INTRODUCTION

Photo 1. Long Lake, Fond du Lac County.

Long Lake, Fond du Lac County, is an approximate 454-acre drainage lake (including Tittle Lake) with a maximum depth of 47 feet and a mean depth of 22 feet (Photo 1). In 2010, the Long Lake Preservation Association, Inc. (LLPA) contracted with Onterra, LLC to conduct a three-year aquatic invasive species (AIS) monitoring and control project. Specifically, the objective of this project is to monitor and assess herbicide treatments aimed at controlling the non-native invasive plants curly-leaf pondweed (*Potamogeton crispus*; CLP) and Eurasian water milfoil (*Myriophyllum spicatum*; EWM) from 2011-2013. Completed in 2013, the project was successful at locating and treating areas of CLP from 2011-213 and then populations of EWM in 2013. In 2013, EWM specimens from Long Lake were sent to the Annis Water Resources Institute at Grand Valley State University in Michigan for DNA analyses, and their results revealed that Long Lake contains both pure strain EWM and hybrid water milfoil (*M. sibiricum X spicatum*; HWM). Hybrid water milfoil is a cross between EWM and the indigenous northern water milfoil. Subsequent use of "EWM" within this report collectively includes, but does not differentiate between, EWM and HWM.

At the end of the three-year AIS monitoring and control project, the LLPA had remaining funds within the Wisconsin Department of Natural Resources (WDNR)-funded AIS-Established Population Control Grant, and along with additional funds requested from the WDNR through an amendment, they were able to extend the project into a fourth year to fund AIS monitoring and control through 2014. Currently, the LLPA is in the process of updating their management plan. The new plan would update the existing plan; add important components such as a shoreland assessment, a written stakeholder survey, and a survey of course woody habitat in the lake. The updated plan would also create new thresholds and triggers for the continued control of CLP and EWM within Long Lake.

The goal of CLP management in Long Lake is to reduce the treatable acreage of CLP. This is accomplished through repeat treatments aimed at depleting the base of turions (vegetative reproductive structures) that have built up in the sediments over time. In 2013, approximately 48.6 acres of CLP around the lake were targeted with liquid endothall at rates of 2.5 – 3.0 ppm active ingredient (ai). Surveys following the treatment indicated that the 2013 CLP treatment on Long Lake was met with success with CLP being reduced within the majority of the treatment areas. However, many viable turions produced in previous years were likely still present within the sediment in these areas. It is unknown exactly how long turions can remain viable in the sediment, but it is believed to be at least 3-5 years. For this reason, all of the areas that were treated in 2013 were proposed to be retreated in 2014 (Map 1). Multiple years of treatment over these same areas will likely need to occur to kill CLP sprouting from previously deposited turions. In total, 48.6 acres were initially proposed for treatment in 2014 (Map 1).

EWM was first observed in Long Lake in 2002. However, point-intercept surveys conducted by the WDNR in 2007 and 2010 indicated that the population was very small. Late-Summer EWM Peak-Biomass Surveys conducted by Onterra ecologists aimed at locating at mapping occurrences of EWM in 2011 revealed that there were no areas of EWM that warranted treatment in 2012. However, with the early ice-out and exceptionally warm summer in 2012, areas of EWM in Long Lake were found to have expanded from 2011.

The LLPA felt that the risk of not treating the new colonies of EWM in 2013 allows the potential of further expansion that ultimately threatens the ecological health of Long Lake. Acting aggressively at the potential initial stage of EWM becoming colonized within the lake may provide the greatest opportunity for implementation of the control strategy. The goal of this program is to reduce the amount of EWM within the lake to more manageable levels – perhaps levels that on an annual basis require minimal or no use of herbicides and can be appropriately controlled using hand removal methods. Using this rationale, approximately 3.9 acres of EWM were targeted with herbicides in Long Lake in 2013. Post-treatment surveys indicated that the treatment was highly successful, with EWM being reduced in all of the treatment areas. None of the areas treated for EWM in 2013 were proposed to be retreated in 2014; however, approximately 3.0 acres of EWM in the southern portion of the lake were proposed to be treated in 2014 (Map 1).

2014 PROPOSED TREATMENT STRATEGY

CLP Treatment 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 implementing successful control strategies utilizing 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, U.S. Army Engineer Research and Development Center (USAERDC), and private consultants. Based on their preliminary findings, lake managers have adopted two main treatment strategies; 1) whole-lake treatments, and 2) spot treatments.

Whole-lake treatments are those where the herbicide is applied to specific sites, but the goal of the strategy is for the herbicide to reach a target concentration when it equally distributes throughout the entire volume of the lake (or lake basin, or within the epilimnion of the lake or lake 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, effective herbicide concentrations for whole-lake treatments are significantly less than required for spot treatments. Whole-lake treatments are typically conducted when the target plant is spread throughout much of the lake or basin. Whole-lake herbicide treatment strategies have not been used on Long Lake.

Spot treatments, the strategy utilized on Long Lake since 2011, 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. Herbicide application rates for spot treatment are formulated volumetrically, typically targeting CLP with liquid endothall at 1.5-4.0 ppm active ingredient (ai). This means that sufficient endothall is applied within the *Application Area* such that if it mixed evenly with the *Treatment Volume*, it would equal 1.5-4.0 ppm ai. 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 1.5-4.0 ppm ai, it is understood that actually achieving 1.5-4.0 ppm ai within the water column is not likely due to dissipation and other factors.

