1.0 INTRODUCTION

Upper Gresham Lake, Vilas County, is a deep headwater drainage lake with a maximum depth of 29 feet and a mean depth of 12 feet (Photo 1). Upper Gresham Lake is considered a Priority Navigable Waterway (PNW) for containing a self-sustaining muskellunge population. This headwater lake ultimately drains via Gresham Creek which flows into the Trout River and eventually the Manitowish Chain of Lakes. Upper Gresham Lake is a Priority Navigable Waterway (PNW) for containing self-sustaining muskellunge populations.

Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) was first discovered in Upper Gresham Lake in 2001, and its presence was confirmed during surveys conducted by the Wisconsin Department of Natural Resources (WDNR) in 2005.

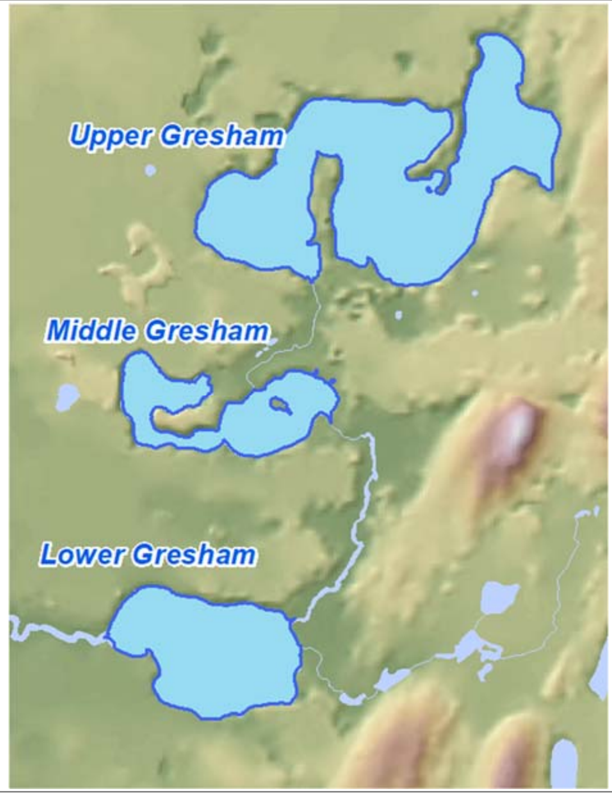


Figure 1.0-1. Gresham Lakes, Vilas County.

1.1 Recent EWM Management & Planning

The Gresham Lakes Association (GLA), in cooperation with the Town of Boulder Junction completed a *Comprehensive Management Plan* for Upper, Middle, and Lower Gresham Lakes in 2009. Within that plan, EWM control and monitoring goals were outlined. Spatially-targeted herbicide spot treatments targeting EWM occurred from 2007-2013. Hand-harvesting operations in 2014 and 2015 were moderately effective in reducing the density of EWM within the targeted areas, however, the lake-wide rate of EWM population increase in Upper Gresham Lake exceeded the rate at which hand-removal can keep the population suppressed.

Following an increasing EWM population, the GLA chose to pursue two 2,4-D spot treatments in 2018. This strategy utilized updated Best Management Practices (BMPs) for 2,4-D spot treatments, including targeting larger areas and attention to basin-wide herbicide concentrations. These treatments were met with mixed results. A more-protected site yielded approximately two years of reduced EWM and a more-exposed site yielded less than a year of reduced EWM. The 2018 trial 2,4-D spot treatments did not entirely meet expectations.

The GLA created an updated and WDNR-approved *Comprehensive Management Plan for Upper Gresham Lake* that was finalized in January 2020. The GLA created a management goal to: *Maintain Lowered EWM Population Through Active Management.* When a Late Season AIS Survey documents colonized EWM populations that are *dominant or greater in density*, an herbicide spot treatment would be considered for the following early-spring. Herbicide spot treatment techniques would be implemented if the colonies have a size/shape/location where management is anticipated to be effective.

No management of EWM was completed in 2019 and 2020 in an effort to assess the population during a period of no active management. During the winter of 2020/2021, the GLA started considering an herbicide treatment for the spring of 2021. After discussions with WDNR and Onterra, the GLA opted to postpone herbicide management until spring 2022, allowing for additional planning steps to occur as well as the ability to seek WDNR grant funds. The GLA successfully applied for and received a WDNR AIS Control Grant during the fall 2021 cycle to assist with funding their 2022 EWM management strategy and 2022-2023 monitoring program. This report the final deliverable for the 1-year grant-funded project (ACEI-297-22).

1.2 2022 EWM Management Strategy

Following a period of discussion, the GLA chose to pursue an herbicide spot treatment aimed at reducing the prevalence of nuisance-causing conditions within select areas of the lake. Building off their 2018 2,4-D spot treatment trails, the GLA understood that the small size and exposed/off-shore nature of the EWM colonies on Upper Gresham Lake make them difficult scenarios to hold sufficient herbicide concentrations and exposure times (CETs) to result in multi-year control. Consistent with their recently approved management plan, the GLA elected to move forward in selecting an herbicide with reportedly short concentration and exposure time requirements.

The GLA selected four trial sites on the lake to conduct the ProcellaCOR™ herbicide spot treatments in 2022 (Map 2). Each of the sites were constructed by applying a 40-60-foot buffer around the EWM colonies that were to be targeted. The preliminary 2022 would target the highest EWM occurrences in high-use areas. Only one site in each "basin" was targeted to minimize the role additive impacts may result in basin-wide herbicide levels. The manufacturer of the herbicide (SePRO) confirmed a traditional dosing strategy of 4.0 prescription dose units (PDU's) in association with the proposed treatment. The maximum application rate of this formulation of ProcellaCOR™ is 25 PDU. Additional details of the planning and development of the proposed 2022 strategy were included within the *2021 EWM Monitoring & Control Strategy Assessment Report* issued in January 2022.

