### INTRODUCTION

Long Lake, Fond du Lac County, is an approximate 417-acres drainage 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 herbicide treatments assess aimed at controlling the non-native invasive plants curly-leaf pondweed (Potamogeton crispus; CLP) Eurasian and water milfoil (Myriophyllum spicatum; EWM) from 2011-2013.



Photo 1. Long Lake, Fond du Lac County.

The goal of the CLP control project is to reduce the treatable acreage of CLP within Long Lake. 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 2012, approximately 50.8 acres of CLP around the lake were targeted with liquid endothall at rates of 2.0 - 2.5 ppm active ingredient (ai). Surveys following the treatment indicated that the 2012 CLP treatment on Long Lake was met with mixed results; some areas saw reductions in CLP presence while others did not. Emerging research indicates that in order for an application of 2.0 ppm ai endothall to be effective at controlling CLP, this concentration likely needs to be maintained for at least 8-12 hours (or longer). That length of exposure time is very difficult to achieve, especially in smaller treatment areas. The 2012 treatment areas that did not see good CLP control likely had more rapid herbicide dissipation rates and the plants were not exposed to the herbicide for a long enough period to cause mortality. As is discussed in the next section, the 2013 CLP treatment strategy involved increasing the dose of endothall in an effort to compensate for the rapid dissipation rate.

EWM was first observed in Long Lake in 2002. Whole-lake point-intercept surveys were conducted by the Wisconsin Department of Natural Resources (WDNR) in 2007 and 2010. EWM was not located at any of the sampling points during the 2007 survey and only located at two sampling locations during the 2010 survey. EWM occurrences were mapped during the summer of 2011 and 2012 to determine changes in the abundance and density of this non-native species within Long Lake. No EWM was located in 2011 that warranted treatment and no EWM treatment occurred in 2012. During Onterra's 2012 EWM survey, EWM was found to have increased in occurrence and density from 2011. The 2012 growing season was considered a banner year for EWM; the early ice-out in the inland lakes, coupled with exceptionally warm water and air temperatures, made for ideal conditions to spur EWM growth. This may help to explain why EWM, which was sluggish to spread in Long Lake in years past, grew quite well within the lake in 2012.



# 2013 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, and was the strategy utilized on South Twin Lake in 2009 and 2010.

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. 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 rebounds by the end of the summer.

As mentioned, the 2012 CLP treatment on Long Lake was met with mixed results. While some of the 2012 treatment areas were successful at causing CLP mortality before they were able to produce turions, it is unknown exactly how long turions formed from previous years can remain viable in the sediment, but it is believed to be at least 3-5 years. For this reason, the 2013 control strategy proposed that all areas treated in 2012 be retreated again in 2013 (Map 1). Multiple years of treatment over these same areas will need to occur to kill CLP sprouting from previously deposited turions. In addition, new areas of colonized CLP located on the lake's eastern shoreline were proposed for treatment. In total, approximately 52.9 acres of CLP were proposed for treatment in 2013 (Map 1).

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. In general, treatment areas that are less than five acres are proposed to be treated with liquid endothall at a rate of 3.0 ppm active ingredient (ai), while treatments greater than five acres will be treated at a rate of 2.5 ppm ai. Due to



its narrow width, treatment site J-12 (7.9 acres) was proposed to be treated at 3.0 ppm ai. These rates are slightly (0.5 ppm ai) higher than application rates prescribed in 2012. The intent of this higher dosing is to achieve higher mortality rates of the CLP within these treatment areas. A few of the proposed treatment sites are 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 rate. 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. 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.

The initial treatment strategy is relatively aggressive, with all areas of colonized EWM were considered for treatment. The LLPA's treatment threshold (trigger) would also extend to immediately adjacent areas of EWM mapped with point-based techniques and other areas of the lake that contained dense aggregations of EWM mapped in this manner. Using this rationale, approximately 14.5 acres of EWM treatment were proposed for 2013 (Map 3). Treatment sites A-13, C-13, and E-13 are relatively small and/or narrow and are therefore were proposed to be targeted with granular 2,4-D at its maximum application rate of 4.0 ppm acid equivalent (ae). The proposed dose for the other sites were slightly less (3.5 ppm ae) due to being either larger in size and/or confined (B-13 and D-13). The herbicide treatment strategy was proposed to be implemented when surface water temperatures are 50 –  $60^{\circ}$ F and ideally in conjunction with the endothall treatments, especially in areas where the application areas of the two herbicides overlap. In whole-lake treatments, it is believed that the simultaneous exposure to endothall and 2,4-D may provide increased control of EWM.

