INTRODUCTION

Long Lake, Fond du Lac County, is an approximately 454-acre drainage lake (including the northwest basin known as 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. The objective of this project was to monitor and assess herbicide treatments aimed at controlling the non-native invasive plants curly-leaf



Photo 1. Long Lake, Fond du Lac County.

pondweed (*Potamogeton crispus*; CLP) and Eurasian water milfoil (*Myriophyllum spicatum*; EWM) from 2011-2013. 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.

The LLPA completed an update of their management plan in 2015 (*Long Lake Comprehensive Management Plan, Onterra, March 2015*). The updated plan created new thresholds and triggers for the continued control of CLP and EWM within Long Lake. The LLPA has outlined an aggressive approach to CLP management within their *Comprehensive Management Plan* 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 handremoval. The LLPA has just begun initiating this aspect of their control program.

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. 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 2014 were proposed to be retreated in 2015 (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, 22.1 acres were initially proposed for treatment in 2015 (Map 1). No areas of EWM warranted herbicide control in 2015. The LLPA outlined an EWM control strategy within the management plan which involved targeting EWM with spot-treatments or hand-harvesting as appropriate. The EWM population in Long Lake was monitored in 2015 through surveys conducted in June and in August.



2015 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.

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.

One of the 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 mixed results observed in previous spot treatments in Long Lake indicate that the herbicide may not have reached an adequate concentration-exposure time to cause plant mortality. With this knowledge, proposed 2015 treatment areas that were less than 5 acres were proposed to be treated with liquid endothall at an increased rate of 3.0 - 3.5 ppm ai, while treatments of greater than five acres would be treated at a rate of 2.5 ppm ai. Following design of the 2015 control strategy, the makers of endothall (United Phosphorus Inc, UPI) made a general recommendation that spot treatments should try to be 5 acres or larger to retain adequate concentrations and exposure times (CETs) required for control. This recommendation would be reflected in the final recommendation following the pretreatment survey.

PRETREATMENT CONFIRMATION AND REFINEMENT SURVEY

On April 27, 2015, Onterra ecologists conducted the Pretreatment Confirmation and Refinement Survey on Long Lake. During this survey, the presence of CLP 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. A temperature and dissolved oxygen profile that was taken indicated that the lake was mixing with temperatures of $9.0^{\circ}C - 11.0^{\circ}C$ ($48^{\circ}F - 52^{\circ}F$) and dissolved oxygen near 10.0 mg/L throughout the water column (Figure 2).

Following this survey, three CLP treatment sites were removed due to insufficient levels of CLP observed within these areas. The extents of the remaining three treatment areas were not modified, and actively growing CLP was observed throughout these areas. Two of the sites were approximately 5 acres each and met the UPI recommendations. The third site was slightly less than 3 acres, but was tucked in a protected part of the lake where CETs were likely to be met. In total, the final 2015 CLP treatment was reduced from the proposed 22 1 acres to 12.8 a



treatment was reduced from the proposed 22.1 acres to 12.8 acres (Map 1).

The treatment was conducted by Aquatic Biologists, Inc on May 5, 2015. The applicator reported a near-surface water temperature of approximately 13.7°C (56.7°F) and southeast winds of 0-5 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 3). These data indicate that winds were



predominantly easterly at the time of treatment and averaged between six and ten mph in the first 10 hours after treatment.



MONITORING METHODOLOGIES

The objective of an herbicide treatment strategy is to maximize target species (CLP) 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 Monitoring

It is difficult, if not impossible, to assess the efficacy of a single year of treatment on a lake's CLP population. Curly-leaf pondweed 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 point-intercept sub-sample locations located within CLP treatment areas (Figure 4). Data has been collected annually in the spring prior to the herbicide treatments from At each of these locations, the 2012-2015. presence (or absence) of CLP was recorded. The surveys were replicated annually during June of 2013 - 2015 to correspond with the peak growth stage of CLP. The presence of native aquatic plant species was also recorded during the June 2013 - 2015 surveys. Comparing these data from year to year allows for a statistical comparison of native aquatic plant occurrence. Comparing the spring pretreatment point-intercept survey data with the June post treatment data is a little more difficult to determine CLP control due to factors of natural die off (senescence) discussed above. But certainly if CLP exist within the treatment areas following treatment, a failed treatment is likely to have occurred.



Qualitative 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. 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 locations that were considered as *Small Plant Colonies* (<40 feet in diameter), *Clumps of Plants*, or *Single or Few Plants*.

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 subsample point-intercept data, following multiple years of treatments, the qualitative should show reductions in CLP acreage and density over time. The 2015 CLP treatment sites were mapped in 2011 at the start of the current control project and a qualitative assessment cannot be made until a future year in which no CLP treatment occurs in the sites and a true understanding of the CLP population can be made. Based upon a pre-determine success criterion, an effective CLP treatment program would include a 75% reduction of CLP as demonstrated by a decrease in at least one 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 involving anticipated whole-lake impacts. In Long Lake, endothall 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 over the course of numerous days following treatment.

