1.0 INTRODUCTION

Lilly Lake, Kenosha County, is an approximately 85-acre seepage lake with a maximum depth of 22 feet and a mean depth of 11 feet (Figure 1.0-1). This oligo-mesotrophic lake has a relatively small watershed when compared to the size of the lake. Lilly Lake contains many native plant species, of which muskgrasses were the most common plant. Eurasian water milfoil (EWM) has been present in Lilly Lake since at least 1976, with Hybrid Eurasian water milfoil (HWM) being verified in 2014. Within this report the collective populations of EWM and HWM are referred to as HWM unless specified otherwise. Numerous control efforts have targeted the HWM population within Lilly Lake, including spot herbicide treatments occurring every year from 2004 to 2019.

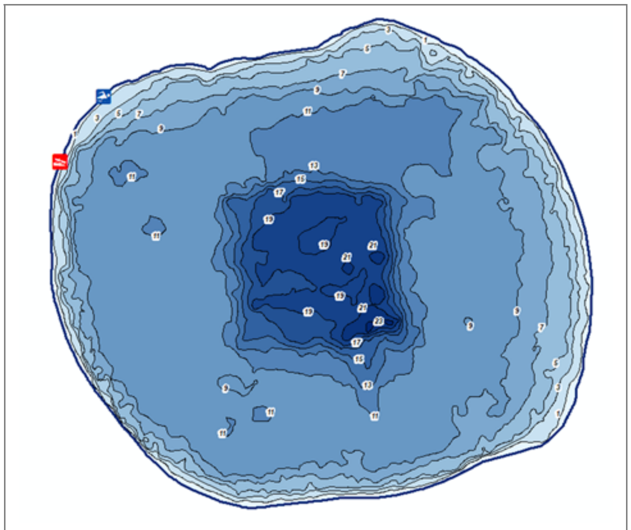


Figure 1.0-1. Lilly Lake, Kenosha County.

1.1 Historic AIS Management & Planning

The Lilly Lake Protection & Rehabilitation District (LLPRD) has been managing Lilly Lake's HWM population on an annual basis primarily targeting the population with 2,4-D spot treatments. While some of these historical treatments have approached whole-lake levels, these treatments have at best resulted in seasonal control and have failed to achieve longer-term, multi-year control of the HWM population. It is likely that the long history of annual use of 2,4-D has selected for an HWM population that is more resistant to this herbicide. The surveys completed in 2020 and 2021 found that HWM was widespread and well established in Lilly Lake, with the densest colonies found in shallow, near-shore areas where they interfere with lake access, recreation, and aesthetics.

As a part of the district's plan development, the group learned about the realistic management of HWM with herbicides and why annual use of a single herbicide is not ecological sound and often not fiscally efficient. In the district's 2021 *Aquatic Plant Management Plan*, a new approach to HWM management was included that utilizes a relatively new herbicide, ProcellaCOR™, in an effort to achieve longer-term control of the HWM population. While florpyrauxifen-benzyl is a similar mode of action to 2,4-D (auxin hormone mimic), differences in molecular configuration and binding affinity are thought to generate a different enough response in the plant to minimize this potential.

The LLPRD's *Aquatic Plant Management Plan* (2021) outlines an HWM population control goal to reduce or eliminate large, contiguous, monotypic colonies of HWM. The preliminary HWM control plan would be developed off the previous year's Late-Season HWM mapping survey. The LLPRD conducted HWM monitoring in 2021 without grant funds to serve as a pretreatment dataset in anticipation of an early-season 2022 herbicide treatment.

The district contracted with Onterra and paid for a whole-lake point-intercept survey in 2021 to quantify the HWM and native plant populations ahead of the proposed 2022 treatment. A late-season HWM peak-biomass survey was also completed to obtain an up-to-date picture of the HWM population and to aid in the construction of herbicide application areas. The 2021 point-intercept survey showed HWM increased in occurrence from 37% in 2020 to 52% in 2022. The HWM late-summer mapping survey showed that the acreage of colonized HWM increased from 8.0 acres in 2020 to 24.0 acres in 2021. Most of this increase was attributable to an increase in areas of scattered HWM; however, approximately 11.0 acres contained dominant, highly dominant, or surface-matted HWM (Map 1).

1.2 2022 HWM Management & Monitoring Strategy

The LLPRD developed a 2022 EWM control strategy to target large and dense HWM occurrences in high-use areas, understanding that HWM control is likely to extend outward and potentially lake-wide from this treatment. The final treatment design is displayed on Map 2 and included the application of ProcellaCOR at 4.0 PDU's (Prescription Dose Units) at three treatment sites totaling 10.5 acres. This proposed dosing rate was confirmed by experts from SePRO, the manufacturer of ProcellaCOR™. This strategy was also incorporated into a successful WDNR AIS Large-Scale Population Control Grant application, providing state-share assistance in carrying out the effort. This report marks the first report deliverable of ACEI-295-22, with *year after treatment* monitoring in 2023 also being a part of this overall project.

The herbicide treatments monitoring plan included comparative Late-Season HWM Mapping Surveys, anticipating little to no HWM present within application areas during the *year of treatment* and only low-density occurrences during the *year after treatment*. Because the whole lake is anticipated to be impacted by the control measure, quantitative monitoring would occur at the whole-lake scale through annual point-intercept surveys. Point intercept surveys would occur consistent with WDNR guidance, being completed the *year prior to treatment* (2021 without grant funds), *year of treatment* (2022), and *year after treatment* (2023). Quantitative success criteria of the herbicide treatment would be a 70% reduction in HWM littoral frequency of occurrence comparing the *year prior to treatment* (2021) dataset to the *year after treatment* (2023). The *year of treatment* post treatment assessment will assist with early determinations of success as well as a better understanding of overall native plant impacts.

1.3 Pre-Treatment Confirmation and Refinement Survey

Onterra ecologists completed the pre-treatment confirmation and refinement survey on May 19, 2022. Parameters such as plant growth stage, water temperature, and water depth were investigated to confirm the final treatment strategy. During this visit, Onterra staff delivered the equipment and monitoring supplies related to the herbicide concentration monitoring efforts being completed by volunteers.

Photo 1.3-1. EWM plants from May 19, 2022 pre-treatment survey on Lilly Lake. Photo by Onterra, LLC

This survey was conducted using a combination of survey methods (visual, rake tows, submersible camera), but largely consisted of visual observations as most of the EWM was visible from the surface. Water temperatures at mid-depth in the application areas was 67°F. Using an optical probe, the pH was measured at 8.6 in site A-22 and 8.7 in site C-22. New EWM growth was apparent on the target plants and appeared to be in an active growth stage ideal for treatment (Photo 1.3-1).

No alterations to the originally proposed strategy were made as a result of the pre-treatment survey. Onterra encouraged that the application follows proper spot-treatment guidelines for a successful treatment including treatment occurring during a period of low winds.

The herbicide application was completed during the morning of May 23, 2022 by Schmidt's Aquatic, LLC. The applicator noted light northeast winds (1-2 mph) within the application area at the time of treatment. The surface water temperature reading was 63°F.

2.0 2022 AQUATIC PLANT MONITORING RESULTS

It is important to note that two types of surveys are discussed in the subsequent materials: 1) point-intercept surveys and 2) HWM mapping surveys. Overall, each survey has its strengths and weaknesses, which is why both are utilized in different ways as part of this project. The point-intercept survey provides a standardized way to gain quantitative information about a lake's aquatic plant population through visiting predetermined locations and using a rake sampler to identify all the plants at each location. The survey methodology allows comparisons to be made over time, as well as between lakes. It is common to see a particularly plant species, such as HWM, very near the sampling location but not yield it on the rake sampler. Particularly in low-density colonies such as those designated by Onterra as *highly scattered* and *scattered*, large gaps between EWM plants may exist resulting in EWM not being present at a particularly pre-determined point-intercept sampling location in that area.

