1.0 INTRODUCTION

Lost Lake, Vilas County, is a shallow (mixed) lowland drainage lake with a maximum depth of 24 feet and a surface area of 552 acres. This eutrophic lake has a relatively large watershed when compared to the size of the lake (20:1) and has a water residence time of approximately 6 months. Stella Lake and Found Lake both flow into Lost Lake with a water control structure (i.e. dam) on Lost Lake artificially maintaining a slightly higher water level (Figure 1).

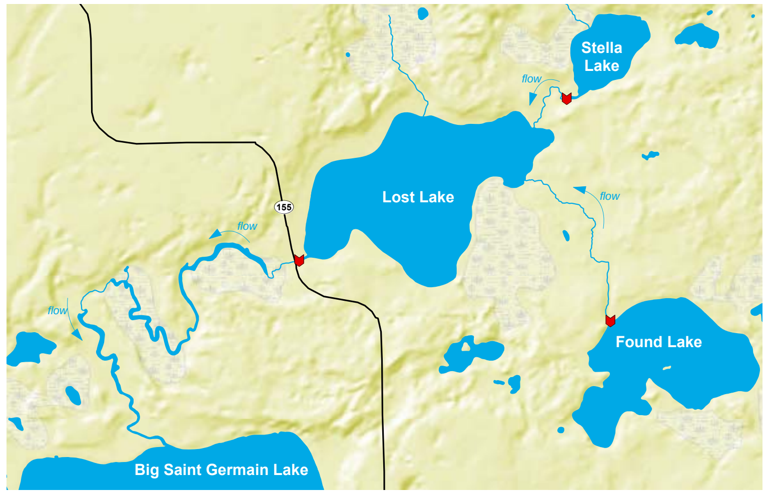

Figure 1. Lost Lake, Vilas County

Following the discovery of Eurasian watermilfoil (EWM) in 2013 and curly-leaf pondweed (CLP) in 2014, the Lost Lake Protection and Rehabilitation District (LLPRD) initiated an Aquatic Invasive Species (AIS) early detection and response framework with increased AIS population monitoring. Partial funding for the monitoring and management strategy (2013-2018) was received through two Wisconsin Department of Natural Resources (WDR) AIS-Early Detection and Response (EDR) grants. This report discusses the AIS management and monitoring efforts conducted in 2018 on Lost Lake which included an approximately 29.5-acre herbicide spot-treatment that targeted CLP. This report marks the final deliverable for the Phase II AIS-EDR (AIR-199-16).

2.0 EURASIAN WATERMILFOIL

Eurasian watermilfoil populations on Lost Lake were initially targeted through professional hand-harvesting activities (2013-2015). The hand-harvesting provided modest reductions in the areas where the hand-harvesting occurred, but the EWM population increases were greater than the amount of EWM that was being removed each year. Once the population exceeded a scale where these activities were thought to be applicable, the LLPRD opted to discontinue further active management until it understands if the EWM population will continue to increase or if the population will plateau at a level where the ecosystem function is not altered and navigation, recreation, and aesthetics are not impeded. EWM population monitoring in 2016-2017 confirm that the EWM population remained relatively stable and continued to be present at low densities (Map 1).

The EWM population in 2018 was found to be of a similar footprint as previous surveys with the majority of the population located in the eastern bay of the lake (Map 1). The majority of the colonized population of EWM was of *highly scattered* or *scattered* density ratings. In the 2018 EWM mapping survey, several additional low-density occurrences of EWM were located in various other locations including the southern and western ends of the lake in areas where it was not documented in previous surveys.

3.0 CURLY-LEAF PONDWEED

Curly-leaf pondweed is a European exotic first discovered in Wisconsin in the early 1900's that has an unconventional lifecycle giving it a competitive advantage over our native plants. The plants begin rapidly growing almost immediately after, if not before, ice-out and by early-summer they reach their peak growth. As they are growing, each plant produces numerous turions (asexual reproductive structures) which break away from the plant and settle to the bottom following the plant's senescence in early July (Photo 1). The deposited turions lie dormant until autumn when a portion of them sprout to produce small winter foliage, and they remain in this state until spring foliage is produced. The portion of turions that do not sprout can remain dormant for at least 5 years (likely longer) and still sprout (Johnson et al. 2012).

Photo 1. Curly-leaf pondweed turion. From Lost Lake, 2015.

The advanced growth in spring gives the plant a significant head start over native vegetation. In certain lakes, CLP can become so abundant that it hampers recreational activities within the lake. In instances where large CLP populations are present, its mid-summer die-back can cause significant algal blooms spurred from the release of nutrients during the plants' decomposition (James et al. 2002). However, in some lakes, mostly in northern Wisconsin, CLP appears to integrate itself within the community without becoming a nuisance or having a measurable impact to the ecological function of the lake. Acknowledging that possibility for Lost Lake, the LLPRD did not reactively conduct active management on the CLP population in 2014-2016, rather monitored the population dynamics.

The CLP population in Lost Lake was found to have dramatically increased from 2014 to 2016 (Map 2). Much of the CLP population in the western bay expanded to form large, continuous, and dense colonies in 2016 (Figure 2). A total of 17.9 acres of colonized CLP was mapped during the June 2016 survey, all of which was described of as being of *dominant* (yellow polygon) or greater densities. Approximately 2.0 acres of the CLP was described as *surface matting* (red polygon), the highest density rating used in the qualitative mapping methodology. An additional 6.7 acres of CLP was described as *highly dominant* (orange polygon) during the 2016 survey. These highly visible, very dense CLP colonies completely dominate the aquatic plant population and can significantly inhibit navigation for boaters until the plant dies back in early July. For reasons not completely

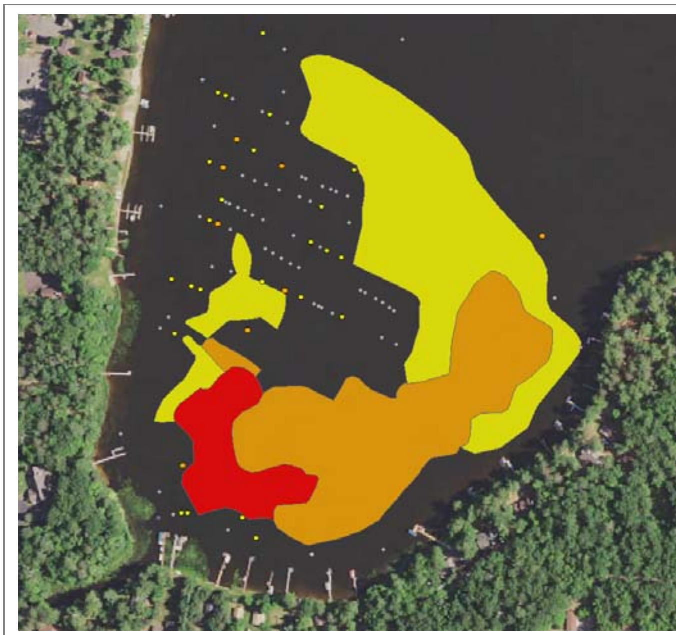

Figure 2. 2016 CLP Population from western basin of Lost Lake. Refer to Map 2 for legend of symbology.

known, the CLP population on Lost Lake has been documented to persist much later in the growing season than other waterbodies.

3.1 CLP Management Strategy Development

The theoretical goal of CLP management is to kill the plants each year before they are able to produce and deposit new turions. Not all of the turions produced in one year sprout new plants the following year; many lie dormant in the sediment to sprout in subsequent years. This results in a sediment turion bank being developed. A control strategy for an established CLP population includes multiple consecutive years of treatments of the same area to deplete the existing turion bank within the sediment without replacement of turions.

During the late-fall/winter of 2016-17, there were a number of correspondences between the district and Onterra discussing the possibility of conducting an herbicide control strategy during the spring of 2017. Factors such as environmentally toxicity of the treatment including likely native plant impacts, the need for multiple subsequent annual treatments, and likely regulatory opposition where weighed heavily. Because CLP had only been present in Lost Lake for a few years, there was speculation that the turion base may be small and if a control program is initiated at that time, may not require as many successive treatments as a more established population would. Following these discussions, the LLPRD board of directors supported pursuing an herbicide spot treatment targeting the largest and densest population of CLP during the spring of 2017. The preliminary strategy was outlined within the *2016 AIS Monitoring & Control Strategy Assessment Report* originally distributed in mid-February 2017.

The WDNR provided written review of the preliminary plan approximately a month later and subsequently initiated a round-table meeting at the Town of Saint Germain Community Center on April 10, 2017. Based upon the WDNR's review comments and discussion held at the April meeting, updates to the monitoring strategy were made.

The LLPRD and the third-party applicator selected by the district, Clean Lakes, applied for a WDNR permit during late-March 2017 to target the CLP within 29.5 acres in the western bay with liquid endothall at 2.0 ppm active ingredient (ai), a typical herbicide spot-treatment dose targeting this species. The EPA-approved endothall label (Aquathol® K) recommends 1.5 to 3.0 ppm ai for spot treatments of CLP although the label approves application up to 5.0 ppm ai. The 2017 Lost Lake herbicide application equates to 5.3% of the lake's acreage and 3.8% of the lake's mixing volume (lake is polymictic), below thresholds that would indicate potential whole-lake impacts from the treatment.

The permit was approved by the WDNR on May 17, 2017 and the herbicide treatment occurred on May 24, 2017. The original intent was to alter the dam operations during the herbicide treatment in attempt to keep the herbicide from being flushed downstream before it could impact the CLP as well as reduce potential downstream impacts of the herbicide on vulnerable growth stages of wild rice. However, the heavy amounts of rain during this timeframe greatly limited the ability to impact discharge rates through manipulation of the dam's spillway.

Monitoring results of the 2017 herbicide treatment are included within the *Lost Lake 2017 AIS Monitoring and Control Strategy Assessment Report* (Jan18), which was provided to WDNR, Vilas County, Great Lakes Indian Fish and Wildlife Commission (GLIFWC), and LLPRD. The report indicated that the treatment appeared to have effectively controlled a single years' worth of growth prior to turion formation within the targeted area. The report also investigated reductions in the native plant community on Lost Lake over time and possible association to the 2017 herbicide efforts.

At the LLPRD's January 4, 2018 First Quarter District Meeting, the LLPRD board of directors (BOD) voted unanimously to conduct another endothall spot treatment in the western bay of the lake during the spring of 2018 with the same monitoring strategy implemented in 2017.

3.2 2018 CLP Monitoring Methodologies

The theoretical objective of an herbicide treatment strategy is to maximize target species (CLP) mortality while minimizing impacts to 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.

Qualitative Aquatic Plant Monitoring

Using sub-meter GPS technology, CLP locations have been mapped on Lost Lake since discovery in 2014 (Map 2). The qualitative mapping is completed typically when the target species is at its peak growth stage and can be seen from the water surface. Observations are recorded to represent points on the lake (*single or few plants*, *clumps of plants*, or *small plant colonies*) or larger beds, which are delineated with polygons and given density ratings (*highly scattered* < *scattered* < *dominant* < *highly dominant* < *surface matted*). Submersible video and/or rake tows may be used for determining colony extents, but these methods are not appropriate as the sole method for determining density.