Water samples were collected at two sites (Map 1, Figure 5) at time intervals of approximately 1, 2, 3, 4. 6, 10, 14 and 24 hours after treatment (HAT) using an integrated sampler. The samples were fixed (preserved) with acid and shipped to the Wisconsin State Lab of Hygiene (SLOH) where the herbicide analysis is completed.

TREATMENT MONITORING RESULTS

Herbicide Concentration Monitoring Results (CLP)

As discussed previously, liquid endothall was applied in three sites totaling 12.8 acres at dosages between 2.5 - 3.5 ppm ai (Map 1). Herbicide concentration monitoring data collected after the treatment showed initial concentrations to be much higher than the application rate from the samples collected at 1 HAT to 4 HAT (Figure 6). The application rate within site C-15 and sampling site LF1 was 3.0 ppm ai (2.13 ppm ae) compared to an observed concentration of 6.00 ppm ae at 1 HAT (Figure 6). Samples collected at 2 HAT, 3 HAT and 4 HAT at LF1 were all above the initial target application rate of 2.13 ppm ae. Concentrations decreased below 1.00 ppm ae by 10 HAT and remained present within the site at low concentrations in the sample collected at 24 HAT (Figure 6).

Herbicide concentration data collected from sampling site LF2 within treatment site E-15 also showed higher than expected concentrations between 1 HAT and 3 HAT. Herbicide was applied at the site at a dosage of 2.5 ppm ai (1.77 ppm ae)



and samples collected from 2 HAT (2.9 ppm ae) and 3 HAT (3.6 ppm ae) were higher than expected (Figure 6). Herbicide concentrations were below 1.00 ppm ae by 6 HAT and remained present in the site at very low concentrations through the last sampling interval at 24 HAT (Figure 6).





Aquatic Plant Monitoring Results

Efficacy

During the April 2015 pre-treatment sub-sample point-intercept survey, seven (9.7%) of the 72 sampling locations contained CLP (Figure 7). Following the treatment, CLP was found on two sampling locations (2.8%) during the June 2015 survey. The reduction in CLP LFOO between the spring and June 2015 surveys suggest that the CLP population was at least somewhat controlled by the spring 2015 treatment. But as discussed above, this includes the disclaimer about difficulties of understanding CLP control with these data.

In analyzing the sub point-intercept data going collected in treated areas since 2012, a downward trend in the CLP population is evident (Figure 7). In the spring of 2012, CLP had a LFOO of 26.4% and was 31.9% in the spring of 2013. After several consecutive years of treatment, the number of viable



Figure 7. Frequency of occurrence of CLP from sub-sample point-intercept locations within Long Lake treatment areas. Dashed lines represent CLP spot treatments.

turions in the lake sediment should be depleted resulting in fewer and fewer CLP plants on the point intercept survey. The LFOO's of CLP from both spring and June surveys has decreased over the



course of the treatment program and only a 2.8% LFOO was recorded during the June 2015 survey (Figure 7).

Curly-leaf pondweed was mapped during a June 9-10 Early Season AIS Survey (ESAIS). During the June survey, the 2015 herbicide treatment areas were visited to conduct quantitative monitoring (sub point-intercept survey points) and to qualitatively map CLP in the sites to assess the spring 2015 treatment. Within site A-15, a combination of point-based CLP occurrences consisting of single or few plants, clumps of plants and small plant colonies were located in the northern end of the site, and no colonized CLP was mapped in the site (Map 2).

Within site C-15, only one *single or few* CLP occurrence was located in the site suggesting successful control in 2015 (Map 2). Only a few *single or few* CLP occurrences were located within site E-15 during the June survey however, a small *scattered* colony and additional single plants were located just outside of the treatment site (Map 2). Curly-leaf pondweed was located widely scattered throughout the littoral areas the lake during the June 2015 survey (Map 2).

Selectivity

The littoral frequencies of occurrence of native aquatic plant species available from the June 2013, June 2014 and June 2015 surveys are shown in Figures 8-9. Only those species which exhibited a 5% or greater LFOO in at least one of the surveys are displayed. Two species, (muskgrasses and clasping-leaf pondweed) displayed a statistically valid decrease from 2014-2015. Clasping-leaf pondweed is believed to be susceptible to herbicide treatments and the decline in this species to 0% in June 2015 is worth noting concern. Muskgrasses are actually not plants, but macroscopic algae that are particularly tolerant to herbicide control strategies. It is not clear if the reductions observed are related to the herbicide treatment or other environmental factors. Eleven other species did not show a statistically different LFOO between the June 2014 and June 2015 surveys (Figures 8-9). Additional aquatic plant monitoring in subsequent years may expose trends that represent population changes caused by the treatment program and not simply document inter-annual population dynamics. Changes observed within the native plant populations within the herbicide treatment sites should not be extrapolated to also include the lake-wide population since the spot-treatment herbicide use pattern does not cause whole-lake impacts.





statistically valid change in occurrence from previous survey and red outline in 2015 indicates statistically valid change compared to 2013 (Chi-squared $\alpha = 0.05$). (n=72)



Long Lake Preservation Association, Inc.