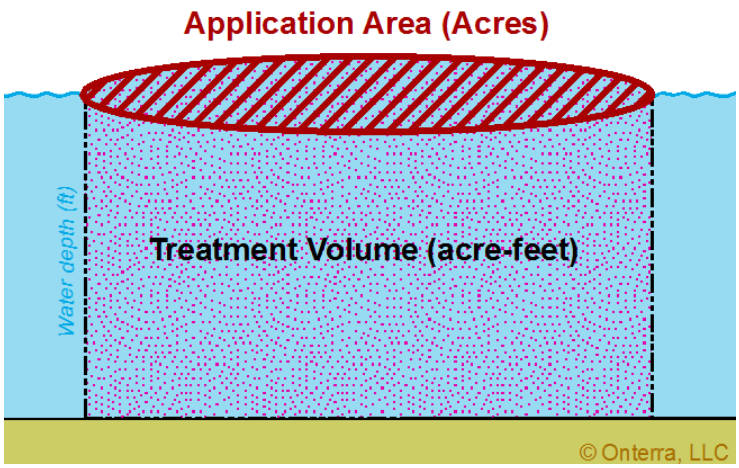


Figure 1. Herbicide Spot Treatment diagram.

Ongoing research indicates that herbicide quickly dissipates and dilutes from spot treatments, especially small spot treatments (less than 5 acres). In order for mortality of the target plants to occur, the short exposure time (often hours) needs to be offset by the plants being exposed to a high herbicide concentration. Like terrestrial herbicide applications, spot treatments are used by lake managers to strategically target a specific colony of a target plant. However, obtaining effective herbicide concentration and exposure times has proven difficult in many instances. In these cases, the treatment results in seasonal control such that the target plants are greatly injured by the treatment, but fully rebound by the end of the summer.

As mentioned, the 2013 CLP treatment on Long Lake was met with success. However, past treatments, like those conducted in 2012, were often met with mixed results. One of the major objectives of this project is to monitor the treatment effectiveness and 'tune' or refine the treatment strategy in order for the most effective results to be achieved. The lack of success in previous treatments indicates that the herbicide did not reach an adequate concentration-exposure time to cause plant mortality. With this knowledge, proposed 2014 treatment areas that were less than 5 acres were proposed to be treated with liquid endothall at an increased rate of 3.0 ppm ai, while treatments of greater than five acres would be treated at a rate of 2.5 ppm ai.

However, there were two exceptions to this proposed strategy: site A-14 (3.0 acres) was proposed to be treated at a slightly higher rate of 3.5 ppm ai due to its narrow width and higher water movement in this area given its proximity to the mouth of Watercress Creek; and site J-14 (7.9 acres) was also proposed to be treated at a high rate of 3.0 ppm ai due to its narrow width. A few of the proposed treatment sites were approaching a point at which the herbicide application areas are too small to consistently predict if the endothall will cause CLP mortality, regardless of the dose. Therefore, potential treatment sites less than 0.5 acres were not proposed for treatment due to their extremely small size and unlikely nature of being successful.

EWM Treatment Strategy

As discussed, EWM was found to have increased in Long Lake from 2011 to 2012. After deliberation, the LLPA decided to initiate an aggressive EWM herbicide control strategy starting in 2013 that was met with success. To continue this success, approximately 3.0 acres of EWM were proposed to be applied with liquid 2,4-D at a rate of 3.0 ppm acid equivalent (ae). Because this area was also being targeted for CLP, liquid endothall was also proposed to be applied at a rate of 2.5 ppm ai. While some of the areas treated for EWM in 2013 were applied with 2,4-D at the maximum allowable rate of 4.0 ppm ae, the 2,4-D application rate was reduced for 2014 because it is understood that the simultaneous exposure of EWM to 2,4-D and endothall would be sufficient to cause mortality to the target plants.

PRETREATMENT CONFIRMATION AND REFINEMENT SURVEY

On May 13 and 14, 2014, Onterra ecologists conducted the CLP and EWM Pretreatment Confirmation and Refinement Survey on Long Lake. During this survey, the presence of CLP and EWM within the proposed treatment sites was confirmed and the treatment area extents were verified both from the surface and via a submersed video camera in deeper water.

Following this survey, nine CLP treatment sites were removed due to insufficient levels of CLP observed within these areas. The extents of the remaining six treatment areas were not modified, and CLP was observed throughout these areas. In total, the final 2014 CLP treatment was reduced from the proposed 48.6 acres to 19.9 acres (Map 1). However, while site E-14 was originally proposed to be treated following assessment during the pre-treatment survey, water temperatures were higher within this site (70.4°F) when compared to the other sites (64°F – 68°F). These higher temperatures increased the growth of native aquatic plants, and due to concerns that these plants may be adversely impacted from the treatment; site E-14 was also removed. The removal of E-14 reduced the final CLP treatment acreage to 14.5 acres (Map 1). The presence of EWM was also confirmed within the single proposed EWM treatment site, and the extents of this area were not modified yielding a final EWM treatment area of 3.0 acres.

