orthophotography: NAIP, 2010
Aquatic Plants: Onterra, 2011
**Map Date:** February 13, 2012
**Filename:** *Map3_ForestFDL_EWM_T2012_Cond1.mxd*

### Legend

- Highly Scattered *(None)*
- Scattered *(None)*
- Dominant
- Highly Dominant *(None)*
- Surface Matting *(None)*
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- PI Location with EWM *(August 2011)*
- 2011 Final Treatment Areas
- 2012 Proposed Treatment Areas

# Map 3
## Forest Lake
Fond du Lac County, Wisconsin

## 2011 EWM Locations & Proposed 2012 Treatment Areas v.1

# Draft: Forest Lake, Fond du Lac County, Herbicide Residual Summary, 2011
## 10 November 2011

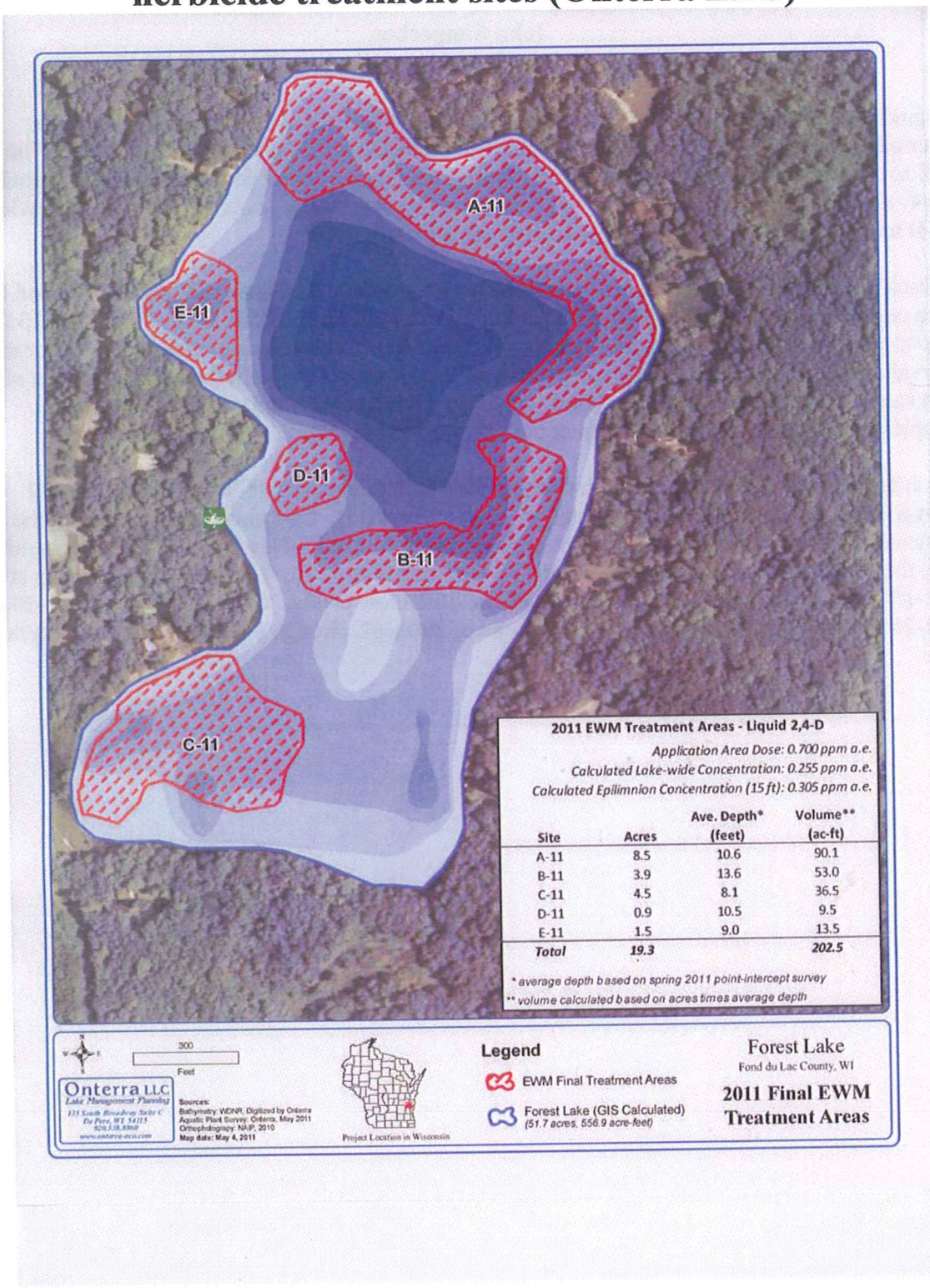
**John Skogerboe**
**US Army Engineer Research and Development Center (ERDC)**

