INTRODUCTION

Long Lake, Vilas County, is an 872-acre drainage lake with a maximum depth of 95 feet and mean depth of approximately 30 feet (Map 1). Connected via the approximately 1.25-mile-long Thoroughfare Creek, Big Sand Lake flows into Long Lake. Big Sand Lake is arguably the first lake in Vilas County to contain Eurasian water milfoil (EWM), with official records of this plant occurring in the lake in 1990. Long Lake flows through the Deerskin River into Scattering Rice Lake of the Eagle River Chain of Lakes.

In 2000 the presence of EWM was verified by the Wisconsin Department of Natural Resources (WDNR) from Long Lake, although it was suspected of inhabiting the system for years before this date. In 2006, the WDNR completed a point-intercept aquatic plant survey, locating EWM in approximately 26% of the littoral area of the lake (< 18ft). Onterra was contracted by the Long Lake of Phelps Lake District (LLPLD) during the late-summer of 2007, where the majority of the littoral zone contained colonized EWM of which approximately 25 acres was found to be *surface matted*.

The LLPLD has been actively managing the EWM population through strategically targeted herbicide spot treatments and hand-removal. In order to build off the success of previous years' control efforts, the Long Lake Comprehensive Management Plan (July 2013) outlines an aggressive approach to EWM management for 2013-2017. This strategy includes a treatment threshold (trigger) to initiate treatment in areas containing colonized EWM and adjacent areas of EWM mapped with point-based techniques, with areas containing *Small Plant Colonies* being targeted for treatment if possible. 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 implement the EWM management program outlined within the July 2013 Comprehensive Lake Management Plan.

WDNR LONG-TERM EWM TRENDS MONITORING RESEARCH PROJECT

Starting in 2005, WDNR Science Services began conducting annual point-intercept aquatic plant surveys on a set of lakes to understand how EWM populations vary over time. This was in response to commonly held beliefs of the time that once EWM becomes established in a lake, its population would continue to increase over time. Because the state of Wisconsin's waters are managed for multiple uses (Statue 281.11), the WDNR wanted to understand if EWM populations would increase and cause either 1) ecological impacts to the lake and/or 2) reductions in ecosystem services (i.e. navigation, recreation, aesthetics, etc.) to lake users. As outlined in *The Science Behind the "So-Called" Super Weed* (Nault 2016), EWM population dynamics on lakes is not that simplistic.

Like other aquatic plants, EWM populations are dynamic and annual changes in EWM frequency of occurrence have been documented in many lakes, including those that are not being actively managed for EWM control (no herbicide treatment or hand-harvesting program). The data are most clear for unmanaged lakes in the Northern Lakes and Forests Ecoregion (Figure 1). Some lakes, such as Handcock Lake, maintained low EWM populations over the study averaging 2.3% between 2008 and 2015. At these low levels, there are likely no observable ecological impacts to the lake and are no reductions in ecosystem services to lake users. The EWM population of Handcock Lake has increased in recent years to 5.2% in 2015 and over 10% in 2016 (preliminary data not shown in Figure 1).

December 2016 1 On



Eurasian water milfoil populations in other lakes, such as Bear Paw Lake and Little Bearskin Lake trended to almost 25% only to decline to approximately 5% by the end of the study period. There are many factors that could contribute to the decline in the EWM population of these lakes, including climactic conditions and water quality parameters. Little Bearskin is known to contain a robust population of milfoil weevils, and this native insect may be having an impact on the EWM population within the lake. Boot Lake is a eutrophic system with low water clarity (approx. 3-ft Secchi depth) due to naturally high phosphorus concentrations. It is hypothesized that water clarity conditions in some years may favor EWM growth whereas in other years it may keep the population suppressed. Extreme changes in EWM populations like those observed on Weber Lake have also been documented. The EWM population in 2010-2011 was approximately 20% before spiking above 50% in 2012. Then the population declined back to approximately 15% in 2014 and 2015.