Continued monitoring of the CLP population over time using this methodology will allow an understanding of population dynamics within the system in the context of the control actions that have taken place. The results of these surveys will also be used to determine if additional areas in the lake are to be targeted for active management techniques.

Quantitative Aquatic Plant Monitoring

For spot treatment strategies like the 2017 and 2018 treatments on Lost Lake, quantitative evaluation methodologies generally follow WDNR guidance in which point-intercept data are collected within the treatment area before and after the spring treatment. Quantitative sampling utilizing a point-intercept grid over the treatment area is typically only completed on treatment areas 10 acres or larger to allow for sufficient sampling points to assure confidence in statistics generated from the results. Quantitative sampling can be completed on smaller treatment areas, but greater differences in pre- and post-data must be documented to bring about confidence in the statistical analysis.

Quantitative data collected annually immediately before the treatment takes place allows for a determination if the CLP population is being reduced in the area over time. To assess the CLP population, a sub-sample point-intercept survey would be conducted within the herbicide application area by sampling 101 locations at a resolution of 35 meters (Figure 3, left frame). This survey would be completed annually during the spring pre-treatment survey and be used to evaluate the overall control program. These data are not used to understand the efficacy of a single treatment.

Because many native aquatic plants are not actively growing at the time of the spring pre-treatment survey, a separate point intercept dataset would be used to assess the native aquatic plant community in response to the herbicide treatment. Whole-lake point intercept surveys were conducted on Lost Lake in 2007, 2010, 2014, and 2017. A subset of these data comprised of the 40 points with a resolution of 75 meters in the western bay that is within and around the herbicide application area will be compared (Figure 3, right frame). A replicate point-intercept survey conducted in 2018 allows an understanding of how the aquatic plant community changed over this timeframe within the treatment area (red dots) and lake wide (entire dataset).

Based on subset data from previous point-intercept surveys, the five most abundant plant species within the western bay were flat-stem pondweed, coontail, common waterweed, northern watermilfoil, and fern-leaf pondweed. Onterra's experience is that flat-stem pondweed, northern watermilfoil, and fern-leaf pondweed are particularly vulnerable to early-season endothall treatments whereas coontail is more resilient and common waterweed is unimpacted or has even shown to have population increases.

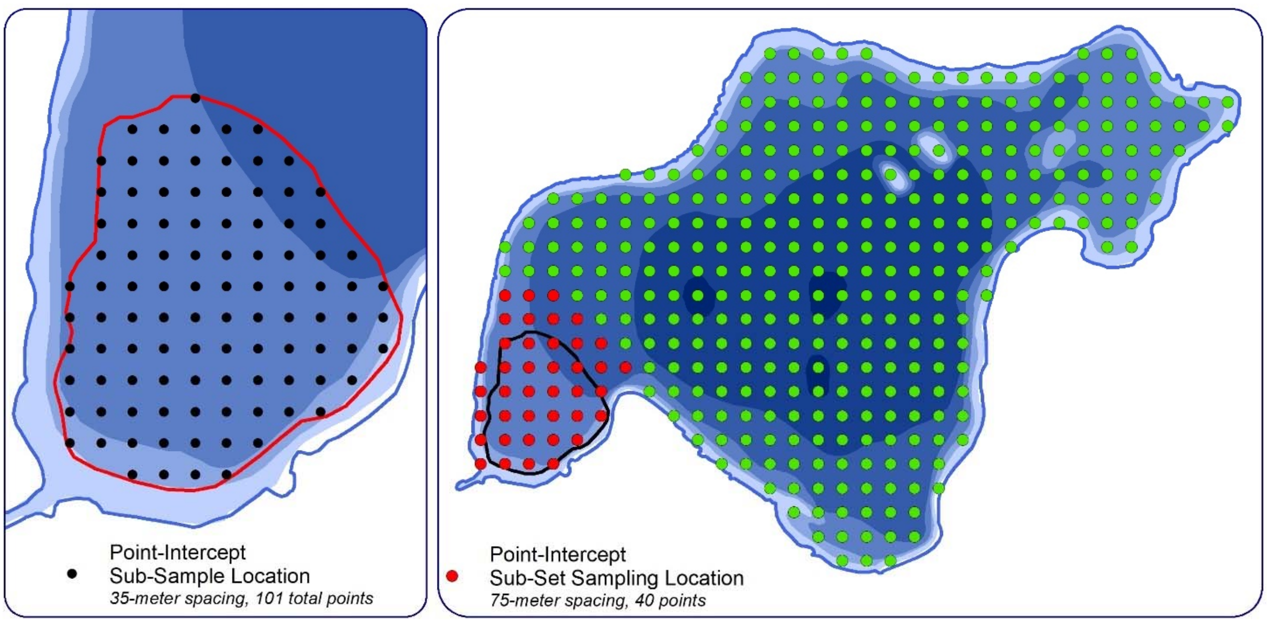

Figure 3. Lost Lake quantitative monitoring plan for the 2017 & 2018 herbicide treatments.

Herbicide Concentration Monitoring

An herbicide concentration monitoring plan was developed jointly by Onterra and the WDNR. LLPRD volunteers were given equipment and instruction by Onterra on how to collect and preserve water samples from Lost Lake that would be analyzed by the Wisconsin State Laboratory of Hygiene for concentrations of endothall. Some modifications were made between the 2017 and 2018 monitoring plans in an effort to balance costs along with obtaining sufficient data to gain further understanding of the dissipation and degradation rates. For the 2018 monitoring plan, the sampling intervals were extended out to 14 days after the treatment, additional samples were collected from the center of the lake and downstream sampling sites, and the number of sampling sites located within the direct application area was reduced from three sites to two. Water samples were collected with a 6-foot integrated sampler at two locations in the treatment area and the deep hole location in the center of the lake (outside of the application area). Samples were collected manually at two locations downstream of Lost Lake including near the outlet and further downstream in Lost Creek (Map 3). The sampling interval matrix and sampling site details are displayed on Figure 4.

Lost Lake Herbicide Sample Sites				
Site	**Station ID**	**Latitude**	**Longitude**	**Sample Depth**
L1 - Deep Hole	643081	45.96495	-89.482789	Integrated Sampler (0-6 ft)
L2 - Application Area	10048321	45.962808	-89.494945	Integrated Sampler (0-6 ft)
L4 - Application Area	10048323	45.960792	-89.495732	Integrated Sampler (0-6 ft)
L5- Outlet	10048324	45.959511	-89.498846	Manual
L6 - Lost Creek	10009240	45.956574	-89.524549	Manual

Interval	L1	L2	L4	L5	L6
2 HAT		X	X	X	X
4 HAT		X	X	X	X
8 HAT		X	X	X	X
1 DAT		X	X	X	X
3 DAT	X	X	X	X	X
5 DAT	X	X	X	X	X
7 DAT	X	X	X	X	X
14 DAT	X	X	X	X	X

X = sample to be collected (36 total samples)
HAT = Hours After Treatment
DAT = Days After Treatment

Figure 4. Herbicide concentration monitoring plan for the 2018 endothall treatment on Lost Lake.

An article by Nault et al. 2017 investigated 28 large-scale herbicide treatments in Wisconsin and found that "herbicide dissipation from the treatment sites into surrounding untreated waters was rapid (within 1 day) and lakewide low-concentration equilibriums were reached within the first few days after application." This research indicates that lake-wide concentrations of herbicides generally reach equilibrium within an entire lake by roughly 3 days after treatment. Said another way, the concentration measured in the center of the lake at three days after treatment has been shown to be reflective of the concentration measured anywhere else in the lake at that time. Therefore, the sampling design for the 2018 spot treatment on Lost Lake included collection of herbicide concentration samples in the center of the lake beginning at 72 hours (3 days) after treatment. The data collected in the center of the lake would serve as a surrogate for the likely herbicide concentrations in non-treated parts of the lake. Onterra suggested adding another herbicide concentration monitoring location in the eastern basin of the lake and the WDNR indicated it was not necessary as the center of the lake location provides sufficient information regarding the concentration outside of the direct application area.

3.3 2018 CLP Pretreatment Survey

On May 22, 2018, Onterra ecologists conducted the Spring Pre-treatment Confirmation and Refinement Survey on Lost Lake. A temperature profile indicated the that water temperatures were between 65-70°F throughout the water column. A secchi disk reading of 5.7 feet was recorded during the survey and the crew noted the water appeared brownish in color. As a part of the survey, the crew conducted a sub-sample point-intercept survey of the proposed treatment area and located CLP on approximately 47.5% of sample locations (Figure 5). Native plant growth was minimal with low amounts of common waterweed present in the site. The majority of the CLP population was not visible from the viewpoint of the boat deck so the survey crew deployed a submersible camera to investigate the site (Photo 2). Through the aid of the submersible camera, actively growing CLP was confirmed throughout the proposed application area with most plants being approximately 1-3 feet in height. Minimal native vegetation was observed during the submersible camera viewing. Based on water temperatures and the stage of CLP/native plant growth observed during the survey, Onterra advised the district that the treatment should occur as soon as the permit is finalized by the WDNR and the applicator could be mobilized.

Photo 2. Curly-leaf pondweed observed during a May 22, 2018 survey on Lost Lake. Photo by Onterra.

The herbicide permit application was submitted on March 31, 2018 by the LLPRD. Following review by the WDNR and GLIFWC, additional consultation between the WDNR and the Lac du Flambeau Band of Lake Superior Chippewa Indians occurred. Following this process, the permit was approved by the WDNR on June 15, 2018. With the delay in the implementation of the herbicide control strategy, Onterra volunteered to replicate the pretreatment data closer to the treatment date. The 101 sub-sample point-intercept locations within the herbicide application area on Lost Lake were re-sampled the day before the treatment on June 20, 2018. This provided a comparison with the survey conducted approximately a month previous. The data indicate that CLP abundance increased from May 22 to June 20 (Figure 5). Onterra hypothesized that the increase in CLP frequency was related to lateral rhizomatic population expansion as opposed to additional turion sprouting.

During the May 22 pretreatment survey, the only native plant observed was common waterweed at 6 locations (Figure 6). An increase in native plant occurrence was observed during the June 20 pretreatment survey, but continued to be a relatively low population of the species present.

The final herbicide treatment included the application of liquid endothall over 29.5 acres of Lost Lake and was completed on June 21, 2018.