While the point-intercept survey is a valuable tool to understand the overall plant population of a lake, it does not offer a full account (census) of where a particular species exists in the lake. During the HWM mapping survey, the entire littoral area of the lake is surveyed through visual observations from the boat (Photo 2.0-1). Field crews supplemented the visual survey by deploying a submersible camera along with periodically doing rake tows. The HWM population is mapped using sub-meter GPS technology by using either 1) point-based or 2) area-based methodologies. Large colonies >40 feet in diameter are mapped using polygons (areas) and are qualitatively attributed a density rating based upon a five-tiered scale from *highly scattered* to *surface matting*. Point-based techniques were applied to AIS locations that were considered as *small plant colonies* (<40 feet in diameter), *clumps of plants*, or *single or few plants*.

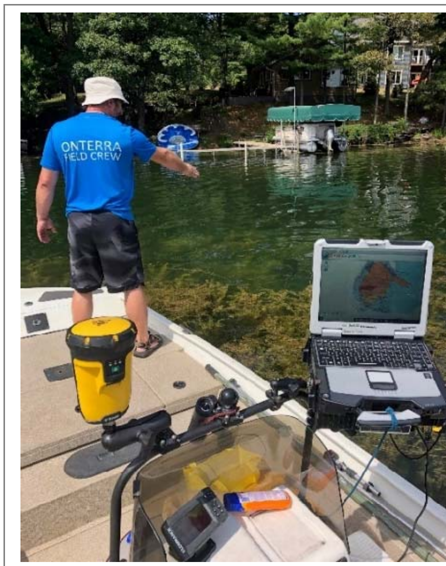


Photo 2.0-1. EWM mapping survey on a Wisconsin lake. Photo credit Onterra.

2.1 Quantitative Monitoring: Whole-Lake Point-Intercept Survey

A whole-lake point-intercept aquatic plant survey was conducted in Lilly Lake by Onterra on August 4, 2022 (Photo 2.1-1). Whole-lake point-intercept surveys have also been completed in 2008, 2020, and 2021 and the results and comparative analysis of the 2022 study is included in the following text. Aquatic plants have been found growing to a maximum depth of 19-20 feet in the point-intercept surveys, which indicates that the littoral zone spans essentially the entire lake.

Photo 2.1-1. Point-intercept survey on a WI lake. Photo credit Onterra.

Species List

In total, 31 species have been recorded from Lilly Lake over the course of these three surveys, with 21 having a submergent growth form (Table 2.1-1). The completion of an emergent and floating-leaf plant mapping survey in 2020 documented additional species growing in near-shore areas that were not documented in subsequent surveys. The list also contains the species' scientific name, common name, status in WI, and its coefficient of conservatism. The latter is discussed in more detail below. Changes in this list over time, whether it is differences in total species present, gains and losses of individual species, or changes in growth forms that are present, can be an early indicator of changes in the ecosystem.

Table 2.1-1. Aquatic plant species located in Lilly Lake during 2020, 2021, & 2022 surveys. Emergent species located during other vegetation surveys are exulted from this list.

Scientific Name	Common Name	Status in Wisconsin	Coefficient of Conservatism	2020	2021	2022
Chara spp.	Muskgrasses	Native	7	X	X	X
Eleocharis acicularis	Needle spikerush	Native	5		X	
Elodea canadensis	Common w aterw eed	Native	3	X	X	X
Heteranthera dubia	Water stargrass	Native	6	X	X	X
Myriophyllum sibiricum x M. spicatum	Hybrid w atermilfoil	Non-Native - Invasive	N/A	X	X	X
Najas flexilis	Slender naiad	Native	6	X	X	X
Najas guadalupensis	Southern naiad	Native	7	X	X	X
Nitella spp.	Stonew orts	Native	7		X	X
Potamogeton amplifolius	Large-leaf pondw eed	Native	7			X
Potamogeton amplifolius x P. praelongus	Large-leaf x w hite-stem pondw eed hybrid	Native	N/A	X	X	X
Potamogeton crispus	Curly-leaf pondw eed	Non-Native - Invasive	N/A	X	X	I
Potamogeton gramineus	Variable-leaf pondw eed	Native	7			X
Potamogeton illinoensis	Illinois pondw eed	Native	6	X	X	X
Potamogeton nodosus	Long-leaf pondw eed	Native	5	I		
Potamogeton praelongus	White-stem pondw eed	Native	8	X	X	X
Potamogeton richardsonii	Clasping-leaf pondw eed	Native	5			X
Potamogeton robbinsii	Fern-leaf pondw eed	Native	8	X	X	X
Potamogeton strictifolius	Stiff pondw eed	Native	8	X	X	X
Potamogeton zosteriformis	Flat-stem pondw eed	Native	6	X	X	X
Sagittaria graminea	Grass-leaved arrow head	Native	9		X	
Stuckenia pectinata	Sago pondw eed	Native	3	X	X	X

Frequency of Occurrence

Frequency of occurrence describes how often a certain aquatic plant species is found within a lake. Obviously, all of the plants cannot be counted in a lake, so samples are collected from pre-determined areas. In the case of the whole-lake point-intercept survey completed on Lilly Lake; plant samples were collected from plots laid out on a grid that covered the lake. Using the data collected from these plots, an estimate of occurrence of each plant species can be determined. The occurrence of aquatic plant species is displayed as the *littoral frequency of occurrence*. Littoral frequency of occurrence is used to describe how often each species occurred in the plots that are within the maximum depth of plant growth (littoral zone), and is displayed as a percentage.

A total of 17 aquatic plant species were encountered directly on the rake during the 2022 whole-lake point-intercept survey. Due to the difficulty of identifying in the field, the occurrences of large-leaf pondweed, white-stem pondweed, and a hybrid white-stem/large-stem pondweed are combined for analysis purposes. Muskgrasses (67.9%), southern naiad (55.0%), and fern-leaf pondweed (41.6%) were the most frequently encountered species (Figure 2.1-1) in the 2022 point-intercept survey. A single highly-injured hybrid watermilfoil plant was found at one sampling location resulting in an occurrence of 0.5%.

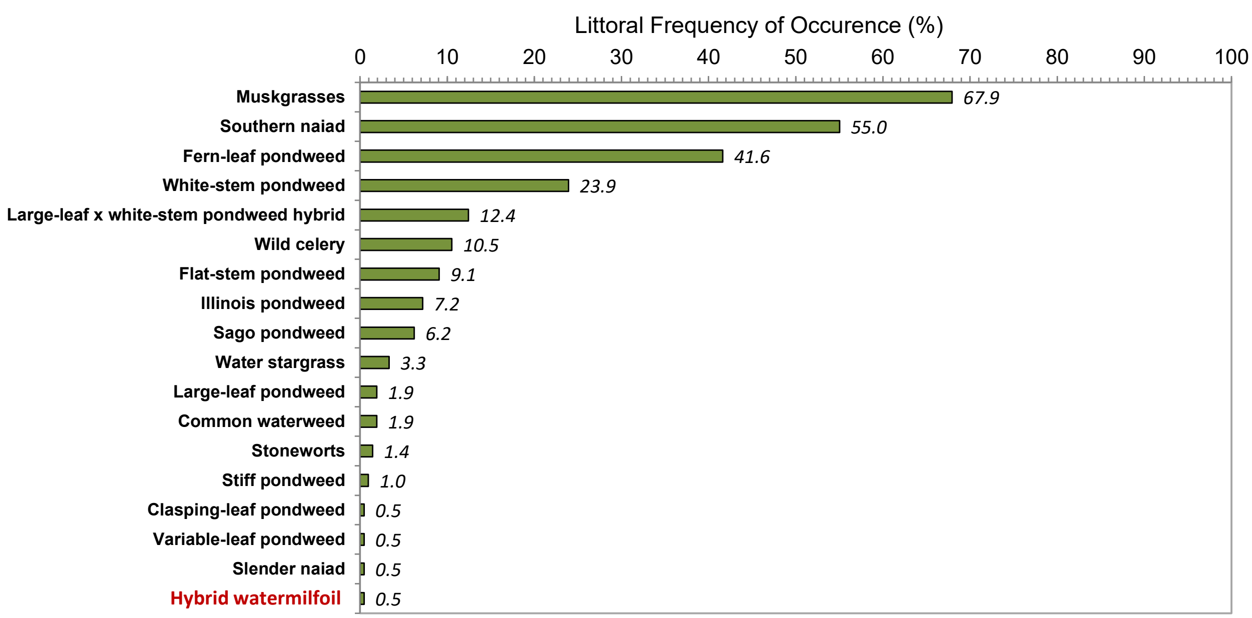


Figure 2.1-1. Lilly Lake 2022 littoral frequency of occurrence of aquatic plant species. Created using data from the 2022 whole-lake point-intercept survey.

