INTRODUCTION

Photo 1. Kentuck Lake, Vilas-Forest Counties.

Kentuck Lake, Vilas and Forest Counties, is a 957-acre drainage lake with a maximum depth of 40 feet. Kentuck Lake flows through Kentuck Creek into the Brule Creek, and eventually into the Brule River and Menominee River on its way to Lake Michigan.

In 2011, established Eurasian water milfoil (EWM, *Myriophyllum spicatum*) was discovered in the lake. During the summer of 2012, Onterra was contracted by the Kentuck Lake Protection and Rehabilitation District (KLPRD) to conduct EWM surveys on the lake later that summer. GPS coordinates relating to EWM locations were provided to Onterra to aid in theAugust 2012 AIS survey. Unfortunately, Onterra field crews located more EWM on Kentuck Lake than was likely believed to exist by the district. As a result, a preliminary treatment strategy targeting 17.4 acres was proposed for treatment in the spring of 2013 (Map 1). A liquid formulation of 2,4-D was chosen for sites A-13 and B-13, whereas a granular formulation of 2,4-D was chosen for C-13.

In February 2013, with Onterra's guidance, the KLPRD received a Wisconsin Department of Natural Resources (WDNR) Aquatic Invasive Species (AIS) Early Detection and Response (EDR) Grant to cover the costs of a 2 year monitoring and herbicide control project on Kentuck Lake. This report details the activities conducted during the first full year of the project.

PRETREATMENT CONFIRMATION AND REFINEMENT SURVEY

On May 22, 2013, Onterra ecologists conducted the EWM Pretreatment Confirmation and Refinement Survey on Kentuck 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.

During the survey, the EWM appeared brown from the surface, but after getting it up on a rake, it was obvious that it was green and presumed to be actively growing signifying proper timing for the treatment to occur. Based on upon this survey, a few modifications were recommended to the treatment strategy. Sufficient EWM was located lakeward from A-13 to justify extending the treatment area boundaries to encompass these occurrences. Because of this deeper expansion and perhaps because the lake levels were a bit higher than during the late-summer 2012, the average depth of this site was increased from 5 feet to 7 feet for herbicide dosing purposes. The extents of sites B-13 and C-13 were also slightly modified to appropriately target the EWM occurrences found within these sites.

The treatment was conducted by Clean Lakes, Inc. in the early afternoons of June 3, 2013 (A-13 and B-13) and June 5, 2013 (C-13). Clean Lakes, Inc. utilized their LitLine® technology during the liquid 2,4-D treatment which incorporates the use of injection hoses to deliver herbicides deeper into the water column where plants are growing. A conventional broadcast spreader was used during the granular 2,4-D application. The applicator reported a near-surface water temperature of approximately 62-63°F and winds at 3.5-5.5 mph on June 3, 2013 and 7 mph on June 5, 2013.

MONITORING METHODOLOGIES

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

Quantitative Aquatic Plant Monitoring

To monitor the treatment's efficacy, point-intercept sub-sample data are collected within treatment areas in the spring of 2013 prior to the treatment and again in August following the treatment. Prior to the treatment, only the presence of AIS was documented at each location as native aquatic plants are not actively growing at that time. This will allow an understanding if the treatment was effective at reducing EWM occurrence. In Kentuck Lake, quantitative evaluation was made through the collection of data at 104 point-intercept sub-sample locations all located within the areas of EWM where herbicide was directly applied (Figure 3).

Qualitative CLP Monitoring

Using sub-meter GPS technology, EWM locations were mapped during the later-summer the year prior to treatment (August 2012) and again in the late-summer following the treatment (August 2013). The EWM 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*. Based upon a pre-determined success criterion, an effective treatment would include a 75% reduction of HWM as demonstrated by a decrease in two density ratings (e.g. *Highly Dominant* to *Dominant*).

Herbicide Concentration Monitoring

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

Water samples were collected at six sites (Map 1) at time intervals of approximately 1, 2, 4, 6, 8, 24, 48, 72, and 120 hours after treatment (HAT) using an integrated 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 *Kentuck Lake, Forest and Vilas County, 2,4-D Concentration Monitoring Summary, 2013* (October 21, 2013) with more detail regarding the herbicide concentration monitoring sampling study on Kentuck Lake. The information within Appendix A is referenced within the following section.