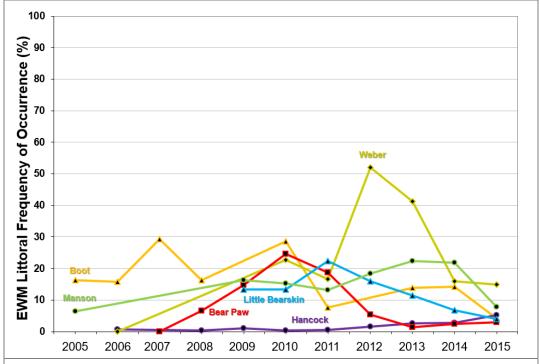


Figure 1. Littoral frequency of occurrence of EWM in the Northern Lakes and Forests Ecoregion without management. Data provided by and used with permission from the WDNR Bureau of Science Services.

The results of the study clearly indicate that EWM populations in unmanaged lakes can fluctuate greatly between years. Following initial infestation, EWM expansion was rapid on some lakes, but overall was variable and unpredictable (Nault 2016). On some lakes, the EWM populations reached a relatively stable equilibrium whereas other lakes had more moderate year-to-year variation. Some lake managers interpret these data to suggest that in some circumstances, it is not appropriate to manage the EWM population as in some years the population may become less. However, even a lowered EWM population of approximately 10% exceeds the comfort level of many riparians because it is potentially approaching a level than may be impactful to the function of the lake as well as not allowing the lake to be enjoyed by riparians as it had been historically.

Some lake groups, like the LLPD, choose to manage the EWM population to keep it at a lowered level. For reference, in the EWM population in Long Lake was at 26% in 2006 and 2.2% in 2012. A point-intercept survey for Long Lake is scheduled for 2017, the final year of the current AIS-EPC Grantfunded project. In continuing an aggressive approach to EWM control in 2016, both herbicide control and professional hand harvesting methods were recommended.

2016 EWM CONTROL 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.

In order to successfully control EWM in a laboratory setting, ongoing research suggest that exposure times of 2,4-D at maximum application rates (4.0 ppm ae) need to be approximately 9 hrs. There are many factors that influence exposure time, including treatment area size (larger = longer), shape (broad = longer) and location (protected parts of lake = longer) along with obvious factors such as wind and wave action. The 2016 strategy proposed to use a combination of liquid 2,4-D and endothall. It is believed that conducting a treatment using a combination of 2,4-D and endothall can have an additive and potentially, synergistic effects, that shorten the exposure time required for control. One site totaling 14.0 acres in Long Lake was proposed to be treated with a combination of liquid 2,4-D and endothall at application rates of 4.0 ppm acid equivalent (ae) and 1.5 ppm active ingredient (ai), respectively (Map 1).

Additionally, seven areas totaling almost 2 acres were proposed to be targeted via professional hand-removal in 2016 (Map 2). Many Waters, LLC's implementation of DASH system in 2014-2015 proved to be effective at removing small, but dense colonies of EWM.

PRETREATMENT CONFIRMATION & REFINEMENT SURVEY

On May 17, 2016, Onterra ecologists conducted the EWM Spring Pretreatment Confirmation and Refinement Survey of Long Lake. During this survey, all the proposed control sites (herbicide and hand harvest) were visited. Sufficient EWM warranting treatment was confirmed within the proposed herbicide treatment area. Proposed hand harvesting site F-16 was removed from the control strategy as no EWM was located in the site. From this survey, the final herbicide treatment acreage was kept at the originally proposed 14.0 acres and the proposed hand removal areas were reduced from 1.97 acres to 1.88 acres (Map 2). Onterra provided the LLPLD and the professional hand harvesting firm with the spatial data containing the EWM locations to aid in the hand removal efforts.