2015 EWM MONITORING RESULTS

The EWM population in Long Lake was monitored in 2015 through mapping surveys conducted during June and August. The first monitoring event on Long Lake in 2015 was the Early Season Aquatic Invasive Species Survey (ESAIS). This late-spring/early-summer survey provides an early look at the lake and in addition to mapping CLP, provides a good opportunity to locate EWM occurrences in the lake while the growth stage of most of the native plant population is relatively low. The EWM locations identified during the June survey are refined during the late summer survey when the plants have grown to their peak biomass level. On June 9-10, 2015, Onterra ecologists conducted the ESAIS Survey on Long Lake. This survey indicated that EWM population was at relatively low levels in the lake and no large continuous colonies were present (Map 3).

The EWM was re-evaluated during the EWM Peak-biomass Survey conducted on August 13, 2015. Overall, less EWM was found during the August 2015 survey than in June possibly as a result of native plants competing for space and resources (Map 4).

CONCLUSIONS AND DISCUSSION

Overall, the 2015 CLP herbicide treatment on Long Lake appears to have been successful in controlling the CLP population within the targeted areas. No colonized areas of CLP were located within the 2015 treatment areas and quantitative data indicate that the occurrence of CLP remains low within areas that have been targeted for control. Curly-leaf pondweed was found in many areas throughout the littoral zone during the June 2015 survey but at low densities that are not causing impact to the ecosystem nor recreational impediments to lake users.

While worth expanded discussion, the higher than anticipated endothall concentrations observed are not overly alarming. CLP appears to have been impacted in these areas and only 2 out of 11 native species found to be statistically different from June 2014 to June 2015. These findings are consistent with other CLP spot treatments that have been monitored.

As noted in the herbicide concentration monitoring section, the samples collected following the treatment were found to contain unexpectedly high herbicide concentrations. In most similar concentration monitoring of spot treatments, samples collected rarely reach application targets due to rapid dissipation. Factors such as water flow and wind activity have been noted to speed up dissipation. It is perplexing to observe the high concentrations noted here in light of flow in these areas and the relatively high wind speeds (5-10 mph) following the application. In an effort to understand why the herbicide concentration values were found to be so high following the treatment, Onterra contacted the Wisconsin State Lab of Hygiene (WSLH) where the samples were analyzed to inquire about the lab results for the samples from Long Lake. The WSLH confirmed the quality control process in the initial lab tests and re-ran several samples in early March 2016 which confirmed the initially reported values as accurate. With no reason to believe that the reported values are incorrect, other explanations for the higher than expected concentration values are considered.

Calculations that were made to determine the appropriate amount of product to use for the 2015 spot treatments were confirmed and the Aquatic Plant Management Herbicide Treatment Record (Form 3200-111) indicated that the application was conducted as planned. One hypothesis is that slightly uneven application lead to "hot spots" of concentrated herbicide in which the monitoring sites were



located. A WDNR supervisor was onsite during the application and did not see anything out of the ordinary.

Another hypothesis is that the herbicide could have been slow to dissipate away from the application area following the treatment because water exchange was minimal around the time of the treatment. As discussed above, the wind conditions around the time of treatment suggest that there was likely some amount of wind driven water exchange during and after the treatment as winds were approximately 5 mph during the application and increased to between 5-10 mph in the hours following treatment (Figure 3). It is also possible that herbicide applied to one site could have migrated into another site. While not uncommon to see, typically herbicide dissipation into a site from a neighboring treatment site is coupled with herbicide dissipation out of the site from the original application.

Consistent with the strategy outlined within *Long Lake Comprehensive Management Plan, Onterra, March 2015*, each of the three 2015 treatment areas is proposed to be retreated again in 2016. Based on the CLP population identified during the June 2015 survey, two additional areas are considered for herbicide control in 2016 and include expanding site C-15 southward totaling 18.0 acres and the addition of site D-15 on the west shore of the lake (Map 5). These areas, totaling approximately 30.5 acres, are proposed to be treated with liquid endothall at rates of 2.0-3.5 ppm ai (Map 5). The results of the 2016 Pretreatment Confirmation and Refinement Survey will ultimately determine the final treatment acreage, particularly if insufficient CLP warranting treatment is observed in parts (or all) of the treatment sites. An ESAIS survey will be conducted in June 2016 to assess the herbicide treatment areas and to map CLP throughout the lake.

Given the low density of EWM within Long Lake in 2015, no herbicide treatment targeting EWM is proposed for 2016. A control strategy utilizing professional hand-harvesting may be the most appropriate method for controlling the current EWM population in Long Lake. An Early Summer AIS Survey (ESAIS) will be conducted in 2016 from which a hand-harvesting strategy would derive. Onterra will provide the hand-harvesting firm with the spatial data from the June survey to aid the removal efforts. Following any hand removal efforts, a Late-Summer EWM Peak Biomass Survey will qualitatively assess the hand harvesting efforts.