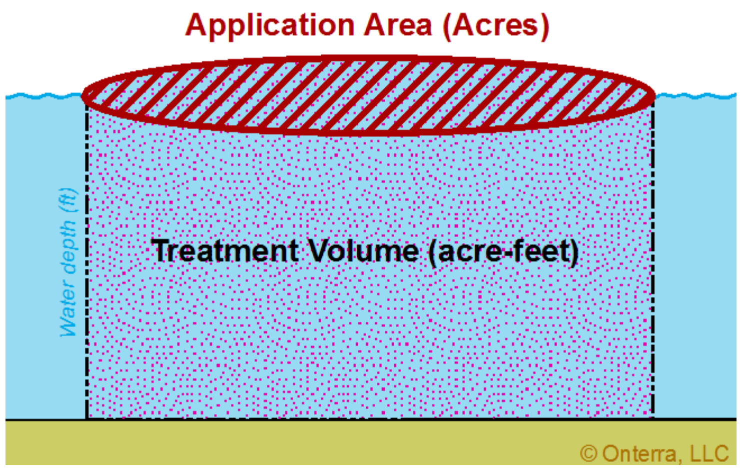


Figure 1. Herbicide Spot Treatment diagram.

Herbicide application rates were formulated volumetrically, targeting 2,4-D at 3.0-4.0 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 1). While lake managers may propose that a particular volumetric dose be used, such as 3-4.0 ppm ae, it is understood that actually achieving 3-4.0 ppm ae within the water column is not likely due to dissipation and other factors. Figures 1 and 4 of Appendix A clearly indicate that in fact target herbicide concentrations within both the granular and liquid application areas never met dosing levels (3-4.0 ppm ae or 3,000-4,000 ug/L ae).

Granular 2,4-D, which is typically used in spot-treatment scenarios, was chosen for site C-13. Ongoing research clearly indicates that the herbicide concentrations and exposure times of large (> 5 acres each) treatment sites are higher and longer than for small sites. Research also indicates that higher herbicide concentrations and exposure times are observed in protected parts of a lake compared with open and exposed parts of the lake. These two factors lead to the use of liquid 2,4-D being applied on sites A-13 and B-13. It was theorized that the close spatial relationship of these two sites would also aid in obtaining sufficient herbicide concentrations and exposure times to cause EWM control. While 2-4-D concentrations where higher within each of the liquid treatment sites for the first few hours after treatment, a uniform herbicide concentration within this bay occurred by approximately 6 HAT as evidenced by the results from K1 (Figure 3 of Appendix A). This confirms that spatial proximity of these sites likely contributed to higher and longer herbicide concentrations being observed within these these sites.

Figure 5 of Appendix A clearly indicates that higher 2,4-D concentrations were achieved in the water column within the liquid treatment sites compared to the granular sites. As discussed above, the granular treatments were conducted in an exposed part of the lake with only a single physical barrier (southern shoreline) keeping the herbicide from diluting horizontally out of the application area. The liquid treatments were conducted within a more-protected site that had physical barriers (shorelines) that aided in keeping the herbicide from migrating out of this location. From information gathered to date on similar treatments, the protective nature and large size of the liquid treatment sites are likely the primary reasons why herbicide concentrations were higher in these areas compared with the granular site.

That being said, ongoing research is not able to demonstrate that granular herbicides maintain higher herbicide concentrations in spot treatment scenarios, and may actually be showing the opposite. 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 in herbicide measurements within the water column. Preliminary data indicate that surprisingly high 2,4-D concentrations exist near the sediment-water interface (porewater) in association with granular treatments. Some herbicide applicators and industry professionals have observed what has been interpreted as successful granular treatments in instances where measured herbicide concentrations within the water column would have suggested otherwise. In these cases, it has been theorized that the higher porewater 2,4-D concentrations have been the mechanism that caused the successful treatment. However, it is not known if there is a mode of action for the EWM plants to uptake the herbicide at this location within the water column, away from the foliage which is suspected as being the primary uptake location. Ongoing research is occurring on this topic.

Aquatic plant Monitoring Results

On August 14, 2013, Onterra ecologists visited Kentuck Lake to complete the post-treatment assessments of the 2013 treatment. This included conducting the point-intercept sub-sampling survey, as well as mapping the EWM occurrences within the lake.