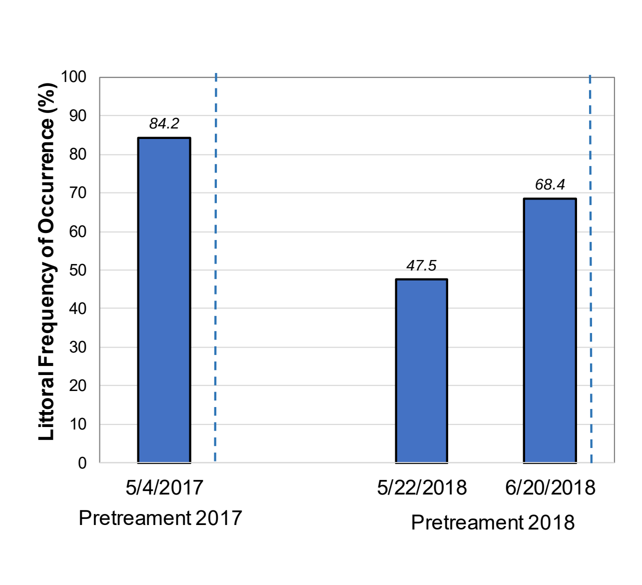

Figure 5. Pretreatment CLP LFOO in the 2017-2018 sub-sampling survey. Sampling locations shown in Figure 3, left frame (N=101). Blue lines indicate endothall treatments.

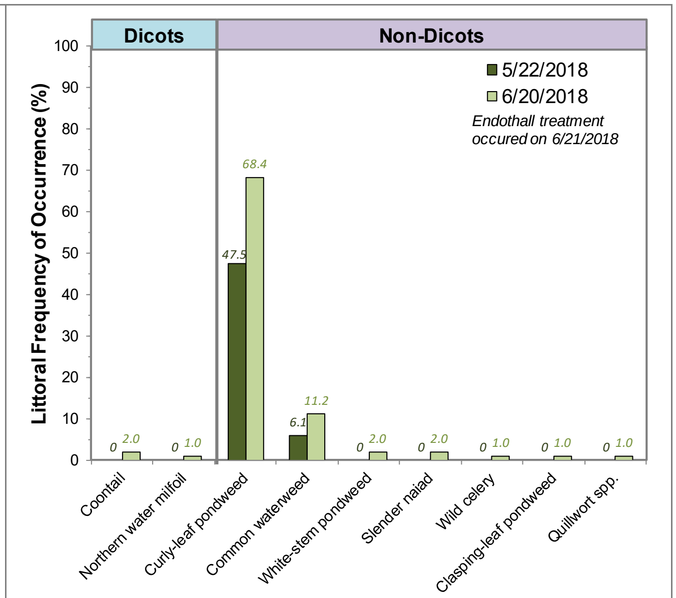

Figure 6. Pretreatment Aquatic Plant LFOO in the 2018 sub-sampling surveys. Sampling locations shown in Figure 3, left frame (N=101).

3.4 2018 Post Treatment Survey Results

<u>Herbicide Concentration Data</u>

Endothall is an aquatic herbicide that is applied as either a dipotassium salt or an amine salt. These active ingredients break down following application to endothall acid, the form that acts as an herbicide (Netherland 2009). The 2017 and 2018 treatments of CLP on Lost Lake used the dipotassium salt at a concentration of 2.0 ppm active ingredient (ai). When broken down into the acid, 2.0 ppm ai equates to 1.42 ppm acid equivalent (ae). The WI State Laboratory of Hygiene is able to test water samples for endothall using an ELISA (enzyme-linked immunosorbent assay) method and reports the results as acid equivalent.

The herbicide concentration data from Lost Lake indicate that the concentration in the treatment area was near the target (1.42 ppm ae), in the samples collected on 2, 4, and 8 hours after treatment (HAT), but were reduced by 24 HAT (Figure 7, left frame). The endothall concentrations were initially higher in 2018 than in 2017, but similar at 3 days after treatment (72 HAT). A longer herbicide collection period occurred in 2018 based on the data recorded in 2017. Minimal endothall was found in the 2018 samples collected on 120 HAT (5 days after treatment) and 168 HAT (7 days after treatment), whereas no herbicide was detected in the 2018 application area in the final sampling interval at 14 days after treatment (omitted from Figure 7).

Minimal endothall was present at 2 and 4 HAT samples collected from the L5 sampling location on the downstream side of the dam in Lost Creek (Figure 7, right frame) in 2018. This observation was similar to 2017. By 8 and 24 HAT during 2018, endothall concentrations were just under 1 ppm and were closer to levels that were observed within application area in the hours immediately after the treatment. The 2018 concentrations were higher than those measured in 2017, but generally follow the same concentration curve. Detectable levels of endothall were present through 7 DAT in 2018 and no herbicide was detected on 14 DAT at the outlet sampling location (L5).

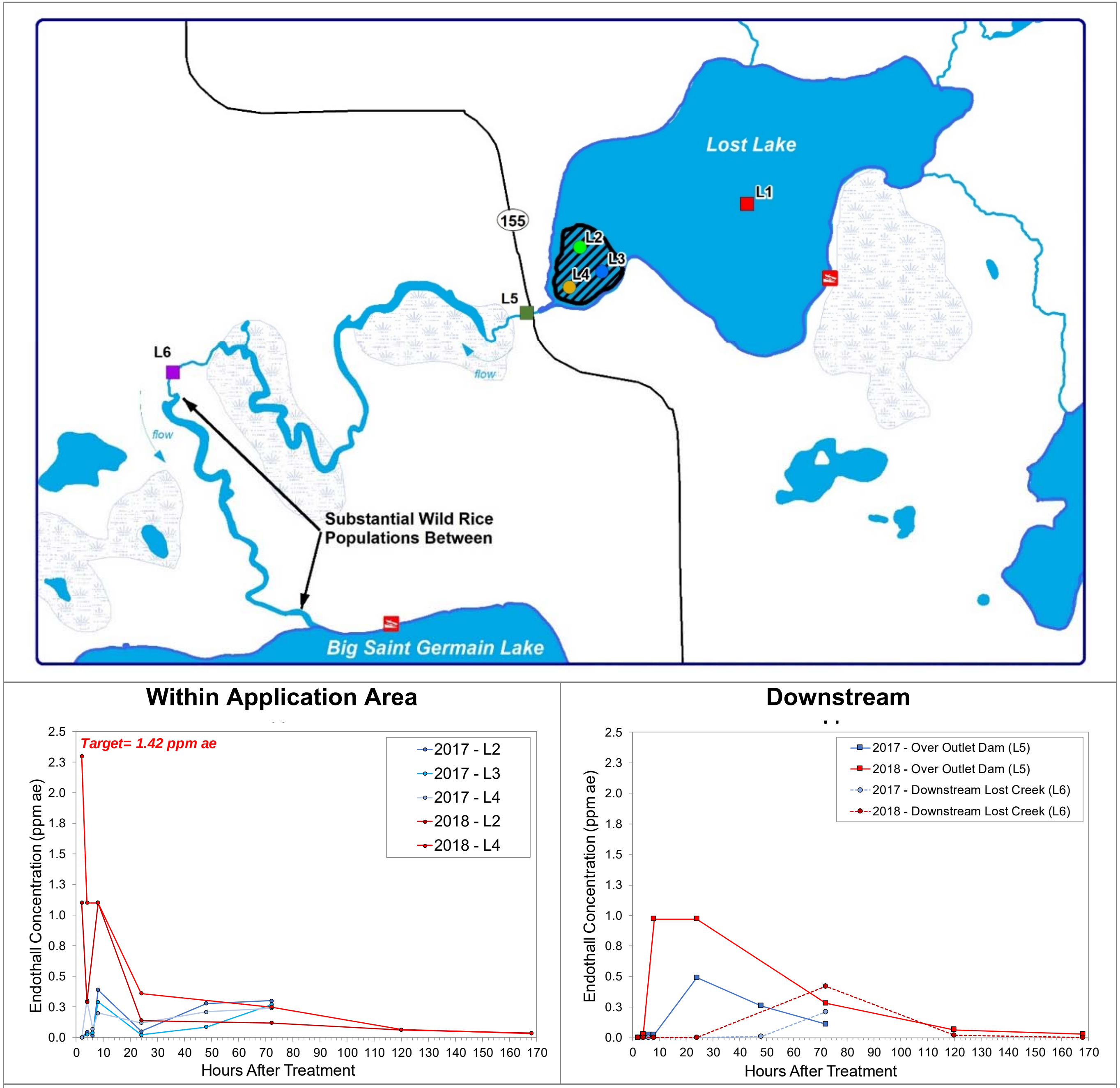

Figure 7. Lost Lake herbicide concentration monitoring results from the 2017-2018 endothall treatment. Samples collected in 2018 from each monitoring site at 336 HAT (14 DAT) all had no detection of endothall and are not displayed on this figure. Samples were not collected from site L3 during the 2018 monitoring. General location of wild rice population provided by GLIFWC.

The samples that were collected from the L6 downstream sampling location in Lost Creek showed endothall was not detected in the first 24 hours after treatment. A sample collected 3 days after treatment showed low-level herbicide concentrations of (0.42 ppm ae) and by the time of the next collection at 5

DAT, the concentration had declined to just 0.019 ppm ae (Figure 7, right frame). Endothall was not detected in samples collected on 7 and 14 DAT in Lost Creek.

The endothall concentrations that were documented in Lost Creek are lower than what the published literature documents as having impacts to wild rice (Nelson et al. 2003). The laboratory research has documented reduced wild rice seedling biomass at the lowest endothall concentration it tested (sustained 0.71 ppm ae for 72 hours), which is approximately 40% higher concentration and a likely longer exposure time as documented in this area in 2018. Young and mature wild rice growth stages did not have reduced biomass at the lowest tested concentration (0.71 ppm ae). While it depends on the specific weather conditions of a given year, early-season herbicide treatments that occur in early-May are most likely to have exposure to recently germinated wild rice (seedlings). The 2017 treatment was conducted in late-May (May 22) and the 2018 treatment was conducted in late-June (June 21), potentially both when wild rice plants have progressed past the seedling growth stage and are therefore less vulnerable to the impacts from endothall.

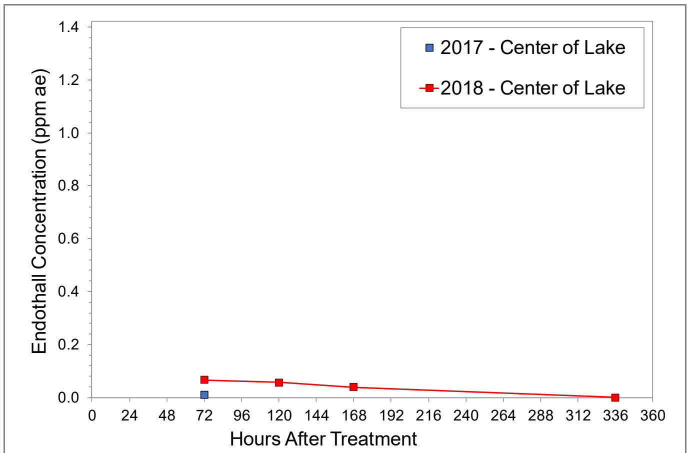

Figure 8. Center of the lake herbicide concentration monitoring results from the 2017-2018 endothall treatment.

