INTRODUCTION

Eurasian water milfoil (EWM, *Myriophyllum spicatum*) was first documented in Long Lake in 2000. Since 2008, the Long Lake of Phelps Lake District (LLPLD) has been actively managing and reducing the EWM population through strategically targeted herbicide spot treatments. With assistance from Onterra, the LLPLD was successfully awarded a Wisconsin Department of Natural Resources (WDNR) Aquatic Invasive Species (AIS) Established Population Control Grant in February 2013 to aid in funding the management of EWM within Long Lake from 2013-2017.

During the 2012 Late-Summer EWM Peak-Biomass Survey, Onterra ecologists located approximately 3.3 acres of colonized EWM within the lake. However, in order to build of the success of the previous years' treatments, it was decided that a more aggressive approach to EWM management would be initiated from 2013-2017, where all areas of colonized EWM would be considered for treatment. In addition, this treatment threshold (trigger) would also extend to immediately adjacent areas of EWM mapped with point-based techniques, with areas mapped as *small plant colonies* being targeted if possible. Using this rationale, approximately 48 acres were initially proposed to be treated in the spring of 2013 (Map 1).

PRETREATMENT CONFIRMATION & REFINEMENT SURVEY

On May 29, 2013, Onterra ecologists conducted the EWM Spring Pretreatment Confirmation and Refinement Survey on Long Lake. During this survey, all the proposed treatment sites were visited along with areas treated in 2012 that were not proposed for retreatment. Sufficient EWM warranting treatment was confirmed in all of the originally proposed treatment areas. Treatment sites E-13 and F-13 were expanded slightly to encompass EWM observed outside of the proposed treatment area, while site G-13 was slightly reduced as EWM was not observed within a portion of this site. From this survey, the proposed treatment acreage was increased slightly from 48.3 acres to 49.8 acres (Map 1).

Although the EWM appeared reddish brown from the surface during the May 29 survey, closer examination of the plants revealed that they were green and actively growing. Onterra recommended that the treatment occur as soon as logistically possible. The treatment was conducted by Clean Lake's, Inc. during the morning of June 4, 2013. The applicator reported 2-4.5 mph winds out of the south during the application.

HAND-HARVESTING CONTROL METHODS

During the summer of 2013, the LLPLP contracted with Aquatic Plant Management, LLC to conduct hand-harvesting of EWM in select areas. Professional hand-harvesters first visited the lake in July 2013. Some EWM was removed during this event, but the crew opted to return to the lake in late-August when EWM was higher in the water column and more easily locatable. During the subsequent site visit, they spent the majority of their efforts in the southern portion of treatment site D-13, and also pulled approximately 30 plants from the northern portion of treatment site G-13.

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 AIS colony density ratings before and after the treatments.

Quantitative Aquatic Plant Monitoring

Because the spot treatment strategy was utilized again on Long Lake in 2013, quantitative evaluation methodologies follow WDNR protocols in which point-intercept data are collected within treatment areas both the summer before and the summer immediately following the spring treatment. In Long Lake, quantitative evaluation was made through the collection of data at 101 point-intercept sub-sample locations all located within the areas where herbicide was directly applied (Figure 1). At each of these locations, EWM and native aquatic plant species presence and rake fullness were documented.

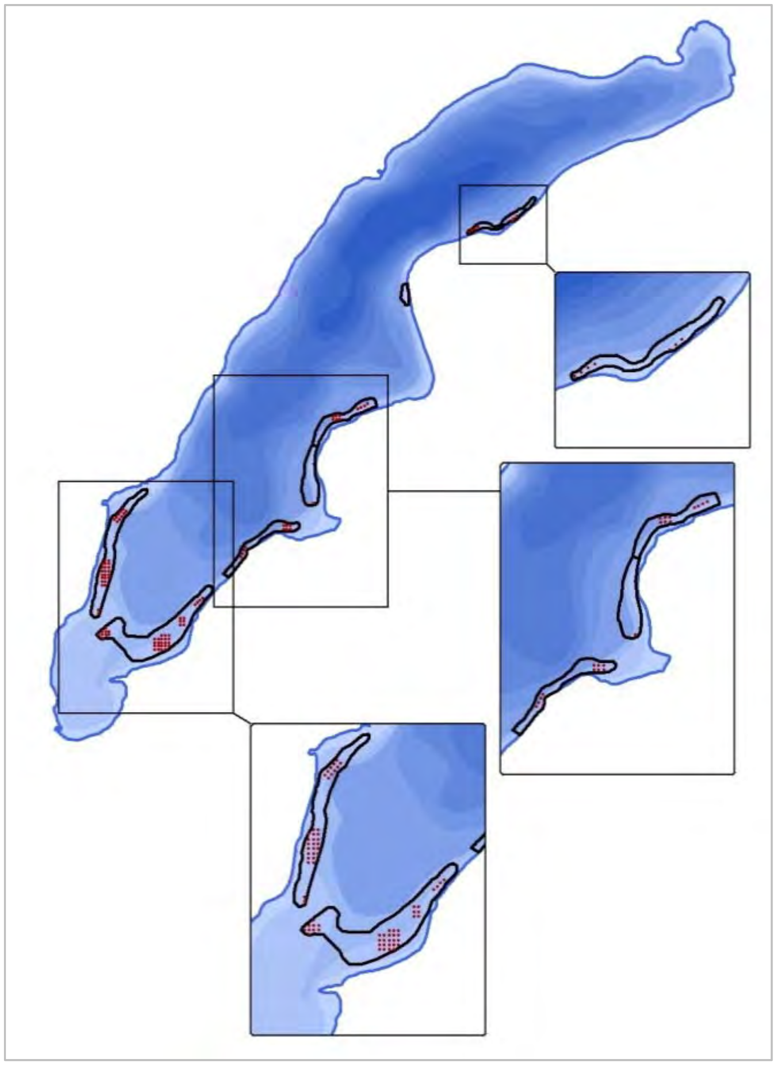


Figure 1. 2013 Quantitative treatment monitoring plan for Long Lake, Vilas County.