Figure 2.1-2 compares the littoral frequency of occurrence for the most commonly encountered aquatic plant species located in point-intercept surveys between 2020-2022 in Lilly Lake. Hybrid Eurasian watermilfoil exhibited a statistically valid 99.1% decrease in occurrence between 2021 and 2022 following the herbicide treatment management strategy. The only other species that had a statistically valid population decrease was water stargrass. A full matrix that displays the littoral frequency of occurrences for all species sampled during the point-intercept surveys is included in Appendix A.

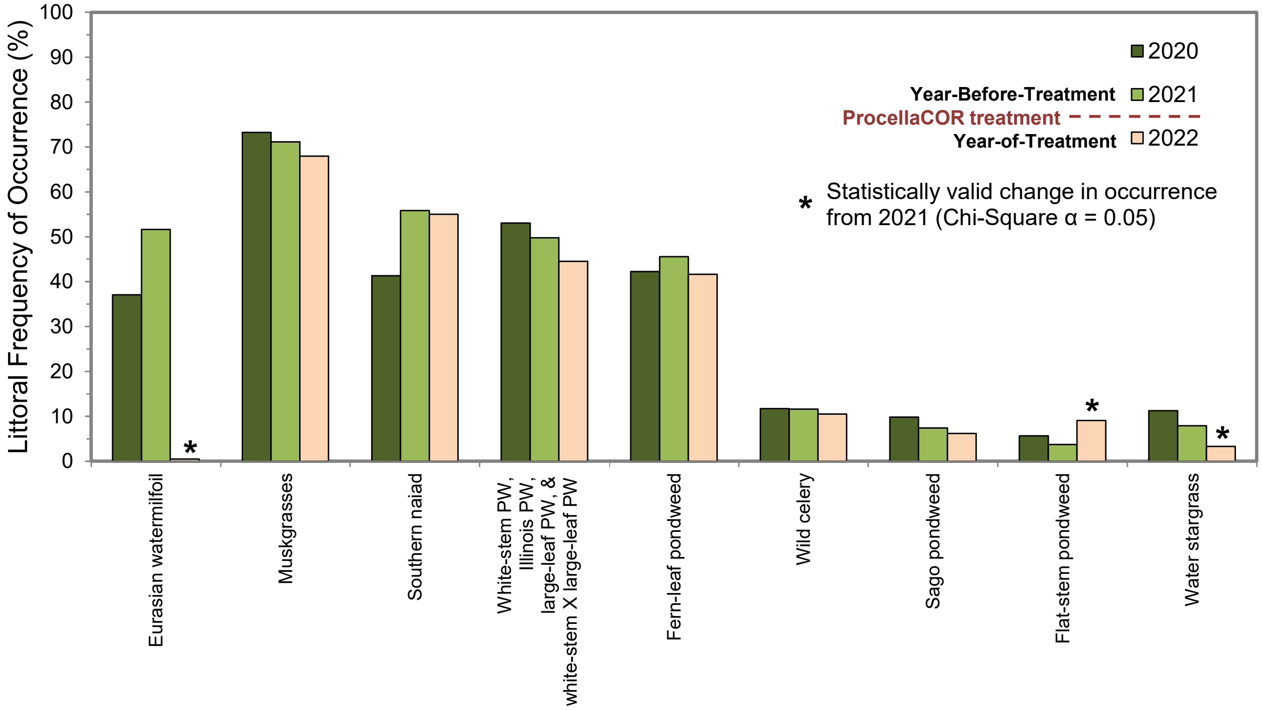


Figure 2.1-2. Lilly Lake 2020-2022 littoral frequency of occurrence of aquatic plant species. Created using data from the 2020, 2021, and 2022 whole-lake point-intercept surveys.

Floristic Quality Assessment

The floristic quality of a lake's aquatic plant community is calculated using its native *species richness* and their *average conservatism*. Species richness is the number of native aquatic plant species that were physically encountered on the rake during the point-intercept survey. Average conservatism is calculated by taking the sum of the coefficients of conservatism (C-values) of the native species located and dividing it by species richness. Every plant in Wisconsin has been assigned a coefficient of conservatism, ranging from 1-10, which describes the likelihood of that species being found in an undisturbed environment. Species which are more specialized and require undisturbed habitat are given higher coefficients, while species which are more tolerant of environmental disturbance have lower coefficients. Higher average conservatism values generally indicate a healthier lake as it is able to support a greater number of environmentally-sensitive aquatic plant species. Low average conservatism values indicate a degraded environment, one that is only able to support disturbance-tolerant species.

On their own, the species richness and average conservatism values for a lake are useful in assessing a lake's plant community; however, the best assessment of the lake's plant community health is determined when the two values are used to calculate the lake's floristic quality. The floristic quality is calculated using the species richness and average conservatism value of the aquatic plant species that were solely encountered on the rake during the point-intercept surveys (equation shown below). This assessment allows the aquatic plant community of Lilly Lake to be compared to other lakes within the region and state.

$$\text{FQI} = \text{Average Coefficient of Conservatism} * \sqrt{\text{Number of Native Species}}$$

Data collected during the aquatic plant surveys was also used to complete a Floristic Quality Assessment (FQA) which incorporates the number of native aquatic plant species recorded on the rake during the point-intercept survey and their average conservatism. The data used for these calculations does not include any incidental species (visual observations) but only considers plants that were sampled on the rake during the survey. Figure 2.1-3 displays the species richness, average conservatism, and floristic quality of Lilly Lake along with ecoregion and state median values.

Lilly Lake's native plant species richness values have ranged from 13 in 2020 to 16 in 2021 and 2022 compared to the median values for lakes within the SWTP ecoregion (15) and lakes across Wisconsin (19). However, Lilly Lake's average species conservatism of 6.2 in 2020 and 6.3 in 2021-2022 falls above the SWTP median value of 5.4 and near the statewide value of 6.3. This indicates that Lilly Lake has a higher number of environmentally sensitive species (higher C-values) when compared to most lakes within the SWTP ecoregion. Using the species richness and average conservatism values, Lilly Lake's Floristic Quality Index was 22.4 in 2020, and 25.2 in 2021 and 2022 falling above the median value for lakes in the SWTP ecoregion (21.1) and below the median value for lakes statewide (27.2).

