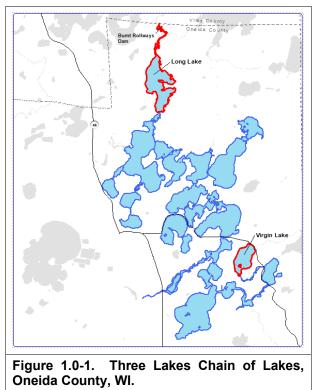
1.0 INTRODUCTION

The Three Lakes Chain is a flowage covering over 6,100 acres and 23 waterbodies in north-eastern Oneida County, Wisconsin (Figure 1.0-1). The chain is partially retained by the Burnt Rollways Dam at the northernmost end of Long Lake. The Three Lakes Waterfront Association (TLWA), along with partners the Town of Three Lakes, Oneida County, and Wisconsin Department of Natural Resources (WDNR) completed an eight-phase comprehensive management planning project in 2020.

Eurasian watermilfoil (EWM) was first discovered in the Three Lakes Chain in 2006 when several beds were located in the Eagle River channel immediately upstream of the Burnt Rollway Dam. The resulting aquatic invasive species (AIS) early detection and response project on the channel, spurred an 8-phase comprehensive management planning effort on the chain that was completed in 2020. AIS prevention, monitoring, and control are a primary aspect of the chain's comprehensive



management plan. The management plan focuses on manual removal methods for EWM control, but also outlines an herbicide management strategy framework if EWM populations exceed levels that can be managed with hand-harvesting.

During studies completed on Townline Lake in 2015, single EWM plants were located and subsequently harvested from one site in the lake. Semi-annual monitoring of Townline Lake was conducted from 2016-2020 with no additional EWM being located. Early detection and response efforts appear to have successfully eliminated EWM from Townline Lake.

The TLWA, in partnership with the Town of Three Lakes, applied for and subsequently was awarded a WDNR small scale AIS control grant during the November 2020 grant cycle. The three-year project (2021-2023) includes aspects related to monitoring and managing EWM in Long Lake and Virgin Lake.

1.1 Recent EWM Management Summary

In July 2020, TLWA volunteer AIS monitors located a small bed of EWM in a new location in Long Lake near its outlet channel towards the Eagle River Chain. The newly discovered EWM location was subsequently targeted with hand harvesting efforts led by the Three Lakes Dive Team (TLDT). A professional mapping survey in late-summer 2021 delineated a relatively small *highly scattered* colony approximately 80' in diameter along with a number of additional *single or few plants* occurrences in the vicinity. This site was given high priority for continued monitoring and EWM harvesting efforts in 2021 and 2022.

EWM was first found in Virgin Lake in 2010. Small herbicide spot treatments were completed as a part of two AIS-EDR projects as well as professional hand-harvesting efforts. In 2017, the TLWA formed the TLDT with their own funds and donations from local businesses. The TLDT has spent hundreds of hours since 2017 harvesting EWM in Virgin Lake. Monitoring surrounding the harvesting efforts in recent years has shown that the EWM population has been approximately maintained at about the same density in most of the targeted areas. In 2020-2021, the EWM population in an area south of the island showed signs of an increasing population with some colonized areas forming. In 2021, while exploring the possibility of conducting an herbicide treatment in this site, the TLWA adopted a strategy to exclude this area of Virgin Lake from the TLDT's efforts allowing the divers to focus harvesting efforts in Long Lake and elsewhere around Virgin Lake.

The *Three Lakes Chain 2021 AIS Report* (April 2022) discussed the EWM monitoring and management activities that took place in Long Lake and Virgin Lake during 2021. Monitoring surveys completed during 2021 indicated that the hand harvesting efforts in Long Lake were able to inhibit the EWM population from expanding within the targeted areas. The EWM population in Virgin Lake expanded slightly during 2021, with approximately six acres of colonized EWM delineated during the September mapping survey. Understanding that a potential 2022 herbicide treatment would cause large impacts to aspects of the Virgin Lake aquatic plant community, specifically to northern watermilfoil which is one of the most dominant native plant populations in the lake, the TLWA opted to postpone treatment until the EWM population was higher to better justify the likely collateral impacts predicted. Ultimately, the TLWA elected not to pursue an herbicide treatment EWM management strategy in Virgin Lake in 2022 but would re-consider this management option in 2023 based on data from another year of EWM monitoring in the lake and after reviewing the results of additional case studies that further the understanding of ProcellaCORTM use outcomes and aquatic plant selectivity in Wisconsin lakes.

The TLWA planned a coordinated hand harvesting EWM management strategy in Virgin Lake and Long Lake in 2022 that included a combination of professional hand harvesting efforts as well as efforts led by the Three Lakes Dive Team. This report details the EWM monitoring and management activities that took place during 2022 on Long Lake and Virgin Lake.

2.0 2022 AIS MANAGEMENT & MONITORING RESULTS

A pair of EWM mapping surveys were used within this project to coordinate and qualitatively monitor the hand-harvesting efforts. The first monitoring event in 2022 was the Early Season Aquatic Invasive Species Survey (ESAIS). This late-spring/early-summer survey provides an early look at the lake to help guide the handharvesting management to occur on the system. While EWM is usually not at its peak growth at the time of the ESAIS survey, the water is typically clearer during the early summer allowing for more effective viewing of submersed plants, and EWM is often growing higher in the water column than many of the native aquatic plants at that time of year. Following the hand-harvesting, Onterra ecologists completed the Late-Summer EWM Mapping Survey, the results of which serve as a post-harvesting assessment of the hand-removal efforts.



Photograph 2.0-1. EWM mapping survey on a Wisconsin lake.

During an EWM mapping survey, the entire littoral area of the lake is surveyed through visual observations from the boat (Photograph 2.0-1). Field crews supplement 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*.

2.1 Early Season AIS Survey

Long Lake:

On June 22, 2022, Onterra crews surveyed all of Long Lake's littoral areas including the Burnt Rollways channel to the dam. In Long Lake, crews delineated a *highly scattered/scattered* density EWM colony on the north end of the lake in the same area as it has been known for the past few years (Map 1). Several additional *single or few plant* occurrences were also marked in the vicinity. The core area of colonized EWM spanned approximately 350 feet long by 75 feet wide and encompasses an area of just under ½ acre. Crews marked two *single or few plant* occurrences within the Long Lake channel. No EWM was found on the west shore south of the boat landing of Long Lake where a few plants were spotted in 2021. No other EWM occurrences were located around Long Lake during the survey.

<u>Virgin Lake:</u>

On June 23, 2022, Onterra survey crews completed the ESAIS survey on Virgin Lake. The survey results in Virgin Lake indicate an increased EWM population compared to 2021. The largest concentration of EWM was directly south of the island and increased in density since the previous survey (Map 2). More areas in this site consisted of *dominant* or *highly dominant* density ratings, while the rest of the area in the vicinity remained in lower densities of *scattered* or *highly scattered*. Elsewhere around the lake, a *scattered* colony was present in an and around an emergent bulrush community on the northeast side of the lake, and many more isolated *single or few plants* occurrences were marked around other littoral areas of the lake.

Onterra provided the spatial data from the ESAIS survey results to the TLWA Dive Team in the form of a GPS compatible basemap which is used to guide the harvesting efforts.