The treatment was conducted by Clean Lakes on June 3, 2014. The applicator reported a near-surface water temperature of approximately 68°F and southwest winds of 2.5-6.7 mph at the time of application.

Wind speed and direction data were also obtained from a weather station in nearby Campbellsport, WI, approximately 15 miles from Long Lake (Figure 2). These data indicate that winds were predominantly westerly at the time of treatment. These data indicate that wind-driven water movement in Long Lake during the application where highest, with relatively calm winds occurring approximately 3 hours after the treatment was completed.

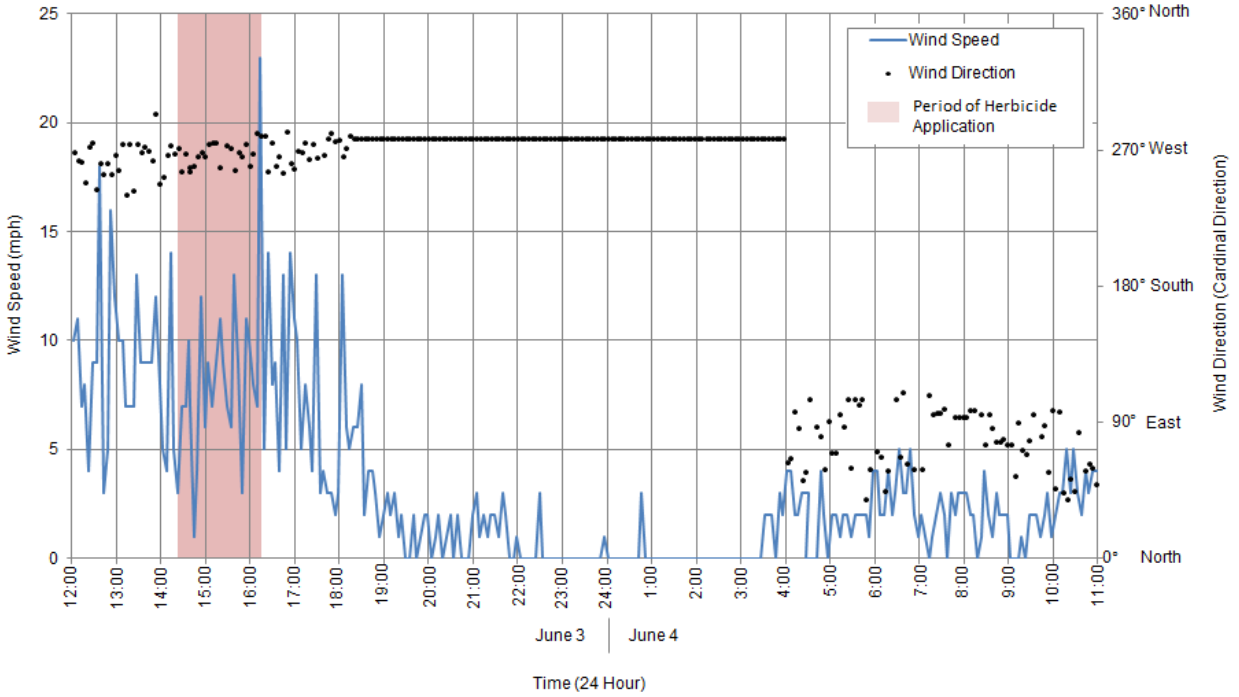


Figure 2. Wind speed and direction approximately 4 hours before and 14 hours after herbicide was applied to the Long Lake 2014 treatment areas on June 3, 2014. Graph created using data from Weather Underground Station

MONITORING METHODOLOGIES

The objective of an herbicide treatment strategy is to maximize target species (CLP and 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 CLP & EWM Monitoring

It is difficult, if not impossible, to assess the efficacy of a single year of treatment on a lake's CLP population. CLP naturally senesces (dies back) in early summer, making it is difficult to determine if a reduction in CLP following a spring treatment was caused by the treatment, natural senescence, or both. However, quantitative sub-sample point-intercept data collected annually in the spring prior to treatment within treatment areas allows for a determination if the CLP population is being reduced over time. The goal of CLP management is to annually kill the plants before they are able to produce and deposit new turions, and thus, overtime, deplete the existing turion bank within the sediment. Over the course of multiple annual CLP treatments, these annual sub-sample point-intercept surveys should quantitatively document a reduction in CLP occurrence as the turion base is depleted.

In Long Lake, quantitative evaluation was made through the collection of data at 115 point-intercept sub-sample locations located within CLP treatment areas from 2012-2014 (Figure 3). At each of these locations, the presence (or absence) of CLP was recorded. The presence of native aquatic plant species were recorded during the June 2013 and June 2014 surveys. Comparing these data from year to year allows for a statistical comparison of CLP and native aquatic plant occurrence and a quantitative determination of the CLP population over time.