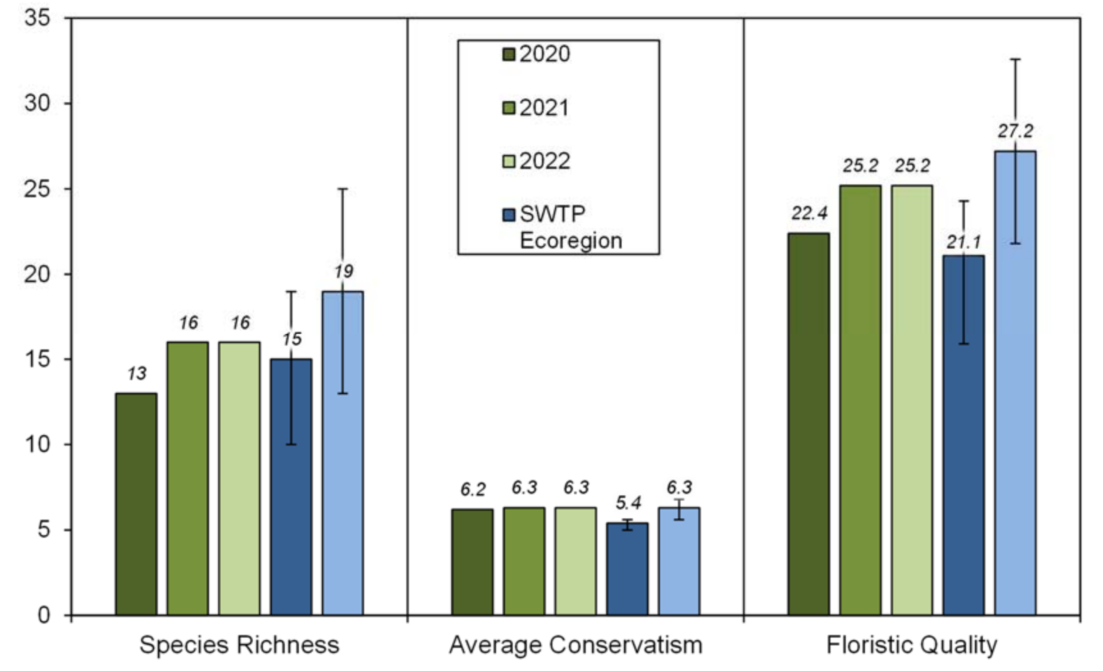


Figure 2.1-3. Lilly Lake Floristic Quality Assessment. Created using data from point-intercept surveys. Analysis following Nichols (1999) where SWTP = Southeastern Wisconsin Till Plains - Lakes Ecoregion.

Species Diversity

Species diversity is often confused with species richness. Species richness is simply the number of species found within a given community. While species diversity utilizes species richness, it also takes into account evenness or the variation in abundance of the individual species within the community. For example, a lake with 10 aquatic plant species that had relatively similar abundances within the community would be more diverse than another lake with 10 aquatic plant species were 50% of the community was comprised of just one or two species.

An aquatic system with high species diversity is more stable than a system with a low diversity. This is analogous to a diverse financial portfolio in that a diverse aquatic plant community can withstand environmental fluctuations much like a diverse portfolio can handle economic fluctuations. Some managers believe a lake with a diverse plant community is also better suited to compete against exotic infestations than a lake with a lower diversity. However, in a recent study of 1,100 Minnesota lakes, researchers concluded that more diverse communities were not necessarily more resistant or resilient to invaders (Muthukrishnan et al. 2018).

If a lake has a diversity index value of 0.90, it means that if two plants were randomly sampled from the lake there is a 90% probability that the two individuals would be of a different species. The Simpson's Diversity Index value from Lilly Lake is compared to data collected by Onterra and the WDNR Science Services on lakes within the Southeastern Wisconsin Till Plains ecoregion and on lakes throughout Wisconsin (Figure 2.1-4). While a method for characterizing diversity values of fair, poor, etc. does not exist, lakes within the same ecoregion may be compared to provide an idea of how Lilly Lake's diversity values rank. Lilly Lake's Simpson's Diversity Index value has been stable at 0.85-0.86 over the course of the three point-intercept surveys spanning 2020-2022, falling between the ecoregion median and upper quartile.

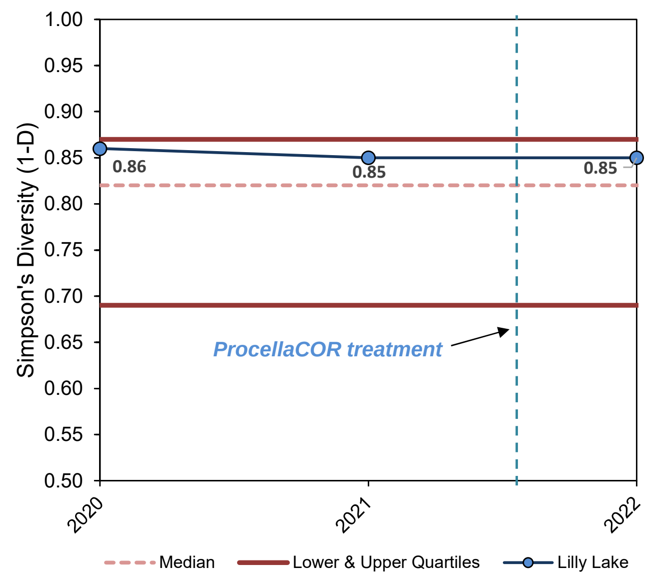


Figure 2.1-4. Lilly Lake Simpson's Diversity Index. Created using data from point-intercept surveys.

2.2. Qualitative Monitoring: HWM Mapping Surveys

Qualitative monitoring compares the late-summer EWM mapping survey population mapped during 2021 (pre-treatment) and late-summer 2022 (post-treatment). Onterra ecologists conducted the Late-Summer HWM Mapping Survey on Lilly Lake on October 5, 2022. The purpose of the survey was to search for and map all occurrences of HWM in the lake. The crew completed a visual meander survey around the lake without detecting any HWM. Following the visual survey, the crew deployed a submersible camera in some of the deeper areas of the lake to search for short-statured plants. The crew also took several rake tows in former HWM colonies around the lake to search for surviving HWM plants. All of these methods resulted in not finding any HWM in Lilly Lake. The crew noted that native plants appeared green and healthy with many native pondweeds present.

The acreage of colonized HWM that has been mapped in late-summer surveys between 2020-2022 is displayed on Figure 2.3-2. In 2020, 8.0 acres were delineated, whereas 24.0 acres were delineated in the 2021 mapping survey. Approximately 10.6 acres of HWM was comprised of *dominant*, *highly dominant* or *surface matted* densities in the 2021 survey. Following the 2022 herbicide treatments, no colonized HWM was located in Lilly Lake. It is important to note that Figure 2.2-1 only accounts for HWM that is mapped with area-based mapping (polygons) and does not account for any occurrences mapped with point-based attributes such as *single plants*, *clumps of plants*, or *small plant colonies*.

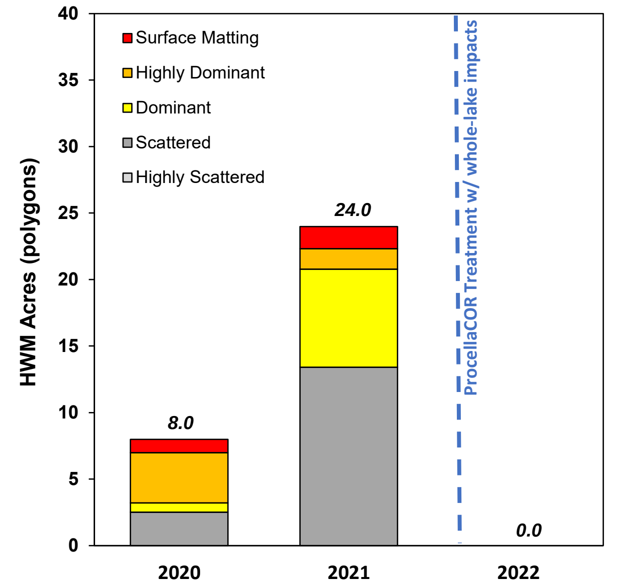


Figure 2.2-1. Acreage of mapped HWM colonies on Lilly Lake from 2020-2022. Data from Onterra Late-Summer EWM Mapping Surveys.

2.3 Herbicide Concentration Monitoring

The herbicide concentration monitoring plan associated with the treatment was developed by Onterra and the WDNR, with the intent of gaining sufficient data to aid in understanding the concentrations of florpyrauxifen-benzyl that were achieved in the hours and days after treatment. Samples were collected three total sites following treatment – two within application areas, and one site located in the deep hole area of the lake. Samples were collected at nine time intervals after treatment beginning at 3 hours after treatment (HAT), with additional samples collected at 9, 24, and 48 HAT as well as 4, 7, 14, 21, and 28 days after treatment (DAT). Samples were collected by a volunteer member of the LLPRD and upon completion of the sampling, were shipped to EPL Bio Analytical Services in Niantic Illinois for analysis. EPL was identified by the WDNR as being one of the few labs in the country that is able to detect florpyrauxifen-benzyl at levels below one part per billion. A copy of the herbicide concentration monitoring plan is included as Appendix B.