2.2 Hand-Harvesting Activities

The TLWA continued with a two-tiered EWM hand harvesting strategy in 2022 utilizing both professional services and a volunteer effort led by the local Three Lakes Dive Team. The TLWA contracted with Aquatic Plant Management, LLC (APM) to conduct professional hand-harvesting of EWM on one site in Virgin Lake in 2022. Divers from APM conducted Diver Assisted Suction Harvesting (DASH) on Virgin Lake on August 10-12, 2022 and reported a harvest of 49 cubic feet of EWM over 19.4 hours of underwater dive time (Appendix A). Additional details of the harvesting efforts are included in Appendix A.

The TLWA has a dedicated volunteer-based dive team that has been in operation since 2017. The Dive Team completed 15 dives on Virgin Lake during 2022 resulting in the harvest of approximately 305 gallons of EWM. The TLWA reported having spent over 300 hours on Virgin Lake and 200 hours on Long Lake between the Dive Team and its supporting cast in 2022. Additional information



related to the Dive Team's efforts to harvest EWM in Long and Virgin Lakes is available within the TLWA's Fall 2022 newsletter which is posted on their website (<u>http://tlwa.org/</u>).

2.3 Late-Summer EWM Mapping Survey Results

Long Lake:

Onterra field crews completed the Late-Summer EWM Mapping Survey in Long Lake including the channel leading to the Burnt Rollway dam on September 14, 2022. The results of the survey on Long Lake are displayed on Map 3. The inset views on the map highlight the two areas of the lake where EWM was located. Several *single or few plants* and two *clumps of plants* were mapped in the northeast end of the lake where EWM was located in past surveys and in the vicinity of where hand harvesting efforts were focused (top inset). Additionally, the field crews located two *single or few plants* in Long Lake along the west shore, south of a public boat landing, as displayed in the bottom inset view. The primary site where hand harvesting actions occurred is highlighted in Figure 2.3-1 which shows the EWM population in June 2022 (before hand harvesting) and September 2022 (after hand harvesting). The EWM population was reduced within the area targeted with hand harvesting as demonstrated by a reduction of EWM from colonized areas in June 2022, to point-based occurrences in September 2022 after harvesting efforts took place.

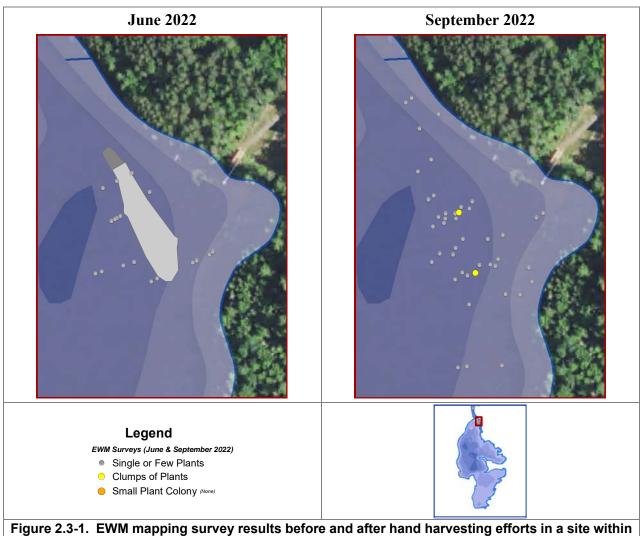


Figure 2.3-1. EWM mapping survey results before and after hand harvesting efforts in a site within Long Lake of the Three Lakes Chain of Lakes. Data from Onterra June & September 2022 surveys.

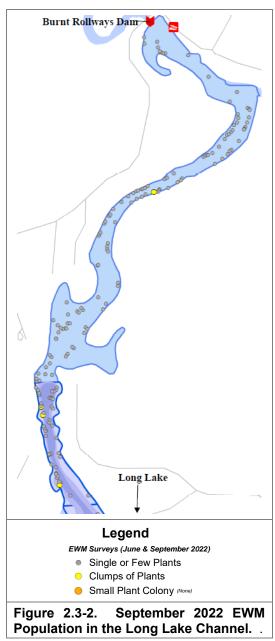


The river section between Long Lake and the Burnt Rollways Dam was surveyed on September 14, 2022. The field crew noted the waters within the channel to be clearer than previous surveys with modest flow rates. The crew recorded nearly 200 *single or few plants* EWM occurrences throughout the river channel as well as four *clumps of plants* (Figure 2.3-2). Although no colonized areas were located in the channel, the EWM population mapped during this visit was greater than has been documented in any previous survey of this area.

Virgin Lake:

The Late-Season EWM Mapping Survey was completed on Virgin Lake on September 15, 2022. The results of the survey are displayed on Map 4. The survey results indicated that the EWM population increased in density in the site south of the island compared to previous surveys (Figure 2.3-3). The EWM in this portion of the lake was mapped with all five of the different density ratings used in Onterra's mapping methodology including one part of the site that was *surface matted* at the time of the survey. Another smaller colonized area was mapped just west of the island. The remainder of Virgin Lake contained a modest EWM population with generally sparse *single or few plants* or *clumps of plants* documented during the survey.

The EWM population appears to have been reduced at the site within an emergent bulrush plant community on the northeast side of the lake which has been a focus of hand harvesting efforts in recent years (Figure 2.3-4). Professional harvesting efforts occurred during three days in the site in 2022, with additional subsequent clean-up efforts by the Three Lakes Dive Team. Following the harvesting efforts, only *single or few plant* occurrences



were located in the site where *a scattered* density colony and many more *single plants* as well as a *small plant colony* had been present earlier in the year during the June ESAIS survey.



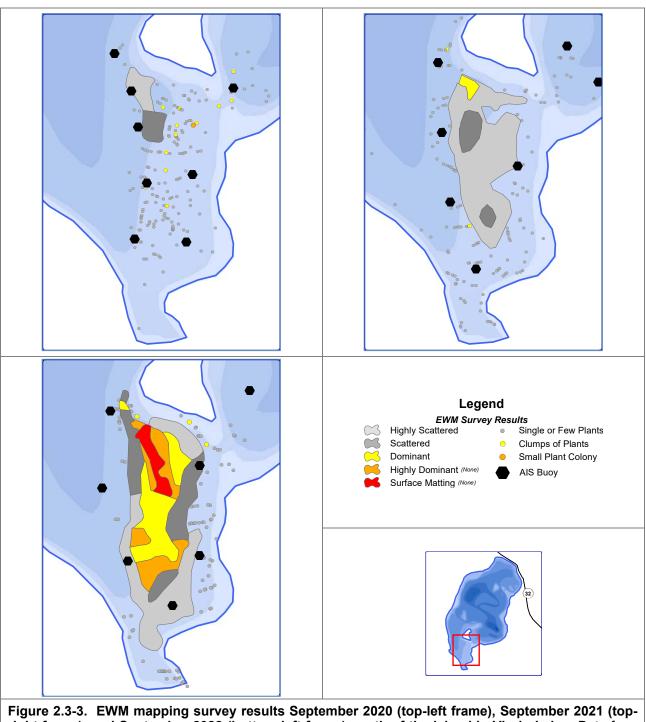
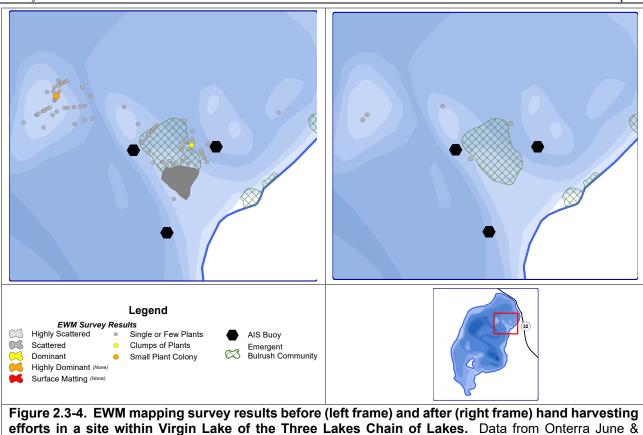


Figure 2.3-3. EWM mapping survey results September 2020 (top-left frame), September 2021 (topright frame), and September 2022 (bottom-left frame) south of the island in Virgin Lake. Data from Onterra Late-Summer EWM Mapping surveys.