# PRETREATMENT CONFIRMATION AND REFINEMENT SURVEY

On May 15, 2013, Onterra ecologists conducted the CLP and EWM Pretreatment Confirmation and Refinement Survey on Long Lake. Based upon a temperature profile collected during the survey, Long Lake was found to be just beginning to stratify, with water temperatures ranging from approximately 58°F near the surface to 48°F near the bottom (Figure 1). Dissolved oxygen was approximately 11 mg/L near the surface and 9 mg/L near the bottom. 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, CLP treatment sites F-13 and G-13 were removed due to insufficient levels of CLP observed in these areas. Treatment site A-13 was expanded slightly to encompass CLP observed outside of the proposed treatment area, while L-13 was reduced as CLP was not observed in a portion of the proposed treatment area. In total, the final CLP treatment was reduced to 48.6 acres from the proposed 52.9 acres (Map 1). Proposed EWM treatment sites A-13, B-13, and D-13 were removed



from treatment as little to no EWM could be observed in these areas. In addition, proposed EWM site C-13 was expanded slightly. In total, the final EWM treatment was reduced from the proposed 14.5 acres to 3.9 acres (Map 3).

The treatment was conducted by Aquatic Biologists, Inc. on May 22, 2013. The applicator reported a near-surface water temperature of approximately 68°F and north winds of 0-5 mph at the time of application.

# 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

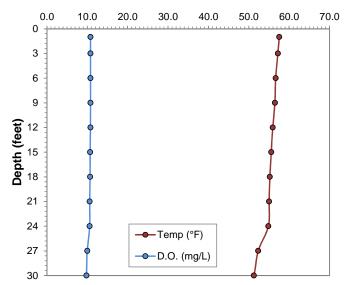


Figure 1. Pretreatment temperature and dissolved oxygen (D.O.) profile collected in North Twin Lake. Collected on May 29, 2013.

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 172 point-intercept sub-sample locations, all located within 2012 CLP treatment areas in both the springs (April) of 2012 and 2013 (May) prior to treatment (Figure 2). At each of these locations, the presence (or absence) of CLP was recorded. The presence of native aquatic plant species were not recorded as most of these plants are not actively growing at this time of year. Comparing these data from year to year allows for a statistical comparison of CLP occurrence and a quantitative determination of the CLP population over time.

In order for Long Lake to participate in the US Army Corps of Engineers (USACE) and WDNR herbicide concentration monitoring study, they required an additional sub-sample point-intercept survey be conducted in June of 2013 following the treatment in an attempt to quantify the treatment's efficacy. However, as discussed, while CLP is generally at or near its peak growth in June, it must be



noted that changes in CLP's occurrence from the spring 2013 pre-treatment survey to the June 2013 post-treatment survey cannot solely be attributed to the treatment and may be due to natural senescence. The June 2013 posttreatment sub-sample point-intercept survey included documenting CLP's presence at 115 sub-sampling locations that fell within 2013 CLP treatment areas that also had an herbicide concentration monitoring location.

While quantitative evaluation methodologies for EWM spot treatments generally follow WDNR protocols in which point-intercept data are collected within treatment areas both before the summer and the summer immediately following the spring treatment, due to the small size of the 2013 EWM treatment, no quantitative monitoring was conducted as a sufficient number of sampling locations could not have been placed within the treatment sites 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

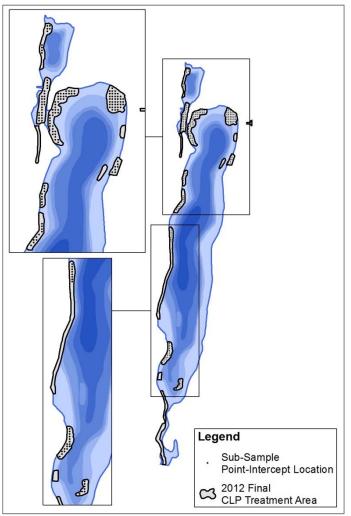


Figure 2. Sub-sample point-intercept survey locations within 2013 treatment areas on North Twin Lake.

peak growth. Because EWM reaches its peak growth in mid to late summer, EWM locations are mapped the year prior to treatment (2012) in late summer and in the late summer immediately following the treatment (2013). 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 - 4).

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*).

# **Herbicide Concentration Monitoring**

Post-treatment herbicide concentration monitoring data have not been received from the USACE at this time. Once these data are received, this information will be sent out separately.

# Aquatic Plant Monitoring Results

## CLP

During the April 2012 sub-sample point-intercept survey, of the 172 point-intercept sampling locations within 2012 treatment areas, 27 (15.7%) contained CLP, and in the May 2013 survey, 33 (19.2%) of the 172 sampling locations contained CLP (Figure 3). While six more sampling points contained CLP in the spring of 2013 compared to the spring of 2012, the occurrence of CLP between the two surveys was not statistically different (Chi-square  $\alpha = 0.05$ ). As discussed earlier, these surveys do not directly measure the efficacy of a given treatment. Even though the occurrence of CLP within these areas was not reduced from 2012 to 2013, it does not indicate an unsuccessful treatment in 2012; rather, it indicates that there were viable turions still present in the sediment that sprouted plants in 2013. Management of CLP populations that have an established turion bank generally require multiple years (3-5) of treatment before annual reductions in occurrence are observed.