The EPL Lab reports the concentration in parts per billion (ppb) of the initial parent active ingredient in ProcellaCOR™ (florpyrauxifen-benzyl, SX-1552), as well as an acid metabolite (SX-1552-A) which is the immediate by-product that it breaks down into.

Figure 2.3-1 and Table 2.3-1 display the concentrations of florpyrauxifen-benzyl from the three monitoring locations. For reference, the dosing rate of 4.0 PDU (prescription dosing units) equates to 7.7 ppb of florpyrauxifen-benzyl. Site L1 was placed in application area A-22 and site L2 was placed in application area C-22. A third monitoring site (L3) was placed in the center of the lake. Florpyrauxifen-benzyl concentrations were initially higher in the application areas at 3 HAT compared to the center of the lake prior to herbicide mixing. By 48 HAT, concentrations at all three monitoring sites were approximately the same at about 0.2-0.3 ppb. At 4 DAT, concentrations decreased to between 0.1-0.2 ppb, and decreased below 0.1 ppb in all samples by 7 DAT. All samples collected at 14 DAT, 21 DAT, and 28 DAT were below detection limits for florpyrauxifen-benzyl.

In an effort to understand the lake-wide herbicide concentration following dispersion and dissipation away from the herbicide application area, samples were collected from the deep hole location in the middle of Lilly Lake (site L3). Concentrations at site L3 are expected to be reflective of the lake-wide concentration following treatment. Studies of this nature conducted to date indicate herbicide mixes and reaches equilibrium within the mixing water volume by approximately 24-48 HAT. On Lilly Lake, the ProcellaCOR™ concentration reached a lake-wide equilibrium of 0.31 ppb at 2 days after treatment. At 4 DAT, the concentration at site L3 was 0.179 ppb and had declined to levels below detection limits by 7 DAT.

Table 2.3-1. Florpyrauxifen-benzyl (SX-1552) concentrations at three monitoring locations following a May 2022 ProcellaCOR™ herbicide treatment in Lilly Lake.

	Florpyrauxifen-benzyl (SX-1552) ppb								
	3 HAT	9 HAT	24 HAT	48 HAT	4 DAT	7 DAT	14 DAT	21 DAT	28 DAT
L1	1.804	0.961	0.741	0.194	0.1373	0.006	0.000		
L2	1.25	0.297	0.190	0.230	0.0903	0.040	0.000		
L3	0.000	0.278	0.250	0.309	0.179	0.0000	0.0000	0.0000	0.0000

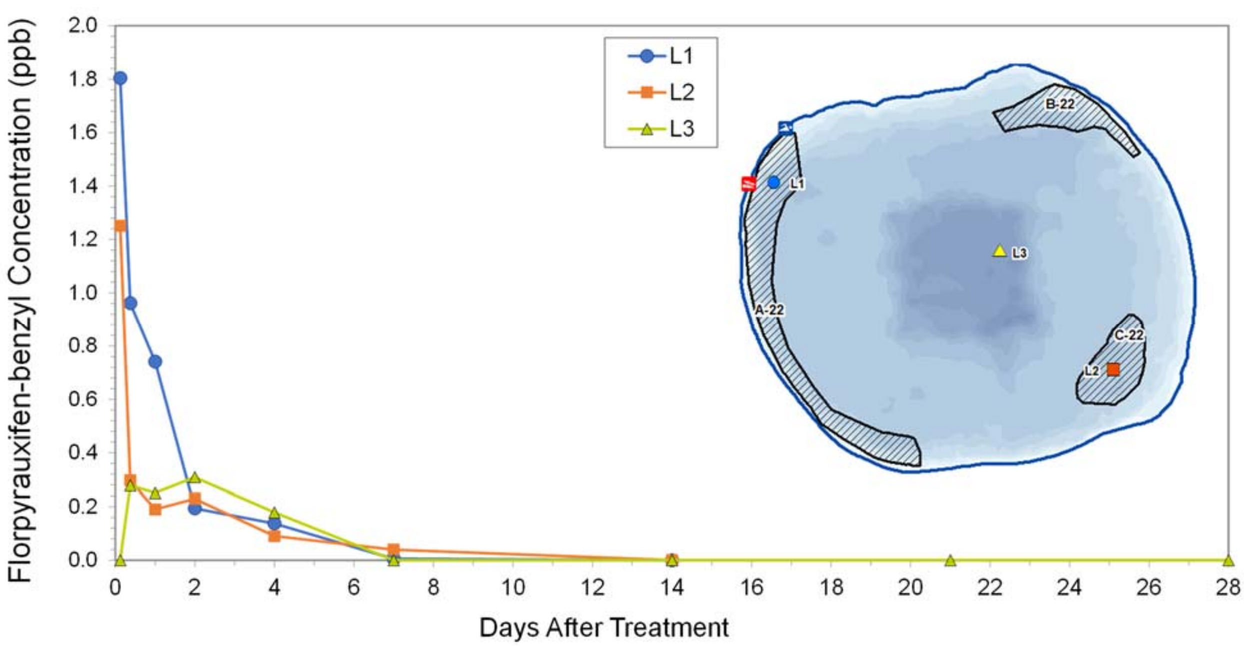


Figure 2.3-1. Florpyrauxifen-benzyl (SX-1552) concentrations measured at three monitoring locations following a May 2022 ProcellaCOR™ herbicide treatment in Lilly Lake.

The primary breakdown product of florpyrauxifen-benzyl is florpyrauxifen acid. Florpyrauxifen acid has been shown to persist in the lake longer than the active ingredient. This chemical metabolite is reported to have activity as an herbicide on aquatic plants, albeit to a lower degree than the active ingredient. It is unclear at this time the exact role that the acid metabolite may play in contributing to EWM reductions, particularly in areas not located directly within the herbicide application area.

Concentrations of the acid metabolite (florpyrauxifen acid, SX-1552-A) are displayed on Table 2.3-2 and Figure 2.3-2. Note that the y-axis on Figure 2.3-2 extends to 0.6 ppb so that the data can be more easily viewed and is a different axis height than Figure 2.3-1 (2.0 ppb). Concentrations of the acid metabolite were initially low in the earliest sampling intervals before exhibiting a steady increase through approximately 7 DAT as the active ingredient was converting into this form. After 7 DAT, samples were analyzed from only site L3 through the remainder of the sampling program to 28 DAT. Concentrations of the acid metabolite were measured at 0.226 ppb at 14 DAT and 0.129 ppb at 21 DAT. At 28 DAT, the center of the lake acid metabolite concentration was measured at 0.129 ppb and it is unknown how much longer the acid persisted in the lake above detection limits.

Table 2.3-2. Florpyrauxifen acid (SX-1552-A) concentrations measured at three monitoring locations within Lilly Lake following a May 2022 ProcellaCOR™ herbicide treatment.

Florpyrauxifen-benzyl acid metabolite (SX-1552-A) ppb									
	3 HAT	9 HAT	24 HAT	48 HAT	4 DAT	7 DAT	14 DAT	21 DAT	28 DAT
L1	0.091	0.000	0.145	0.110	0.109	0.171			
L2	0.000	0.000	0.000	0.033	0.071	0.226			
L3	0.000	0.000	0.000	0.041	0.148	0.158	0.226	0.129	0.129