1.3 Pretreatment Confirmation and Refinement Survey

Onterra ecologists completed the pretreatment confirmation and refinement survey on June 6, 2022. The main purpose of this site visit was to collect the pretreatment sub-sample point-intercept data to help understand the change in occurrence of EWM and native plants following the treatment. Parameters such as plant growth stage, water temperature, and water depth were also 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 from the GLA.

This survey was conducted using a combination of survey methods (visual, rake tows), but largely consisted of visual observations as the EWM was visible from the surface. Water temperatures were 63.5°F at mid-depth of application areas. Using an optical probe, the pH was measured at 8.2. New EWM growth was apparent on the target plants and appeared to be in an active growth stage ideal for treatment. There were high amounts of coontail (*Ceratophyllum demersum*), flat-stem pondweed (*Potamogeton zosteriformis*), fern pondweed (*P. robbinsii*), and other pondweeds within the treatment areas. No alterations to the originally proposed strategy were made as a result of the pretreatment 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 early-morning hours of June 17, 2022 by Schmidt's Aquatic, LLC. This slightly delayed treatment date facilitated the collection of pretreatment native plant data just prior to treatment. The applicator noted northwest winds of 2-3 mph at the time of treatment. The surface water temperature reading was 65°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) EWM 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 (Photo 2.0-1). 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 EWM, 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.

Photo 2.0-1. Point-intercept survey on a WI lake. Photo credit Onterra.

Photo 2.0-2. EWM mapping survey on a Wisconsin lake. Photo credit Onterra.

The point-intercept survey can be applied at various scales. The point-intercept survey is most often applied at the whole-lake scale. The whole-lake point-intercept survey was last conducted on Upper Gresham Lake in 2017 and is tentatively planned for 2023. If a smaller area is being studied, a modified and finer-scale point-intercept sampling grid may be needed to produce a sufficient number of sampling points for comparison purposes. This sub-sample point-intercept survey methodology is often applied over herbicide application sites. This type of sampling is used within this project for the herbicide application areas and is discussed in Section 2.1 below.

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 EWM mapping survey, the entire littoral area of the lake is surveyed through visual observations from the boat (Photo 2.0-2). Field crews supplemented the visual survey by deploying a submersible camera along with periodically doing rake tows. The EWM 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*.

Overall, each survey has its strengths and weaknesses, which is why both are utilized in different ways as part of this project.

2.1 Quantitative Monitoring: Sub-Sample Point-Intercept Survey

A quantitative monitoring plan for this treatment includes a total of 88 sub-sample point-intercept sampling locations that are contained within the four treatment application areas (Figure 2.1-1). The quantitative assessment would be completed through the comparison of the sub point-intercept survey from mid-June 2022 (*year of pretreatment*), late-summer 2022 (*year of post treatment*), and 2023 (*year after treatment*). Figure 2.1-2 compares the occurrence of EWM from comparing pre-treatment (June 2022) to *year-of-post treatment* (September 2022) and shows that in each of the four treatment sites, the occurrence was reduced to 0% after treatment.

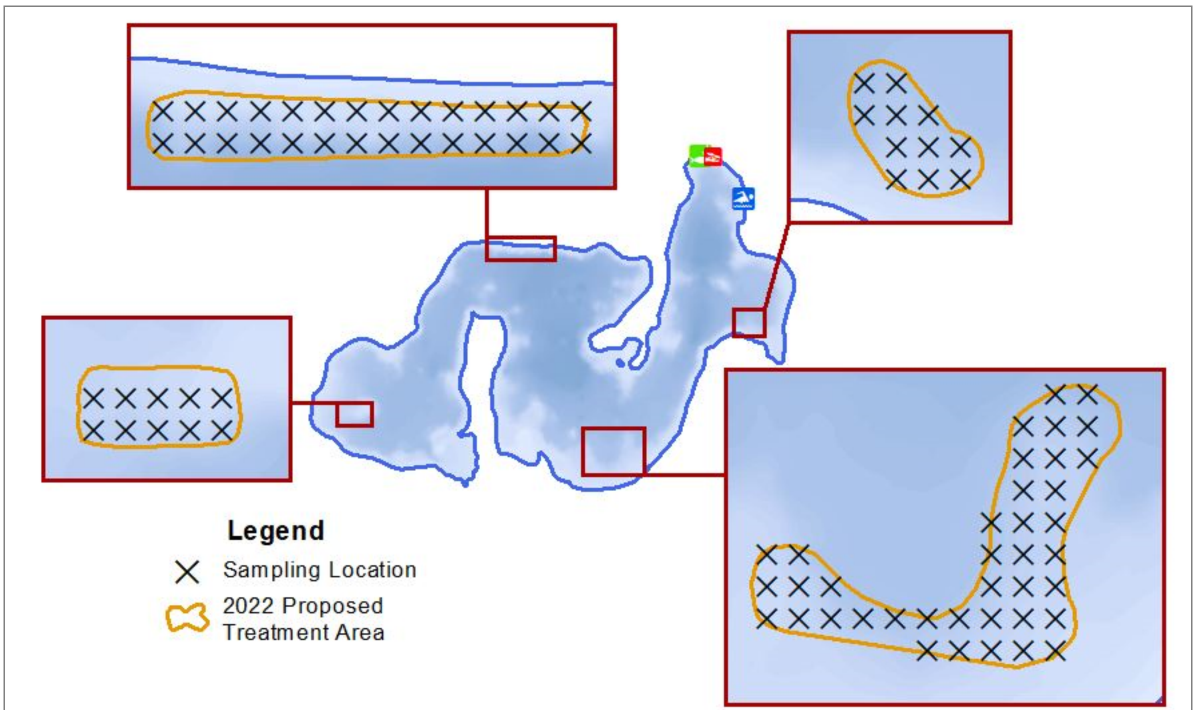


Figure 2.1-1. Upper Gresham Lake Quantitative Monitoring Plan for 2022 Herbicide Treatments. 20m spacing, n=88.