The scientific literature and Onterra's experience suggest that Lost Lake should have had an equilibrium concentration within the entire waterbody by roughly 3 days after treatment. At the center of the lake in 2018, endothall concentrations were 0.066 ppm ae at 3 DAT and concentrations were slightly lower during 5 DAT (0.057 ppm ae) and 7 DAT (0.039 ppm ae) (Figure 8). No endothall was detected from the site on 14 DAT. These concentrations are higher than observed in 2017, where a single sample from the center of the lake at 3 DAT yielded 0.009 ppm ae). The average concentration from the three sampling intervals in 2018 was 0.054 ppm ae. For whole-lake CLP treatments the manufacturers of endothall (UPI) recommend target concentration of recommend whole-lake target concentrations of 0.53 ppm ae (0.75 ppm ai) to 0.71 ppm ae (1.0 ppm ai). This is an order of magnitude (10X) greater than the measured concentrations from Lost Lake. Based on the endothall concentrations observed in the center of the lake, the impacts of the spot treatment are anticipated to be confined to the approximate area of the application area.

Efficacy (CLP Control)

In a typical year, the herbicide treatment would occur in early- to mid-May and an Early-Season AIS Survey would map the CLP during late-June when it is expected to be at its peak growth stage. This allows four to six weeks between the treatment and the mapping survey, sufficient time for the effects of the herbicide to be realized. If CLP remains present in the application area 4-6 weeks after treatment, it may be assumed that the treatment was not successful. Because the 2018 herbicide application did not occur until June 21 with natural senesce likely within a few weeks, the 2018 mapping survey does not allow an assessment of the 2018 management actions. The 2018 mapping survey located only 1.4 acres of colonized CLP outside of the area targeted for control, indicating that the strategy is essentially targeting the entire CLP population (Map 3).

Selectivity (native plant impacts)

Aquatic plant communities are dynamic, and the abundance of certain species can fluctuate from year to year depending on climatic conditions, herbivory, competition, water levels, and disease among other factors, and fluctuations in the abundance of species are to be expected over time. Herbicide treatments, can also impact non-target native plant abundance.

Analysis of the Lost Lake aquatic plant community is provided within *Lost Lake Comprehensive Management Plan* (Draft Nov18) as well as the *Lost Lake 2017 AIS Monitoring and Control Strategy Assessment Report* (Jan18). These data show a reduced aquatic plant abundance within the lake (Figure 9).

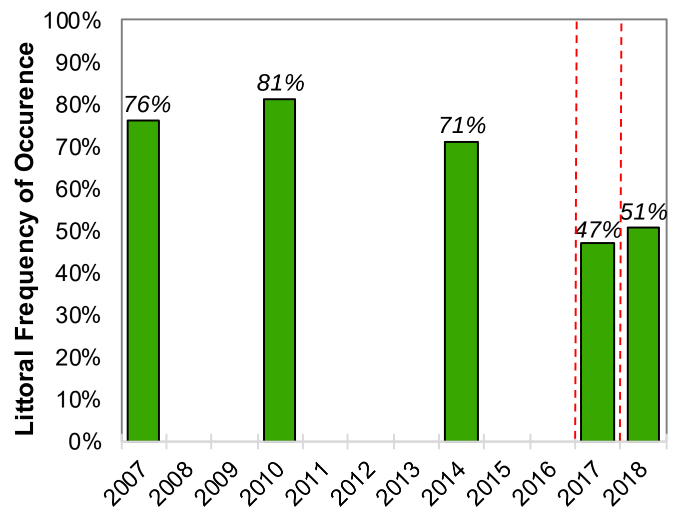

Figure 9. Point-intercept sampling locations within aquatic vegetation. Red dashed lines indicate western basin herbicide spot treatment.

Using the presence/absence data from each years' point-intercept survey, an interpolation model (kringing) was created that explores the areas of Lost Lake that have a high likelihood of containing vegetation in a given year. The model shows the footprint of aquatic vegetation from Lost Lake increased from 2007 to 2010, largely in the lakeward direction. Aquatic vegetation at depth resided in 2014 to an area similar, but perhaps a little smaller than observed in 2010. The models from 2017 and 2018 shows less aquatic vegetation in deeper waters, with most of the vegetation being observed in near shore areas. Vegetation reductions were also observed in the far eastern part of the lake.

Large-scale reductions in aquatic plants are often associated within changes in water clarity within a lake. Lost Lake's water clarity can vary significantly from year to year, with some annual growing season Secchi disk readings averaging 9 or more feet while other years, like 2017, averaging 4.4 feet. The water clarity of Lost Lake is largely driven by free-floating algae but also impacted by dissolved humic substances and organic acids which give the lake a light tea color in some years (30 SU in 2017).

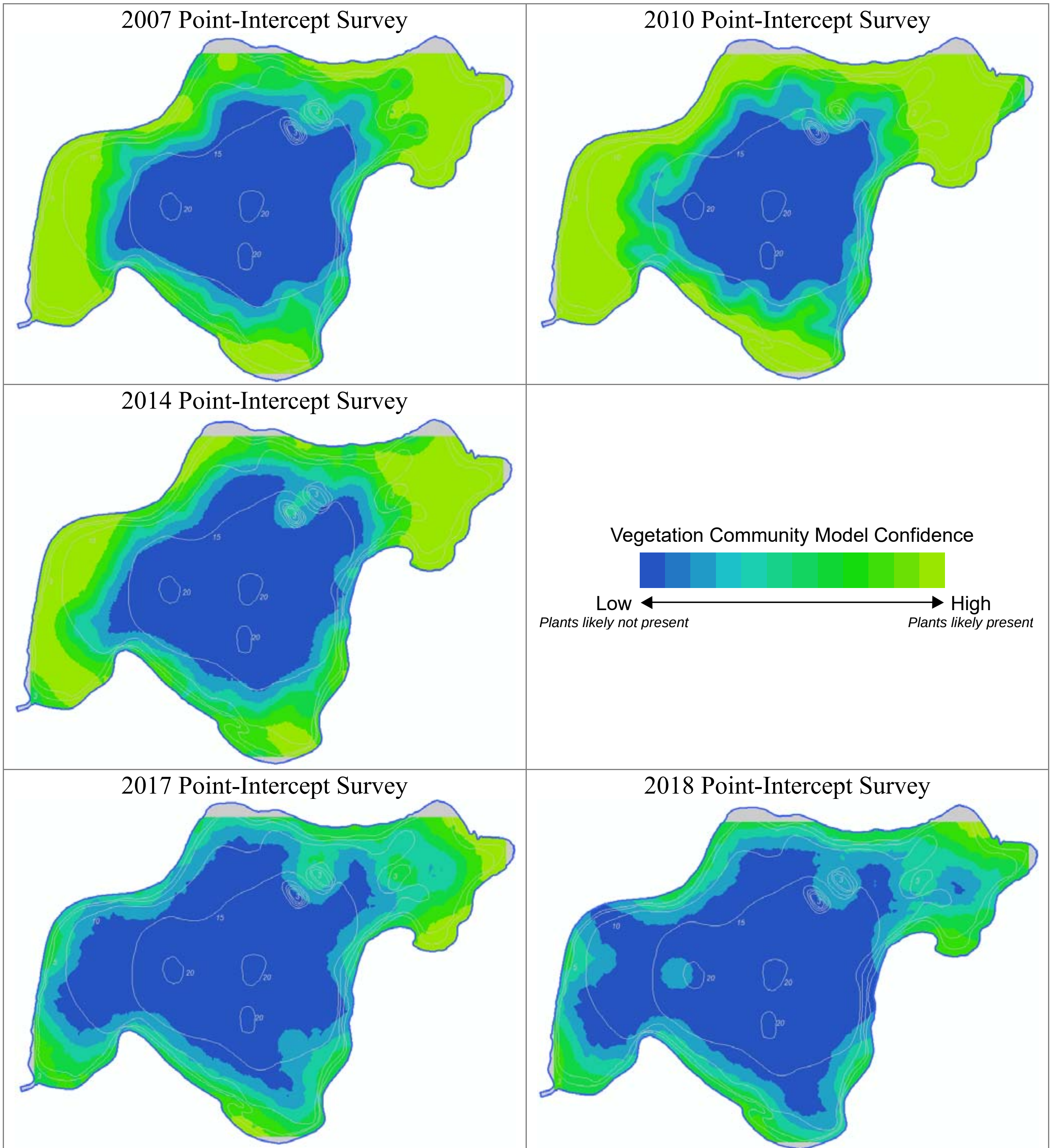

Figure 10. Distribution of aquatic vegetation in Lost Lake point-intercept surveys. Modeled vegetation likelihood based upon interpolation of presence/absence vegetation at point-intercept locations. Grey areas indicate no data.

The maximum depth of aquatic plants found from the point-intercept surveys has reduced by four feet during the most recent point-intercept survey (Figure 11). Figure 12 shows that aquatic plant abundance in the 2-3 foot range of the lake was the highest in 2018, but little vegetation was observed greater than 8 feet deep in 2017 and 2018. Some of the greatest abundance of aquatic plants during 2007, 2010, and 2014 was found in waters of 8 to 14 feet.

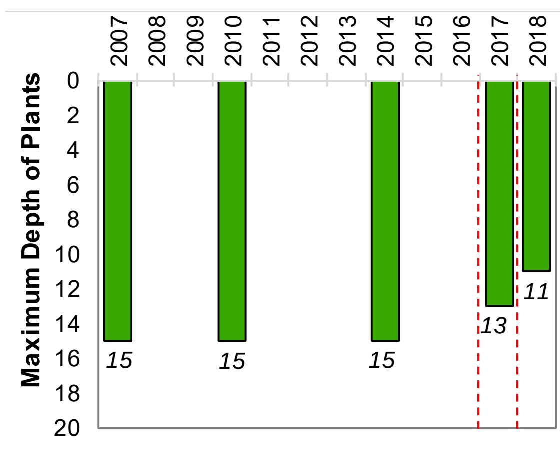

Figure 11. Maximum depth of plants from point-intercept surveys. Red dashed lines indicate western basin herbicide spot treatment.

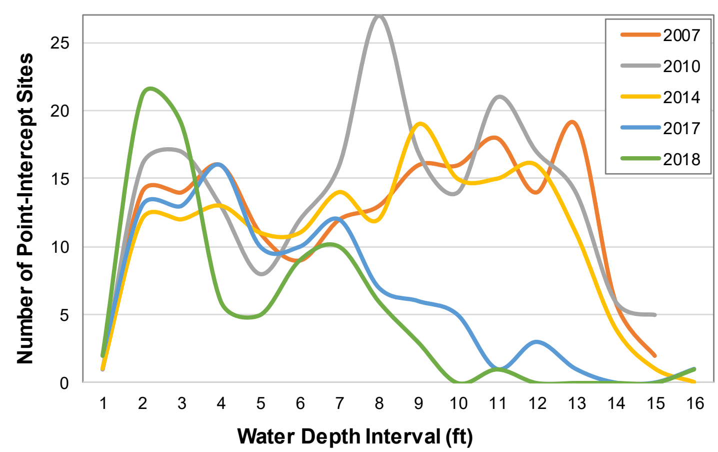

Figure 12. Depth distribution of aquatic plants from point-intercept surveys.