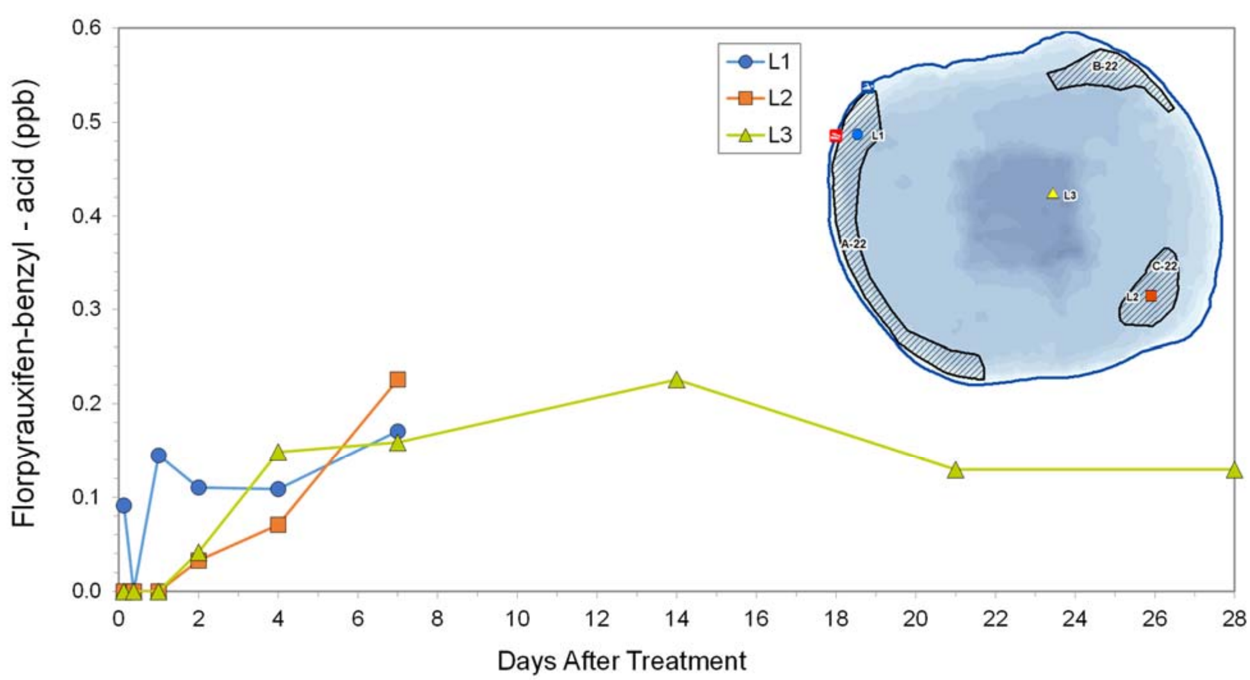


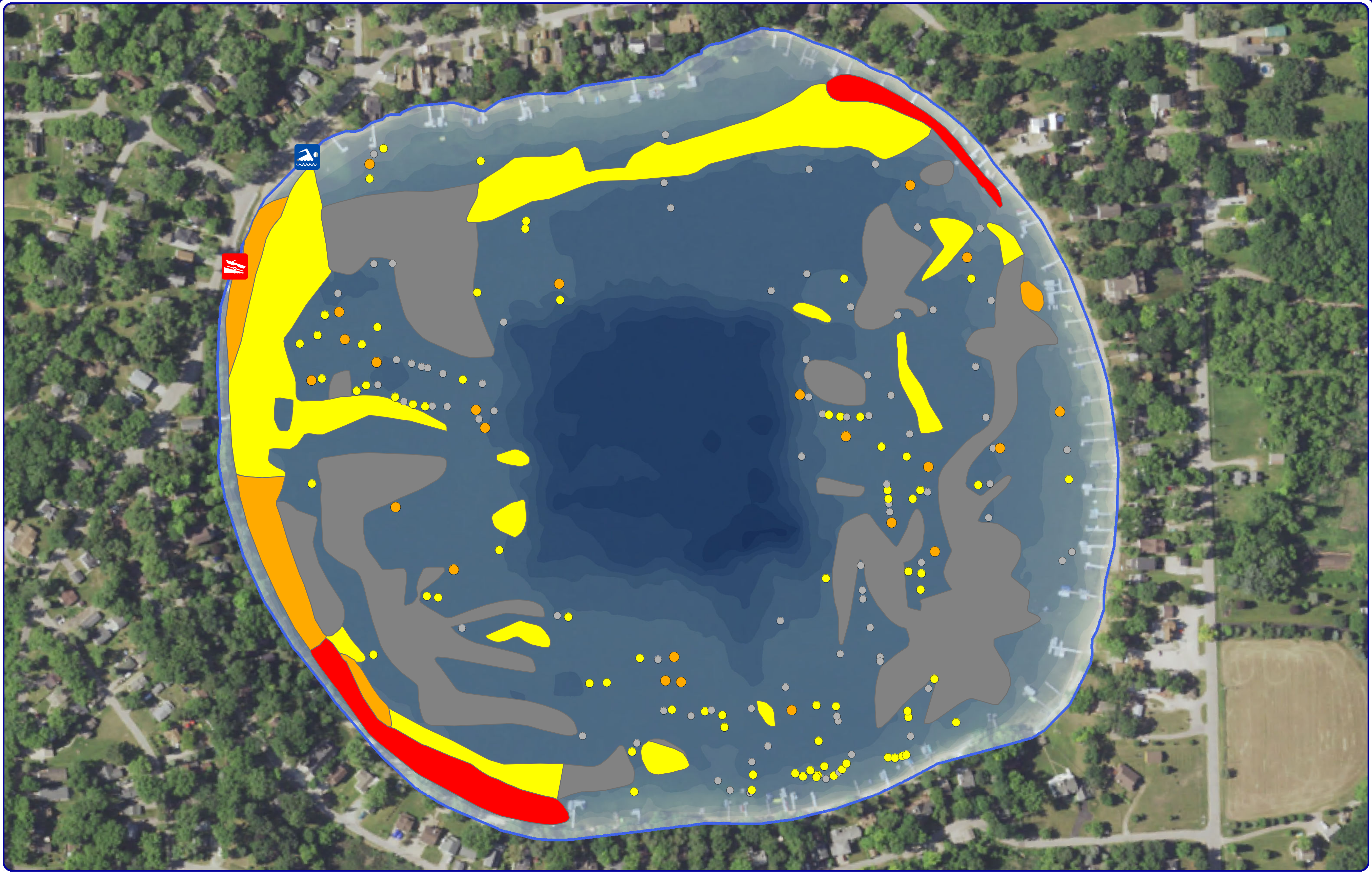
Figure 2.3-2 Florpyrauxifen acid (SX-1552-A) concentrations measured at three monitoring locations following a May 2022 ProcellaCOR™ herbicide treatment in Lilly Lake.

3.0 CONCLUSIONS AND DISCUSSION

The LLPRD's current AIS Control Grant (ACEI-295-22) contains cost coverage for the treatment and monitoring occurring in 2022, and for the *year after treatment* monitoring in 2023. The 2022 herbicide treatment strategy was designed to target specific colonies of HWM where herbicide was directly applied, however, Onterra also anticipated that lake-wide impacts to HWM were likely based on the treatment design. The only detectable HWM occurrence post treatment in Lilly Lake in 2022 was an injured, but growing, individual found on the rake sampler at a single point-intercept sampling point. This sampling location was from the northwest part of the lake near the boat landing growing in 10-feet of water. The initial EWM control results area appear promising, but the *year after treatment* results in 2023 will allow for an understanding if the plants were greatly injured for a season (i.e. seasonal impacts) or if the root crowns were indeed controlled and rebound does not occur.

Native aquatic plant monitoring following the 2022 treatment showed limited impacts to the non-target plant community. Onterra's experience monitoring numerous ProcellaCOR™ treatments indicates that native pondweeds are not particularly susceptible to this chemistry while water stargrass has shown some level of impact. Additional aquatic plant monitoring is planned in 2023 through the completion of a whole-lake point-intercept survey. Quantitative success criteria of the overall 2022 herbicide treatment project would be a 70% reduction in HWM littoral frequency of occurrence comparing the *year prior to treatment* dataset (2021) to the *year after treatment* (2023).

The results from the herbicide concentration monitoring component largely aligned with expectations. The herbicide concentrations of the active ingredient was measured at approximately 0.2-0.3 ppb by 48 HAT when the herbicide had mixed throughout the lake and was reduced to below detection by 14 DAT. As the active ingredient was degraded into its acid metabolite form, these levels increased and peaked at roughly 7 DAT. This chemical form gets further broken down and degraded to 0.13 ppb at the final sampling interval at 28 DAT. It is unknown how long the acid metabolite remained above detection limits after the final monitoring interval.