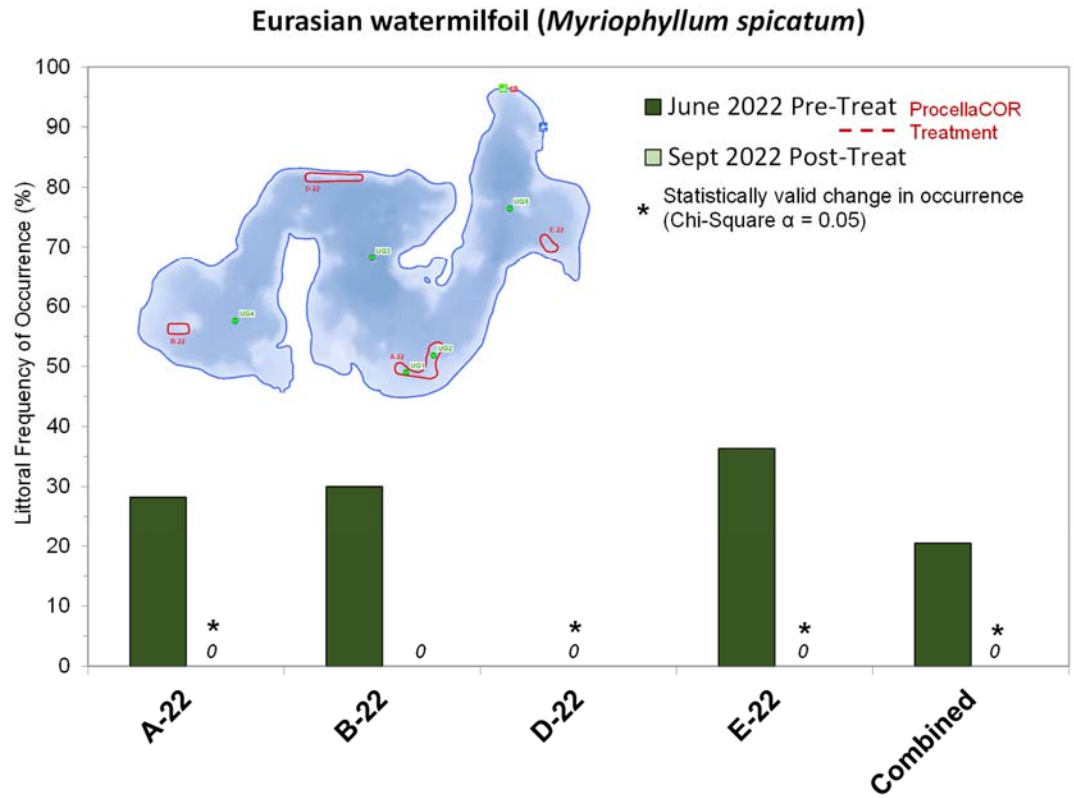


Figure 2.1-2. Littoral frequency of occurrence of EWM from sub-sample point-intercept surveys in Upper Gresham Lake. Asterisk indicates statistically valid change from pre- to post-treatment surveys.

Four of the commonly encountered native species are highlighted in Figure 2.2-2. Fern-leaf pondweed was fairly common within the sites and this species did not show valid changes in occurrence between the two surveys at any site or when all data are pooled together (Figure 2.1-3, top-left frame). Coontail was present in each site during the pretreatment survey with occurrences between 7.7-20.0%. In the post treatment survey, the occurrence of coontail not statistically different in any individual site or the collective sites combined. Coontail was present in three of the four sites in the post treatment survey. The occurrence of flat-stem pondweed was relatively unchanged between the pre- and post treatment surveys (Figure 2.1-3, bottom-left frame).

Wild celery was documented in just one site (B-22) in the pretreatment survey, but was common in sites A-22, B-22, and D-22 in the post treatment survey (Figure 2.1-3, bottom-right frame). This is most likely a reflection of the timing of the surveys in which these species were likely at an early growth stage during the mid-June pretreatment survey timing compared with an advanced growth stage during the September post treatment surveys. Therefore, these data are simply picking up on the increased biomass trajectory of this species during a growing season. That being said, wild celery was clearly not negatively impacted by the treatment, which is an important discovery of these data. A table that displays the occurrences for all species is included in Appendix A.