In June 2013, 16 (13.9%) of the 115 sampling locations contained CLP within treatment areas where herbicide concentration was measured. Comparing the June 2013 occurrence of CLP from these same sampling locations from the spring 2012 and spring 2013 surveys indicates its occurrence was not statistically different from either of these surveys (Figure 4). While the post-treatment sub-sample point-intercept survey has to occur in June to coincide with the peak growth of CLP, a flaw of this survey is that it is done relatively soon after the herbicide application. Generally, the plants need at least a month or more before the effects of the herbicide are apparent. However, if the survey is conducted any later, the plants are also beginning to naturally senesce. So, in other words, the fact that a statistically valid reduction in CLP occurrence was not detected in June 2013 does not mean the treatment was eventually effective at causing CLP mortality.

While the occurrence of CLP from the June 2013 post-treatment sub-sample point-intercept survey was not statistically different from the May 2013 pre-treatment survey, the qualitative mapping data indicated reductions of CLP density within the treatment areas compared to 2012 (Map 1 and 2). In the southern portion of the lake, treatment areas K-13, M-13, N-13, T-13, and S-13 all contained colonized areas of *scattered* CLP in 2012, while little to no CLP could be found in these areas in June of 2013. The large *scattered* colony within site E-13 was also reduced, while very little CLP could be located in sites H-13, I-13, or J-13. Treatment sites A-14, C-14, and D-14 also saw qualitative reductions in CLP occurrence. The qualitative reductions observed within the 2013 treatment areas indicate CLP was impacted or beginning to be impacted by the 2013 treatment.



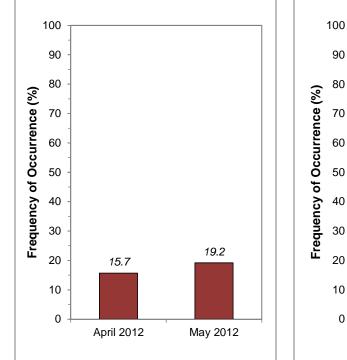
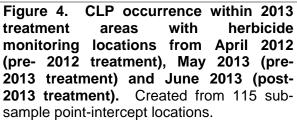


Figure 3. CLP occurrence within 2012 with treatment areas herbicide monitoring locations 2012 2012 from April (pretreatment) and May 2013 (pre- 2013 treatment). Created from 172 subsample point-intercept locations.



22.6

May 2013

13.9

June 2013

17.4

April 2012

10

0

#### EWM

Long Lake was also surveyed for EWM during the June 2013 Early-Season AIS Survey. This survey indicated that within the 2013 treatment areas, 100% of the EWM acreage treated had been reduced by at least one density rating (Map 3 and 4), exceeding the predetermined qualitative success criterion (75% reduction). The *dominant* EWM colony within C-13 was reduced to a small number of *single or* few plants and clumps of plants, while no EWM could be located within treatment site E-13 where a scattered EWM colony had been prior to treatment. No colonized areas of EWM were located anywhere in Long Lake in 2013, and the highest occurrence of EWM was located in channelized area of the southernmost portion of the lake.



### CONCLUSIONS AND DISCUSSION

As discussed, it is difficult to determine the efficacy of a given CLP treatment given the unique lifecycle of this plant. While the occurrence of CLP was not statistically different from the 2013 preand post-treatment point-intercept surveys, the qualitative survey indicated the treatment was successful at reducing the density of CLP within the 2013 treatment areas. Many viable turions produced in previous years are likely still present within the sediment in these areas. As mentioned, 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, the 2014 control strategy proposes that all areas treated in 2013 are proposed to be retreated in 2014 (Map 2). Multiple years of treatment over these same areas will need to occur to kill CLP sprouting from previously deposited turions. In total, 48.6 acres are initially proposed for treatment in 2014 (Map 2). These areas will be focused on during the 2014 spring pretreatment survey and may be refined based on those survey results. If sufficient CLP is not located within a particular area during this survey, it will be removed from the final treatment plan. Depending on the size and depth of the treatment area, liquid endothall is proposed to be applied at a rate of 2.5 to 3.5 ppm ai.

Overall, the 2013 EWM treatment was met with success, with both areas of colonized EWM within the two treatment areas being reduced. CLP treatment site L-14 (4.6 acres) is also proposed to be treated with liquid 2,4-D at a rate of 3.0 ppm ae to target EWM within this area (Map 4).

This project was scheduled to end with the 2013 report; however, grant funds remain; therefore, the LLAA has decided to extend the project through 2014. To do so, additional grant funds will be requested for the current AIS-Established Population Control Grant, in the form of a grant amendment. Further, the group has decided to move forward with the development of an updated management plan. The proposed management planning project would be much more comprehensive then the lake's original management plan. The LLAA has requested funds for the completion of the management through an AIS-Education, Planning and Prevention Grant application turned in for the February 1, 2014 grant deadline. To further open up funds for the 2014 treatment on Long Lake through the current AIS grant, the annual monitoring conducted by Onterra, as defined in this report, would be funded through the new management planning project. Finally, the annual report for 2014 would be included within the updated management plan, scheduled for completion in November 2014.