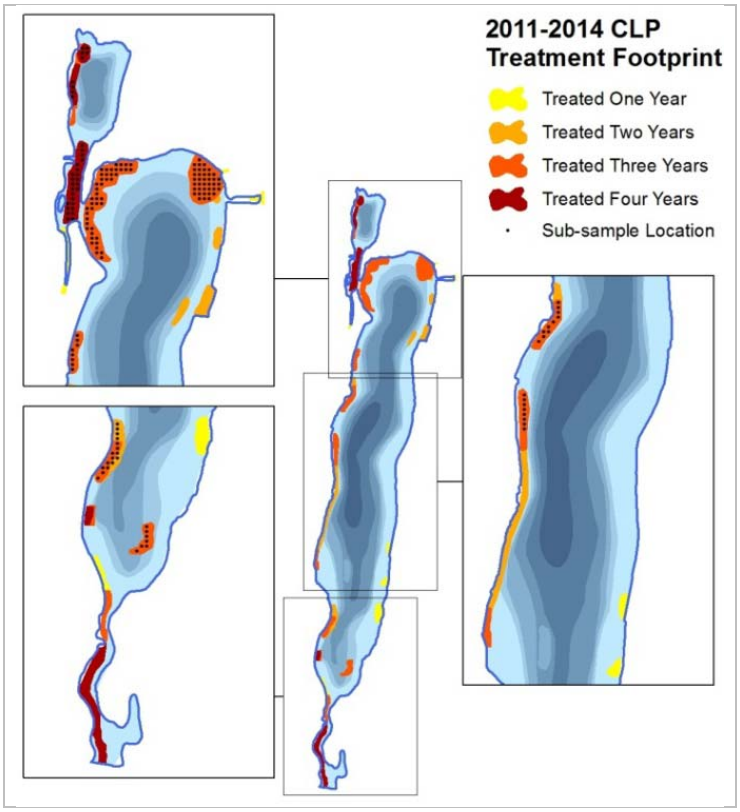


Figure 3. Sub-sample point-intercept survey locations within 2011-2014 CLP treatment areas.

While quantitative evaluation methodologies for EWM spot treatments generally 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, due to the small size of the 2014 EWM treatment, no quantitative monitoring was conducted as a sufficient number of sampling locations could not have been placed within the treatment site to be statistically valid.

Qualitative CLP & EWM Monitoring

As discussed in the quantitative monitoring section, CLP's natural senescence makes it difficult to determine a single treatment's efficacy. However, using sub-meter GPS technology, CLP locations are mapped in late spring/early summer following the treatment when the plants should be at or near their peak growth. Because EWM reaches its peak growth in mid to late summer, EWM locations are mapped the year prior to treatment (2013) in late summer and in the late summer immediately following the treatment (2014). Both the CLP and EWM populations were 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* (Maps 1 – 3).

Qualitative monitoring of CLP herbicide treatments includes comparing spatial data reflecting CLP locations and densities during the peak-growth stages from year to year. As with the quantitative sub-sample point-intercept data, following multiple years of treatments, the qualitative should show reductions in CLP acreage and density over time. Because EWM does not naturally senesce in early summer, the qualitative mapping data can be used to qualitatively assess a given year's treatment. Based upon a pre-determine success criterion, an effective EWM treatment would include a 75%

reduction of EWM as demonstrated by a decrease in at least one density rating (*e.g. Highly dominant to dominant*).

Aquatic Plant Monitoring Results

CLP

During the May 2013 pre-treatment sub-sample point-intercept survey six (5.2%) of the 115 point-intercept sampling locations within areas of CLP treated at least once since 2011 contained CLP (Figure 4). Following the treatment in June 2014, the occurrence of CLP within these areas was not statistically different (Chi-square $\alpha = 0.05$) with five (4.3%) of the 115 point-intercept sampling locations containing CLP. As discussed earlier, these surveys do not directly measure the efficacy of a single treatment. Even though the occurrence of CLP within these areas was not reduced from before and after the 2014 treatment, it does not indicate an unsuccessful treatment. Rather, is indicates that there were viable turions still present within the sediment that sprouted plants in 2014 following the treatment. These dormant turions within the sediment are the reason why CLP involves multiple years of treatments over the same areas. Following multiple years of treatments, comparing the occurrence of CLP at its highest in May 2013 of 22.6% to 4.3% in June 2014 indicates a statistically valid reduction of 81%. These data suggest that the multiple years of repeat treatments have reduced Long Lake's CLP turion bank. Without directly measuring the amount of CLP turions within these areas, this cannot be confirmed.

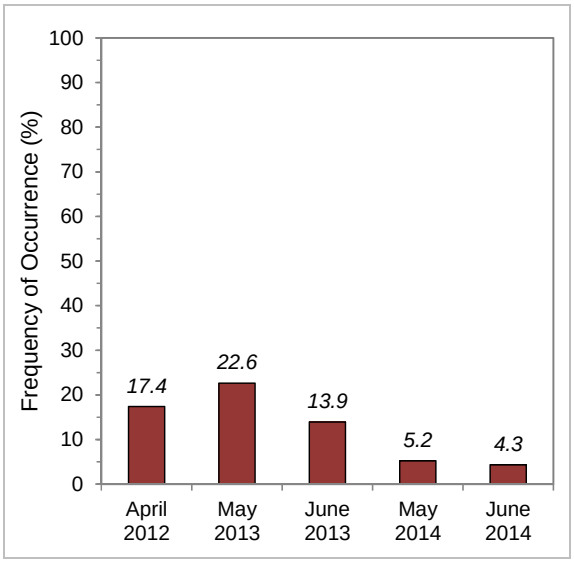


Figure 4. Frequency of occurrence of CLP as determined from 115 sub-sample point-intercept locations within Long Lake treatment areas.