A liquid formulation of 2,4-D was applied to five sites on Forest Lake infested with Eurasian watermilfoil (Figure 1). The herbicide was applied on 18 May 2011 at an application rate of 700 ug/L ae. The herbicide was assumed to completely mix in a very short period of time and degrade slowly due to cold water, and the lake was believed to be stratified at about 15 feet. The lake wide 2,4-D target concentration in the upper 15 feet was 305 ug/L ae.

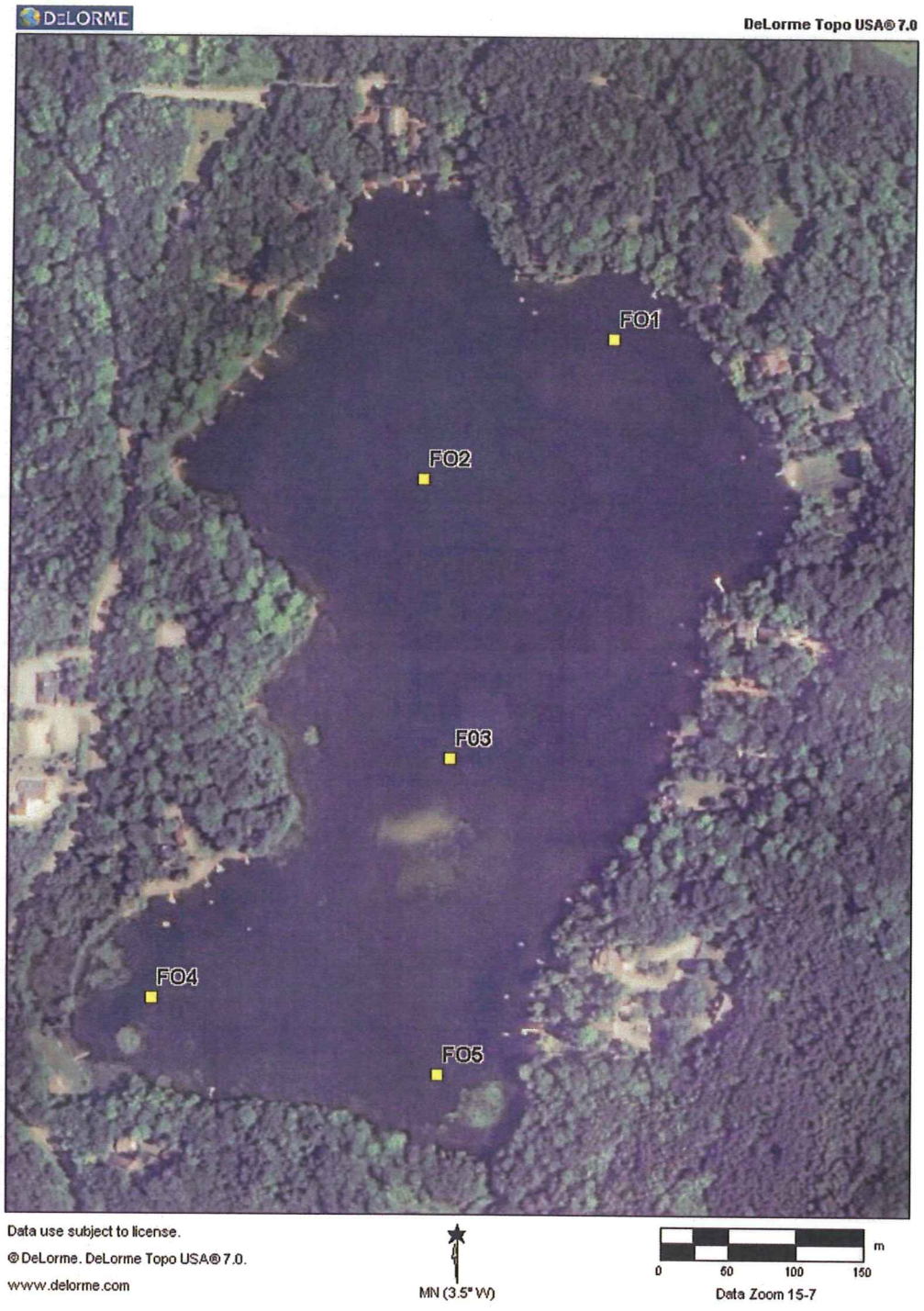
Herbicide residual sample sites were located at five sites (Figure 2). Sites FO1, FO3, FO4, and FO5 were sampled at mid depth, and Site FO2 (deep water site) was sampled at 5, 10, 15, 20, and 25 feet deep to determine the actual depth of mixing. Water residual samples were collected by volunteers at intervals of 0, 1, 3, 6, 9, 13, 20, 27, and 34 days after treatment (DAT). Following completion of each sample interval, 2-3 drops of muriatic acid were added to all samples to fix the herbicide. Samples were then stored in a refrigerator until shipped to the ERDC laboratory for analysis.