Comparing data collected before and after the treatment allows for a statistical comparison of aquatic plant occurrences and a quantitative determination of treatment efficacy within the herbicide application areas. Based upon a pre-determined success criterion, the 2013 herbicide treatment strategy would be deemed effective if the point-intercept data show that the EWM frequency of occurrence within the 2013 treatment sites decreased by at least a statistically valid 50% ($\alpha = 0.05$). It is important to note that changes in aquatic plant frequencies following the herbicide treatment cannot be extrapolated to the lake-wide level, and can only be considered within the areas where herbicide was directly applied and the monitoring occurred.

Qualitative EWM Monitoring

Using sub-meter GPS technology, EWM locations were mapped the year prior to treatment (2012) in late-summer when EWM is at or near its peak growth, and in the late summer immediately following the treatment (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 EWM locations that were considered as *Small Plant Colonies* (<40 feet in diameter), *Clumps of Plants*, or *Single or Few Plants* (Map 1 and 2).

Qualitative monitoring of herbicide treatments includes comparing spatial data reflecting EWM 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 EWM 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 to understand if adequate concentration-exposure times are being met to effectively control the EWM. For this type of monitoring, water samples are collected by trained volunteers from multiple locations within the herbicide application areas over the course of hours following treatment. The samples are fixed (preserved) with acid and shipped to the U.S. Army Engineer Research and Development Center (USAERDC) where the herbicide analysis is completed.

In Long Lake, water samples were collected at seven sites, one in C-13, D-13, F-13, and two in G-13 and E-13 (Appendix A, Figure 2). Water samples were collected by the trained volunteer at time intervals of 1, 2, 4, 6, and 24 hours after treatment.

POST TREATMENT MONITORING RESULTS

Herbicide Concentration Monitoring Results

Appendix A contains the USAERDC draft Long Lake 2,4-D Concentration Monitoring Summary (November 21, 2013) with more detail regarding the herbicide concentration monitoring sampling study on Long Lake. The information within Appendix A is referenced within the following section.

Herbicide application rates were increased in 2013 from rates used in 2012 because results of the 2012 treatments did not meet expectations. The two smallest treatment sites, A-13 and B-13, were applied at maximum label rates for liquid 2,4-D (4.0 ppm ae), as research is indicating herbicide dissipates most rapidly from these smaller sites and a higher concentration is needed to cause EWM mortality. Liquid 2,4-D was applied at a slightly lower rate of 3.5 ppm within the other 2013 treatment areas because these treatment sites were believe to be large enough to maintain a longer exposure time of the herbicide to the EWM plants to cause mortality.

The herbicide concentration data collected from treatment sites C-13 and D-13 indicated the herbicide dissipated from these areas very rapidly. Figure 3 from Appendix A illustrates that herbicide concentration within treatment sites C-13 and D-13 peaked at 0.617 ppm ae and 0.666 ppm ae four hours after treatment (HAT), respectively, and were below the irrigation standard (0.100 ppm ae) by six HAT.

In contrast, the herbicide applied to treatment sites E-13, F-13, and G-13 likely dissipated throughout the entire southern portion of the lake, increasing the exposure time of the herbicide. Appendix A Figure 4 shows that herbicide concentrations within these treatment areas peaked between two and six HAT, and the concentrations remained above the irrigation standard at 24 HAT.

Aquatic Plant Monitoring Results

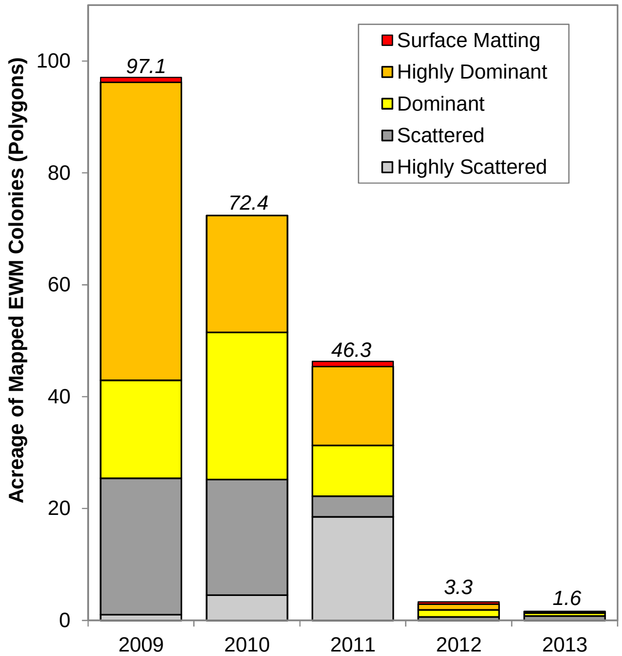


Figure 2. Acreage of mapped EWM colonies on Long Lake from 2009-2013.

Post-treatment surveys were completed by Onterra ecologists on September 3, 2013. Map 2 displays the results of the EWM Peak-Biomass Survey. As illustrated, EWM density and occurrence was reduced within all of the 2013 treatment areas, with the largest reductions occurring in the larger treatment areas in the southern portion of the lake. Ninety-eight percent of the 3.3 acres of colonized EWM mapped in 2012 were reduced by at least one density rating following the 2013 treatment, exceeding the qualitative success criterion (75% of colonial acreage reduced). Not only were these colonized areas of EWM reduced in density, but they were also reduced in size: EWM colonial acreage was reduced from 3.3 acres in 2012 to 1.6 acres in 2013, representing a 98% decrease in EWM colonial acreage since 2009 (Figure 2).