No areas of colonized CLP could be located within any of the 2014 treatment areas (Map 2). However, single plants, clumps of plants, and some small plant colonies were located widely scattered throughout littoral areas around the lake. In addition, a few smaller colonized areas of CLP were located outside of 2014 treatment areas in the southern portion of the lake.

In order to build off their successes, the LLPA has outlined an aggressive approach to CLP management whereas within their *Comprehensive Management Plan – Nov 2014 Draft* whereas:

- All areas targeted the previous year would be considered for treatment. Based upon the pretreatment survey, these areas may be reduced or removed.
- All areas of colonized CLP will be considered for treatment during the following spring. The LLPA's treatment threshold (trigger) would also extend to immediately adjacent areas of CLP with point-based techniques, with areas mapped as *small plant colonies* being targeted if possible.
- Areas containing AIS but not targeted for herbicide control will be considered for hand-removal. The LLPA has just begun initiating this aspect of their control program.

All of the 2014 treatment areas, except M-14 due to its small size (< 2 acres), are proposed to be retreated again in 2015 along with a new area, B-15, that is targeting an area of highly scattered CLP (Map 2). These areas, totaling approximately 22.1 acres, are proposed to be treated with liquid endothall at a rate of 2.5-3.5 ppm ai.

EWM

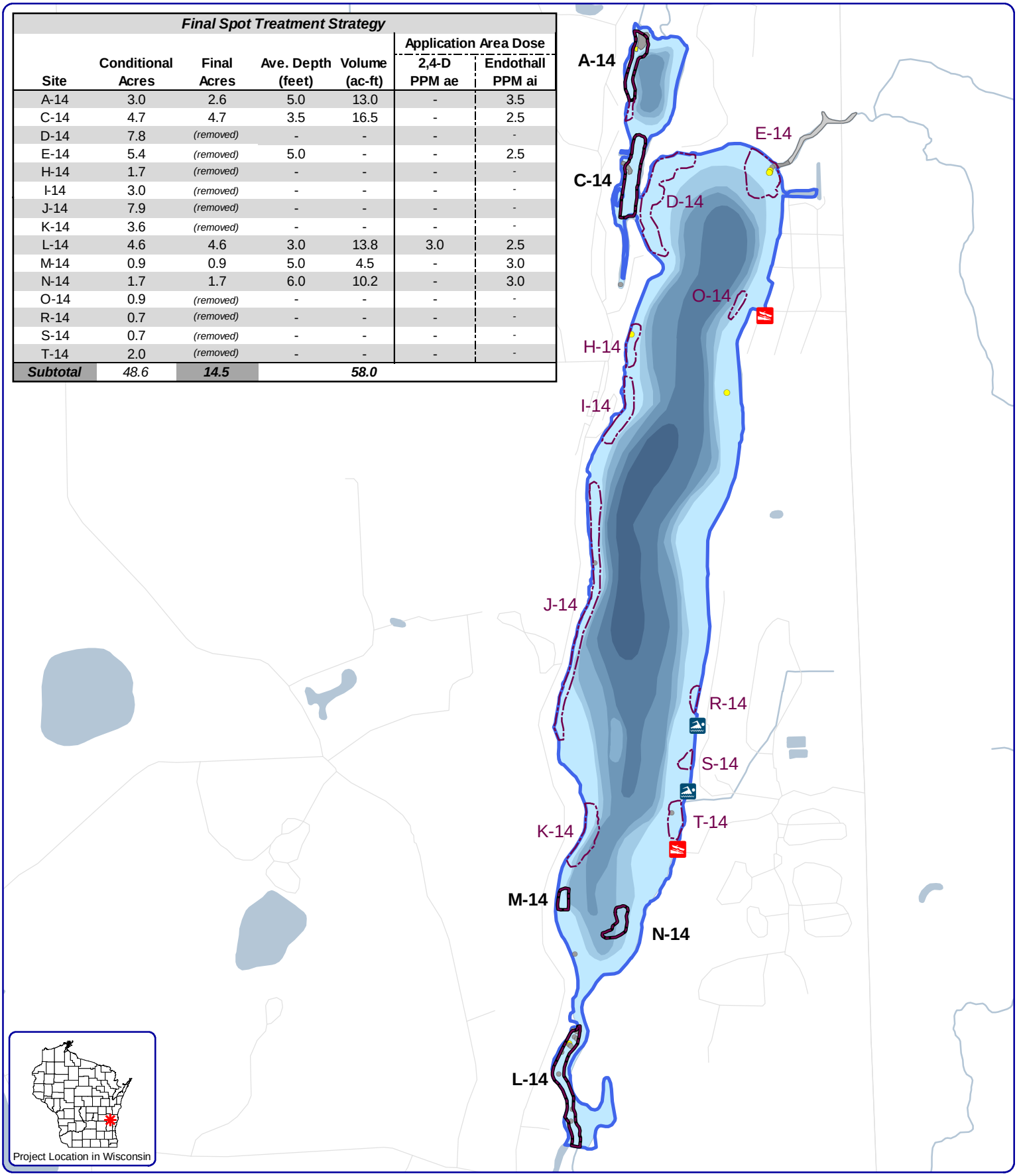
Long Lake was also surveyed for EWM during the June 2014 Early-Season AIS Survey. This survey indicated that EWM within treatment site L-14 was highly successful as no EWM could be located within this area following the treatment (Map 3). No colonized areas of EWM were located anywhere within the lake in 2014, and the highest occurrence of EWM was located within the northern part of Long Lake in the channel that leads to the bay known as Tittle Lake. Given the low density of EWM within Long Lake in 2014, no EWM treatment is proposed for 2015.