Efficacy

The summer 2012 EWM Peak-Biomass Survey indicated approximately 13.4 acres of the lake contained colonized areas of EWM, all of which were targeted by the spring 2013 herbicide control strategy (Figure 2). A small number of additional EWM occurrences were mapped in other parts of the lake using point-based methodologies. It must be noted that the water clarity of Kentuck Lake is quite low in the latter part of the summer and it is acknowledged that some EWM occurrences could have escaped detection. Late-summer Secchi disk transparency values were less than 5 feet during both August 2012 and August 2013 surveys.

The post treatment mapping surveys conducted during the late-summer of 2013 indicated that the EWM population was greatly impacted by the 2013 treatment. During an early-July site visit, Onterra noted a few decrepit-looking plants within the eastern part of A-13. These plants were also observed by trained KLPRD members during informal scuba surveys of this area. By late-August, these plants could not be observed from the surface and did not come up on any of the numerous rake tows conducted in this area as a part of the quantitative sub-sampling effort.

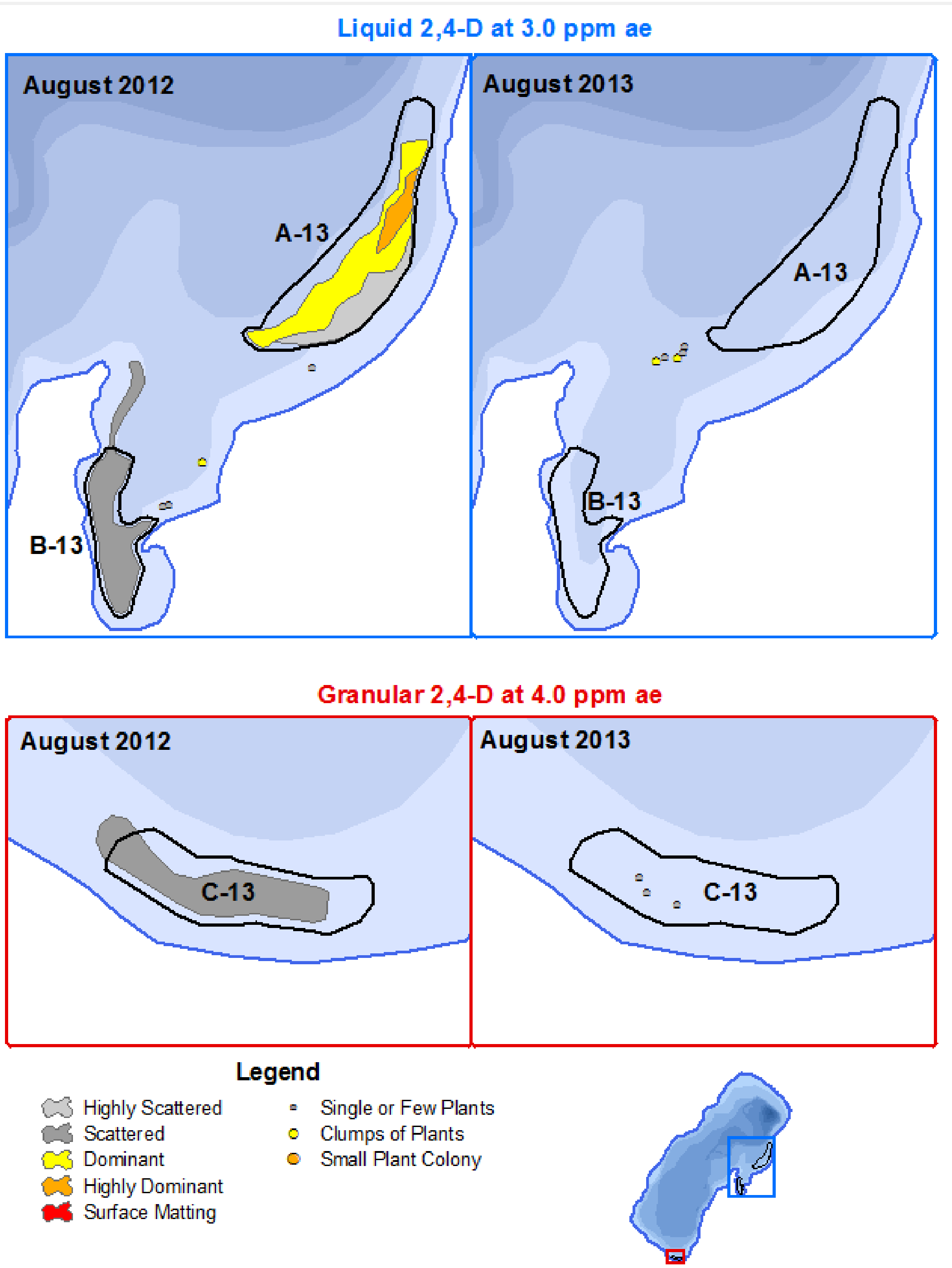


Figure 2. Qualitative EWM monitoring results.