As discussed, quantitative data were collected from 101 sub-sample point-intercept locations within 2013 herbicide application areas before and following the treatment. Prior to treatment in late-summer 2012, approximately 27% of these locations contained EWM, while 5% contained EWM in 2013 following the treatment (Figure 3). This 82% reduction was statistically valid, and greatly exceeds the quantitative success criterion of at least a 50% reduction in occurrence.

EWM rake fullness ratings used to quantify the abundance of EWM when pulled up on the rake were also recorded during the pre- and post-treatment point-intercept surveys. A rake fullness rating of 1, 2, or 3 was recorded, where 1 indicates a minimal amount of EWM on the rake, 2 indicates a moderate amount of EWM on the rake, and 3 indicates the rake is overflowing with EWM. Figure 4 displays the EWM rake fullness ratings from the pre- and post-treatment surveys, and illustrates not only the overall reduction in EWM occurrence, but indicates that the density of EWM decreased as well as no rake fullness ratings of 2 or 3 were recorded in 2013 following the treatment.

Data concerning native aquatic plant species were also collected at the same 101 point-intercept locations within the 2013 herbicide application areas. Figure 3 displays the occurrences of the native plants before and after treatment. As illustrated, no native aquatic plant species were found to have declined within the treatment areas following the treatment; the only plant to exhibit a statistically valid change in occurrence was southern naiad (*Najas guadalupensis*), which increased in occurrence from 6% to 16%.

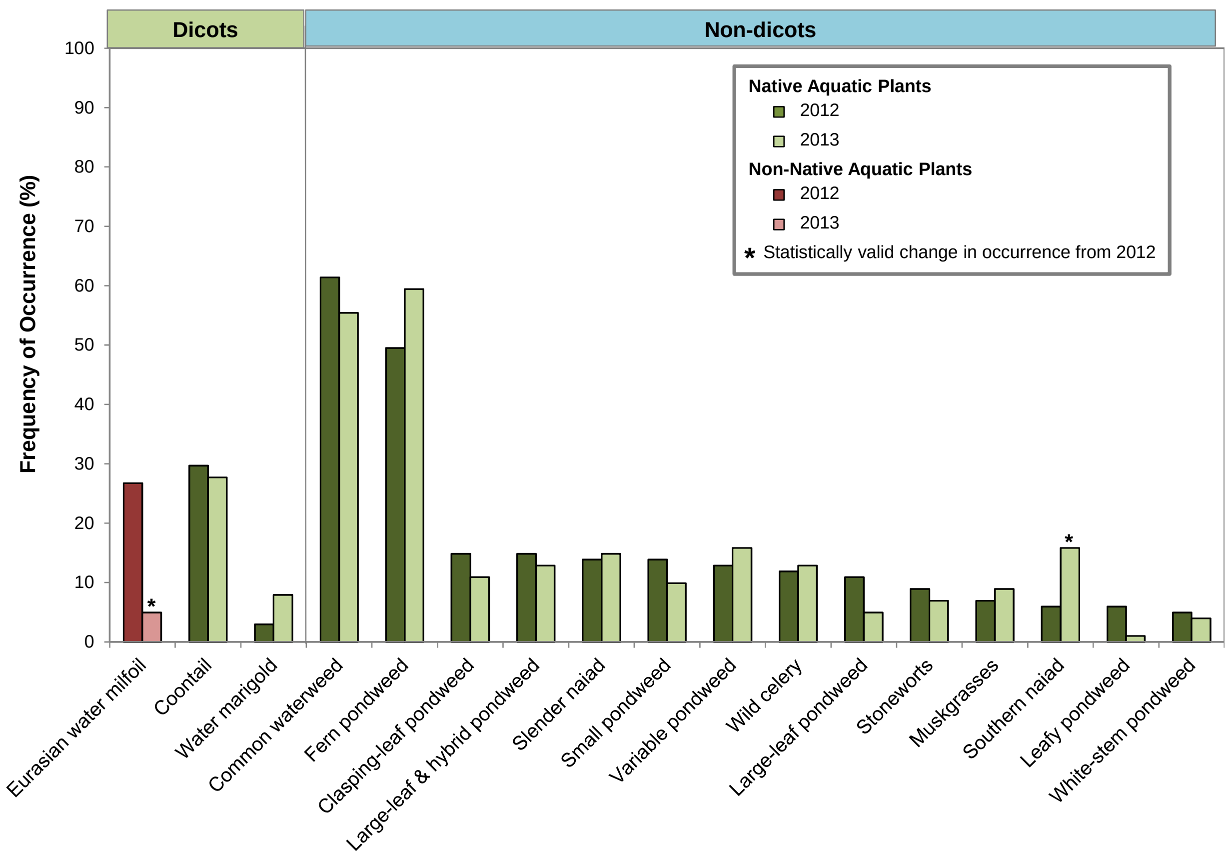


Figure 3. 2012 pre- and 2013 post-treatment occurrence of aquatic plant species within 2013 treatment areas in Long Lake. Only those species with at least an occurrence of 5% in either year are displayed. Created using data from 2012 and 2013 sub-sample surveys.