CONCLUSIONS AND DISCUSSION

Overall, the 2014 CLP and EWM treatment on Long Lake was met with success; no colonized areas of CLP or EWM were located within the 2014 treatment areas and quantitative data indicate that the occurrence of CLP remains low within areas that have been targeted for control. As mentioned, approximately 22.1 acres of CLP are proposed for treatment in 2015; however, the results of the 2015 Pretreatment Confirmation and Refinement Survey will ultimately determine the final treatment acreage. No areas of EWM are proposed to be treated in Long Lake in 2015, as the 2014 treatment proved to be highly successful and no areas of EWM warranting treatment were located. Eurasian water milfoil will be mapped in the summer of 2015 and a treatment strategy for 2016 will be developed if necessary. The LLPA will be seeking a WDNR AIS-Established Population Control Grant in February of 2015 to implement the control/monitoring efforts moving forward.

Final Spot Treatment Strategy

Site	Conditional Acres	Final Acres	Ave. Depth (feet)	Volume (ac-ft)	Application Area Dose 2,4-D PPM ae	Application Area Dose Endothall PPM ai
A-14	3.0	2.6	5.0	13.0	-	3.5
C-14	4.7	4.7	3.5	16.5	-	2.5
D-14	7.8	*(removed)*	-	-	-	-
E-14	5.4	*(removed)*	5.0	-	-	2.5
H-14	1.7	*(removed)*	-	-	-	-
I-14	3.0	*(removed)*	-	-	-	-
J-14	7.9	*(removed)*	-	-	-	-
K-14	3.6	*(removed)*	-	-	-	-
L-14	4.6	4.6	3.0	13.8	3.0	2.5
M-14	0.9	0.9	5.0	4.5	-	3.0
N-14	1.7	1.7	6.0	10.2	-	3.0
O-14	0.9	*(removed)*	-	-	-	-
R-14	0.7	*(removed)*	-	-	-	-
S-14	0.7	*(removed)*	-	-	-	-
T-14	2.0	*(removed)*	-	-	-	-
Subtotal	*48.6*	***14.5***		***58.0***		



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Sources:
Roads and Hyrdo: WDNR
Aquatic Plants: Onterra, June 2013
Map Date: November 11, 2014
Filename: Map1_LongFDL_CLP_T2014Perm1.mxd

Legend

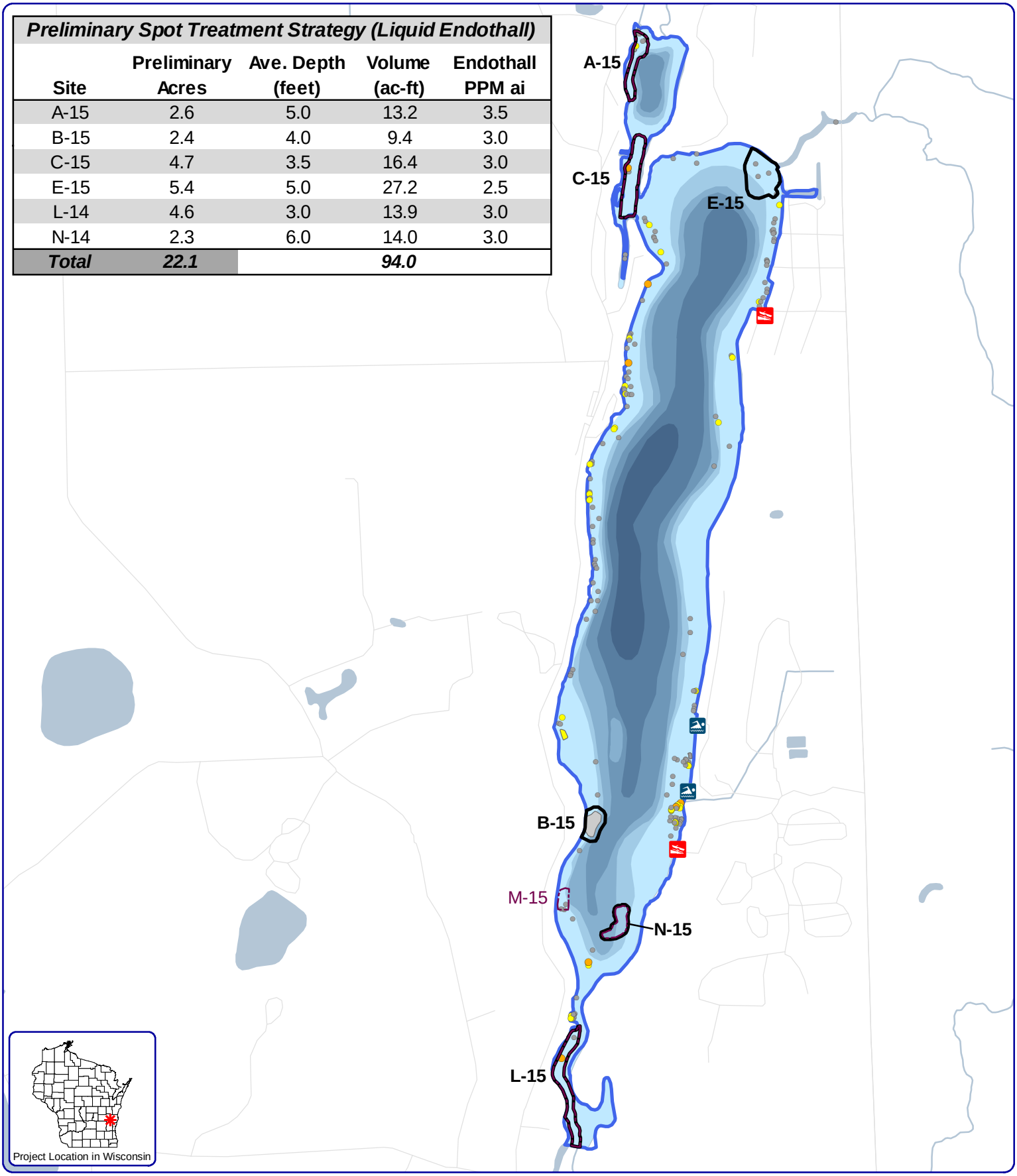
Curly-leaf pondweed (June 2013)