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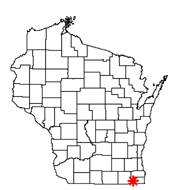
Feet



Project Location in Wisconsin

Legend

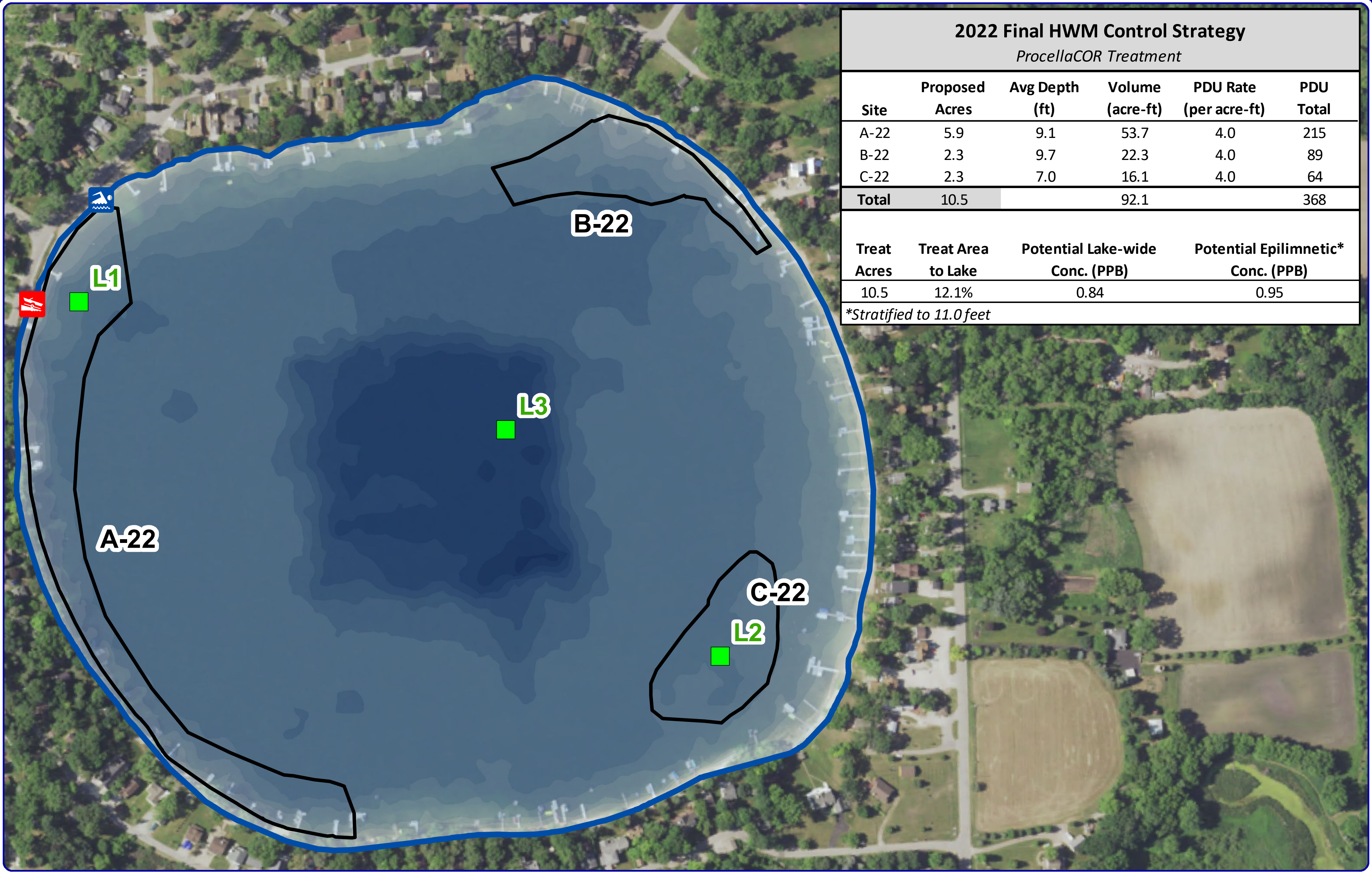
HWM Survey: August 2, 2021

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- Public Boat Landing
- Public Beach

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

Sources:
Hydro: WDNR
Bathymetry: Onterra
Aquatic Plants: Onterra, 2021
Orthophotography: NAIP, 2022
Map Date: August 9, 2021 HAL
Filename:Lilly_EWMPB_Aug21.mxd

Map 1
Lilly Lake
Kenosha County, Wisconsin
August 2021 Hybrid Watermilfoil Survey Results



2022 Final HWM Control Strategy

ProcellaCOR Treatment

Site	Proposed Acres	Avg Depth (ft)	Volume (acre-ft)	PDU Rate (per acre-ft)	PDU Total
A-22	5.9	9.1	53.7	4.0	215
B-22	2.3	9.7	22.3	4.0	89
C-22	2.3	7.0	16.1	4.0	64
Total	10.5		92.1		368

Treat Acres	Treat Area to Lake	Potential Lake-wide Conc. (PPB)	Potential Epilimnetic* Conc. (PPB)
10.5	12.1%	0.84	0.95

Stratified to 11.0 feet

N W E S

400

Feet

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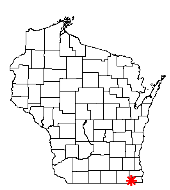
Sources:
Hydro: WDNR; modified by Onterra
Orthophotgraphy: NAIP 2022
Map Date: May 20, 2022 AMS
Map Filename: Lilly_HWM_Prelim3.mxd



Project Location in Wisconsin

Legend

- Final 2022 Herbicide Application Area
- Herbicide Concentration Monitoring Site
- Public Boat Landing
- Public Beach

Map 2

Lilly Lake

Kenosha County, Wisconsin

Final 2022 Hybrid Watermilfoil Control Strategy

A

APPENDIX A

Lilly Lake Littoral Frequency of Occurrence of Aquatic Plants from 2020-2022 Point-Intercept Surveys

Scientific Name	Common Name	LFOO (%)		
		2020	2021	2022
Chara spp.	Muskgrasses	73.2	71.2	67.9
Najas guadalupensis	Southern naiad	41.3	55.8	55.0
Potamogeton praelongus & P. amplifolius & P. p	White-stem pondweed, large-leaf, and white-s	53.1	49.8	44.5
Potamogeton robbinsii	Fern-leaf pondweed	42.3	45.6	41.6
Potamogeton praelongus	White-stem pondweed	42.3	32.6	23.9
Myriophyllum spicatum	Eurasian watermilfoil	37.1	51.6	0.5
Potamogeton amplifolius x P. praelongus	Large-leaf x white-stem pondweed hybrid	17.4	19.1	12.4
Vallisneria americana	Wild celery	11.7	11.6	10.5
Stuckenia pectinata	Sago pondweed	9.9	7.4	6.2
Potamogeton zosteriformis	Flat-stem pondweed	5.6	3.7	9.1
Heteranthera dubia	Water stargrass	11.3	7.9	3.3
Potamogeton illinoensis	Illinois pondweed	0.5	0.5	7.2
Elodea canadensis	Common waterweed	1.4	2.3	1.9
Potamogeton strictifolius	Stiff pondweed	0.9	1.4	1.0
Nitella spp.	Stoneworts	0.0	1.4	1.4
Najas flexilis	Slender naiad	1.4	1.9	0.5
Potamogeton amplifolius	Large-leaf pondweed	0.0	0.0	1.9
Potamogeton crispus	Curly-leaf pondweed	0.5	0.9	0.0
Sagittaria graminea	Grass-leaved arrowhead	0.0	0.9	0.0
Potamogeton richardsonii	Clasping-leaf pondweed	0.0	0.0	0.5
Potamogeton gramineus	Variable-leaf pondweed	0.0	0.0	0.5
Eleocharis acicularis	Needle spikerush	0.0	0.5	0.0