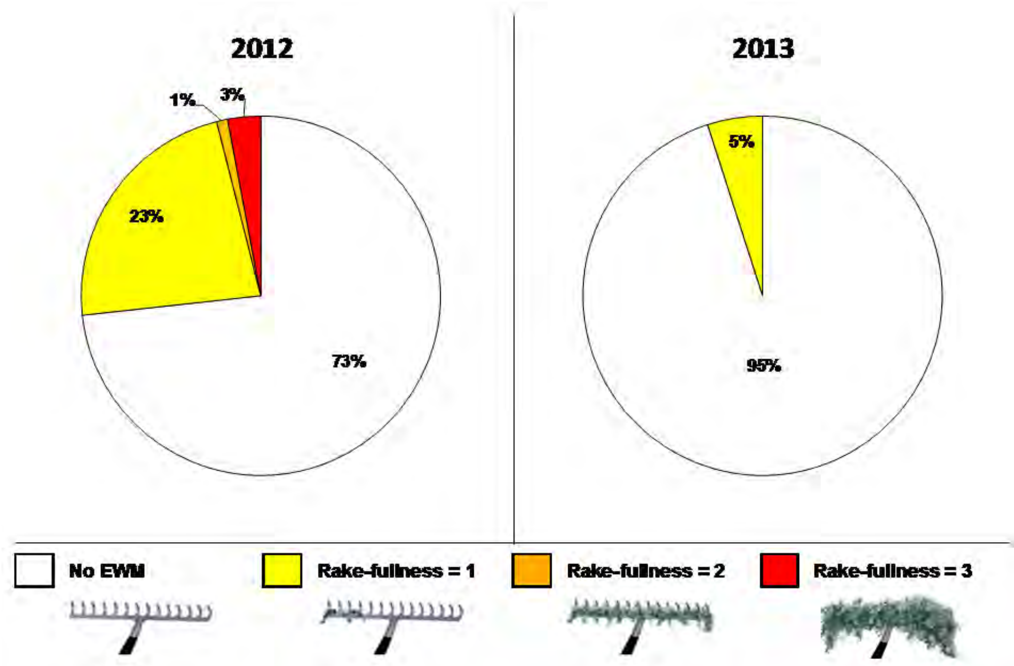


Figure 4. EWM rake fullness ratings from 101 point-intercept sampling locations within 2013 treatment areas on Long Lake. Created using data from 2012 and 2013 sub-sample surveys.

CONCLUSIONS AND DISCUSSION

The 2013 liquid 2,4-D spot treatments in Long Lake were highly successful at reducing EWM, as indicated by the qualitative and quantitative aquatic plant monitoring data. The 2013 treatment was also successful in that no impacts were detected to the native aquatic plant species within the 2013 treatment areas.

As discussed previously, the LLPLD is taking an aggressive approach to EWM management over the course of this five-year project, targeting colonized areas of EWM and adjacent EWM mapped via point-based mapping techniques. Using this strategy, approximately 26.6 acres of EWM are preliminarily proposed for treatment in 2014 (Map 2).

Sites A-14, B-14, and C-14 are all considered small treatment sites and concerns exist whether adequate herbicide concentration and exposure time requirements can be achieved when targeting this small of areas. Sites E-14 may be large in area, but its narrow shape is a liability for achieving sufficient longevity of herbicide concentrations. While this site had successes in 2013 at roughly the same size and shape, the influence of herbicide from other nearby treatment sites may have aided in the efficacy. Similarly, the efficacy of D-14 may be increased through the influence of herbicide dissipating from the upstream E-14.

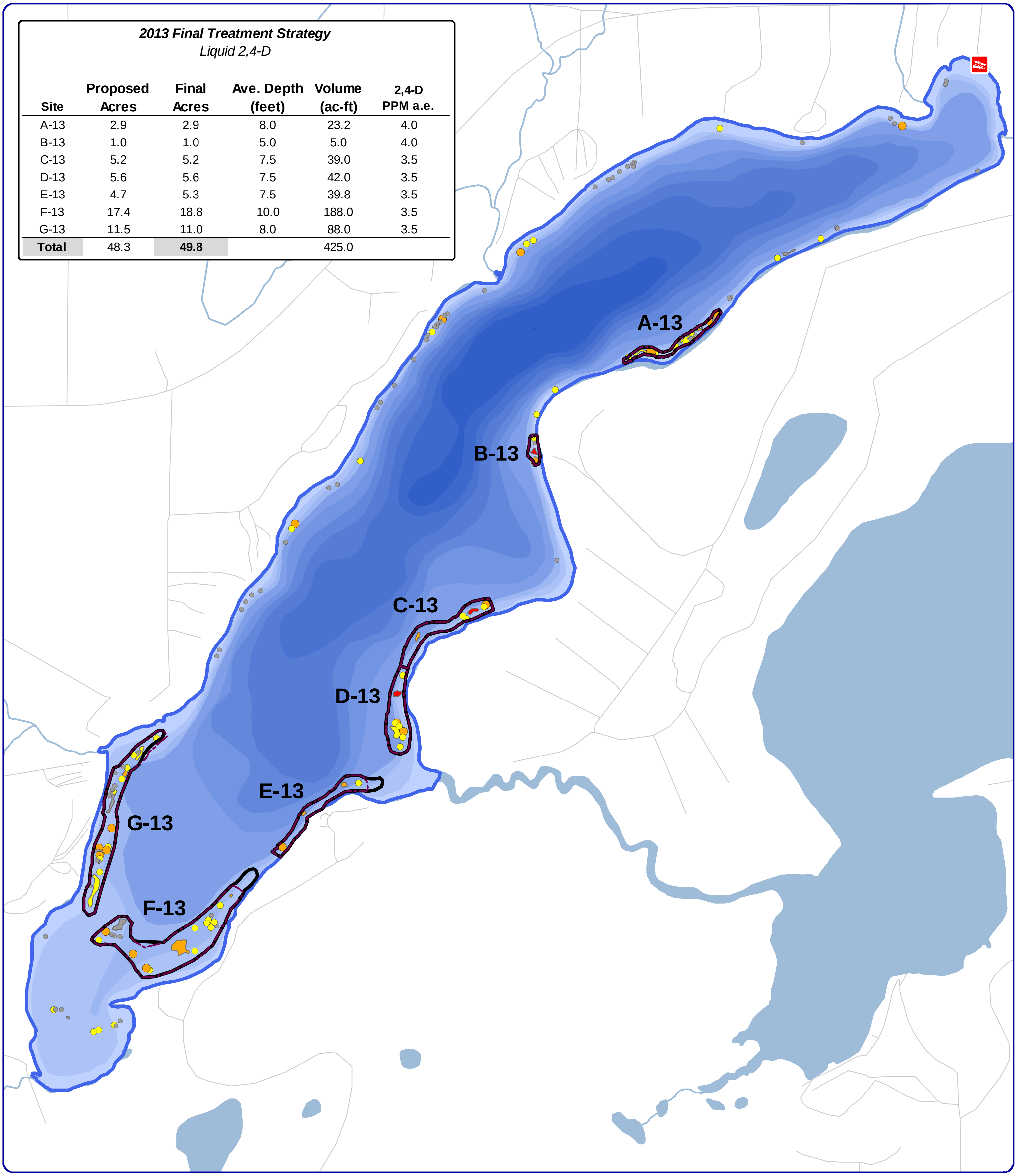
If the lake is stratified to 20 feet around the time of the 2014 herbicide application, the potential epilimnetic concentration would be around 0.050 ppm ae; these concentrations would be far insufficient to cause lake-wide native or non-native plant impacts at anticipated exposure times. A temperature and dissolved oxygen profile will be collected during the 2014 pretreatment survey to better understand the potential role of stratification.