The point-intercept sub-set analysis (corresponding locations shown in Figure 3, right frame) reviews the aquatic plant community within the treatment area (red data) and within the entire lake-wide dataset (blue data) (Figure 13 and Figure 14). Appendix A contains the full matrix of lake-wide point-intercept data results. Within the *2016 Lost Lake AIS Monitoring & Control Strategy Assessment Report*, Onterra indicated predicted that fern-leaf pondweed, flat-stem pondweed, and northern watermilfoil were likely to be impacted by an endothall spot treatment. Onterra's experience is that white-stem pondweed is not typically impacted by early-season endothall treatments even though it emerges early in the growing season and has a relatively high biomass at the time of treatment. Figure 13 will explore the population changes in these four plant species. Of these four species, only white-stem pondweed was located within the higher resolution June 20, 2018 pretreatment point-intercept survey (not present in May 22 survey), and only at 2 of the 101 sampling locations.

Northern watermilfoil populations decreased lakewide from a littoral frequency of occurrence (LFOO) of 38.1% in 2010 to 14.0% in 2014 in absence of management (Figure 8, blue data). Further declines in northern watermilfoil populations were observed following herbicide management in 2017 and 2018. Northern watermilfoil was not observed within the treatment area in 2017 or in 2018 (Figure 8, red data).

The flat-stem pondweed population of Lost Lake has fluctuated greatly over the period of study. No flat-stem pondweed was observed in Lost Lake during 2014 nor 2017 after being the second-most dominant plant species in 2010 (50% LFOO, blue data). In 2018, flat-stem pondweed was sampled during the whole-lake point-intercept survey with a LFOO of 0.6%, whereas none was sampled within the treated area.

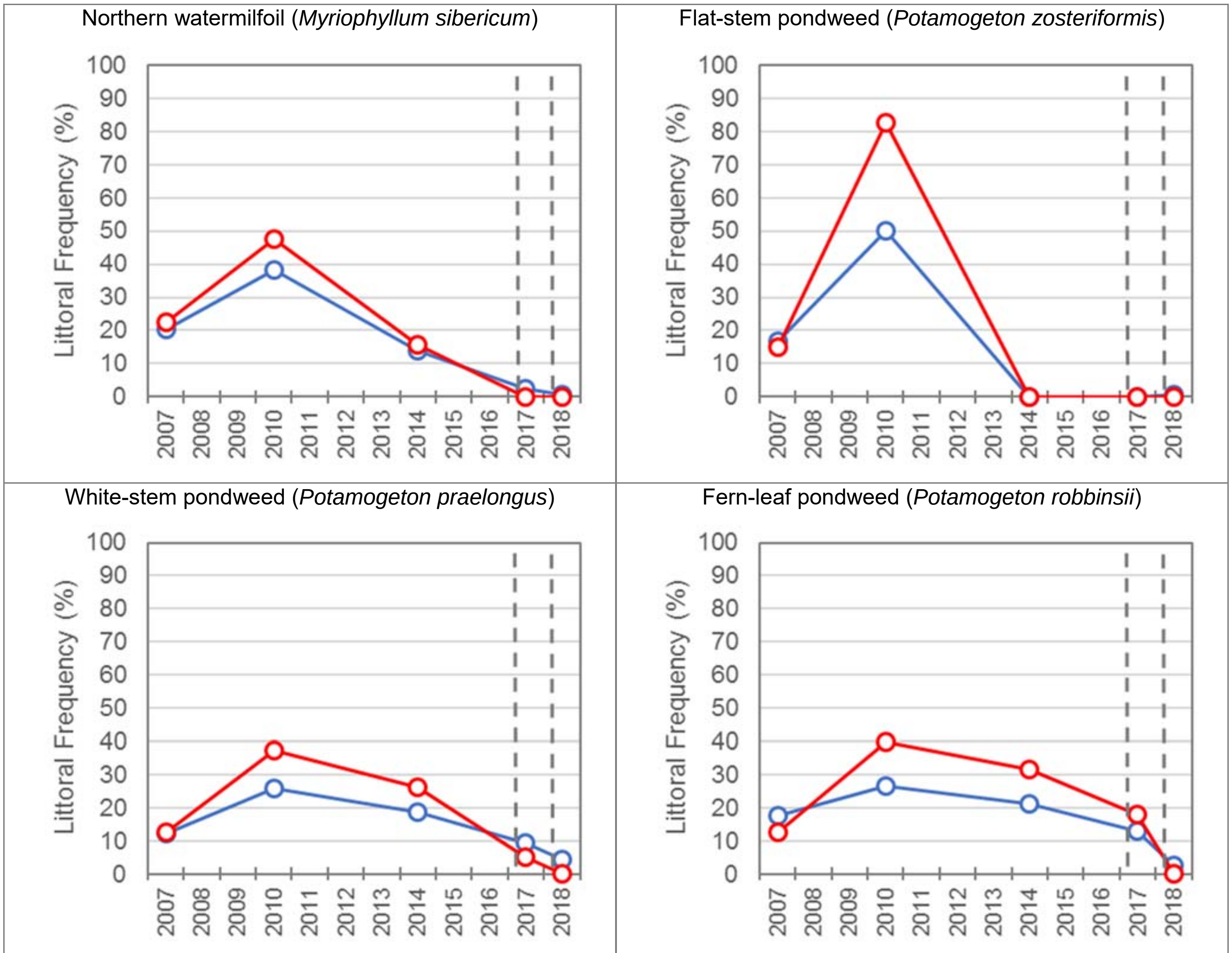

Figure 13. Littoral frequency of occurrence of select native aquatic plant species in Lost Lake. Red = within the treatment area. Blue = entire dataset (lake-wide) Grey dashed line indicates western basin spot herbicide treatments.

Both white-stem pondweed and fern-leaf pondweed lake-wide LFOOs increased in 2010 compared to 2007 (Figure 13, blue data). Both populations also declined from 2010 to 2014. Figure 13 confirms that the populations of these two species were higher in the treatment areas (red data) compared to the entire lake during 2010 and 2014. While a lake-wide decline in white-stem pondweed was observed between 2014 to 2017 (Figure 13, blue data), the decline was greater in the treatment area (Figure 13, red data). Fern-leaf pondweed populations also declined lake-wide from 2014 to 2017, but the declines in the treatment area were similar to the lake-wide declines. In 2018, the lake-wide population of white-stem pondweed declined further to 4.4% and the population within the treated area decreased to 0%. Similarly, for fern-leaf pondweed, the lake-wide population decreased in 2018 to 2.5% and the population in the treated area decreased to 0%. These two species declined greater in the application area than the lake-wide dataset, indicating the declines within the treatment area are likely attributed to the herbicide management actions.

Figure 14 explores the population changes of species Onterra does not typically observe being impacted by early-season endothall spot treatments. Lakewide coontail populations have been steadily declining since 2007 (Figure 14, blue data). The 2014 lake-wide coontail LFOO was 38.7% and reduced to 13.2% in 2017. Almost 66% of the sampling locations with the treatment area contained coontail in 2014 and was reduced to just over 10% in 2017 (Figure 14, red data). This decrease was greater than was observed lake-wide. This trend continued in 2018 as the coontail population was found to have declined in the lake-wide population to 11.3% and had declined to 0% within the treated area.

Common waterweed has been shown to metabolize endothall much quicker than other species (particularly pondweeds) and not translocate the herbicide making it tolerant of endothall treatments (Keckemet and Nelson 1968). For reasons not understood, the lakewide common waterweed population reduced from approximately 37% LFOO in 2010 to 6% LFOO in 2014 (Figure 14, blue data), with only one of the 2014 sampling locations with the treatment area containing common waterweed (Figure 14, red data). The lake-wide LFOO in 2017 was statistically unchanged from 2014 although an increase was observed within the treated part of the lake. In 2018, the lake-wide population of common waterweed was found to have increased to 7.5% and the population was about the same in the treated area in 2018 at 12.5% as in 2017 (12.8%).

Slender naiad has been shown to be particularly susceptible to large-scale 2,4-D treatments during the year of treatment (Nault et al. 2017). The population of this annual species was found to increase within the endothall treatment area and lake-wide from 2014 to 2017 on Lost Lake. Slender naiad was the most dominant plant species in Lost Lake during 2017. However, in 2018, populations of slender naiad declined both lake-wide (11.9%) and within the treated area (2.5%).

Wild celery is typically not impacted by most early-season herbicide treatments as this species emerges later in the year after the herbicide has dissipated/degraded. Wild celery population increases were observed lake-wide in Lost Lake from 4.7% LFOO in 2014 to 20.0% LFOO in 2017 (Figure 14, blue data). In 2018, the lake-wide population of wild celery declined to 11.9% as the population in the treated area remained approximately the same as in 2017 at 5.0%.

Large-leaf pondweed populations have steadily decreased from almost 17% LFOO in 2007 to 4.4% in 2017 and 1.3% in 2018 (Figure 14, blue data). Within the treatment area, only 1 sampling point contained large-leaf pondweed in 2014 before the treatment and no sampling points contained large-leaf pondweed after the treatment in 2017 or 2018 (Figure 14, red data). Clasping-leaf pondweed populations were statistically unchanged lake-wide (Figure 14, blue data) but indicate reduction within the treated part of Lost lake between 2014 and 2018 (Figure 14, red data).

Small pondweed, slender pondweed, and stiff pondweed have all been identified from Lost Lake. These morphologically similar looking species are sometimes referred to as thin-leaved pondweeds. Analysis of these data requires grouping or “lumping” of the species. Only 1 location in Lost Lake contained any of these species in 2007 (Appendix A). The population of thin-leaved pondweeds increased to almost 40% in 2014. No locations contained thin-leaved pondweeds in 2017 or 2018, although slender pondweed was observed incidentally during the 2018 survey.