A temperature/dissolved oxygen profile indicated that the lake was beginning to stratify below approximately 20 feet with near-surface temperatures in the low 50s°F and temperatures in the low to mid 40s°F below 25 feet (Figure 2). Dissolved oxygen was above 9.0 mg/L throughout the water column down to 90 feet (Figure 2). Eurasian water milfoil pulled up on the rake during this mid-May survey revealed that it was actively growing, and Onterra recommended that the treatment occur as soon as logistically possible. The treatment was conducted by Clean Lake's, Inc. on June 7, 2016 using their LittLine® Littoral Zone Treatment Technology — an application system that reportedly minimizes herbicide diffusion by delivering the herbicide closer to the target plant's root system where plant biomass is greatest. The applicator reported northwest winds of 4 mph at the time of application.

Wind speed and direction data were also obtained from a weather station in nearby Watersmeet, MI, approximately 15 miles from Long Lake (Figure 3). These data indicate that winds were variable and ranged in speed from 2-5 mph during herbicide

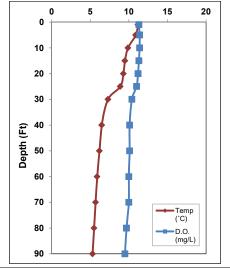


Figure 2. Pre-treatment temperature and dissolved oxygen profile collected on Long Lake. Data collected on May 17, 2016.

application. Over the next 14 hours following application, wind direction was predominantly from the northwest but remained relatively calm with speeds recorded between 0-7 mph. These data indicate that there was likely little wind-driven water movement in Long Lake during and immediately after application that would have increased herbicide dissipation rates.

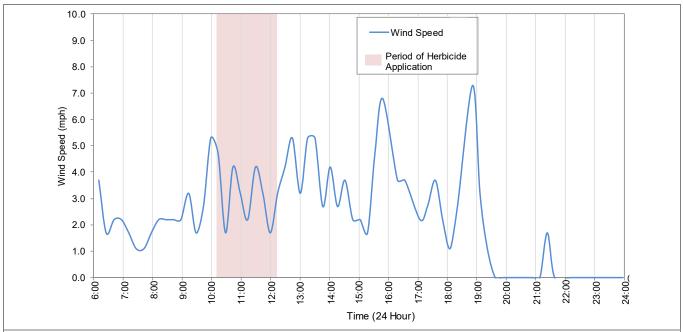


Figure 3. Wind speed and direction approximately 4 hours before and 14 hours after herbicide was applied to the Long Lake 2016 treatment area on June 7, 2016. Graph created using data from Weather Underground Station in Watersmeet MI.

HAND-HARVESTING CONTROL METHODS

In 2016, the LLPLD contracted with Many Waters, LLC to conduct EWM hand-removal within the six selected areas using the Diver Assisted Suction Harvesting (DASH) program. The use of the DASH system allows divers to target larger, denser areas of EWM than they would be able to using just divers alone. Many Waters, LLC removed EWM from the six pre-determined locations over a period of thirteen days in August and October 2016. Table 1 displays the amount of EWM in pounds that was removed from each location. In total, 877 pounds of EWM were removed from six areas using the DASH system (40.75 hours) and an additional 5 pounds were hand removed by scuba divers (4.25 hours) yielding 882 total pounds of EWM removed (Table 1, Appendix A). The 2016 hand removal summary report from Many Waters, LLC is attached with this report as Appendix A.

			six hand-harvesting ters, LLC 2016 (Appe
Site	DASH Hours	s Dive Hours	EWM Removed (lbs)
B-16	3		84
C-16	2.75	2.5	38
D-16	6.25	1.75	127
E-16	4		46
G-16	4.75		152
H-16	20		435

4.25

882

MONITORING METHODOLOGIES

TOTALS

40.75

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.

Using sub-meter GPS technology, EWM locations were mapped the year prior to treatment (2015) in late-summer when EWM is at or near its peak growth, and in the late summer immediately following the treatment (2016). 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*.

Qualitative monitoring of herbicide treatments includes comparing spatial data reflecting EWM locations and densities during the peak-growth stages the summer before the treatment and the summer immediately following the treatment. Based upon a pre-determined success criterion, an effective herbicide treatment would include a 75% reduction of EWM as demonstrated by a decrease in density rating (e.g. *Highly Dominant* to *Dominant*) and frequency of EWM within point-intercept subsampling locations.