Four areas totaling approximately 0.72 acres are proposed to be targeted via hand-removal in 2014 (Map 2). These proposed hand-harvesting areas contain very small, isolated colonies of *dominant* and *highly dominant* EWM. 2013 was the first year the LLPLD utilized professional hand-harvesting services. Along with developing a relationship with a hand-harvesting firm, the LLPLD and Onterra have started developing a more effective way to apply these services. In 2014, it is proposed that the professional hand-harvesting activities focus precisely within these 4 areas. Onterra's late-summer post treatment survey will allow an evaluation of this technique to be made. Continued discussions between the LLPLD, Onterra, and various hand-harvesting firms may result in expansion of the 2014 hand-harvesting efforts towards some of the smaller areas originally slated for herbicide treatment.

2013 Final Treatment Strategy

Liquid 2,4-D

Site	Proposed Acres	Final Acres	Ave. Depth (feet)	Volume (ac-ft)	2,4-D PPM a.e.
A-13	2.9	2.9	8.0	23.2	4.0
B-13	1.0	1.0	5.0	5.0	4.0
C-13	5.2	5.2	7.5	39.0	3.5
D-13	5.6	5.6	7.5	42.0	3.5
E-13	4.7	5.3	7.5	39.8	3.5
F-13	17.4	18.8	10.0	188.0	3.5
G-13	11.5	11.0	8.0	88.0	3.5
Total	48.3	**49.8**		425.0	



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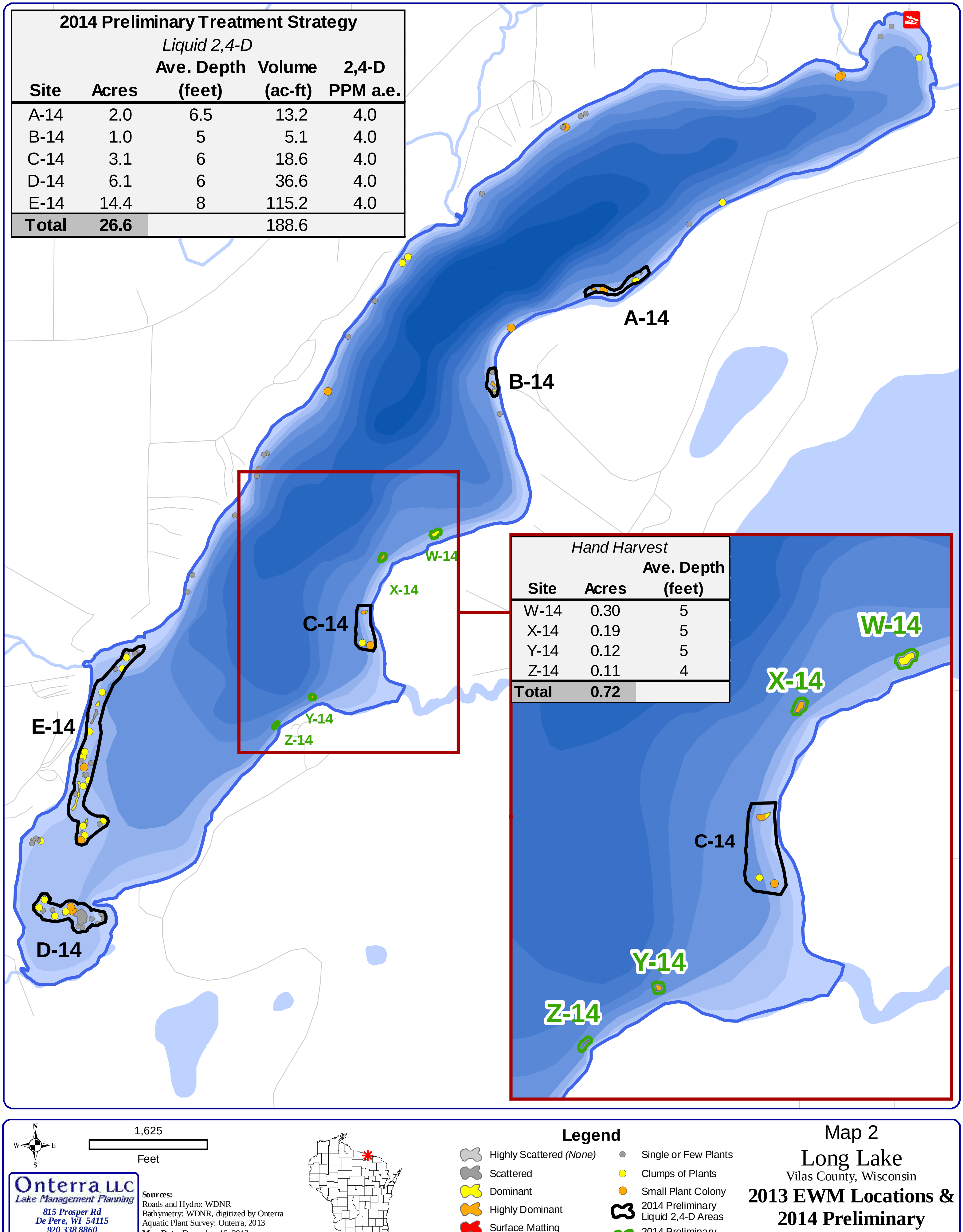
Sources:
Roads, Hydro, and Bathymetry: WDNR
Aquatic Plants: Onterra, 2012
Map Date: December 10, 2013
Filename: Map1_LongV_T2013_Perm1.mxd