Three Lakes Waterfront Association



September 2022 surveys.

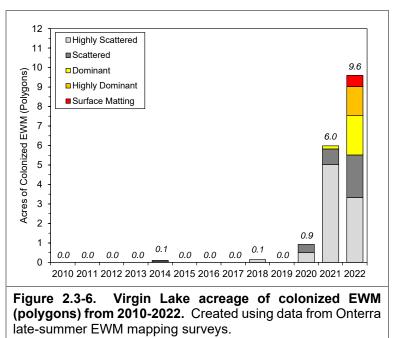
Volunteer EWM monitoring conducted by members of the Three Lakes Dive Team in 2022 documented the presence of a significant amount of EWM plants growing within Julia Creek downstream of Virgin Lake. Some of these plants were approaching the entrance of Julia Creek into Whitefish Lake. GPS coordinate locations and notes recorded by the TLWA volunteer are incorporated into figure 2.3-5 below.

Virgin Lake Julia Creek 2 3	TLWA Volunteer Notes:1. Mouth of creek, occasional plants first couple hundred yards2. Lots of mature plants – some of the small clusters surface matting3. Density seemed to lessen somewhat to perhaps a plant every 10 feet4. EWM population diminished to an occasional plant5. Last marked EWM plant 6. End of surveyed area
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Figure 2.3-5. Locations of EWM within Julia Creek, downstream of Virgin Lake.



Figure 2.3-6 displays the acres of EWM that have been mapped with area-based methodologies (polygons) from 2010-2022 in Virgin Lake. These data do not account for occurrences mapped with point-based methods including *single* or few plants, clumps of plants, and small plant colonies. Even though EWM was present during each of the monitoring years, all occurrences in some years consisted only of point-based occurrences which do not contribute to the acreage totals. Very little colonized EWM was mapped within the lake during the timeframe spanning from 2010-2019. An increase in colonized EWM is apparent from surveys completed from 2020-2022. The 2022 mapping survey



delineated 9.6 acres of colonized EWM including 0.6 acres that consisted of a *surface matted* density rating, while another 1.5 acres was given a *highly dominant* density rating, and 2.0 acres consisted of a *dominant* density rating. Another 5.5 acres consisted of lower-density ratings described as either *highly scattered* or *scattered*.

2.4 Sub-set Point-Intercept Survey Results

On September 13, 2021, Onterra staff completed a sub point-intercept survey within a focus area located at the southern end of Virgin Lake. Sampling locations were spaced at 21-meters apart throughout an approximate 11.5-acre site resulting in 108 total sampling locations. The survey was replicated on September 15, 2022 in order to document the dynamics of the aquatic plant population in the site. A total of 25 native aquatic plant species have been identified within the survey area between the 2021-2022 surveys. Figure 2.4-1 displays the frequency of occurrence of aquatic plants sampled during the 2022 sub-sample point-intercept survey.

Wild celery was the most frequently encountered aquatic plant species in the site being present on 73 of the 108 sampling locations resulting in an occurrence of 67.6%. Eurasian watermilfoil was the second-most frequently encountered species in the survey area with an occurrence of 56.5%. Northern watermilfoil was the third most frequently encountered species with an occurrence of 42.6%. Fern-leaf pondweed (29.6%), common waterweed (18.5%), and clasping-leaf pondweed (17.6%) were also common within the site.

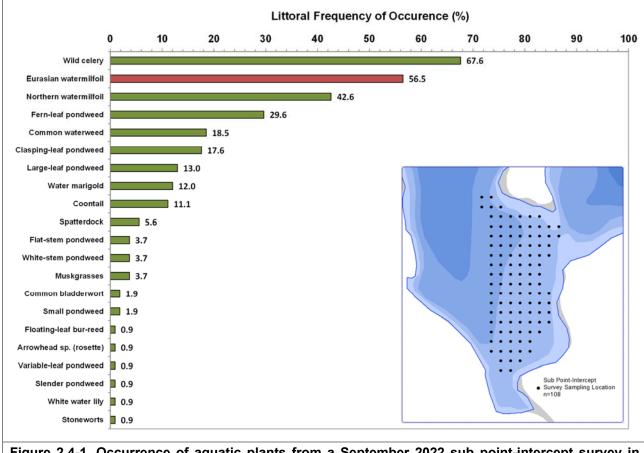


Figure 2.4-1. Occurrence of aquatic plants from a September 2022 sub point-intercept survey in Virgin Lake. (n=108)

Figure 2.4-2 compares the sub point-intercept survey results from 2021 to 2022. Species which exhibited a statistically valid change in occurrence between the two surveys are indicated with an asterisk. Three native species exhibited statistically valid increases in occurrence from 2021 to 2022 including wild celery (+40.4%), fern-leaf pondweed (+88.2%), and large-leaf pondweed (+180%). Meanwhile, eight native species showed valid decreases in occurrence during the same time period including common waterweed (-39.4%), slender naiad (-100%), muskgrasses (-81%), small pondweed (-91.3%), flat-stem pondweed (-73.3%), stoneworts (-93.3%), stiff pondweed (-100%), and water stargrass (-100%). Eurasian watermilfoil exhibited a statistically valid increase from 19.4% in 2021 to 56.5% in 2022 (+190.5%). Many other native species present within the study area did not have a statistically significant change in occurrence between the two surveys including northern watermilfoil.



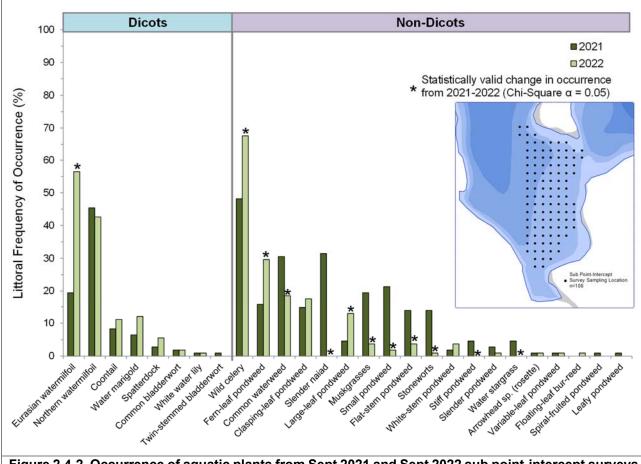
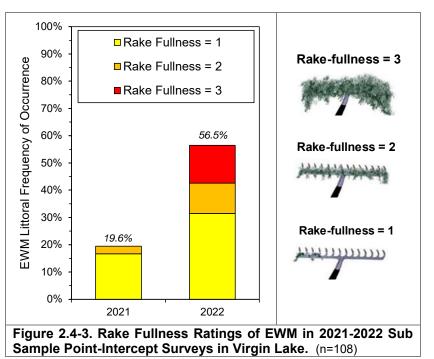


Figure 2.4-2. Occurrence of aquatic plants from Sept 2021 and Sept 2022 sub point-intercept surveys in Virgin Lake. (n=108)

Rake fullness is a semiqualitative measure of aquatic plant biomass that is collected as a part of the point-intercept survey. In the 2021 survey, just sampling locations three contained EWM that was given a rake fullness rating of 2 or greater while 18 other sites were given a rating of 1 (Figure 2.4-3). The replication of the survey in 2022 showed that 34 sampling locations had an EWM rake fullness rating of 1, with 12 points given a rating of 2 and 15 sampling locations having the highest rake fullness rating of 3. These data demonstrate an increased biomass of EWM in the study area between 2021 and 2022.