Onterra LLC
Lake Management Planning

For spot treatment strategies like the 2016 treatment in Long Lake, quantitative evaluation methodologies 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. To assess the EWM population, a sub-sample point-intercept survey was conducted within the herbicide application area by sampling 73 locations at a resolution of 20 meters. This survey was completed during the late summer of 2015 (pre-treatment) and again during the 2016 late-summer EWM peak bio-mass survey (post-treatment) (Figure 4).

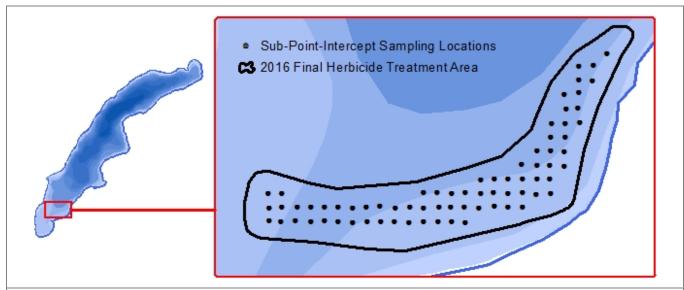


Figure 4. Quantitative monitoring locations for the 2016 herbicide treatment Site A-16 on Long Lake. (20 meter spaced points – 73 total points)

The hand-removal program would be considered successful if the density of EWM within the hand-removal areas was found to have decreased from the late summer 2015 survey to the late summer 2016 survey. Due to the relatively small size of the hand-harvesting target sites, a quantitative assessment was not feasible.

POST TREATMENT MONITORING RESULTS

The Late-Summer EWM Peak-Biomass Survey was conducted on September 13, 2016 to qualitatively assess the herbicide treatment site and hand harvesting efforts as well as to understand the peak growth (peak-biomass) of the EWM population throughout the lake. The field crews noted fair conditions for the survey with a mix of sun and clouds and light to moderate winds.

Herbicide Treatment Results

The 2016 herbicide treatment site was assessed for EWM control during the peak-biomass survey. Site A-16 was reduced from a 9.7 acre *highly scattered* EWM colony in 2015, to only point-based EWM occurrences comprised of *small plant colonies*, *clumps of plants* or *single or few plants* following the herbicide treatment (Figure 5). This 100% reduction in colonized EWM density exceeded the qualitative success criterion for the site.

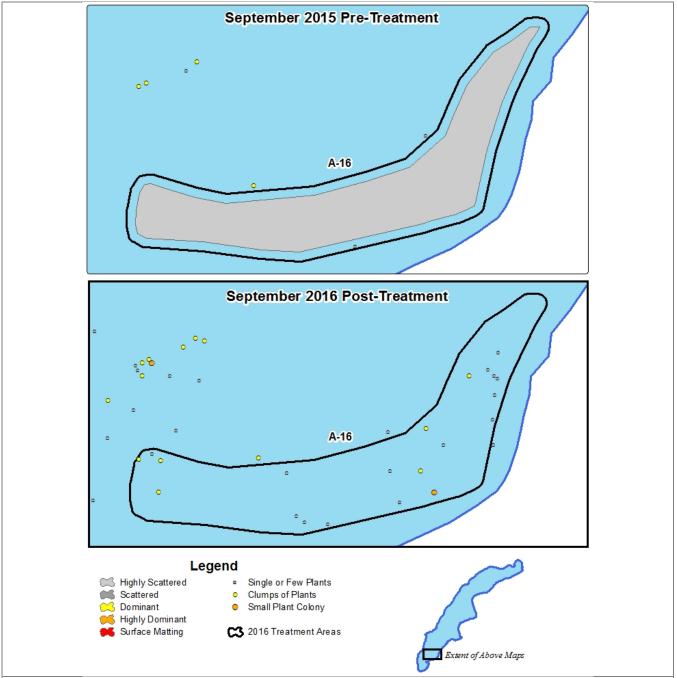


Figure 5. Late Summer 2015 Pre- and Late Summer 2016 Post-herbicide Treatment Area EWM Survey Results.