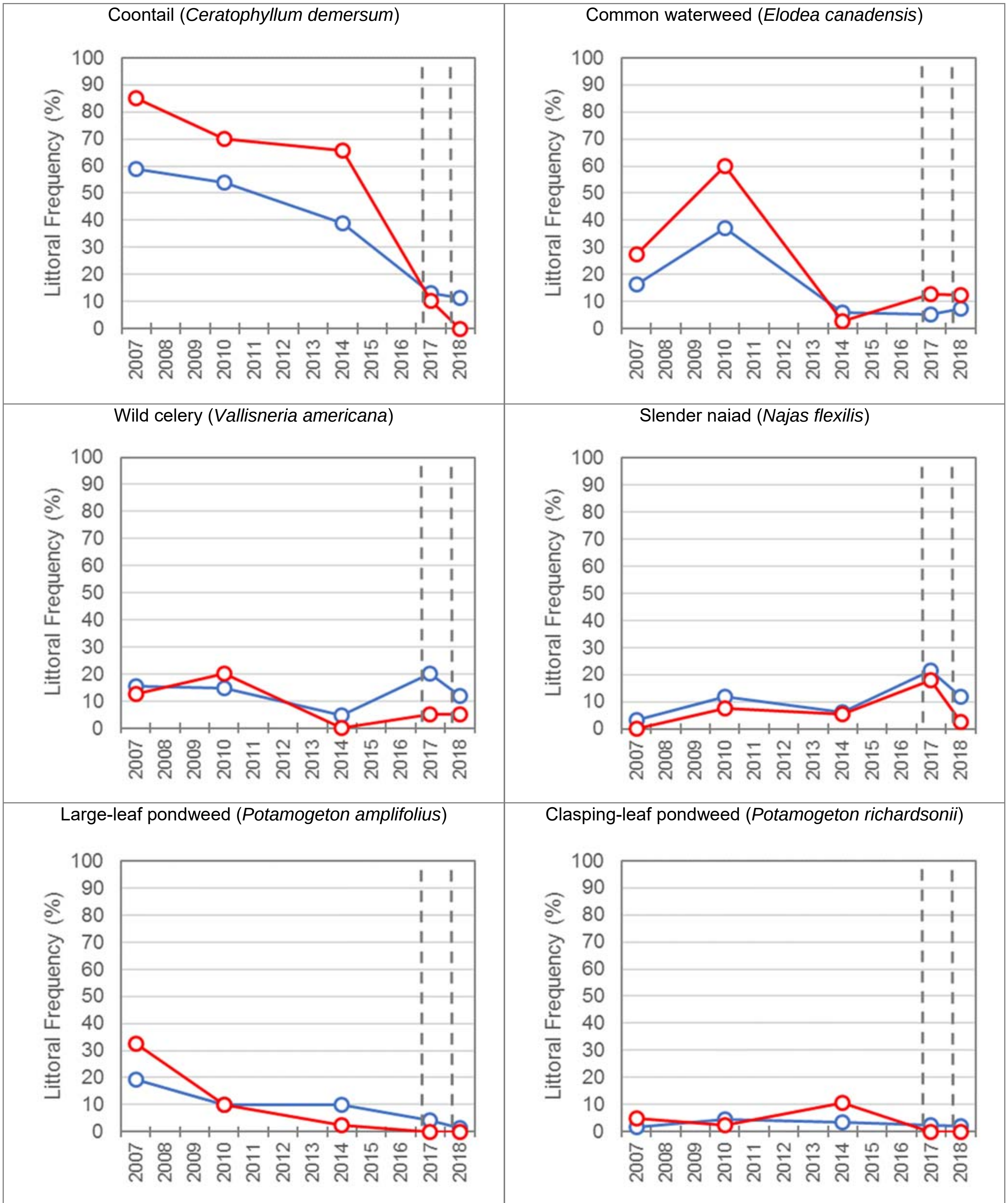

Figure 14. Littoral frequency of occurrence of select native aquatic plant species in Lost Lake. Red = within the treatment area. Blue = entire dataset (lake-wide) Grey dashed line indicates western basin spot herbicide treatment.

4.0 CONCLUSIONS & DISCUSSION

Herbicide concentration monitoring of the 2017 endothall treatment of Lost Lake provided insight for improved design in 2018. The concentration monitoring at all locations in 2018 was below detection at the final sampling period of 14 days after treatment (DAT). The average 2018 endothall concentrations within the application area were 1.12 ppm ae for the first 8 hours after treatment. These concentrations were likely sufficient to control CLP and were higher than observed in 2017. The higher concentrations may be related to less flow over the dam in 2017 compared to 2018. The 2018 data again confirm that the primary direction of herbicide dissipation was to the west over the dam into Lost Creek. The measured concentrations from Lost Lake near sensitive wild rice populations peaked at 0.42 ppm ae at 3 DAT with bracketed sampling at 1 DAT and 5 DAT containing no herbicide and low levels of herbicide, respectively. Herbicide concentration and exposure times measured from the center of Lost Lake were well below levels anticipated to impact aquatic plant populations.

Analysis of the aquatic plant community indicate that aquatic plant populations did not increase in 2018, with some metrics indicating further decrease. Broad analysis of these data indicates that the reduction of the aquatic plant community of Lost Lake is largely occurring at depths greater than 8 feet of water. Point-intercept surveys prior to 2017 contained abundance of aquatic plants from 8 to 14 feet, whereas extremely low levels of aquatic plants were observed within Lost Lake greater than 8 feet.

White-stem pondweed, fern-leaf pondweed, coontail, and slender naiad exhibited larger population decreases within the western basin application area than was observed lake-wide. This suggests that the herbicide treatment likely had an impact on these species within the treatment footprint. The pretreatment data from May 22 indicate that none of these species was present at that time but a low population (1.0-2.0%) of three of these species was present during the day before treatment (June 20). Fern-leaf pond was not located within the treatment area during the pretreatment survey nor during the summer point-intercept survey.

In the *Lost Lake Comprehensive Management Plan* (Draft Nov2018), the LLPRD outlined a strategy to manage the early population of CLP with herbicide spot treatment. Because CLP has only been present in Lost Lake for a few years, some theorize that the turion base may be small and if a control program is initiated at this time, may not require as many successive treatments as a more established population would. Continued pretreatment monitoring the pretreatment point-intercept sub-sample locations will confirm if reduced turion sprouting occurs over time.

Johnson et al. (2012) investigated nine midwestern lakes that received five consecutive annual large-scale endothall treatments to control CLP. The greatest reductions in CLP frequency, biomass, and turions was observed in the first two years of the control program, but continued reductions were observed following all five years of the project. The LLPRD's preliminary strategy anticipates targeting the same location for another two years (2019-2020). As outlined within the draft Plan, if the pretreatment sub-sample survey contains less CLP than 30% in 2019-2020, consideration for postponement of the herbicide strategy would be given by the LLPRD. The LLPRD believes that this threshold for management guidance attempts to balance tolerance of CLP at lower levels/densities while continuing to manage for an overall reduced CLP population within lake. Further, this would guide herbicide management when the financial costs and collateral impacts of the treatment are commensurate with the level of CLP population reduction achieved. The LLPRD has applied for a permit with WDNR to carry forward this management action pending the results of the pretreatment survey.

The LLPRD has also applied for an AIS-Established Population Control Grant to fund the continued monitoring and control strategy from 2019-2021. The monitoring strategy includes continued annual pretreatment monitoring, annual summer sub-set point-intercept surveys in the western basin of the lake, and annual volunteer-based herbicide concentration monitoring. A whole-lake point-intercept survey is planned for the final year of the project.

The application would occur before water temperatures greatly exceed 60°F, as endothall uptake rates have been shown to be higher at these water temperatures (Dr. Cody Gray, personal comm.). This timing also corresponds to the period before viable turion formation is likely to occur on CLP, which is important for the overall goal of the management strategy (i.e. control CLP before turions are produced). Conducting herbicide treatments earlier in the growing season are also thought to be more protective of the native plant community. However, the timing of the treatment would be postponed until after the Lac du Flambeau Band of Lake Superior Chippewa Indians has finished their spring open-water spear harvest and when downstream wild rice populations are anticipated to have advanced past the growth stage that is most sensitive to endothall treatment. The impacts of precipitation events prior to and during the treatment will continue to be monitored, particularly as longer residence time may justify a decrease in application rate down to 1.5 ppm ai.

4.0 LITERATURE CITED

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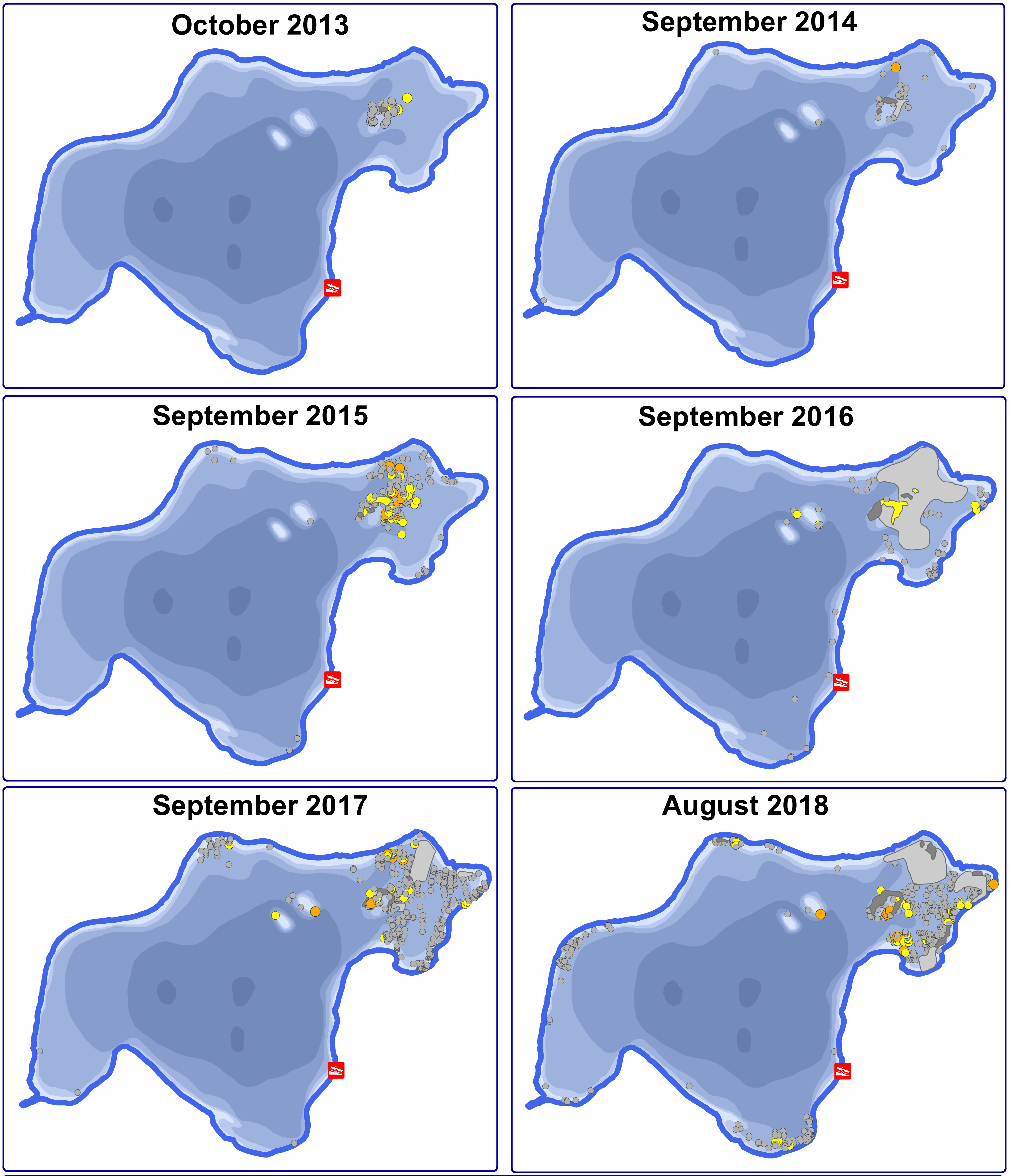
APPENDIX A