3.0 CONCLUSIONS AND DISCUSSION

Monitoring surveys completed during 2022 served to evaluate the coordinated EWM hand harvesting program on Virgin and Long Lakes, while also monitoring the aquatic plant population dynamics within a focus area in Virgin Lake.

Surveys in Long Lake show that EWM has not established in wider areas of the main body of the lake since its discovery a few years ago. Hand harvesting efforts appear to have been successful in inhibiting EWM from expanding in the lake, and show a reduced population in the main area of the lake where harvesting actions took place. A significant amount of individual EWM plants were located throughout much of the Long Lake channel in the September 2022 survey. It is suspected that growing conditions were favorable for EWM in this part of the system during 2022 and may be related to the clearer than usual water and lowered flow rate at the time of the mapping survey. Continued monitoring and harvesting efforts will be instrumental in keeping the EWM population in Long Lake at a low level and limiting its spread within the lake.

The EWM population increased within Virgin Lake in 2022, in particular in the area south of the island, as demonstrated by comparative EWM mapping surveys and sub-sample point-intercept surveys. A combination of professional DASH and TLWA Dive Team efforts were effective at maintaining a low EWM population around the remainder of Virgin Lake including a site located within an emergent bullrush community on the northeast end of the lake. Hand harvesting efforts have continued to aid in maintaining low EWM populations around the lake and inhibiting EWM from establishing in new areas of the lake.

The main colonized area of EWM in Virgin Lake expanded in area and density during 2022. The late-summer EWM mapping survey documented a significant portion of this site comprised of *highly dominant* or *surface matted* densities. These particularly dense areas are resulting in reduced recreational use in this area of lake, particularly for boating or swimming. Data from the sub-sample point-intercept survey collected from this site showed EWM increased significantly between 2021 and 2022 while many native species decreased in occurrence indicating that EWM may be imparting some competitive influence on the native plant population.

The presence of many EWM plants growing in Julia Creek downstream from Virgin Lake was documented by TLWA volunteers in 2022. Although not unexpected, concern exists that EWM may eventually enter into Whitefish Lake by way of the inlet of Julia Creek. EWM was not located in Whitefish Lake during the most recent professional monitoring surveys that took place during 2020. The TLWA is encouraged to monitor EWM in the river channel during 2023 and attempt to hand pull the EWM if possible.

3.1 2023 EWM Monitoring & Management Strategy Development

Management Goal # 2 within the TLWA's WDNR-approved Comprehensive Management Plan is to "Control Existing AIS" with one of the associated actions being to control EWM through the use of herbicides if the area of EWM is too large to effectively hand pull and large enough for herbicides to be expected to be successful. In 2023, the TLWA will pursue an integrated approach to EWM management that would include a spot-herbicide treatment in Virgin Lake and a continued hand harvesting effort within Long Lake and other sites within Virgin Lake. By targeting the largest EWM colonies in Virgin Lake with herbicides, the Dive Team will be able to direct their efforts elsewhere

around Virgin Lake with also being prepared to hand harvest any remnant or re-establishing EWM within the herbicide site during the years after treatment.

Diver Assisted Suction Harvesting/Hand Harvesting

Much of the EWM population in Virgin and Long Lake consists of relatively small and low-density occurrences that are of a reasonable size and scale that is applicable to a hand harvesting management strategy. The TLWA will use the EWM mapping survey data that was collected in September 2022 to determine an initial hand harvesting strategy for 2023. The TLWA should prioritize harvesting efforts in Long Lake to inhibit the EWM population from expanding to new areas of the lake. It may be challenging to harvest the numerous individual EWM plants throughout much of the Long Lake channel, but these occurrences should also be given consideration for removal in 2023 if the Dive Team finds it to be feasible given the complexity of water flow in the area. The Dive Team will also consider targeting the isolated occurrences within Virgin Lake, particularly plants not within or directly adjacent to the prospective herbicide management site. Hand removal efforts may also take place in Julia Creek, downstream from Virgin Lake, in an effort to reduce the likelihood of EWM reaching Whitefish Lake via the river. An Early Season AIS survey is scheduled to occur during June 2023 that will serve to update and finalize the hand harvesting strategy for the season. A late-summer EWM mapping survey in 2023 will be used to evaluate the hand harvesting strategy.

The TLWA is in the process of acquiring an Association-owned and operated DASH boat that is expected to be operational in 2023. Having a DASH boat will increase the TLWA's capacity to harvest EWM in the system in a more efficient manner and will be a valuable management tool for the TLWA's long-term EWM management efforts. DASH requires a WDNR permit. Depending on the operating status of the Association's DASH boat, the TLWA may also consider contracting with a professional firm that offers this service once again in 2023, particularly in sites that may be beyond the Dive Team's capabilities to manage. The TLWA has identified one site in Virgin Lake to include in a preliminary 2023 DASH strategy. The preliminary 2023 DASH strategy includes a 2.2-acre site that is located in the northeast end of Virgin Lake within, and adjacent to, a community of emergent bullrush (Map 5). This site has been targeted with a combination of professional and local harvesting efforts in recent years.

ProcellaCORTM Spot Treatment

Many lake managers have shifted away from weak-acid based herbicides such as 2,4-D in spottreatment scenarios as the concentration and exposure time needed to result in EWM control is difficult to achieve without the aid of a barrier curtain. The original scope of the current AIS grant included monitoring and control activities that included a spot treatment with florpyrauxifen-benzyl solid exclusively under the trade name ProcellaCORTM. ProcellaCORTM has been the state's most popular spot-treatment strategy for EWM management in recent years. Onterra has monitored over 50 ProcellaCORTM treatments in Wisconsin since 2019 with data analysis related to herbicide concentration monitoring and native aquatic plant impacts being investigated in the majority of treatments. Analysis of these data have allowed lake managers to better understand the ways in which the herbicide dissipates or mixes within a lake in the hours and days after application. Additionally, aquatic plant monitoring data provides insights as to which native species are typically impacted with ProcellaCORTM treatments.