During the spring prior to the 2013 2,4-D treatment, EWM within the Kentuck Lake treatment sites had a pretreatment frequency of occurrence of 24.8% (Figure 3). During the August 2013 post treatment point-intercept sub-sample survey, EWM decreased to a frequency within the treatment sites of 2.8% (4 occurrences), resulting in a statistically valid 88.5% reduction. This indicates that quantitatively, the 2013 control strategy met the predetermined success criteria by exceeding a 50% reduction.

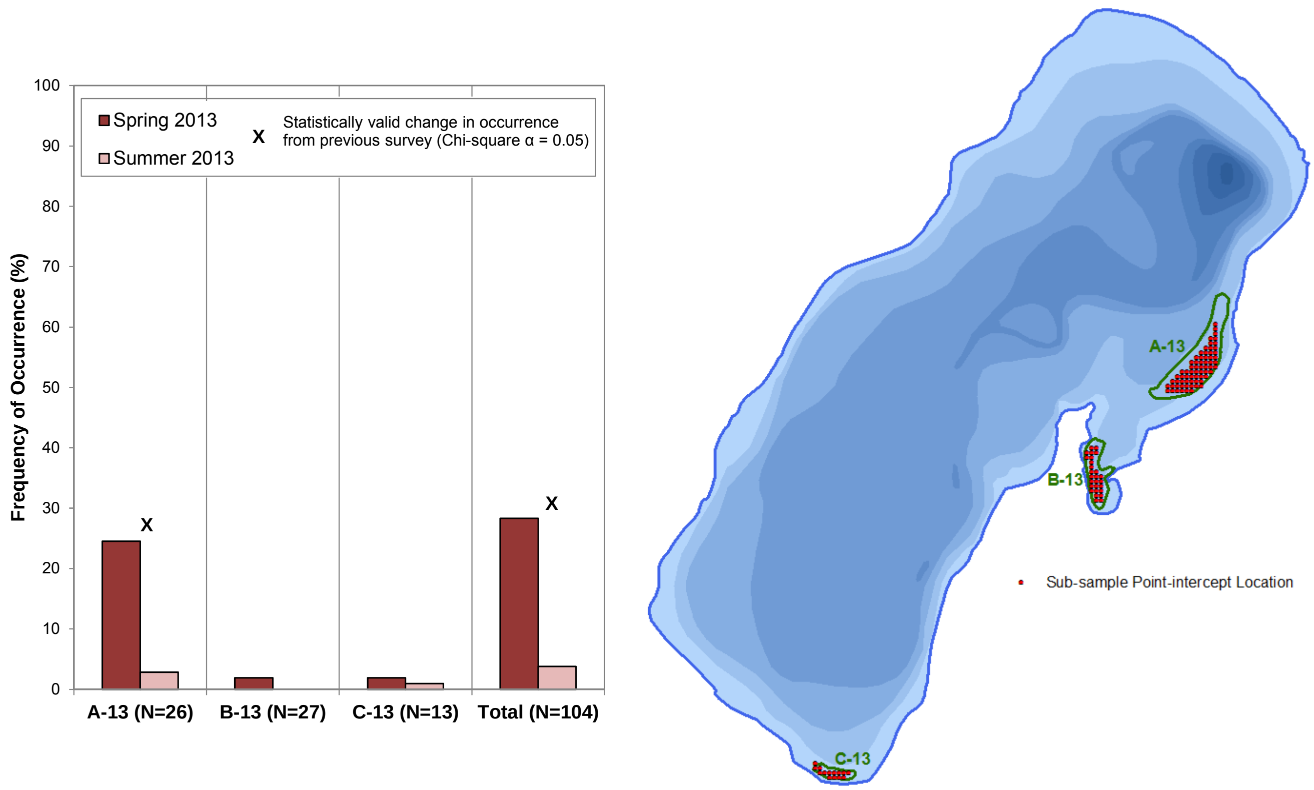


Figure 3. Quantitative AIS Monitoring Results.