Map 1
Long Lake
Vilas County, Wisconsin
2012 EWM Locations & 2013 Final EWM Treatment Areas

2014 Preliminary Treatment Strategy				
Liquid 2,4-D				
Site	Acres	Ave. Depth (feet)	Volume (ac-ft)	2,4-D PPM a.e.
A-14	2.0	6.5	13.2	4.0
B-14	1.0	5	5.1	4.0
C-14	3.1	6	18.6	4.0
D-14	6.1	6	36.6	4.0
E-14	14.4	8	115.2	4.0
Total	**26.6**		188.6	

Hand Harvest		
Site	Acres	Ave. Depth (feet)
W-14	0.30	5
X-14	0.19	5
Y-14	0.12	5
Z-14	0.11	4
Total	**0.72**	



Legend

- Highly Scattered *(None)*
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2014 Preliminary Liquid 2,4-D Areas
- 2014 Preliminary Hand Harvest Areas

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Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR, digitized by Onterra
Aquatic Plant Survey: Onterra, 2013
Map Date: December 16, 2013
Filename: Map2_LongV_T2014_EWM_Prelim1.mxd

Map 2

Long Lake

Vilas County, Wisconsin

2013 EWM Locations & 2014 Preliminary Treatment Strategy v1

Draft: Long Lake, Vilas County, 2,4-D Concentration Monitoring Summary, 2013

21 November 2013

John Skogerboe

Long Lake has an area of 862 acres, a maximum depth of 88 feet, and a mean depth of 30 feet. On 4 June 2013, seven areas totaling 49.8 acres (Figure 1) were treated with a liquid formulation of 2,4-D to control Eurasian watermilfoil (*Myriophyllum spicatum*). The target application rates ranged from 3500 ug/L ae in larger treatment areas (> 5 acres) to 4000 ug/L ae in smaller treatment areas (< 5 acres). The Treatment Report recorded that the water temperature was 59°F (15°C), and the wind was 2 to 4.5 mph from the south. The wind was reported by www.wunderground.com to be 2 mph from the southeast.

Water sample sites were located in treatment areas C, D, E, F, and G to quantify 2,4-D dissipation from the treatment areas (Figure 2).

Water samples were collected from each sample site using an integrated water sample which collects water from most of the water column. Water samples were collected at intervals of approximately 1, 2, 4, 6, and 24 hours after treatment (HAT). 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 2,4-D 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.