Lake managers continue to learn how to successfully implement this form of treatment after being registered for use in Wisconsin only a few years ago. ProcellaCORTM has a high sediment/organic binding affinity (Koc) and relatively short persistence (half-life of < 6 days), so it was thought to stay

where applied better than other chemistries. However; in many of the treatments Onterra has monitored, EWM impacts have been observed extending outside of the application area (i.e through herbicide dissipation), as this chemical has shown activity at even low concentrations and exposure times as it mixes into an area of potential impact. For some treatment scenarios, this could be a bay or basin if not the entire lake. Recent herbicide concentration monitoring analysis conducted around the state indicates that the acid metabolite of ProcellaCORTM, florpyrauxifen acid, often persists in the lake at a low concentration for weeks after the treatment takes place. This chemical is reported to have activity on aquatic plants, albeit to a lower degree than the active ingredient. It is unclear at this time the role that the acid metabolite may play in contributing to EWM reductions, particularly in areas not located directly within the herbicide application area. The WDNR's fact sheet on this chemistry can be found here:

https://dnr.wi.gov/water/wsSWIMSDocument.ashx?documentSeqNo=164039981

Onterra's experience monitoring ProcellaCOR[™] treatments indicates that EWM control has been high with almost no EWM being located during the summer post treatment surveys. Some treated sites have shown EWM population recovery two-years after treatment, while other sites have demonstrated three years and counting of continued EWM reductions to-date. Aquatic plant monitoring data indicates that northern watermilfoil is highly susceptible to ProcellaCOR[™] with frequency of occurrences typically reduced to 0% in the year of treatment with little to no sign of recovery during the year after treatment. Other species that have shown a degree of susceptibility to this chemical include water marigold, coontail, and potentially water stargrass. Pondweed species appear to be unaffected by this herbicide, with some lakes having large increases in species like richardson's pondweed during the years following treatment. Wild celery, slender naiad, native pondweed species, and muskgrasses are common within the sub-sample location in Virgin Lake and these species are not believed to be particularly susceptible to ProcellaCOR[™]. Adjacent populations of floating-leaf species (i.e. water lilies) may shows signs of herbicidal impacts such as leaf twisting, but typically rebound well including in intentional whole-lake treatment scenarios.

Herbicide concentration monitoring completed in the last three years have shed more light on the dissipation of ProcellaCORTM and the concentrations that are being measured within an Area of Potential Impact (AOPI), which is the volume of water in which the herbicide is expected to mix within and ultimately reaching some level of equilibrium. Monitoring studies have indicated that florpyrauxifen-benzyl concentrations can mix within a AOPI within as little as nine hours or less, but often takes closer to 1-2 days after treatment to reach equilibrium. When planning future treatments, lake managers are factoring in the potential AOPI concentrations as opposed to solely the application area dosing rate.

Preliminary Virgin Lake Herbicide Treatment Strategy

Onterra staff met with members of the TLWA Board on November 10, 2022 to discuss the preliminary results of the 2022 monitoring surveys and to discuss a potential herbicide management strategy for 2023. During the meeting, Onterra provided a risk assessment covering the expectations and uncertainties associated with the proposed treatment strategy, much of which is discussed within this report. In response to the higher EWM populations in Virgin Lake, the TLWA opted to pursue an herbicide management strategy for 2023. The TLWA believes the integrated pest management approach of herbicide treatment and hand-harvesting/DASH will position them to tackle the totality of the EWM population in the near future. The TLWA understands the likelihood of northern

watermilfoil impacts within the Virgin Lake application area, and that these impacts are likely to extend outward from the treatment an unknown distance.

To target the colonized EWM south of the island in Virgin Lake, an 11.7-acre application area was constructed and a preliminary dose of 4.0 prescription dosing units (PDU) is being considered (Map 6). A proposed application rate of 4.0 PDU's equates to approximately 7.7 parts per billion (ppb). Onterra will confirm the dosing rate with experts from SePRO, the manufacturer of ProcellaCORTM, in advance of the treatment.

Designing an herbicide management strategy must also consider the potential lake-wide herbicide concentrations within the entirety of the lake. At a proposed application rate of 4.0 PDU, a theoretical lake-wide concentration of 0.16 ppb is calculated. Based upon Onterra's experience, lake-wide concentrations that approach 0.25 ppb of ProcellaCORTM active ingredient (florpyrauxifen-benzyl) have functioned as lake-wide treatments. This means that along with the upfront high concentration in the application area, the entire lake will reach an equilibrium concentration that at an extended exposure (days to a week) could have the potential to impact EWM and sensitive native species throughout the entire lake. The proximity of the herbicide application area to the outlet stream in Virgin Lake, coupled with the island protecting the northern side of the site, may reduce the likelihood of complete herbicide dissipation and mixing on a lake-wide scale. The potential for the herbicide to instead move downstream through the outlet stream is high; however, it is not known if impacts to sensitive aquatic plant species, including EWM, would be expected to occur in downstream areas.

Northern watermilfoil was the second most frequently encountered native species in a 2020 wholelake point-intercept survey (19.8% occurrence) and large contiguous colonies are known to be present in various locations around the lake including along the western and eastern shorelines. It is expected that northern watermilfoil growing within and immediately adjacent-to the proposed application area will be greatly impacted by the treatment. It is unknown whether a potential lake-wide concentration of 0.16 ppb is sufficient to impact northern watermilfoil or other sensitive native species on a lakewide scale. The TLWA has carefully considered the potential for northern watermilfoil to be negatively impacted by the proposed treatment and believes the benefits outweigh the risks associated with pursuing this EWM management strategy. A robust native aquatic plant population present within the proposed treatment site may be more likely to maintain a healthy and diverse plant community following the potential removal of EWM and northern watermilfoil from the site.

Preliminary Virgin Lake Monitoring Strategy

The 2023 EWM treatment monitoring program will include qualitative success monitoring through comparative EWM mapping surveys, herbicide concentration monitoring, and a sub-sample point-intercept aquatic plant survey.

Pretreatment Confirmation and Refinement Survey

Onterra ecologists will conduct a *Pretreatment Confirmation and Refinement Survey* prior to the early-season herbicide application to verify application area extents and inspect the condition of the EWM colonies targeted for treatment through the use of a combination of surface surveys, rake tows, and submersible video monitoring. This meander-based survey would investigate for colonial expansion, reduced occurrence, growth stage of the EWM (and native plants), application area specifies (e.g. average depth & extents), and other aspects that could warrant a modification to the treatment plan. Water temperature and pH data would be collected during the survey to assist with

projecting ideal treatment timing. During this visit, Onterra staff would provide supplies and training to volunteers for conducting herbicide concentration monitoring.

Following the *Pretreatment Confirmation & Refinement Survey*, an email-style report with map(s) of the survey results and finalized treatment plan would be provided to the TLWA, WDNR, and other project partners for review prior to the treatment. Spatial data would be provided to the herbicide applicator in appropriate format. The chosen contractor, in conjunction with the TLWA, will be responsible for completing appropriate permit-related documentation and deliverables to the WDNR.

Herbicide Concentration Monitoring

TLWA volunteers would collect herbicide concentration monitoring during the hours/days following treatment following a sampling regime that will be created through collaborative efforts of the WDNR and Onterra. Samples would be collected at specified time intervals and locations within and outside the application area, likely to include a downstream location on the Julia Creek. Sample collection would be focused on understanding the quantity and longevity of the herbicide active ingredient and the acid metabolite. Properly preserved samples would be overnight delivered EPL Bio Analytical Services where the herbicide analysis is conducted.