2013 CLP Monitoring Results

Curly-leaf pondweed (CLP, *Potamogeton crispus*) was first documented in Kentuck Lake in 1999, but according to the Kentuck Lake Protection and Rehabilitation District (KLPRD), had not been located within the lake again until July of 2012. During the early-summer of 2012, the KLPRD observed a few CLP occurrences within Kentuck Lake, but an accurate understanding of where in the lake the CLP was located from did not exist.

An Early Season AIS (ESAIS) Survey was planned to occur during late-June 2013 to coincide with the peak growth stage of CLP. However, scheduling conflicts due to weather conditions forced Onterra to conduct this survey on July 3, 2013. The conditions were ideal for the survey: full sun, 75°F, and calm winds.

As shown on Map 2, a small number of CLP *Single or Few Plants* and *Clumps of Plants* were observed during this survey. The CLP plants appeared healthy and indicated no signs that the survey was conducted too late in the year. The current CLP population within Kentuck Lake is not forming dense colonies that are negatively impacting the ecology nor the recreational use of the lake. Particularly in northern Wisconsin, not all established CLP populations become problematic to the lake ecosystem. While the CLP may not be causing devastating impacts to Kentuck Lake, that may not hold true for other nearby lakes that may inherit CLP that originated in Kentuck Lake from hitch-hiking on transient watercraft. Within Kentuck Lake's current management planning project, it will be important to develop strategies to continually monitor the CLP population within Kentuck Lake as well as implement strategies to limit its spread to other area waterbodies.

CONCLUSIONS AND DISCUSSION

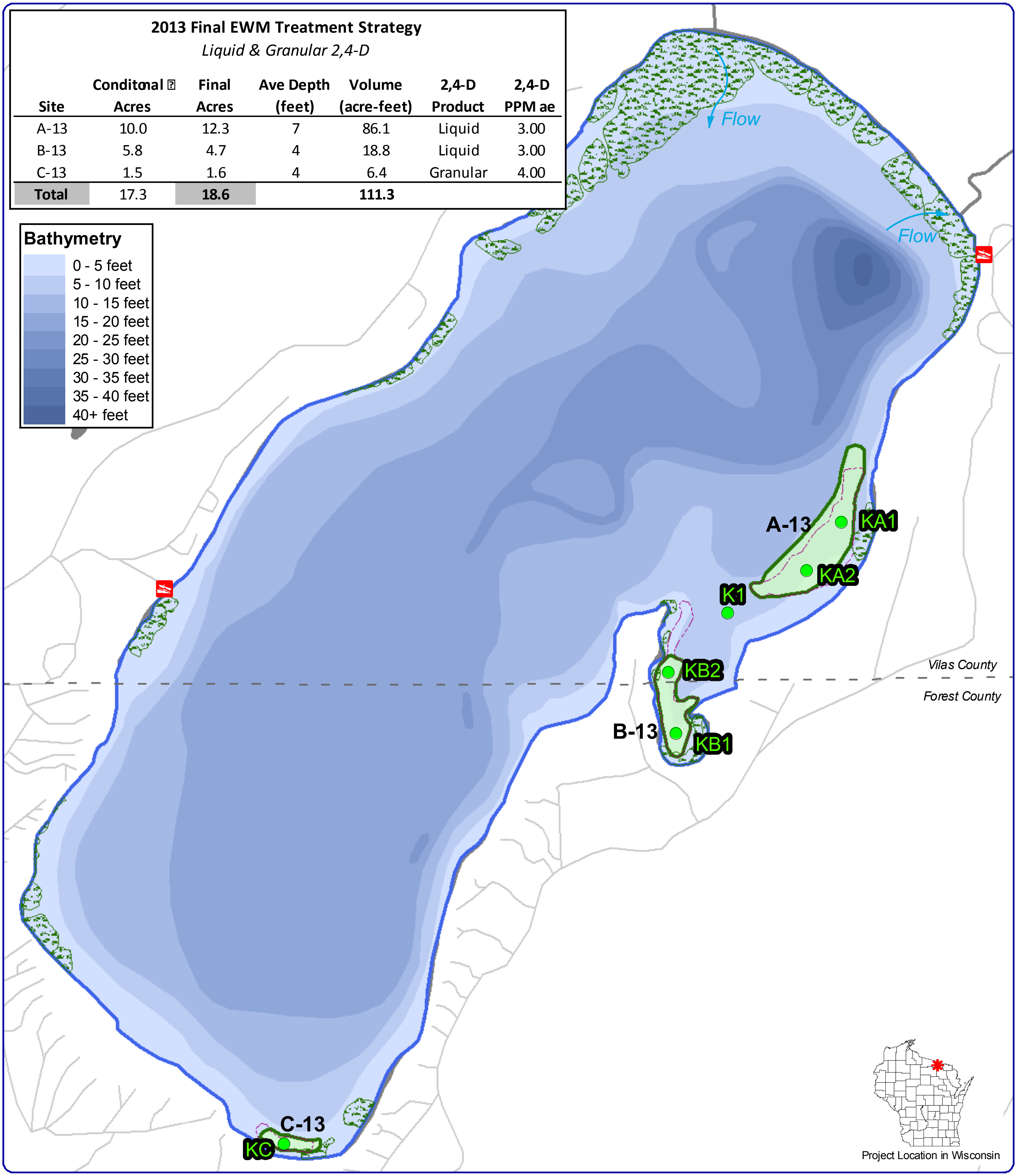
All indications point towards a successful EWM treatment on Kentuck Lake in 2013. Based upon the volunteer-based herbicide concentration monitoring data, herbicide concentrations appeared to be sufficiently high within A-13 and B-13, but slightly lower in the smallest granular treatment site, C-13. Post treatment assessments located little EWM within the treatment sites following the treatment, and the point-intercept sub-sampling data indicated an 88.5% decline in EWM frequency of occurrence within these areas. Continued qualitative monitoring of these sites in 2014 will allow an understanding if the EWM was completely controlled within these sites or if only greatly injured by the 2013 treatment and the population rebounds in 2014 from unaffected root crowns.