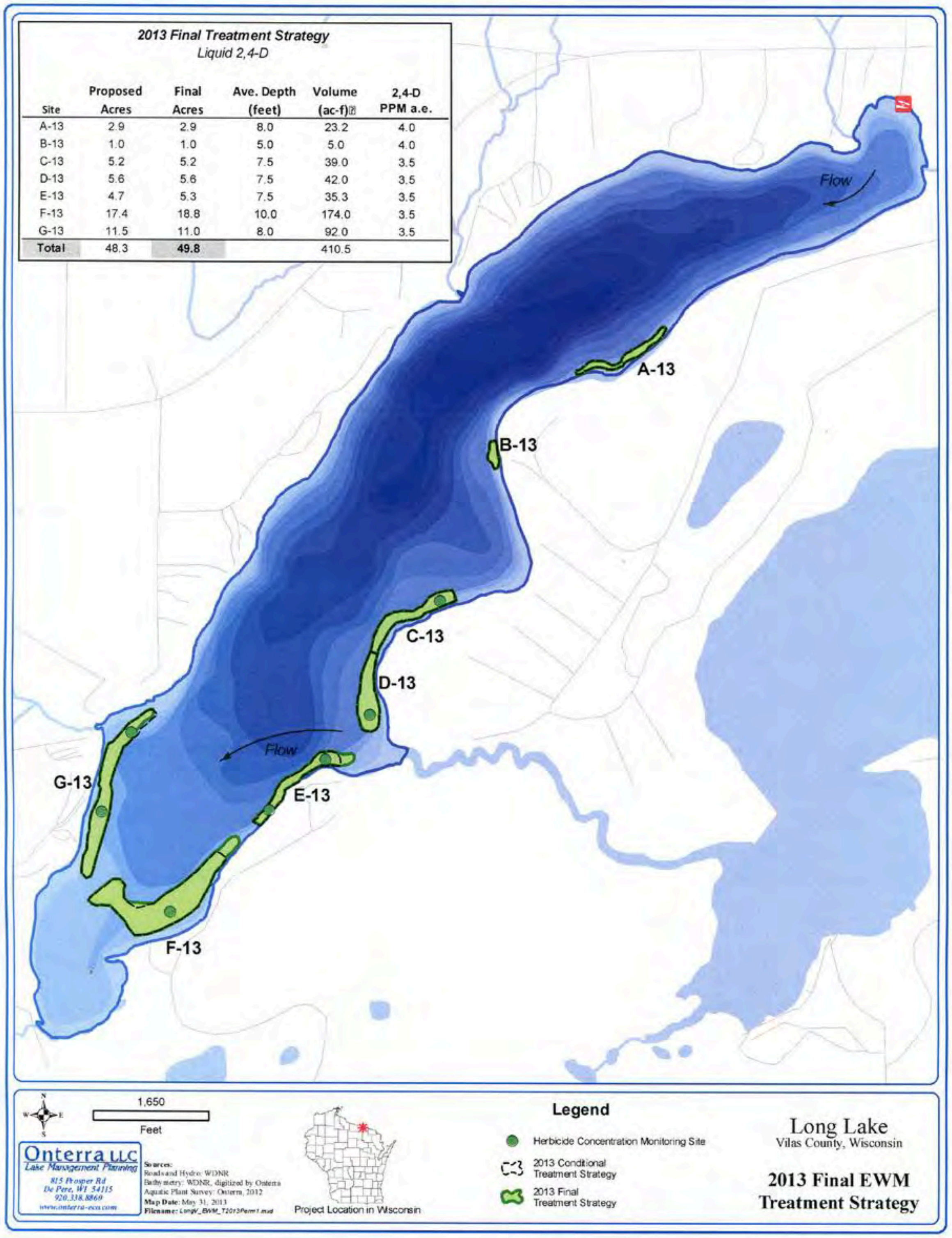
The peak 2,4-D concentrations in samples collected from Sites LC and LD were 617 and 666 ug/L ae at 4 HAT compared to the target concentration of 3500 ug/L ae (Figure 3). Concentrations of 2,4-D in samples from both sites were less than the irrigation standard (100 ug/L ae) by 6 HAT.

The peak 2,4-D concentrations in samples collected from Site LE1 was 1244 ug/L ae at 4 HAT and 1029 ug/L ae from Site LE2 at 6 HAT compared to the target concentration of 3500 ug/L ae (Figure 4). Concentrations of 2,4-D in samples collected at 24 HAT were 419 ug/L ae at site LE1 and 320 ug/L ae at site LE2 compared to the irrigation standard of 100 ug/L ae.

The peak 2,4-D concentration in samples collected from Site LF was 1407 ug/L ae at 4 HAT compared to the target concentration of 3500 ug/L ae. The concentration of 2,4-D in the sample collected at 24 HAT was 791 ug/L ae compared to the irrigation standard of 100 ug/L ae.

The peak 2,4-D concentrations in samples collected from Site LG1 was 1418 ug/L ae at 2 HAT and 1333 ug/L ae from Site LG2 at 4 HAT compared to the target concentration of 3500 ug/L ae. Concentrations of 2,4-D in samples collected at 24 HAT were 181 ug/L ae at site LG1 and 244 ug/L ae at site LG2 compared to the irrigation standard of 100 ug/L ae.

Figure 1. 2013 Long Lake 2,4-D Treatment Areas (Onterra LLC)



2013 Final Treatment Strategy
Liquid 2,4-D

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D-13	5.6	5.6	7.5	42.0	3.5
E-13	4.7	5.3	7.5	35.3	3.5
F-13	17.4	18.8	10.0	174.0	3.5
G-13	11.5	11.0	8.0	92.0	3.5
Total	48.3	49.8		410.5	