Selectivity

During the late-summer 2015 pre-treatment sub point-intercept survey within site A-16, EWM was present at 11 of the 73 (15.1%) monitoring locations (Figure 6). The post-treatment sub point-intercept survey conducted in the late summer of 2016 yielded EWM on the sampling rake at five of the 73 (6.8%) sampling locations. This 54.5% reduction in EWM occurrence falls short of the predetermined quantitative success criteria (75% reduction) for the site.

The littoral frequencies of occurrence of native aquatic plant species available from the September 2015 pre-treatment, and September 2016 post treatment surveys are also shown in Figure 6. Three native plant species exhibited a significant decrease in littoral frequency from 2015 to 2016, while six species remains statistically unchanged. The littoral frequency of occurrence of coontail was decreased by 76.9% (from 17.8% to 4.1%). Coontail, a dicot species, is a largely unrooted plant that entangles itself on vegetation and other structure (rocks, wood, etc). It is theorized that the reductions in coontail may be two-fold, plants may have been impacted directly from the herbicide treatment, and additionally, plants may have been reduced following a loss in suitable habitat where coontail may be have been utilizing EWM and other plants for structure in which to grow. Two non-dicot species that saw a significant reduction from 2015 to 2016 include fern-leaf pondweed (-45.2%) and clasping-leaf pondweed (-100%). Onterra's experience indicates that fern-leaf pondweed is sensitive to early season herbicide treatments, particularly when endothall is a component of the treatment strategy.

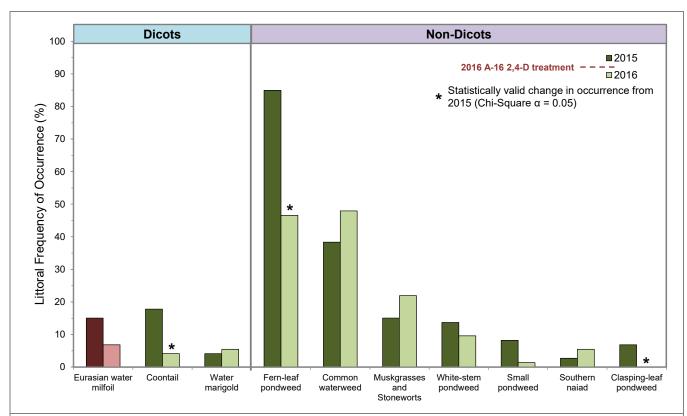


Figure 6. Littoral Frequency of Occurrence of Aquatic Plants From Late Summer 2015 Preand Late Summer 2016 Post-Herbicide Treatment Within Site A-16 (n=73). Only aquatic plants species that had a littoral frequency of greater than 5% in at least one survey are displayed.

Hand-Harvesting Results

Figures 7-9 examine the level of control achieved within the areas where professional hand-harvesting efforts were undertaken in 2016. Additional details of the diving efforts undertaken by Many Waters, LLC in 2016 are included with this document as Appendix A.

B-16: Prior to hand-harvesting, Site B-16 contained a *small plant colony, clump of plants*, and three *single or few* EWM occurrences (Figure 7, top left frame). Many Waters, LLC conducted DASH based EWM removal and reported approximately 84.0 pounds of EWM being removed from the site over three hours of effort (Table 1). Following the late summer 2016 survey, no EWM was located in the site following the hand harvesting efforts (Figure 7, top right frame).

C-16: A small plant colony and single or few plants EWM occurrence was mapped in Site C-16 prior to hand-harvesting (Figure 7, bottom left frame). A total of 5.25 hours of effort (2.75 DASH, and 2.5 of conventional diving) was undertaken by Many Waters within Site C-16 during the summer yielding a combined total of 38 pounds of EWM (Table 1) The post-harvesting survey found just one single or few EWM occurrences in Site C-16 (Figure 7, bottom right frame). Both sites B-16 and C-16 saw effective EWM control exceeding the success criteria through the hand-harvesting efforts conducted in 2016.