Prior to the August 2013 EWM survey, KLPRD members provided GPS location data regarding EWM occurrences within the lake. With these location loaded into Onterra's onboard GPS technology, Onterra staff conducted a full meander of Kentuck lake including focused additional attention on these locations. As shown on Map 3, numerous EWM occurrences were located in Kentuck Lake during the August 2013 survey. Although more wide-spread that thought to have existed, the EWM population within Kentuck Lake was found to be extremely sparse and of low density. Only two small colonies of *Highly Scattered* and *Scattered* EWM were located, totaling less than a third of an acre. As discussed, conducting an effective herbicide control project on small sites can be extremely challenging and the results can be unpredictable. Conducting herbicide control strategies on individual plants or even small colonies will not prove effective unless grouped into a much larger treatment site where sufficient herbicide concentrations and exposure times are more likely to be achieved. The EWM population within Kentuck Lake is currently at too low of levels for herbicide control methods to be effective. Within the upcoming management planning effort, the KLPRD will be able to use the knowledge gained during this project to develop specific thresholds (triggers) that would guide future management actions including herbicide control, volunteer-based hand-harvesting, and professional hand-harvesting methods.

2013 Final EWM Treatment Strategy

Liquid & Granular 2,4-D

Site	Conditonal Acres	Final Acres	Ave Depth (feet)	Volume (acre-feet)	2,4-D Product	2,4-D PPM ae
A-13	10.0	12.3	7	86.1	Liquid	3.00
B-13	5.8	4.7	4	18.8	Liquid	3.00
C-13	1.5	1.6	4	6.4	Granular	4.00
Total	17.3	**18.6**		**111.3**		