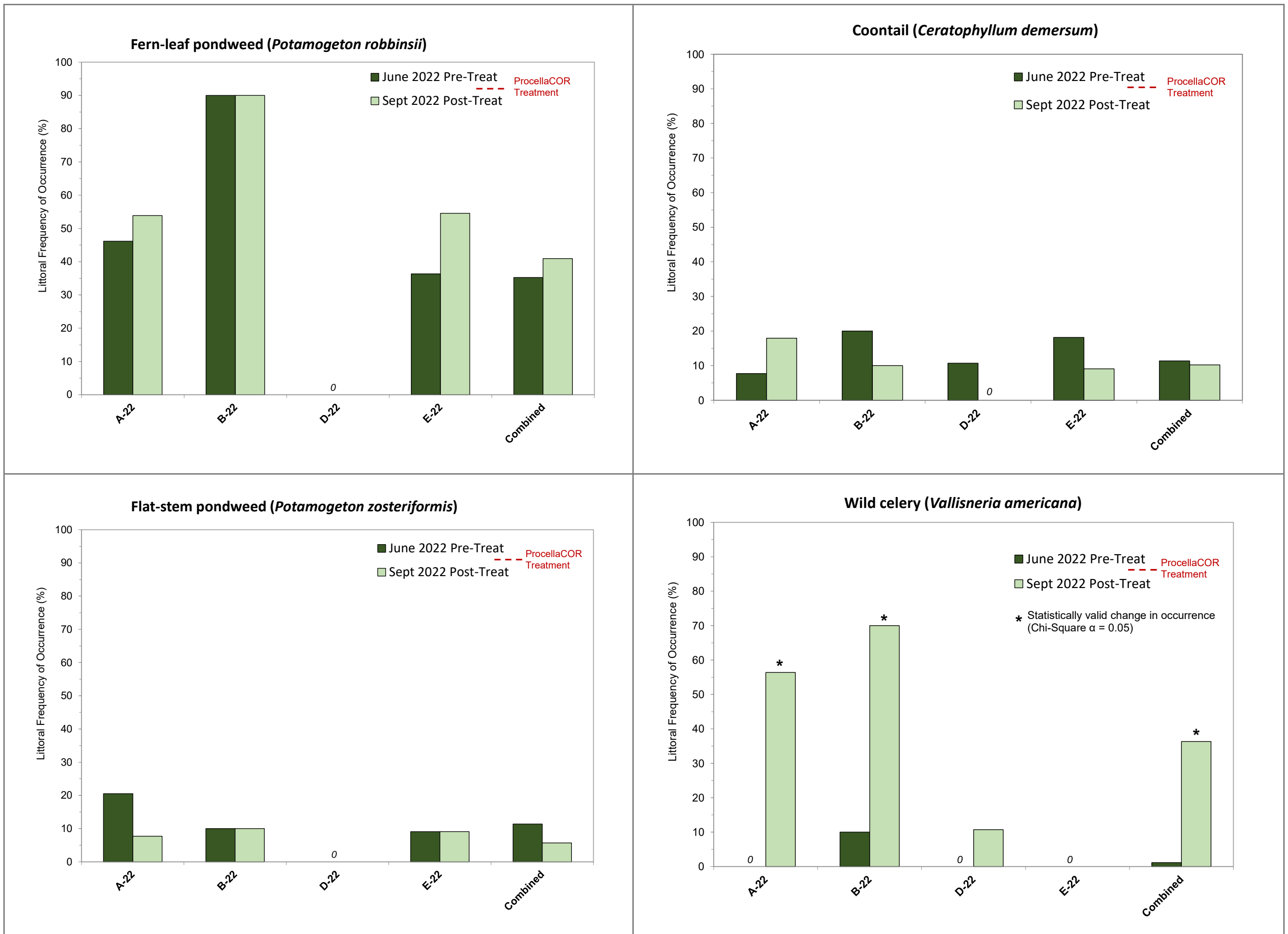


Figure 2.1-3. Littoral frequency of occurrence of select aquatic plant species from point-intercept surveys in Upper Gresham Lake. Asterisk indicates statistically valid change from pre- to post treatment surveys.

2.2. Qualitative Monitoring: EWM Mapping Surveys

Qualitative monitoring compares the late-summer EWM mapping survey population mapped during 2021 (pretreatment) and late-summer 2022 (post treatment). Onterra ecologists conducted the Late-Summer EWM Mapping Survey on Upper Gresham Lake on September 21, 2022. Field crews noted that the water appeared greener than past visits. During the course of the survey, crews encountered only four single EWM plants in the entire lake representing a large decrease compared to the previous survey. Two single plants were located in a small bay to the south of site E-22, and two other plants were located in the western basin of the lake in the vicinity of an emergent plant community Map 2. No EWM plants were located within any of the four herbicide application areas. Crews supplemented the visual mapping survey with the deployment of a submersible camera in select areas of the lake focusing on previously known colonized EWM locations. Native aquatic plants including wild celery, common waterweed, coontail, and thin-leaved pondweeds were visible with the aid of a submersible camera within some of the application areas. In an effort to find surviving EWM plants, crews also took several rake tows within application areas in addition to the tows taken during the sub point-intercept survey described above. Survey crews expressed confidence that the EWM plants were not present rather than escaping detection.

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 from five sites, two of which within treatment area A-22 (UG1 and UG2) and three from locations outside of treatment areas. Samples were collected at seven 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, and 14 days after treatment. Samples were collected by volunteer members of the GLA and upon completion of the sampling, were shipped to EPL Bio Analytical Services in Illinois for analysis. This lab was identified by the WDNR as being able to detect florpyrauxifen-benzyl at lower levels than the herbicide manufacturer's facility – 1 part per billion (ppb). 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 (florpyrauxifen acid, 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 five monitoring locations. The active ingredient was measured at 0.190 ppb at site UG1 and 0.50 ppb at site UG2 during the first monitoring interval collected at 3 HAT. Trace amounts of active ingredient were detected at site UG1 24 HAT, but was not detected at any other sampling interval. No amounts of active ingredient were ever detected at the sampling sites located in untreated areas of the lake (UG3, UG4, and UG5).

Table 2.3-1. Florpyrauxifen-benzyl (SX-1552) concentrations following a June 2022 ProcellaCOR™ herbicide treatment in Upper Gresham Lake.

Florpyrauxifen-benzyl (SX-1552) ppb HAT							
	3	9	24	48	96 (4 DAT)	168 (7 DAT)	336 (14 DAT)
UG1	0.190	0.000	0.048	0.000	0.0000	0.000	
UG2	0.50	0.000	0.000	0.000	0.0000	0.000	
UG3			0.000	0.000	0.000	0.0000	0.000
UG4				0.000	0.000	0.000	
UG5				0.000	0.000	0.000	

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. While all samples were analyzed for the acid metabolite, none was detected from any of the sample locations during the duration of the herbicide monitoring.