D-16: Prior to hand harvesting, Site D-16 contained a *small plant colony, clump of plants*, and numerous *single or few* EWM occurrences (Figure 8). Many Waters reported removing 127 pounds of EWM from the site using both DASH and conventional diving methods in 2016 (Table 1). Following the 2016 hand removal efforts, only three *single or few plant* occurrences were located in the site indicating successful control (Figure 8).

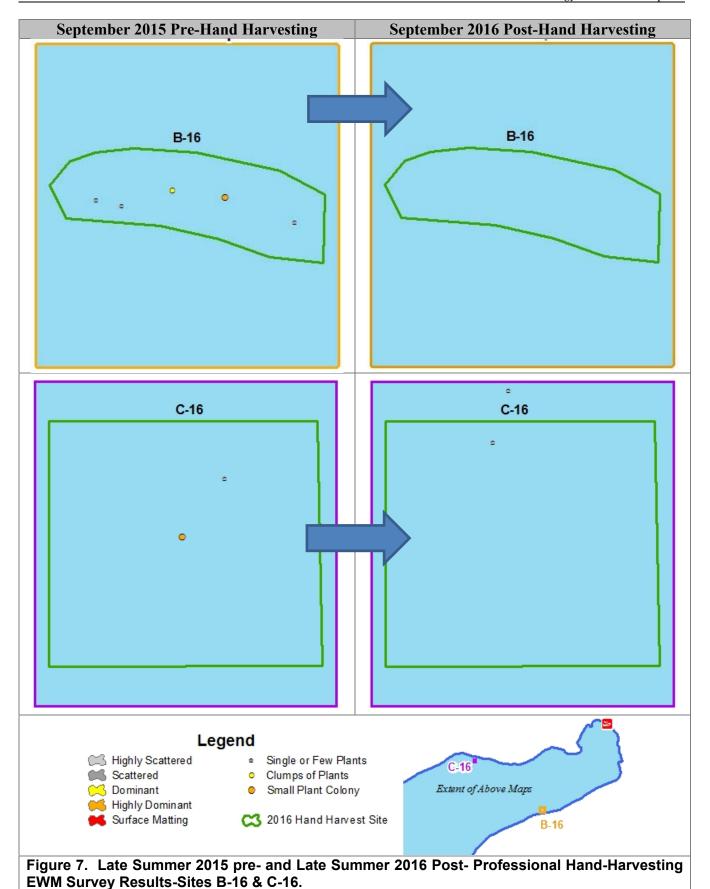
E-16: Site E-16 contained several *clumps of plants* and *single or few plants* prior to hand harvesting. Removal efforts totaling 46 pounds of EWM removed over 4 hours of DASH time resulted in no EWM being located during the post-harvesting survey (Figure 8). Successful EWM control was achieved within Site E-16.

G-16: A *small plant colony* was mapped in site G-16 during the September 2015 pre-harvesting survey (Figure 9). Many Waters spend three hours harvesting EWM with the DASH setup on an August 2 visit and reported removing 84 pounds of EWM from the site. No EWM was located at site G-16 during the September 2016 post-harvesting survey indicating successful control (Figure 9).

H-16: Site H-16 contained a *small plant colony*, three *clumps* of EWM and several *single or few plant* occurrences during the 2015 pre-harvesting survey (Figure 9). Many Waters spent 20 hours of DASH effort in the site over three days in August 2016 and reported removing 435 pounds of EWM from the site. The 2016 post-harvesting survey shows an overall reduction of EWM in the site, however point based EWM occurrences consisting of either *clumps of plants* or *single or few plants* remained present following the removal efforts (Figure 9).

After the completion of the September 2016 mapping survey, Many Waters revisited Long Lake in early October to conduct limited additional hand-harvesting through conventional diving with SCUBA gear. These efforts were directed at Site C-16, D-16 and at other known EWM/HWM locations located in the area between B-16 and D-16. Details of these efforts are available in Appendix A.