B

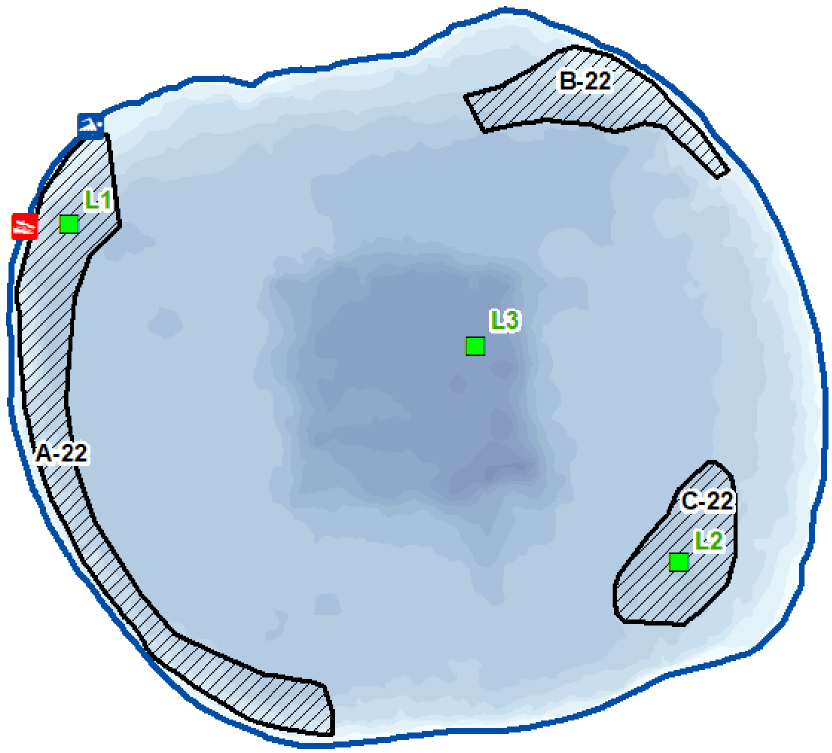
APPENDIX B

Lilly Lake Final 2022 ProcellaCOR™ Herbicide Concentration Monitoring Sampling Plan

Lilly Lake, Kenosha (WBIC: 740900)
2022 Herbicide Sample Plan
Onterra, LLC

Lilly Lake, Kenosha County is an approximately 85-acre seepage lake that has a maximum depth of 23 feet and a mean depth of 10 feet. Florpyrauxifen-benzyl (commercially as ProcellaCOR™) is proposed to be applied to 10.5 non-contiguous acres of the lake in early-summer 2022 to control Hybrid Eurasian watermilfoil. Herbicide concentration sampling will be conducted in order to monitor the herbicide concentrations in the hours and days following the application.

Water samples will need to be collected at the sites and depths listed below. Data are in decimal degrees and the datum is WGS84. Locations of each sampling site are displayed with green squares on the image below.



Lilly Lake Herbicide Sample Sites					
Site Label	**Site Description**	**Station ID**	**Latitude**	**Longitude**	**Sample Depth**
L1	Application Area A-22	10056144	42.564602	-88.216175	Integrated (0-6 feet)
L2	Application Area C-22	10056146	42.561887	-88.209851	Integrated (0-6 feet)
L3	Deep Hole	303126	42.563591	-88.21194	Integrated (0-6 feet)

Please note that a single sample is to be collected before the treatment as a 'control' for the lab analysis. Please collect the pre-treatment sample from site L1 at a time that is most convenient for the volunteer but as close to the treatment date as possible. After the herbicide application is completed, 21 additional samples will need to be collected at nine different time intervals throughout the project and are listed in the table below. Sample collection intervals are listed either as Hours After Treatment (HAT) or Days After Treatment (DAT). Direct communication between the water sample collector and the herbicide applicator is necessary to ensure the collector is prepared to begin three hours after treatment is completed. If a sample cannot be collected at the interval listed below, please collect the sample as soon as reasonably possible and record the change.

Sampling Interval Matrix (X indicates sample to be collected)			
Interval	Application Area		Deep Hole
	Site L1	Site L2	Site L3
Pre-Treatment	X		
3 HAT	X	X	X
9 HAT	X	X	X
24 HAT	X	X	X
2 DAT	X	X	X
4 DAT	X	X	X
7 DAT	X	X	X
14 DAT			X
21 DAT			X
28 DAT			X
HAT = Hours After Treatment, DAT = Days After Treatment			

All water samples will be collected using a six-foot integrated sampler (Photo 1). A video tutorial demonstrating the proper sample collection methodology is available on Onterra's YouTube web page: click here

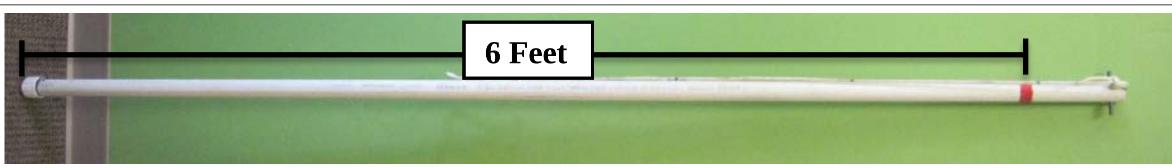


Photo 1. 6-foot Integrated sampling device constructed of PVC tubing.

Due to the extremely low concentrations being measured at the laboratory (<1 part per billion), **it is very important to thoroughly rinse the integrated sampler device and the custom mixing bottle with the water from each sampling site upon arrival at the site**. Water is collected by pushing the integrated sampler straight down to a depth of six feet; or in water shallower than six feet, down to approximately one foot above the bottom sediment. The sampler is brought to the surface and emptied into a customized mixing bottle by pushing open the stop valve at the end of the integrated sampler (Photo 2). Water should be poured from the custom mixing bottle to triple rinse the clear glass bottle. After the clear glass bottle is triple rinsed, it is to be filled for a fourth time with the water from the custom mixing bottle and then carefully poured into the brown glass bottle which has a preservative solution already inside (Photo 3).

Please use a fine-tipped permanent marker to record the date and time the sample is collected on the sticker label of the brown glass bottle. The final sample (in the brown bottle) as well as the emptied clear glass bottle should be carefully placed back within the bubble wrapped pouch to protect from accidental breakage.

While the samples are being collected, they should be kept cold and out of direct sunlight by keeping them in a small cooler on the boat. After collection, all samples should be stored in a refrigerator until shipping.

	
Photo 2. Emptying the water sample from the integrated sampler device into the custom mixing bottle.	**Photo 3. Clear glass mixing bottle and final brown glass bottle.**

Onterra will provide all of the necessary supplies to complete the sampling and provide training to the volunteer(s) collecting the samples. Onterra has a supply of handheld GPS units and integrated sampler devices available to loan out for the duration of the sampling upon request. All other materials, including sampling bottles with labels, a customized mixing bottle and necessary paperwork will be provided.

Please fill out the yellow highlighted fields on the Chain of Custody forms including:

- Sampler: (Volunteer Name)
- Client Sample ID: (example: L1, L2, L3)
- Date sample is collected

When all sampling is complete, the water samples **and** Chain of Custody Datasheets should be shipped by overnight currier to:

EPL Bio Analytical Services
9095 W. Harristown Blvd.
Niantic, IL 62551

Samples should <u>not</u> be shipped on loose ice. Ice packs or frozen water bottles (contained in a zip bag) may be shipped with the samples to keep them cool. Samples should not be shipped on a Friday, but rather refrigerated and shipped on the following Monday.

If you have any questions, please reach out to one of the contacts listed below.

Project specifics, logistics and sampling methods	
Todd Hanke **Onterra, LLC** **thanke@onterra-eco.com** **Cell Phone (920) 360-7233** **Office Phone (920) 338-8860**	**Andrew Senderhauf** **Onterra, LLC** **asenderhauf@onterra-eco.com** **Cell Phone (920) 279-9994** **Office Phone (920) 338-8860**
WDNR Support	
Michelle Nault **WI DNR** **Michelle.Nault@wisconsin.gov** **Office (608) 513-4587**	**Heidi Bunk** **WI DNR – Lakes Biologist** **Heidi.Bunk@wisconsin.gov**
SePro (ProcellaCOR manufacturer)	
Michael Hiatt **SePro Aquatic Specialist** **michaelh@sepro.com**	