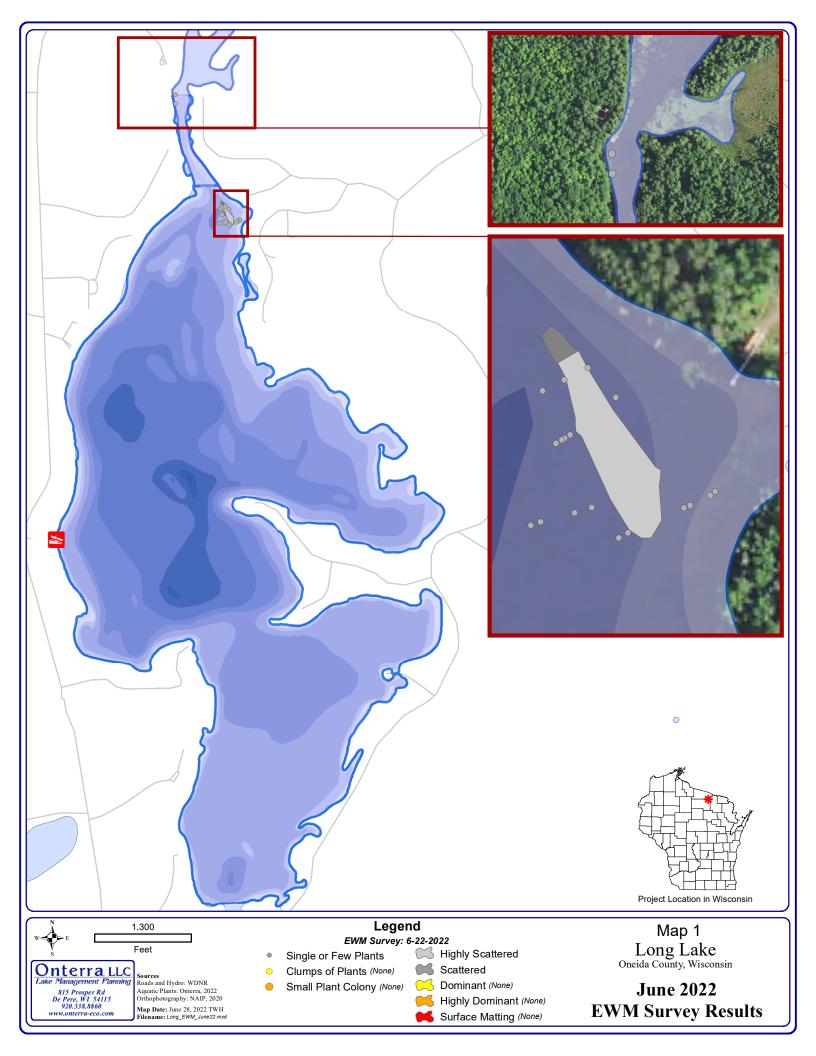
Quantitative Aquatic Plant Monitoring

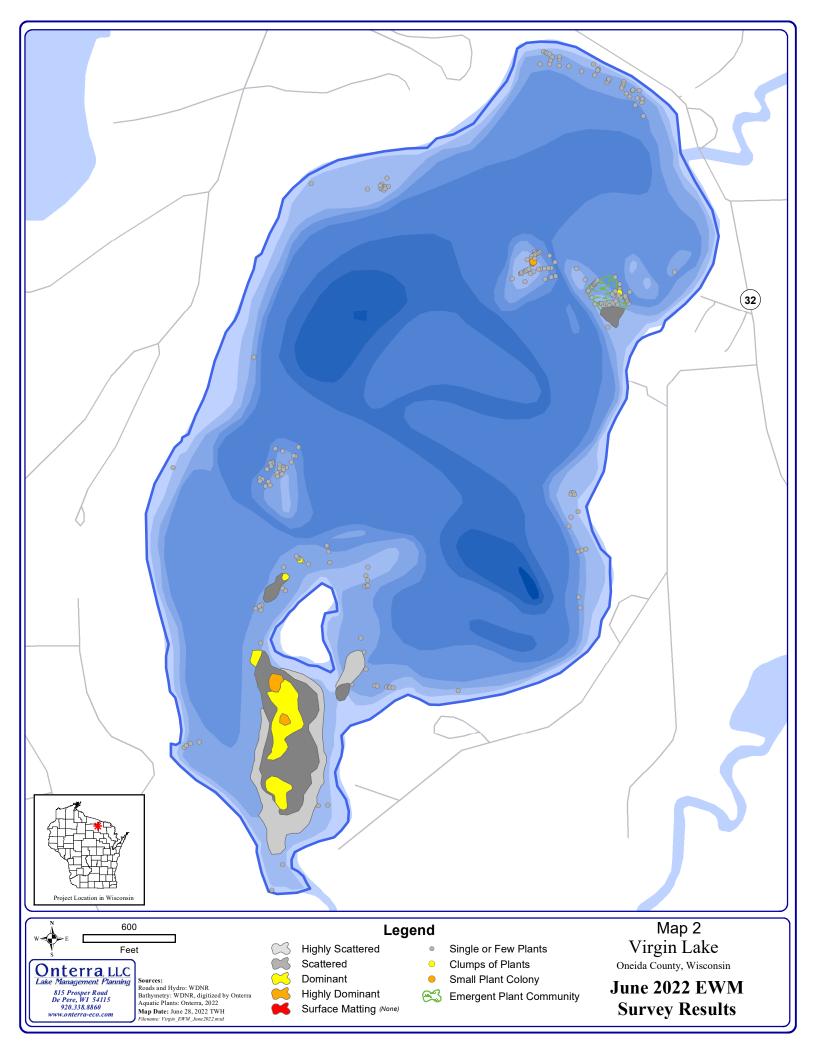
Aquatic plant monitoring is planned in 2023 through the replication of a sub-sample point-intercept survey within the herbicide application area. These data will be compared to previous surveys to understand how native and non-native aquatic plant populations may be impacted by the 2023 management strategy. Consideration of conducting this survey during 2024 to understand the *year after treatment* impacts.