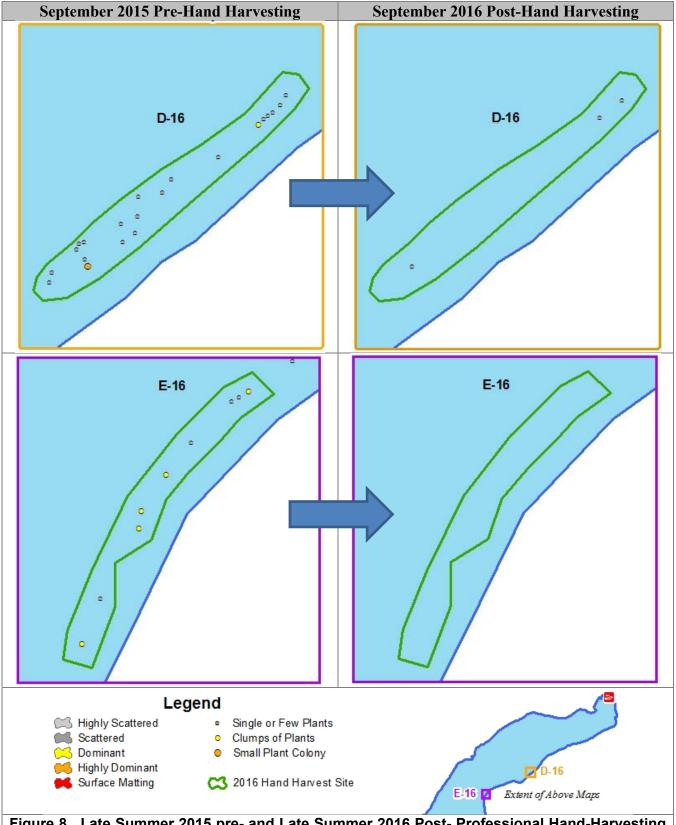


Figure 8. Late Summer 2015 pre- and Late Summer 2016 Post- Professional Hand-Harvesting EWM Survey Results-Sites D-16 & E-16.

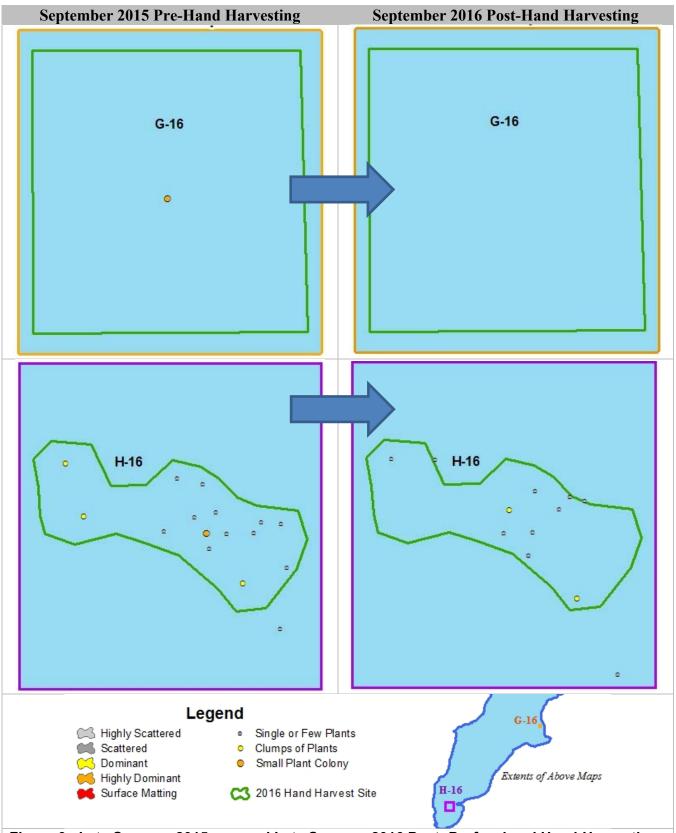


Figure 9. Late Summer 2015 pre- and Late Summer 2016 Post- Professional Hand-Harvesting EWM Survey Results-Sites G-16 & H-16.