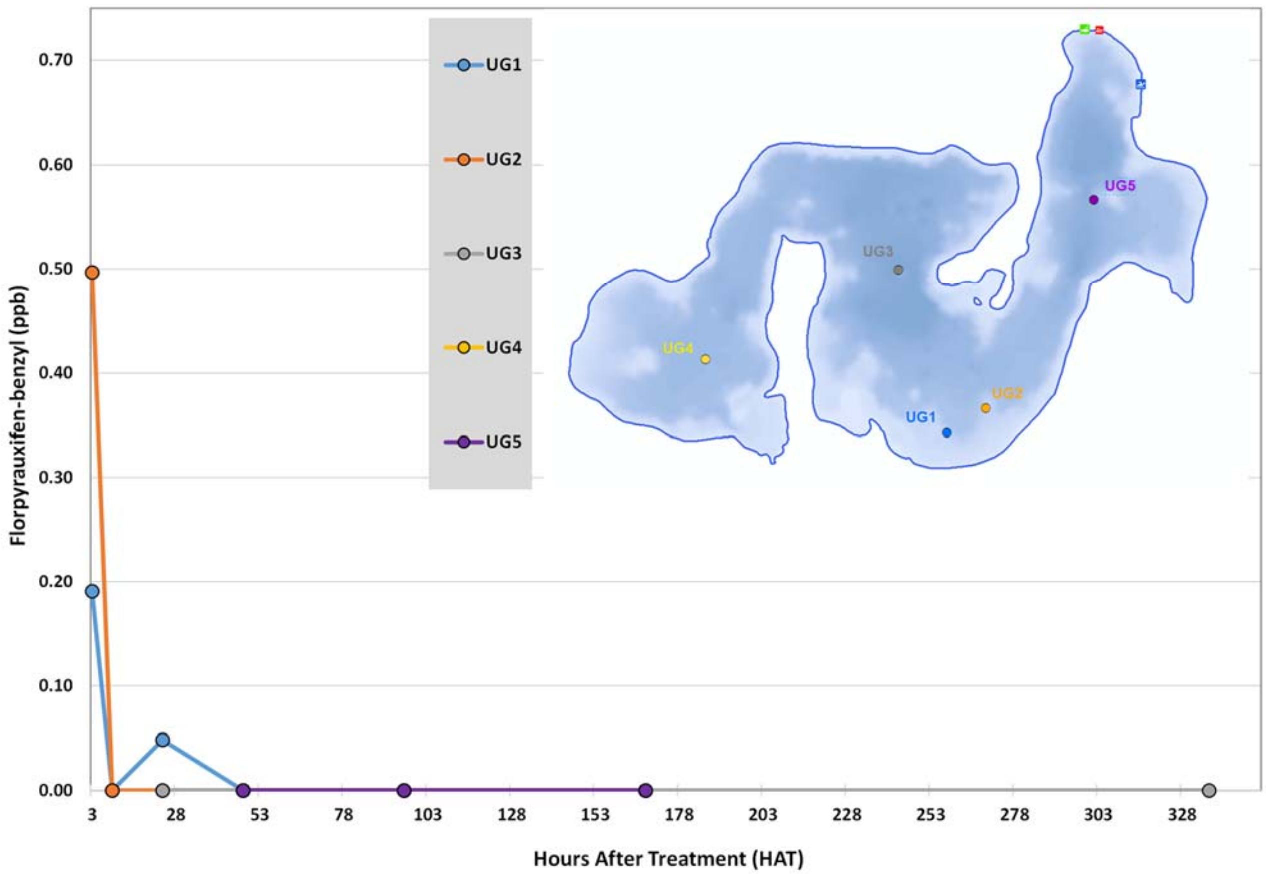


Figure 2.3-1. Florpyrauxifen-benzyl (SX-1552) concentrations measured at five monitoring locations following a June 2022 ProcellaCOR™ herbicide treatment in Upper Gresham Lake.

3.0 CONCLUSIONS & DISCUSSION

The coordination and implementation of the 2022 EWM management strategy were completed as planned for Upper Gresham Lake with collaboration from the GLA, WDNR, Onterra, and other project partners. Volunteer efforts provided by the GLA were instrumental in the completion of the post treatment herbicide concentration monitoring.

Monitoring surveys conducted during 2022 showed a large decrease in EWM throughout all of Upper Gresham Lake. No EWM was detected within any of the four herbicide application areas during post treatment visits to the lake, and just four single EWM plants were spotted anywhere in the lake during a late-summer visual mapping survey. Minimal amounts of the herbicide active ingredient were detected in post treatment sampling and the acid derivative was not detected in any sample throughout the sampling regimen. The herbicide concentration data do not indicate herbicide mixing within the lake and were below levels known to be effective on controlling EWM. Negative impacts to the native aquatic plant population were not detected within the sub point-intercept sampling that took place within the herbicide application areas.

Environmental factors including changes in lake water clarity are known to influence aquatic plant species on an annual basis. Field survey crews noted 'greener' waters during the late-summer survey compared to earlier visits to the lake with a Secchi disk measurement of 5.4 feet recorded on September 21. Other available Secchi measurements from 2022 include a 15-foot reading on May 16 by the CLMN volunteer, and a 6.5-foot measurement recorded on June 6, during Onterra's pretreatment survey visit. It is theorized that reduced water clarity later in the growing season, in combination with the herbicide treatment strategy, may have resulted in the lake-wide decline of EWM. A review of historic Secchi data compiled during the GLA's most recent Management Planning Project indicates that Secchi disk readings have been lowest in April, and increased during May, June, and July, before decreasing in late-summer (Figure 3.0-1). Limited Secchi data available during 2022 suggest that water clarity may have been lower during the growing season compared to historic averages. Reduced water clarity may impact aquatic plant growth including plants growing in deeper waters as less light is available.