	Scientific Name	Common Name	LFOO (%) 2007	2010	2014	2017	2018	2007-2010 % Change	2007-2010 Direction	2010-2014 % Change	2010-2014 Direction	2014-2017 % Change	2014-2017 Direction	2017-2018 % Change	2017-2018 Direction	2007-2018 % Change	2007-2018 Direction
Dicots	*Myriophyllum spicatum*	Eurasian watermilfoil	0.0	0.0	0.0	2.4	8.8		-		-		▲	261.0	▲		▲
Dicots	*Ceratophyllum demersum*	Coontail	58.8	54.0	38.7	13.2	11.3	-8.3	▽	-28.2	▼	-66.0	▼	-14.0	▽	-80.8	▼
Dicots	*Myriophyllum sibiricum*	Northern watermilfoil	20.2	38.1	14.0	2.4	0.6	88.9	▲	-63.1	▼	-82.6	▼	-74.2	▽	-96.9	▼
Dicots	*Bidens beckii*	Water marigold	0.0	2.4	0.9	1.5	2.5		▲	-64.3	▽	72.0	△	71.9	△		▲
Dicots	*Nuphar variegata*	Spatterdock	1.7	0.8	0.4	0.5	1.3	-52.8	▽	-46.4	▽	14.6	△	157.9	△	-25.2	▽
Dicots	*Nymphaea odorata*	White water lily	0.8	0.0	0.0	1.0	1.3	-100.0	▽		-		△	28.9	△	49.7	△
Dicots	*Utricularia vulgaris*	Common bladderwort	0.0	0.0	0.0	0.0	0.6		-		-		-		△		△
Dicots	*Myriophyllum tenellum*	Dwarf watermilfoil	0.0	0.0	0.0	0.0	0.6		-		-		-		△		△
Dicots	*Ranunculus aquatilis*	White water crowfoot	0.4	0.0	0.0	0.0	0.0	-100.0	▽		-		-		-	-100.0	▽
Dicots	*Myriophyllum verticillatum*	Whorled watermilfoil	0.0	0.4	0.0	0.0	0.0		△	-100.0	▽		-		-		-
Non-dicots	*Potamogeton crispus*	Curly-leaf pondweed	0.0	0.0	0.0	4.4	4.4		-		-		▲	0.3	△		▲
Non-dicots	*Potamogeton robbinsii*	Fern-leaf pondweed	17.6	26.6	21.3	13.2	2.5	50.7	▲	-20.0	▽	-38.1	▼	-80.9	▼	-85.7	▼
Non-dicots	*Elodea canadensis*	Common waterweed	16.4	36.9	6.0	5.4	7.5	125.2	▲	-83.9	▼	-9.9	▽	40.7	△	-53.9	▼
Non-dicots	*Potamogeton praelongus*	White-stem pondweed	12.2	25.8	18.7	9.3	4.4	111.7	▲	-27.4	▽	-50.5	▼	-52.5	▽	-63.9	▼
Non-dicots	*Potamogeton zosteriformis*	Flat-stem pondweed	16.8	50.0	0.0	0.0	0.6	197.5	▲	-100.0	▼		-		△	-96.3	▼
Non-dicots	*Vallisneria americana*	Wild celery	15.5	14.7	4.7	20.0	11.9	-5.6	▽	-68.1	▼	327.3	▲	-40.3	▼	-23.1	▽
Non-dicots	*Najas flexilis*	Slender naiad	3.4	11.9	6.0	21.5	11.9	254.2	▲	-50.0	▼	260.3	▲	-44.3	▼	255.5	▲
Non-dicots	*Potamogeton pusillus, P. berchtoldii, & P. strictifolius*	Thin-leaved pondweed spp.	0.4	13.9	38.7	0.0	0.0	3205.6	▲	178.8	▲	-100.0	▼		-	-100.0	▽
Non-dicots	*Isoetes spp.*	Quillwort spp.	8.4	4.4	3.8	10.2	15.1	-48.1	▽	-12.3	▽	167.5	▲	47.3	△	79.6	▲
Non-dicots	*Potamogeton amplifolius*	Large-leaf pondweed	19.3	9.9	9.8	4.4	1.3	-48.7	▼	-1.3	▽	-55.1	▼	-71.3	▽	-93.5	▼
Non-dicots	*Potamogeton pusillus*	Small pondweed	0.4	2.8	27.2	0.0	0.0	561.1	▲	880.4	▲	-100.0	▼		-	-100.0	▽
Non-dicots	*Chara spp.*	Muskgrasses	3.8	2.0	0.9	1.0	12.6	-47.5	▽	-57.1	▽	14.6	△	1189.3	▲	232.6	▲
Non-dicots	*Potamogeton berchtoldii*	Slender pondweed	0.0	0.0	17.9	0.0	0.0		-		▲	-100.0	▼		-		-
Non-dicots	*Sagittaria sp. (rosette)*	Arrowhead sp. (rosette)	0.0	4.8	0.0	5.4	3.8		▲	-100.0	▼		▲	-29.7	▽		▲
Non-dicots	*Potamogeton richardsonii*	Clasping-leaf pondweed	1.7	4.4	3.4	2.4	1.9	159.7	△	-22.0	▽	-28.4	▽	-22.6	▽	12.3	△
Non-dicots	*Potamogeton strictifolius*	Stiff pondweed	0.0	12.7	0.0	0.0	0.0		▲	-100.0	▼		-		-		-
Non-dicots	*Potamogeton gramineus*	Variable-leaf pondweed	4.2	4.0	3.4	1.5	0.0	-5.6	▽	-14.2	▽	-57.0	▽	-100.0	▽	-100.0	▼
Non-dicots	*Filamentous algae*	Filamentous algae	2.1	0.0	5.5	4.4	1.3	-100.0	▼		▲	-20.6	▽	-71.3	▽	-40.1	▽
Non-dicots	*Eleocharis acicularis*	Needle spikerush	3.4	1.2	0.9	1.0	1.3	-64.6	▽	-28.5	▽	14.6	△	28.9	△	-62.6	▽
Non-dicots	*Heteranthera dubia*	Water stargrass	0.8	0.0	0.4	0.5	3.1	-100.0	▽		△	14.6	△	544.7	▲	274.2	△
Non-dicots	*Stuckenia pectinata*	Sago pondweed	0.0	0.8	1.3	0.5	0.0		△	60.9	△	-61.8	▽	-100.0	▽		-
Non-dicots	*Juncus pelocarpus*	Brown-fruited rush	0.8	0.8	0.4	0.0	0.0	-5.6	▽	-46.4	▽	-100.0	▽		-	-100.0	▽
Non-dicots	*Eriocaulon aquaticum*	Pipewort	0.0	0.0	0.0	0.0	1.3		-		-		-		△		△
Non-dicots	*Schoenoplectus acutus*	Hardstem bulrush	0.0	0.0	0.4	0.0	0.6		-		△	-100.0	▽		△		△
Non-dicots	*Nitella spp.*	Stoneworts	0.0	0.8	0.0	0.0	0.0		△	-100.0	▽		-		-		-
Non-dicots	*Lemna turionifera*	Turion duckweed	0.0	0.0	0.0	0.0	0.6		-		-		-		△		△
Non-dicots	*Lemna trisulca*	Forked duckweed	0.0	0.0	0.0	0.0	0.6		-		-		-		△		△
Non-dicots	*Schoenoplectus tabernaemontani*	Softstem bulrush	0.0	0.4	0.0	0.0	0.0		△	-100.0	▽		-		-		-
Non-dicots	*Potamogeton illinoensis*	Illinois pondweed	0.4	0.0	0.0	0.0	0.0	-100.0	▽		-		-		-	-100.0	▽
Non-dicots	*Potamogeton foliosus*	Leafy pondweed	0.4	0.0	0.0	0.0	0.0	-100.0	▽		-		-		-	-100.0	▽
Non-dicots	*Pontederia cordata*	Pickerelweed	0.0	0.4	0.0	0.0	0.0		△	-100.0	▽		-		-		-
Non-dicots	*Lobelia dortmanna*	Water lobelia	0.0	0.4	0.0	0.0	0.0		△	-100.0	▽		-		-		-
Non-dicots	*Fissidens spp. & Fontinalis spp.*	Aquatic Moss	0.0	0.0	0.0	0.5	0.0		-		-		△	-100.0	▽		-

▲ or ▼ = Change Statistically Valid (Chi-square; α = 0.05)
△ or ▽ = Change Not Statistically Valid (Chi-square; α = 0.05)



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Lake Management Planning
815 Prosper Road
De Pere, WI 54115
920.338.8860
www.onterra-eco.com

Sources:
Roads, Hydro, and Bathymetry: WDNR
Plant Surveys: Onterra, 2013-2015
Map Date: January 16, 2019 TWH
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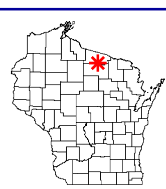