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2014 Proposed CLP Treatment Area
- 2014 Final CLP Treatment Area
- Public Access

Map 1
Long Lake
Fond du Lac County, Wisconsin
2013 CLP Locations & 2014 Final Treatment Areas

Preliminary Spot Treatment Strategy (Liquid Endothall)

Site	Preliminary Acres	Ave. Depth (feet)	Volume (ac-ft)	Endothall PPM ai
A-15	2.6	5.0	13.2	3.5
B-15	2.4	4.0	9.4	3.0
C-15	4.7	3.5	16.4	3.0
E-15	5.4	5.0	27.2	2.5
L-14	4.6	3.0	13.9	3.0
N-14	2.3	6.0	14.0	3.0
Total	***22.1***		***94.0***	



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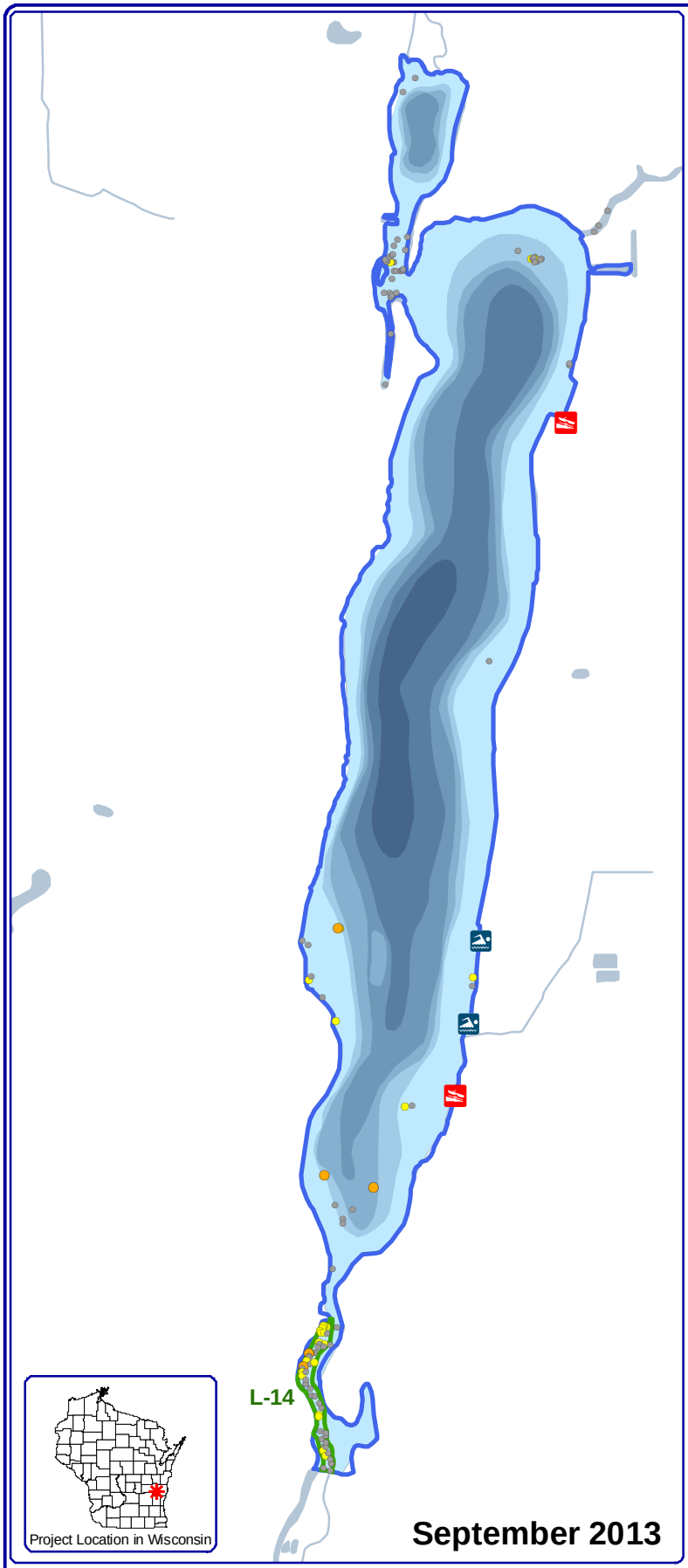
Sources:
Roads and Hyrdo: WDNR
Aquatic Plants: Onterra, June 2014
Map Date: November 11, 2014
Filename: Map2_LongFDL_CLP_T2015_Prelim1.mxd