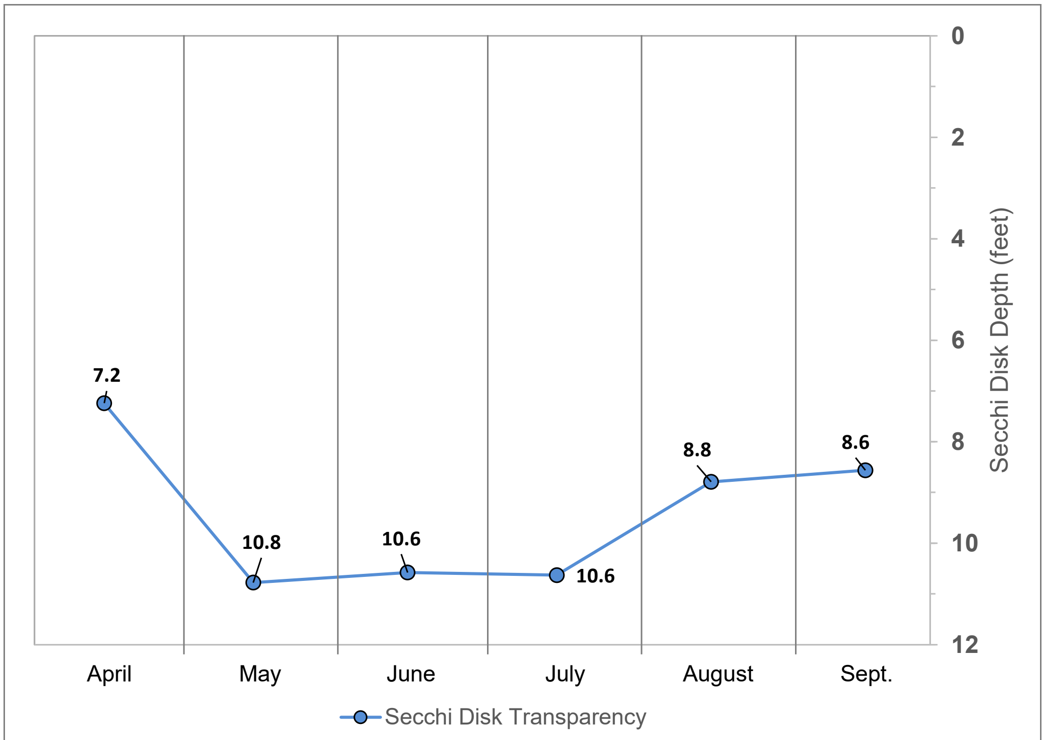


Figure 3.0-1. Historic Average Secchi Disk Measurements by Month in Upper Gresham Lake. Data from SWIMS from 1984-2017.

3.1 2023 Management & Monitoring Strategy Development

Although the GLA originally applied for grant funding for a 2-year project, the project could only be funded for one year due to limited funds available. As outlined in the original project, Onterra recommends continued management and monitoring during 2023. Replication of the late-summer EWM mapping survey will allow for a *year after treatment* evaluation of the 2022 herbicide treatment. These data will also be used to determine an appropriate EWM monitoring or management strategy for 2024. Repeating the sub-sample point-intercept survey within the 2022 herbicide application area will serve to evaluate whether EWM reductions extend through the *year after treatment*, while also assessing the native plant population.

The initial project also included the completion of a whole-lake point-intercept survey in 2023. The whole-lake point-intercept survey will be valuable in assessing the lake-wide aquatic plant population and results are compared to previous or future surveys to monitor aquatic plant populations in the lake. The last point-intercept survey was conducted in 2017. Point-intercept surveys are required to occur at least once every 5 years in order for the GLA to be eligible for AIS Control Grants.