Project Location in Wisconsin

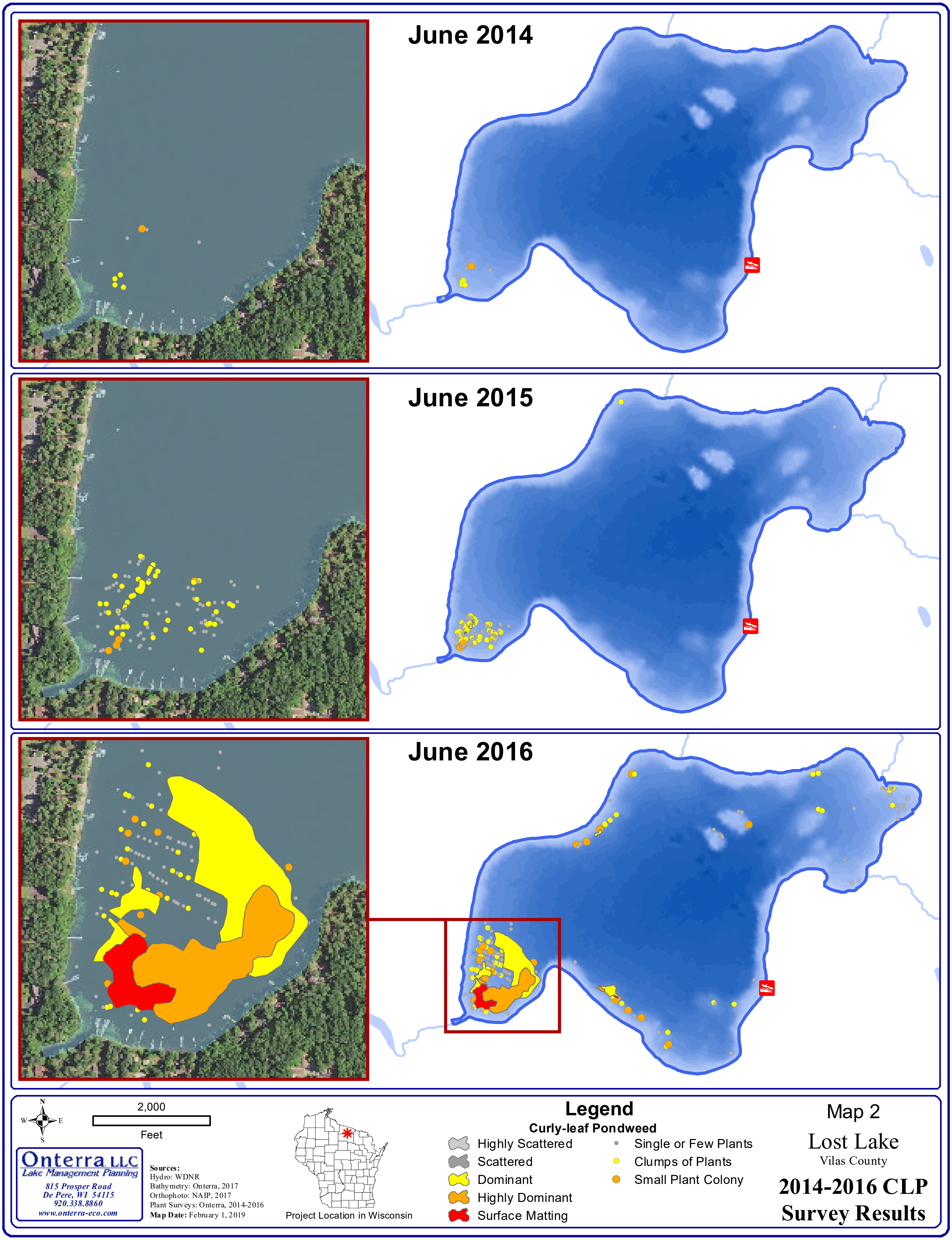
Legend

Eurasian Watermilfoil

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony

Map 1
Lost Lake
Vilas County

2013-2018 EWM Survey Results



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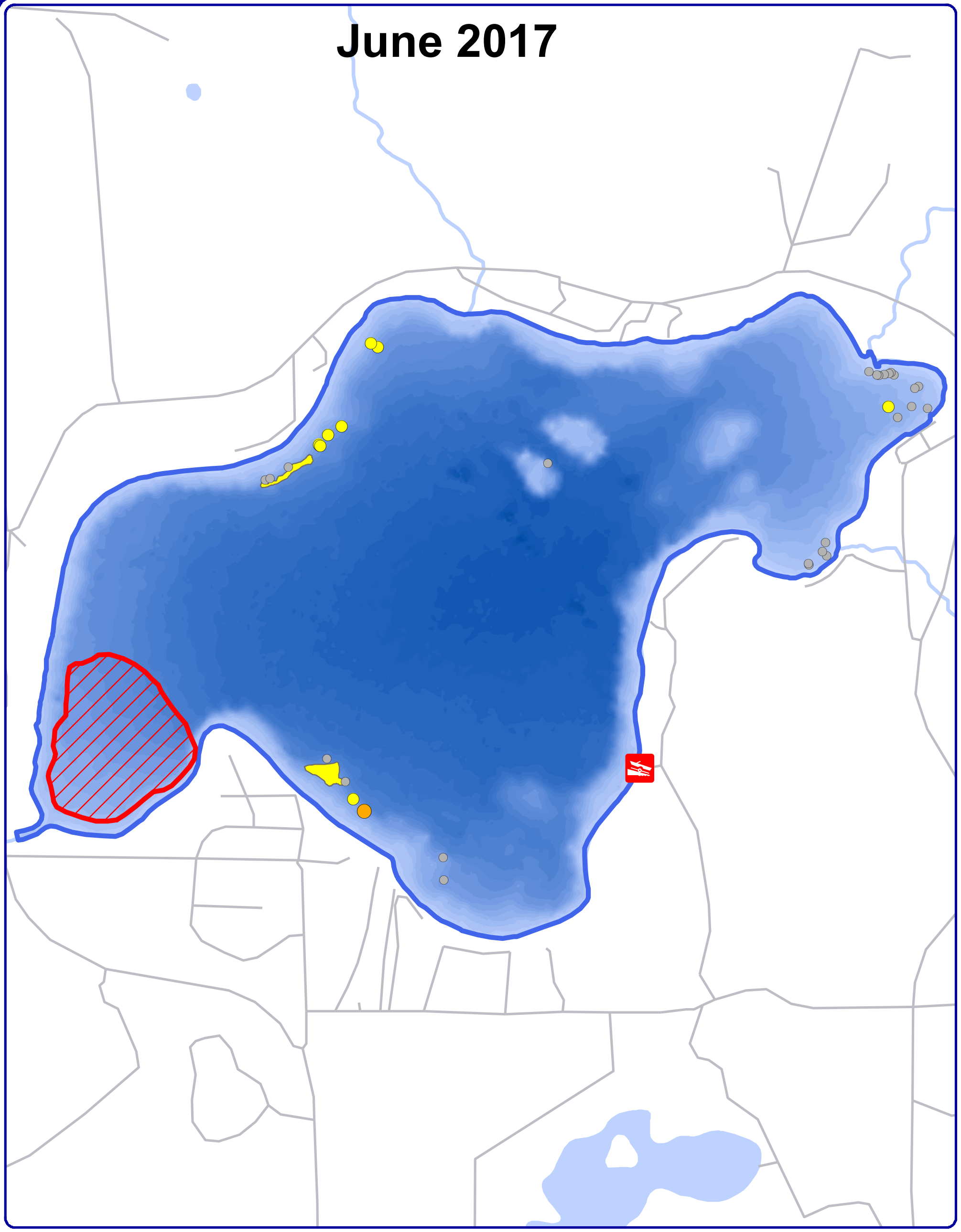
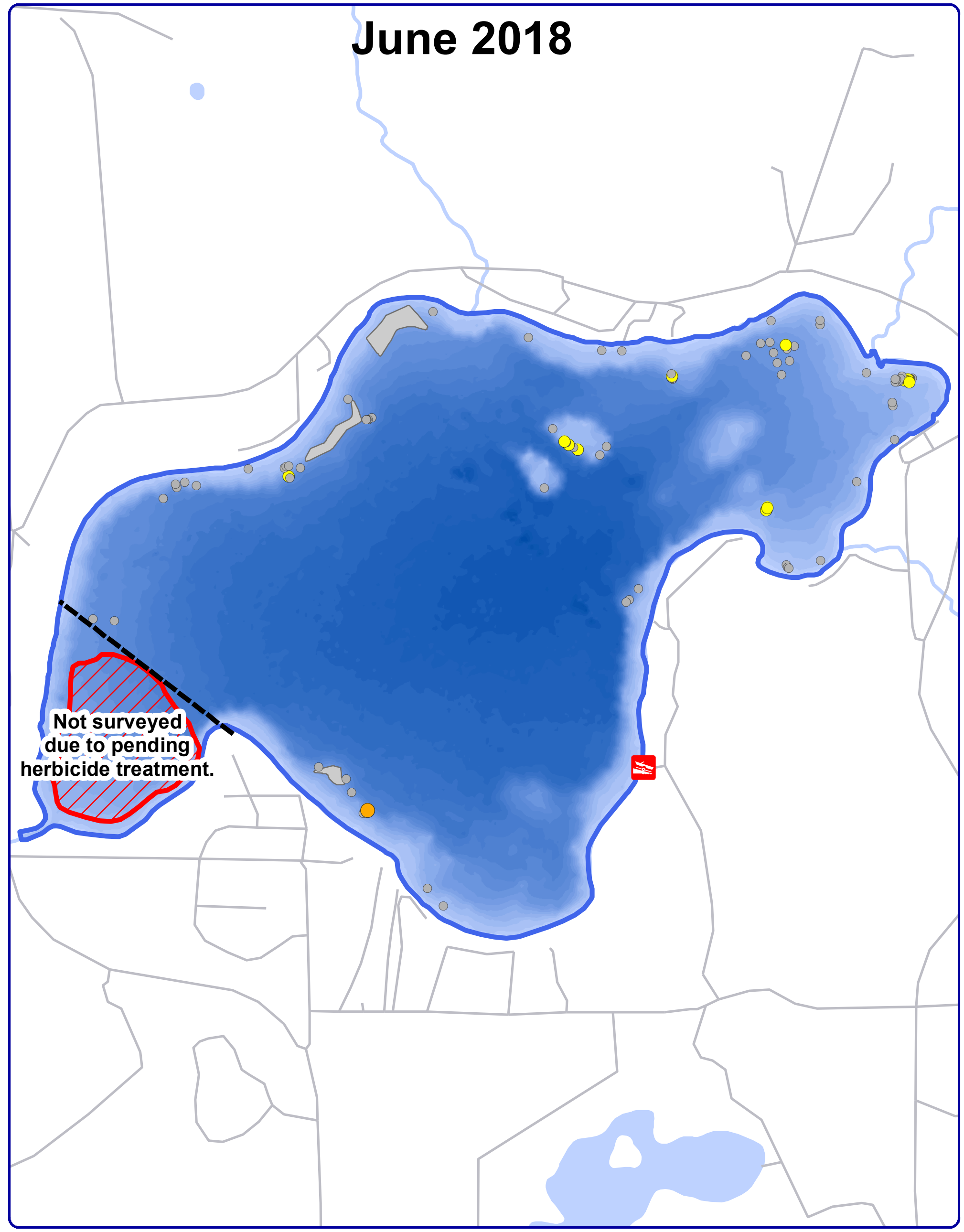
920.338.8860
www.onterra-eco.com
Sources:
Hydro: WDNR
Bathymetry: Onterra, 2017
Orthophoto: NAIP, 2017
Plant Surveys: Onterra, 2014-2016
Map Date: February 1, 2019
Project Location in Wisconsin
Legend
Curly-leaf Pondweed
Highly Scattered
Scattered
Dominant
Highly Dominant
Surface Matting
Single or Few Plants
Clumps of Plants
Small Plant Colony
Map 2
Lost Lake
Vilas County
2014-2016 CLP Survey Results





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www.onterra-eco.com

Sources:
Roads & Hydro: WDNR
Bathymetry: Onterra, 2017;
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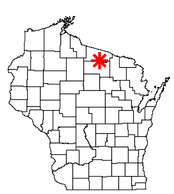
Project Location in Wisconsin

Legend

- Highly Scattered
- Scattered *(none found)*
- Dominant *(none found)*
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clump of Plants
- Small Plant Colony

Map 3
Lost Lake
Vilas County, Wisconsin

June 2017 & June 2018
CLP Survey Results