During the late summer 2016 EWM peak-biomass survey, EWM was found to be present within many areas of the lake (Map 3). The majority of EWM in the lake consists of relatively low density occurrences utilizing point-based mapping methodologies. Areas in which the EWM occurrences were most prevalent include the southern end of the lake as well as the small bay near the Thoroughfare Creek inlet from Big Sand Lake on the east shore of Long Lake. No large colonies were found in 2016 that could be described with a density as *dominant*, *highly-dominant or surface matted*. One approximately 0.1 acre colony of *scattered* EWM was mapped near the inlet location from Thoroughfare Creek and another small 0.1 acres *highly scattered* colony was located along the eastern shore of the lake (Map 3).

CONCLUSIONS AND DISCUSSION

Lake-wide levels of colonized EWM remain relatively low since the successful EWM control over the past several years (Figure 10). Please note that Figure 10 represents the acreage of mapped EWM polygons, not EWM mapped within pointbased methodologies (Single or Few Plants, Clumps of Plants, or Small Plant Taken out of context, this Colonies). figure can be misleading as it relates to the EWM population changes. For instance, large increases in colonized acreage may seem like drastic changes, but actually represent when a collection of point-based EWM occurrences increase in density to the point they require delineation with polygons. Similarly, an increase in pointbased EWM occurrences within a lake would not be represented on this figure.

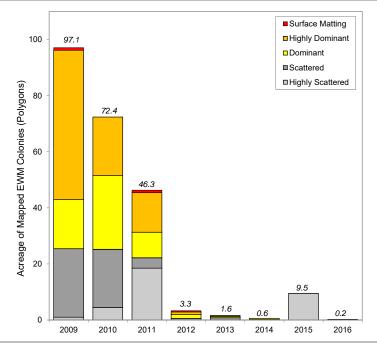


Figure 10. Acreage of mapped EWM colonies on Long Lake from 2009-2016.

On an August 12th, 2016 visit to the lake,

Many Waters, LLC encountered some suspected hybrid water-milfoil plants while conducting hand-harvesting in site D-16. Hybrid water milfoil (HWM) is a cross between the native northern water milfoil (Myriophyllum sibericum) and the invasive Eurasian water milfoil (EWM, Myriophyllum spicatum). The suspected HWM was collected and sent to a lab in lower Michigan (GenPass, LLC) for genetic analysis where they were confirmed to by HWM.

The concept of heterosis, or hybrid vigor, is important in regards to HWM management on Long Lake. The root of this concept is that hybrid individuals typically have improved function compared to their pure-strain parents. Hybrid water milfoil typically has thicker stems, is a prolific flowerer, and grows much faster than pure-strain HWM (LaRue et al. 2012). Data gathered from whole-lake 2,4-D treatments in Wisconsin from 2009-2016 suggest that treatments on lakes with populations of HWM were not as successful when compared to lakes with pure strains of HWM. It appears that most strains of HWM, but perhaps not all, are more tolerant of 2,4-D treatments than pure-strain EWM.

Given the expansion of EWM in the southern basin of Long Lake, it is believed that the population exceeds the level where hand-harvesting is an applicable control technique. It is proposed to monitor the EWM population in this area in 2017, potentially reaching predefined triggers for implementing an herbicide treatment in 2018. But as is discussed earlier in this report, long-term research being completed by the WDNR is showing that EWM has the capacity to fluctuate in its occurrence naturally over time in the absence of any active management strategy. It must be noted that EWM populations do not always simply increase over time which strengthens the rationale for continued monitoring.

Colonized areas of EWM and areas with *small plant colonies* have been prioritized for hand-harvesting in 2017. A total of 2.0 acres has originally been outlined (Map 3) and will be investigated during a late-May/early-June pre-hand-harvesting survey for potential alterations to the strategy. A volunteer based EWM monitoring regimen should be continued in the summer of 2017. Any EWM located by LLPLD volunteers would be recorded on the groups GPS and the data would again be utilized by Onterra during the late summer 2017 Peak Biomass Survey. This late summer survey will also be used to assess the 2017 control activities and guide the 2018 EWM control strategy.