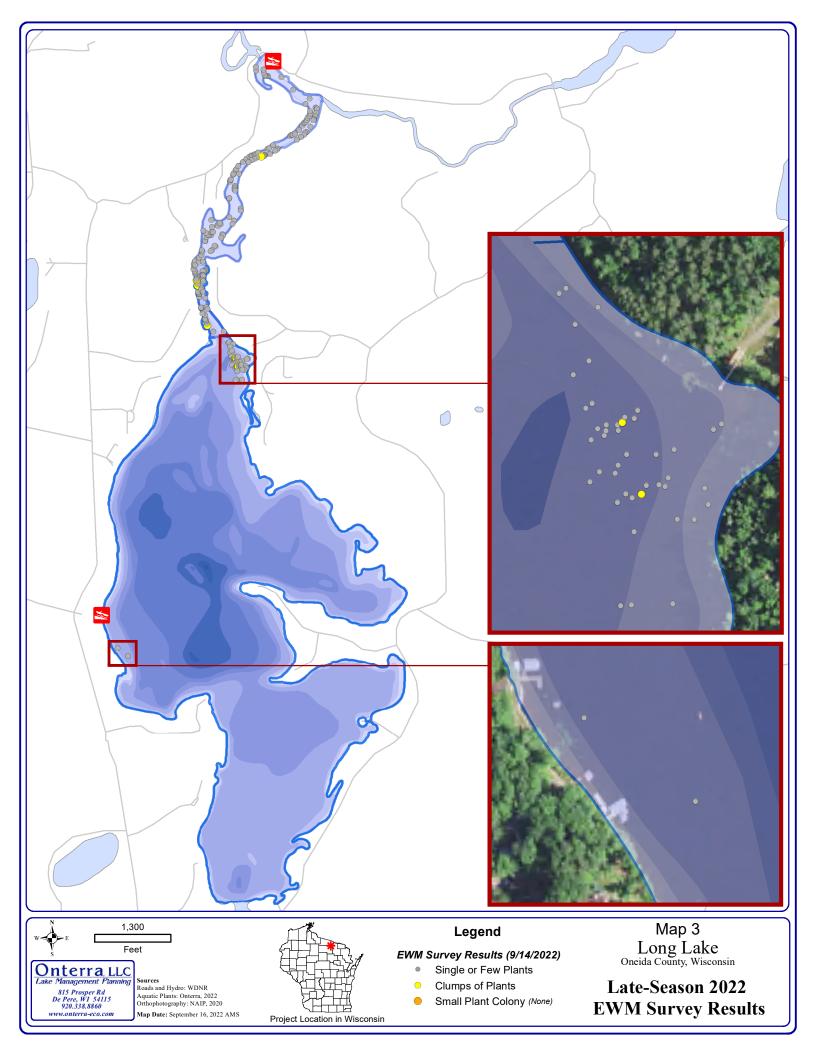
Qualitative EWM Monitoring

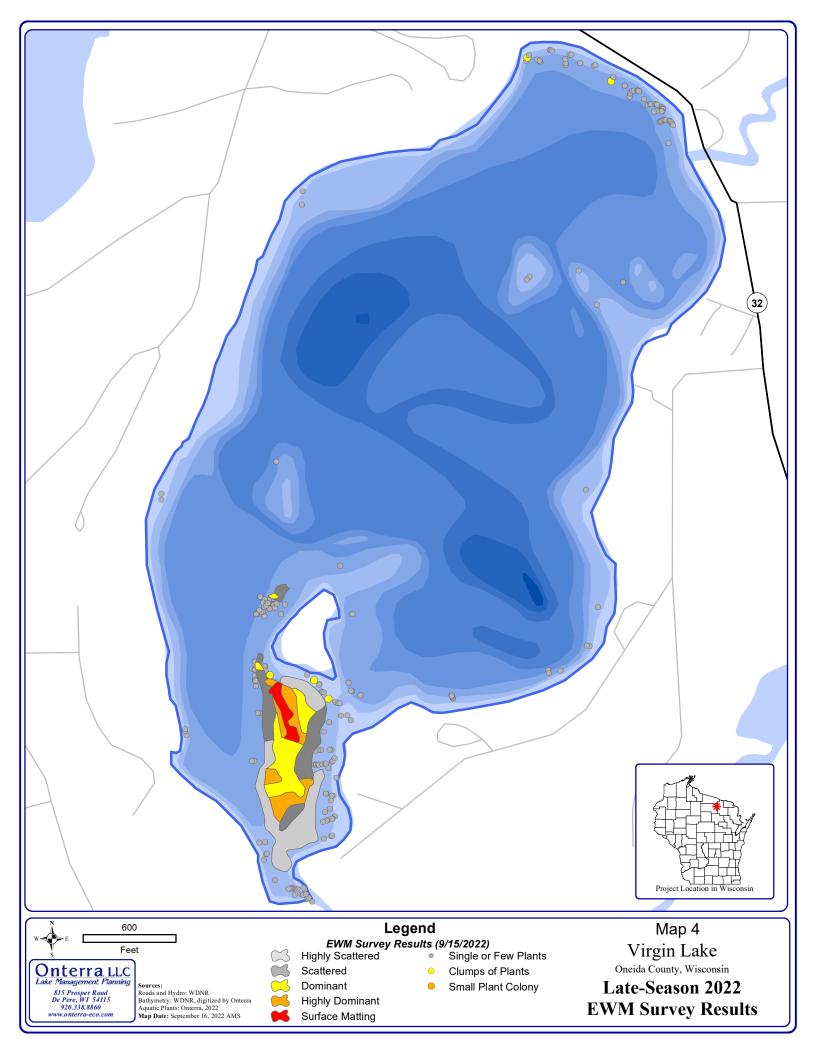
A Late Season EWM Mapping Survey will again be conducted in 2023 to produce the mapping data to document a census of the EWM population within Virgin Lake at the perceived peak growth stage. Comparing these data to previous surveys will help lake stakeholders understand management outcomes. The herbicide treatment would meet control expectations if little to no EWM is present in the application area during the late-summer 2023 survey. Many treatment impacts during the *year of treatment* may be short-lived, so understanding how populations stabilize during the *year after treatment* is important within evaluations. EWM reductions would be expected to extend into 2024 for the treatment to be deemed successful.