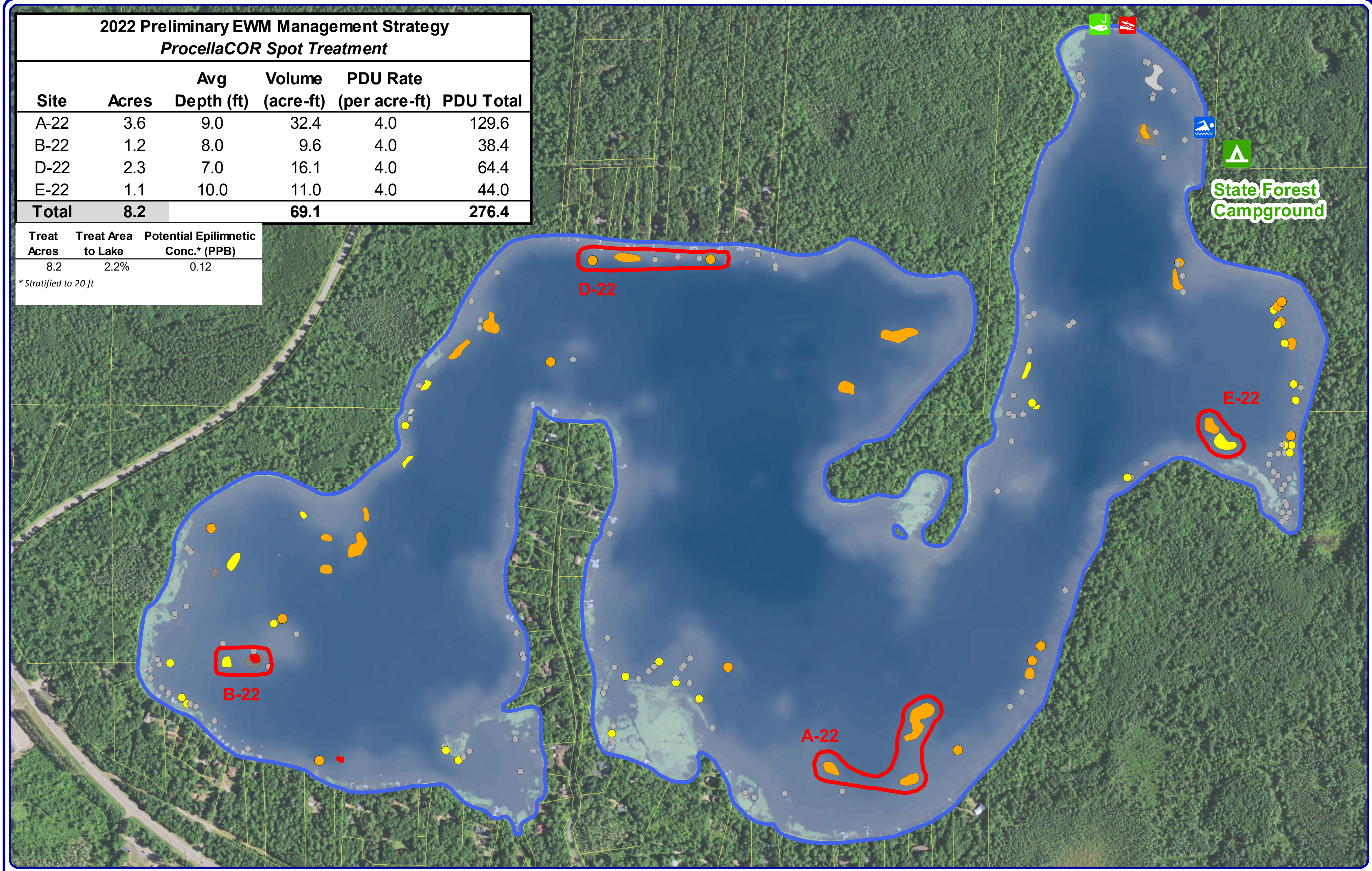
With minimal EWM detected in Upper Gresham Lake during the late-summer 2022 mapping survey, no areas are initially being considered for a hand harvesting management approach in 2023. Should GLA volunteers detect rebounding EWM in the lake during early-summer 2023, consideration for conducting hand harvesting efforts may be made at that time.

2022 Preliminary EWM Management Strategy
ProcellaCOR Spot Treatment

Site	Acres	Avg Depth (ft)	Volume (acre-ft)	PDU Rate (per acre-ft)	PDU Total
A-22	3.6	9.0	32.4	4.0	129.6
B-22	1.2	8.0	9.6	4.0	38.4
D-22	2.3	7.0	16.1	4.0	64.4
E-22	1.1	10.0	11.0	4.0	44.0
Total	**8.2**		**69.1**		**276.4**

Treat Acres	Treat Area to Lake	Potential Epilimnetic Conc.* (PPB)
8.2	2.2%	0.12

** Stratified to 20 ft*



N
W E
S
750
Feet

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

Sources:
Roads & Hydro: WDNR
Bathymetry: Onterra 2017
Parcel Data: WI SPMI, 2020
Orthophoto: NAIP, 2020
EWM Survey: Onterra, 2021
Map date: October 18, 2021 - EJH

Project Location in Wisconsin

Legend

EWM Survey Results **(August 2, 2021)**

- Highly Scattered
- Scattered
- Dominant
- Highly Dominant
- Surface Matting
- Single or Few Plants
- Clump of Plants
- Small Plant Colony
- 2022 Preliminary Treatment Site

Map 1
Upper Gresham Lake
Vilas County, Wisconsin
Preliminary EWM Treatment Strategy v2

N
W E
S
750
Feet

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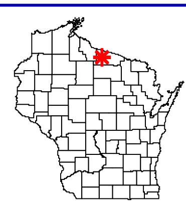
Sources:
Roads & Hydro: WDNR
Bathymetry: Onterra 2017
EWM Survey: Onterra, 2022
Map date: October 27, 2022 JMB



Project Location in Wisconsin

Legend

EWM Survey Results (September 21, 2022)

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

Map 2

Upper Gresham Lake

Vilas County, Wisconsin

2022 Late-Season EWM Survey Results

A

APPENDIX A

Upper Gresham Lake Point-Intercept Survey Littoral Frequency of Occurrence Matrix: June & September 2022

Upper Gresham Lake Point-Intercept Survey Data Matrix

Scientific Name	Common Name	LFOO (%)		June_22-Sept_22	
		June_22	Sept_22	% Change	Direction
Potamogeton robbinsii	Fern-leaf pondweed	35.2	40.9	16.1	△
Vallisneria americana	Wild celery	1.1	36.4	3100.0	▲
Ceratophyllum demersum	Coontail	11.4	10.2	-10.0	▽
Myriophyllum spicatum	Eurasian watermilfoil	20.5	0.0	-100.0	▼
Potamogeton zosteriformis	Flat-stem pondweed	11.4	5.7	-50.0	▽
Potamogeton berchtoldii & P. pusillus	Slender and small pondweed	8.0	5.7	-28.6	▽
Potamogeton pusillus	Small pondweed	5.7	5.7	0.0	-
Potamogeton richardsonii	Clasping-leaf pondweed	2.3	5.7	150.0	△
Chara spp.	Muskgrasses	1.1	6.8	500.0	△
Potamogeton amplifolius	Large-leaf pondweed	1.1	4.5	300.0	△
Potamogeton gramineus	Variable-leaf pondweed	1.1	2.3	100.0	△
Elodea canadensis	Common waterweed	0.0	3.4		△
Potamogeton berchtoldii	Slender pondweed	2.3	0.0	-100.0	▽
Najas flexilis	Slender naiad	0.0	2.3		△
Potamogeton strictifolius	Stiff pondweed	1.1	0.0	-100.0	▽
Potamogeton praelongus	White-stem pondweed	0.0	1.1		△
Potamogeton friesii	Fries' pondweed	1.1	0.0	-100.0	▽
Nitella spp.	Stoneworts	0.0	1.1		△
Heteranthera dubia	Water stargrass	0.0	1.1		△
Eleocharis acicularis	Needle spikerush	1.1	0.0	-100.0	▽
Bidens beckii	Water marigold	0.0	1.1		△