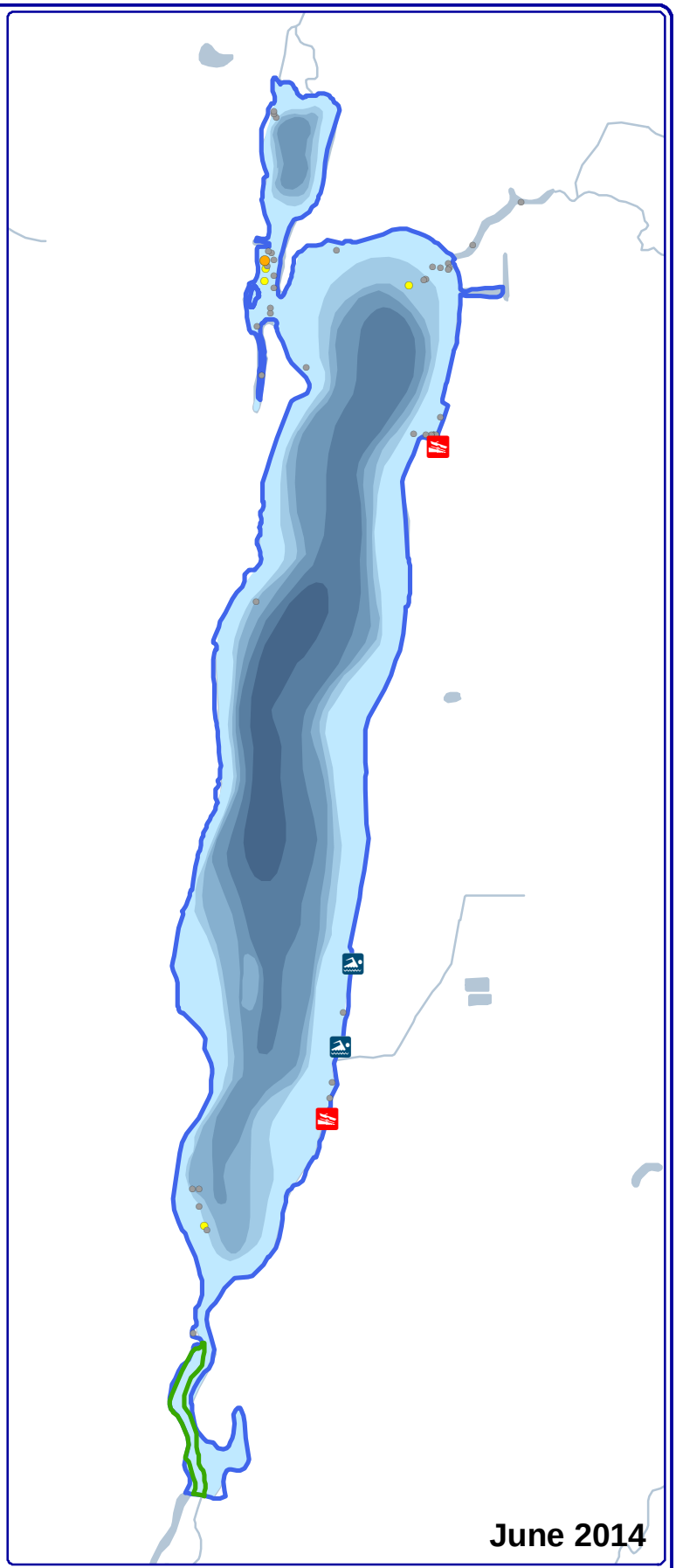
Legend

Curly-leaf pondweed (June 2014)

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2014 Final CLP Treatment Area
- 2015 Preliminary CLP Treatment Area
- Public Access

Map 2
Long Lake
Fond du Lac County, Wisconsin
2015 Preliminary CLP Treatment Strategy





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Sources:
Roads and Hyrdo: WDNR
Aquatic Plants: Onterra, September 2013
Map Date: November 11, 2014
Filename: Map3_LongFDL_EWM_T2014Perm1.mxd

Legend

Eurasian water milfoil (September 2013 and June 2014)

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2014 Final EWM Treatment Area
- Public Access

Map 3
Long Lake
Fond du Lac County, Wisconsin
2013 EWM Locations & 2014 Final Treatment Areas & June 2014 EWM Locations