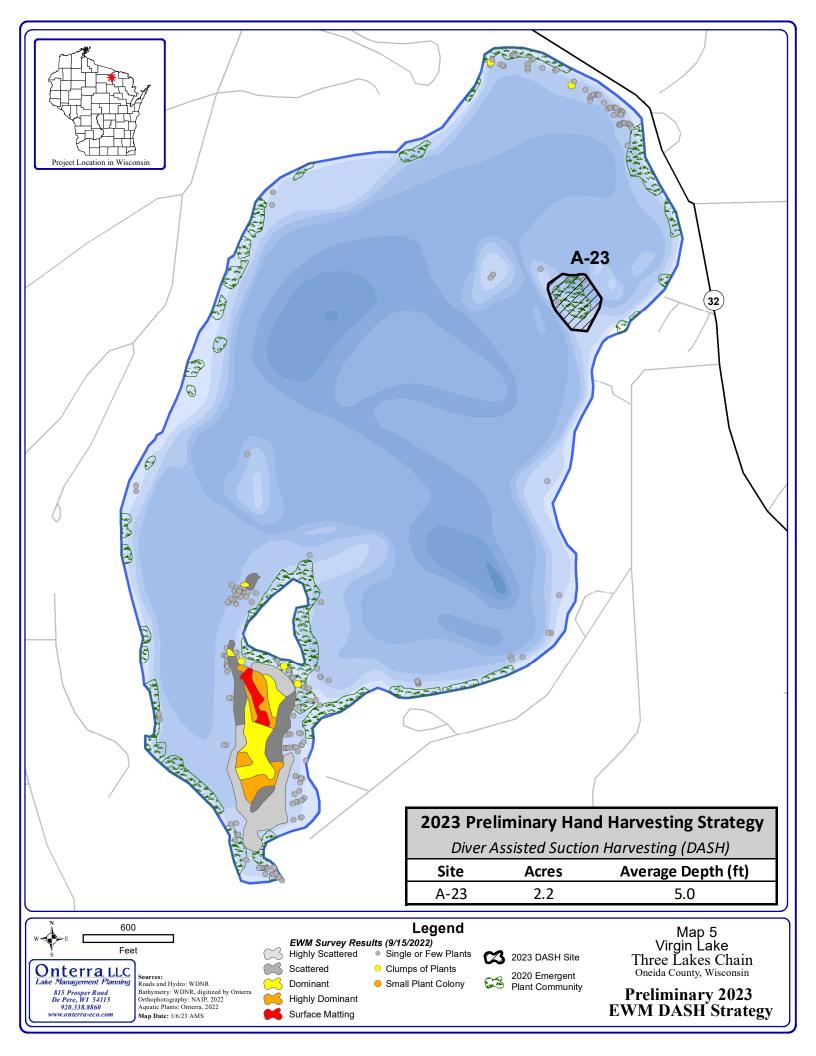


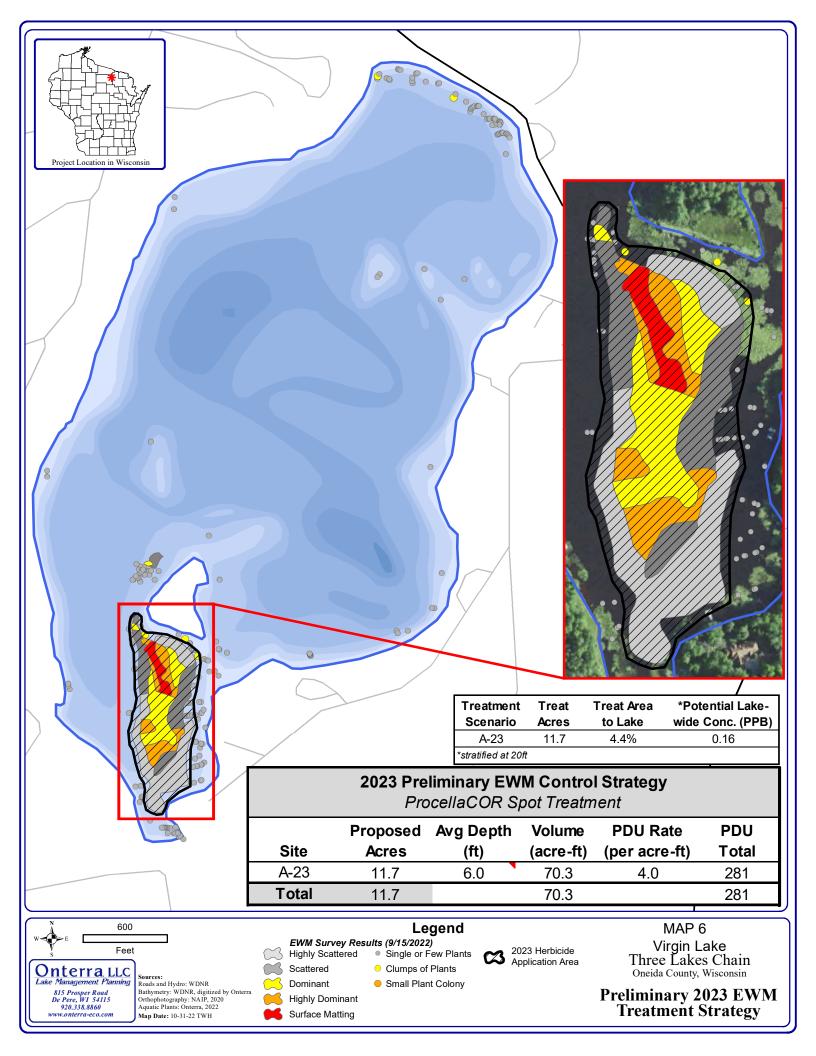












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APPENDIX A

Virgin Lake 2022 EWM Manual Removal Summary Report – Aquatic Plant Management, LLC



Virgin Lake EWM Removal Report 2022

PO Box 1134 Minocqua, WI 54548



Dive Background: In August Aquatic Plant Management LLC (APM) conducted three (3) days of Diver Assisted Suction Harvesting (DASH) for Eurasian Watermilfoil (EWM) on Virgin Lake in Oneida County, WI. The team focused their efforts at one main site in the northern portion of the lake that was near/within a large patch of bullrush. In total APM was able to remove **49.0 cubic feet of EWM** from the Virgin Lake.

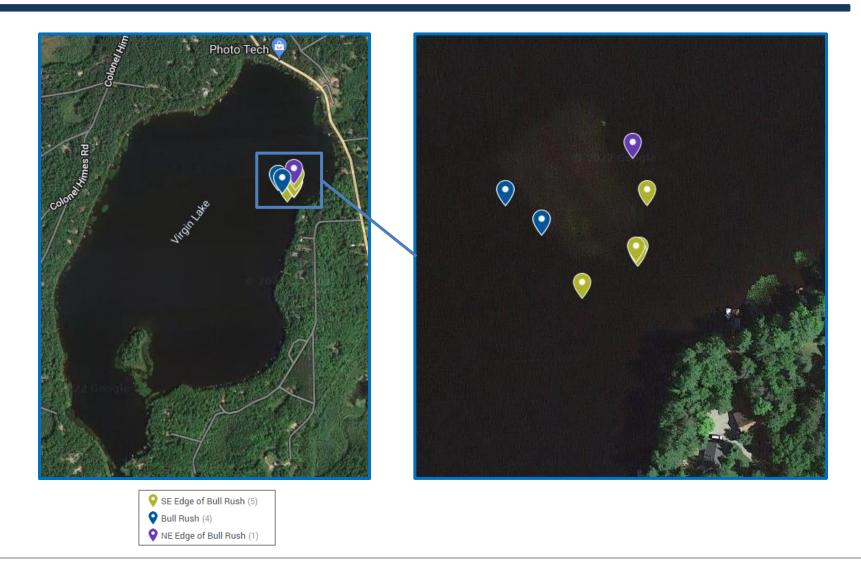
Date	Weather Conditions	Water Temp (F)	Underwater Dive Time (hrs)	AIS Removed (cubic ft)
8/10/2022	Sunny	73	6.8	13.5
8/11/2022	Sunny	72	6.4	13.5
8/12/2022	Cloudy	73	6.3	22.0
Grand Total			19.4	49.0

Dive Location	Avg. Water Depth	# of Dives	Underwater Dive Time	AIS Removed (cubic feet)
Bull Rush	4.0	4	13.2	27.0
NE Edge of Bull Rush	5.0	1	0.8	4.0
SE Edge of Bull Rush	5.4	5	5.4	18.0
Grand Total	4.8	10	19.4	49.0

Dive Highlights and Recommendations: The dive team started on the in the bullrush, before eventually diving around the perimeter of the bullrush patch working counterclockwise. DASH was effective at targeting the EWM infestation that was intermingled with the native bullrush growth. Overall, Virgin Lake should continue to take an Integrated Pest Management (IPM) approach and evaluate different strategies to manage the EWM population on the lake. Continued monitoring and management efforts are important to prevent the spread of EWM throughout Virgin Lake.



Map of Virgin Lake Dive Sites



Aquatic Plant Management LLC



Detailed Diving Activities

Date	Dive Location	Latitude	Longitude	Underwater Dive Time (hrs)	AIS Removed (cubic ft)	AIS Density	Avg Water Depth (ft)	Native Species	Native By- Catch	Substrate Type
8/10/2022	Bull Rush	45.78734	-89.08270	3.17	9.0	Scattered	4.0	Northern Milfoil	0.5	Gravel
8/10/2022	Bull Rush	45.78734	-89.08270	3.58	4.5	Scattered	4.0	Northern Milfoil	0.0	Gravel
8/11/2022	Bull Rush	45.78721	-89.08247	3.17	9.0	Scattered	4.0	Elodea	1.5	Gravel
8/11/2022	Bull Rush	45.78721	-89.08247	3.25	4.5	Scattered	4.0	Elodea	1.0	Gravel
8/12/2022	SE Edge of Bull Rush	45.78693	-89.08221	1.42	3.0	Scattered	6.0	Elodea	1.0	Sand
8/12/2022	SE Edge of Bull Rush	45.78709	-89.08185	1.50	4.5	Scattered	6.0	Elodea	1.0	Sand
8/12/2022	SE Edge of Bull Rush	45.78707	-89.08186	0.42	1.5	Scattered	5.0	Elodea	0.5	Sand
8/12/2022	SE Edge of Bull Rush	45.78709	-89.08187	1.08	4.5	Scattered	5.0	Elodea	1.0	Sand
8/12/2022	SE Edge of Bull Rush	45.78734	-89.08180	1.00	4.5	Scattered	5.0	Elodea	1.0	Sand
8/12/2022	NE Edge of Bull Rush	45.78755	-89.08189	0.83	4.0	Scattered	5.0	Elodea	1.0	Sand
Total	10			19.42	49.0					