N
W E
S

1,200

Feet

Legend

2013 Conditional Treatment Strategy

2013 Final EWM Treatment Strategy

Herbicide Concentration Monitoring Location

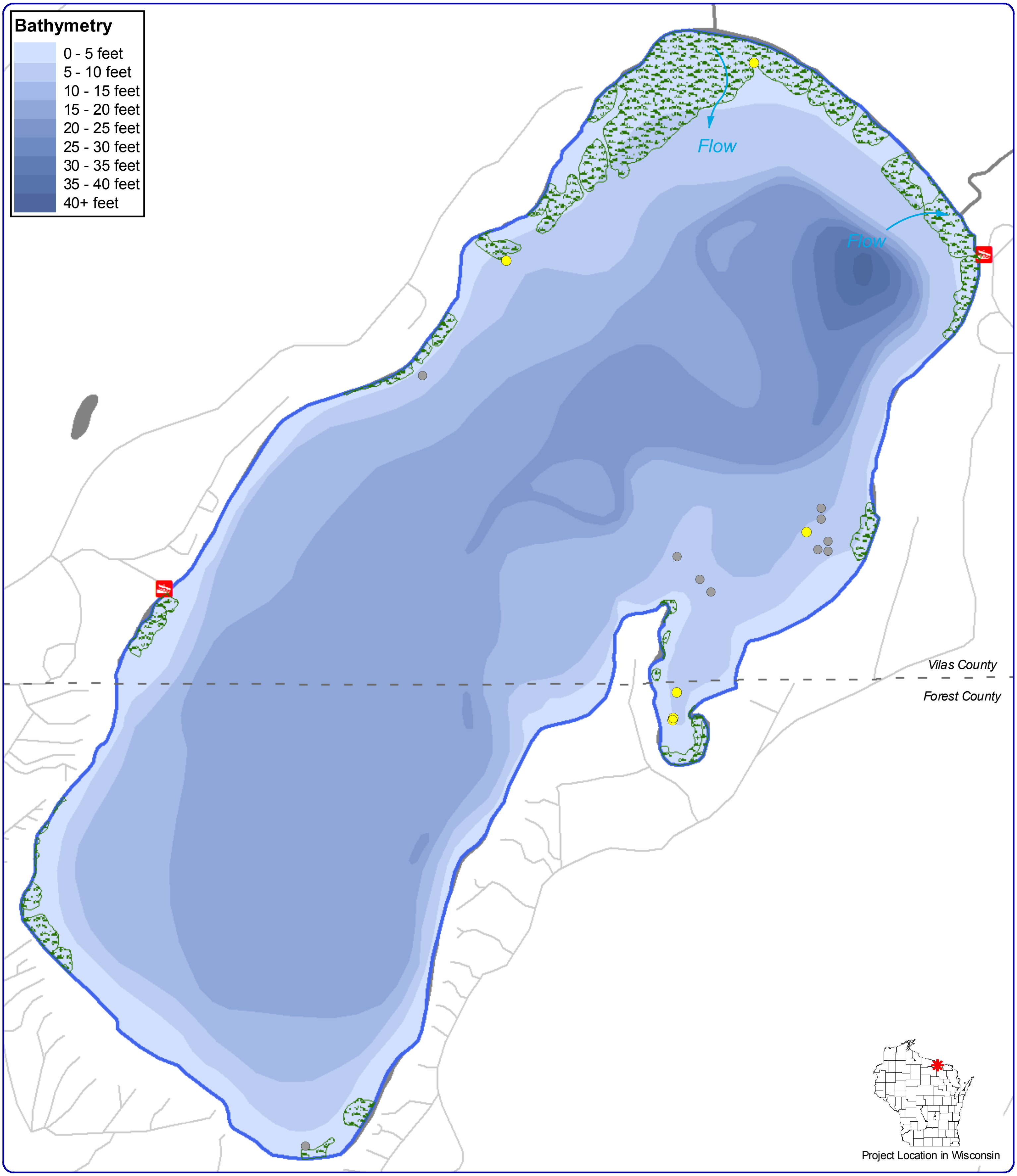
Floating-leaf and/or Emerget Plant Community

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

Sources:
Roads and Hyrdo: WDNR
EWM Survey: Onterra, 2012-13
Bathymetry: WDNR, digitized by Onterra
Map Date: May 23, 2013
Filename: *Map1_Kentuck_EWM_T2013Perm1.mxd*