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

B

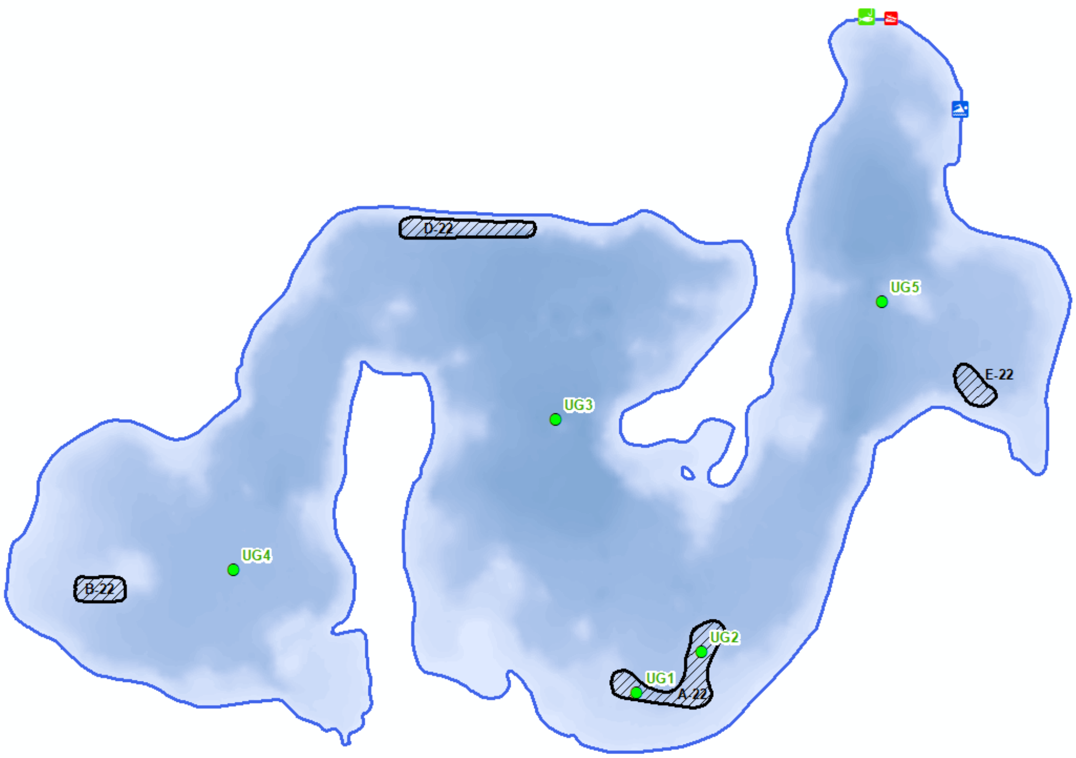
APPENDIX B

Upper Gresham Lake Final 2022 ProcellaCOR™ Sampling Plan

Upper Gresham Lake, Vilas County (WBIC:2330800)
2022 Herbicide Sample Plan
Onterra, LLC

Upper Gresham Lake, located in Vilas County, is a 362-acre drainage lake that has a maximum depth of 32 feet. Florpyrauxifen-benzyl (commercially as ProcellaCOR™) is proposed to be applied to 8.1 noncontiguous acres within the lake in early-summer 2022 to control 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 circles on the image below.



Upper Gresham Lake Herbicide Sample Sites					
Site Label	**Site Description**	**Station ID**	**Latitude**	**Longitude**	**Sample Depth**
UG1	Application Area A-22	10056199	46.06334	-89.73487	Integrated (0-6 feet)
UG2	Application Area A-22	10056200	46.06408	-89.73318	Integrated (0-6 feet)
UG3	Deep Hole	643268	46.068299	-89.73696	Integrated (0-6 feet)
UG4	West AOPI	10056201	46.06560	-89.74538	Integrated (0-6 feet)
UG5	East AOPI	10056202	46.07041	-89.72845	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 UG1 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, 25 additional samples will need to be collected at seven 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	West AOPI	East AOPI
	Site UG1	Site UG2	Site UG3	Site UG4	Site UG5
Pre-Treatment	X				
3 HAT	X	X			
9 HAT	X	X			
24 HAT	X	X	X	X	X
48 HAT	X	X	X	X	X
4 DAT	X	X	X	X	X
7 DAT	X	X	X	X	X
14 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

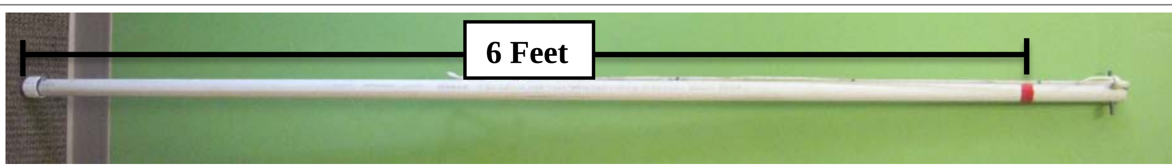


Photo 1. 6-foot Integrated sampling device constructed of PVC tubing.

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: UG1, UG2, UG3, UG4, or UG5)
- 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	
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