Figure 2. 2013 Long Lake 2,4-D Sample Locations

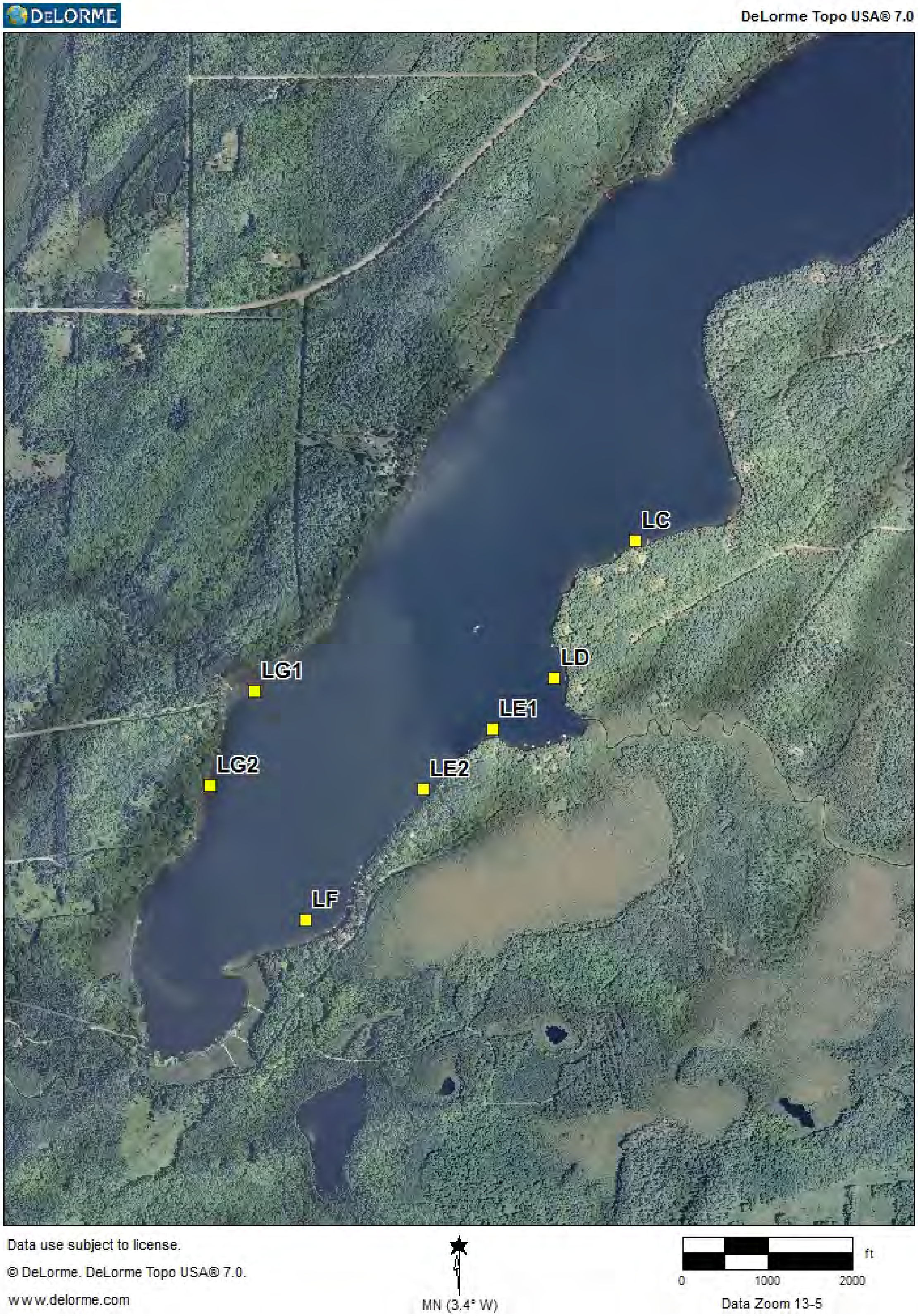

Figure 3

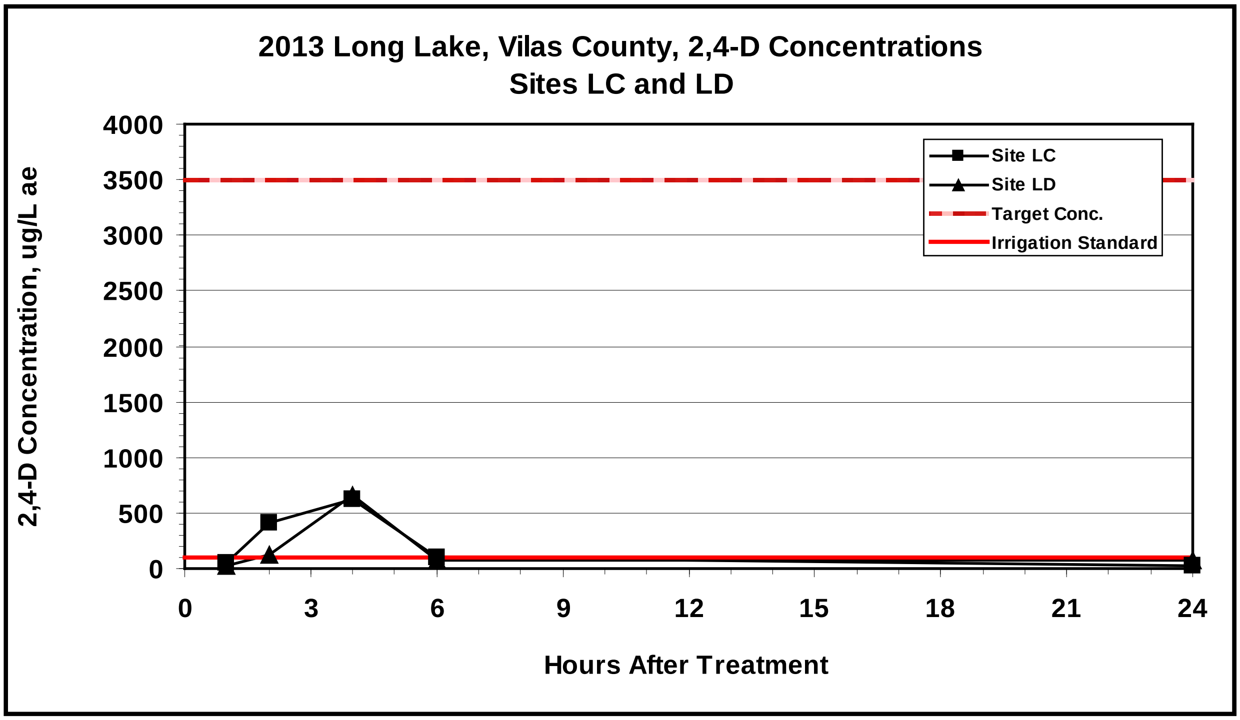

Figure 4