Map 1
Kentuck Lake
Forest County, Wisconsin

2013 Final EWM Treatment Plan



N W E S

1,200

Feet

Legend

CLP Locations ***(Onterra, 7/03/13)***

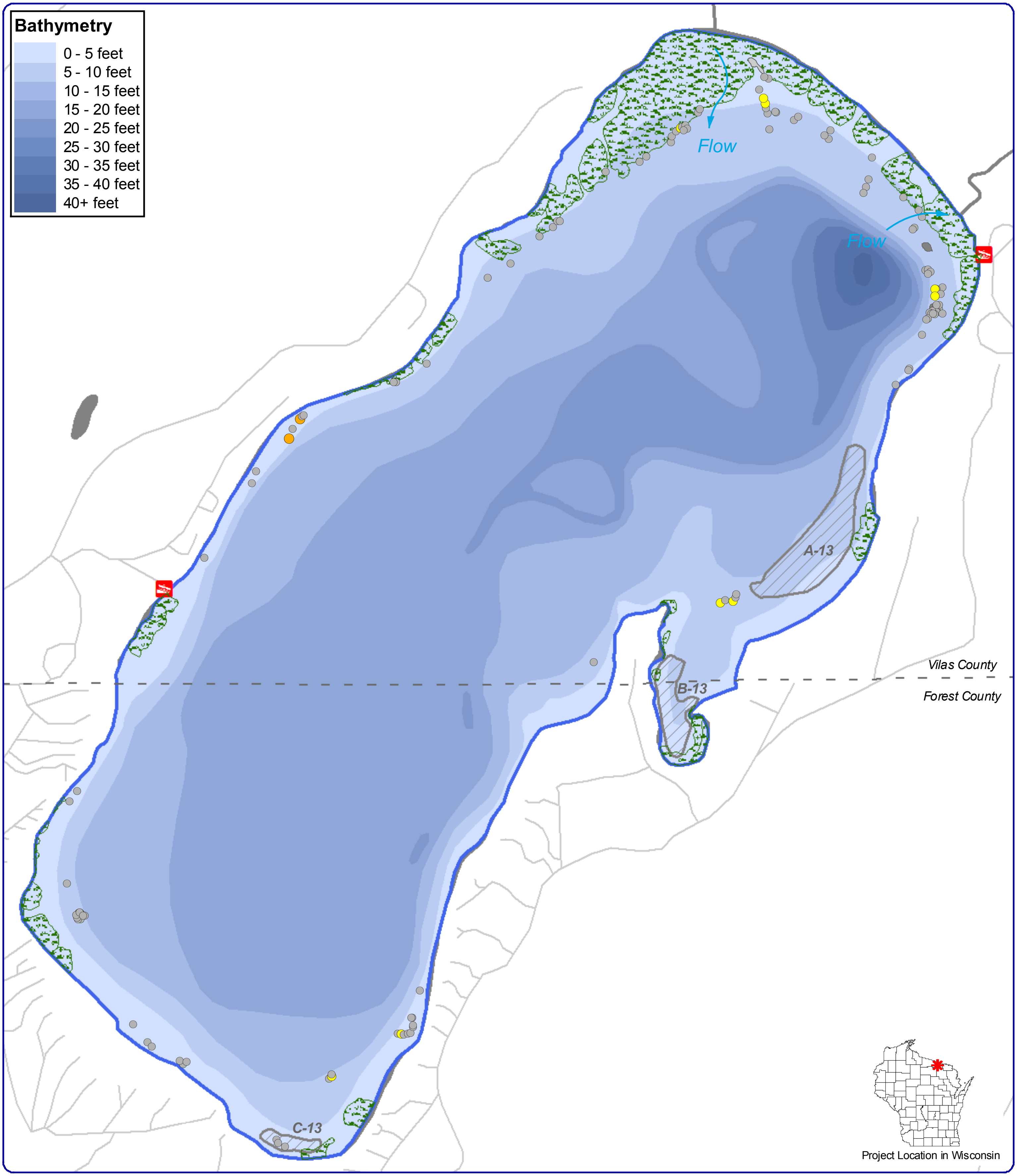
- Single or Few Plants
- Clump of Plants
- Small Plant Colony *(none)*

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

Sources:
Roads and Hyrdo: WDNR
Aquatic Plant Survey: Onterra, 2013
Batymetry: WDNR, digitized by Onterra
Map Date: July 8, 2013
Filename: *Map2_Kentuck_ESAIS_July13.mxd*

Map 2
Kentuck Lake
Vilas-Forest County, Wisconsin

2013 CLP
Survey Results



N
W E
S
1,200
Feet

Legend

Highly Scattered
Scattered
Dominant *(None)*
Highly Dominant *(None)*
Surface Matting *(None)*

Single or Few Plants
Clumps of Plants
Small Plant Colony

2013 EWM Treatment Area

Floating-leaf and/or Emergent Plant Community

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

Sources:
Roads and Hyrdo: WDNR
Aquatic Plant Survey: Onterra, 2013
Batymetry: WDNR, digitized by Onterra
Map Date: July 8, 2013
Filename: *Map2_Kentuck_ESAIS_July13.mxd*

Map 3
Kentuck Lake
Vilas-Forest County, Wisconsin

August 2013 EWM Survey Results