The average 2,4-D concentrations at depths less than 10 ft ranged from 178 to 417 ug/L ae and averaged 290 ug/L ae for the time period 0 to 7 DAT (Figure 3). Concentrations remained $\geq$ the irrigation standard of 100 ug/L ae through approximately 31 DAT. Based on the herbicide residual data, the lake did appear to stratify at around 15 feet deep (Figure 4). The mean concentration at FO2-15 was 155 ug/L ae compared to 290 ug/L ae in shallow depths. Concentrations at FO2-20 and FO2-25 were < 50 ug/L ae compared to the mean of 290 ug/L through 7 DAT in shallower depths.

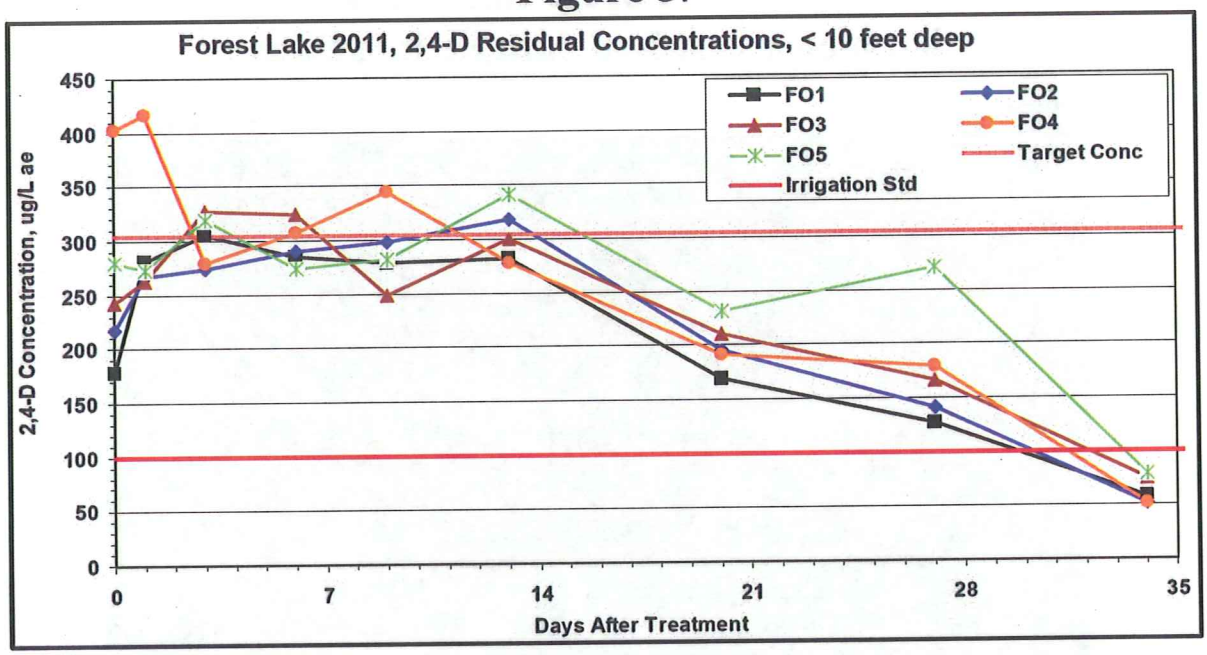
# Figure 1. Forest Lake, 2011 herbicide treatment sites (Onterra LLC)



# Figure 2. Forest Lake, 2011 herbicide residual sample locations



# Figure 3.




# Figure 4.