1.0 INTRODUCTION

The Cloverleaf Lakes are a chain of three spring lakes: Round Lake, Grass Lake, and Pine Lake in Shawano County (Figure 1.0-1). Three submergent non-native aquatic plants species are known to have populations in the Cloverleaf Lakes: Eurasian watermilfoil, curly-leaf pondweed, and starry stonewort.

Eurasian watermilfoil (*Myriophyllum* spicatum; EWM) was first documented in Round Lake in 1992. It was later confirmed via DNA analysis to be a hybrid between EWM and the indigenous northern water milfoil (*M. sibiricum*) in 1994. Curly-leaf pondweed (CLP, (*Potamogeton crispus*), is also present in the system and has periodically



been the target of active management along with continued monitoring. Starry stonewort (*Nitellopsis obtusa*; SSW) was first found in Pine Lake in 2021 and later confirmed from Grass Lake in 2023.

The Town of Belle Plaine and the Cloverleaf Lakes Protection Association (CLPA) have partnered on a number of projects, including Clean Boats Clean Waters (CBCW) staffing and education, enforcement, and cost-sharing on past projects. The CLPA is currently sponsoring all aquatic invasive species monitoring and management activities on the Cloverleaf Lakes.

1.1 Historic HWM Management & Planning

The Cloverleaf Lakes have a history dating back to at least 2004 during which HWM control included nearly annual 2,4-D herbicide treatments. Whole-lake 2,4-D treatments occurred in each lake between 2012 and 2013, with HWM rebound occurring within Grass and Pine Lakes within 2-3 years. Subsequent whole-lake fluridone treatments took place in Grass and Pine Lakes in 2016 and in Round Lake in 2018. In the years since the fluridone treatments, the CLPA has enacted an Integrated Pest Management (IPM) strategy of follow-up efforts largely utilizing professional hand harvesting. Expanding HWM populations in Grass Lake during 2019-2020 culminated in two spot-treatments in 2021 that utilized florpyrauxifen-benzyl, sold exclusively by SePRO corporation as ProcellaCOR. The initial result of the ProcellaCOR treatments was promising during the year of treatment, however HWM rebound was evident by 2022 in the treated sites.

The CLPA conducted a Comprehensive Management Planning project in 2021-2022, being completed and approved by WDNR in June 2022. The Plan set a trigger for considering whole-lake herbicide treatment again in each lake with an integrated pest management strategy that employs the use of hand harvesting as follow up measures after herbicide treatment.

This report details the aquatic plant monitoring activities that occurred during 2023 on the Cloverleaf Lakes including whole lake point-intercept surveys on each lake as well as a late-summer HWM mapping survey. This report also outlines an HWM management and monitoring program for 20204.



2.0 2023 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.

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. The point-intercept survey can be applied at various scales. The point-intercept survey is most often applied at the whole-lake scale. The <u>whole-lake point-intercept survey</u> has been conducted on Cloverleaf Lakes during most years from 2010-2023.

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 (Photograph 2.0-1). Field crews supplement 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 HWM locations that were considered as *small plant colonies* (<40 feet in diameter), *clumps of plants*, or *single or few plants*.



Photograph 2.0-1. EWM mapping survey. Photo credit Onterra.

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

2.1 Point-Intercept Survey

The point-intercept survey of the Cloverleaf Lakes was conducted on August 3, 2023 by Onterra staff. The point-intercept survey method as described by the Wisconsin Department of Natural Resources Bureau of Science Services, PUB-SS-1068 2010 (Hauxwell, et al., 2010) was used to complete the study. The sampling location spacing (resolution) and resulting total number of locations varied by lake and were created based upon guidance from the WDNR (Table 3.4-2). During aquatic plant surveys that have taken place on the system, a total of 44 species of plants have been located in the Cloverleaf Lakes. The most populous native aquatic plant species include muskgrasses (*Chara* spp.), wild celery (*Vallisneria americana*), and naiads (*Najas flexilis & N. guadalupensis*) (Photo 2.1-1).

Table 2.1-1. Cloverleaf Lakes point-intercept resolutions.		
Distance Between		
	Sampling Points	Number of
Lake	(meters)	Sampling Locations
Round	25	174
Grass	40	233
Pine	47	398





In the Cloverleaf Lakes, starry stonewort (SSW) has been located primarily in deeper waters, not visible from surface viewing, and therefore is difficult to understand its system-wide distribution. The point-intercept survey is the best way to understand the population dynamics in this system (Figure 2.1-1).

Starry stonewort was located at six point-intercept survey sampling locations within Pine Lake during the 2021 survey and five completely different locations during the 2022 survey. The 2023 survey found SSW to be present at nine sampling locations in Pine Lake representing a littoral frequency of occurrence of 4.1%. The 2023 findings included two repeat points from past survey and seven new locations around the lake. Figure 3.0-1 displays all locations for which SSW has been identified during the 2021-2023 point-intercept surveys.

Starry stonewort was found at one sampling location in Grass Lake during the 2023 survey, making this the first documented occurrence in the system outside of Pine Lake. The sample was vouchered and confirmed by WDNR. Starry stonewort has not been located within Round Lake to-date.





Figure 2.1-1 displays the locations HWM was located in the Cloverleaf Lakes in 2003. More discussion of HWM and its population will occur in the subsequent lake-by-lake subsections as well a Section 2.2.



Round Lake

In the 2023 point-intercept survey for Round Lake, muskgrasses (69.5%) were the most frequently encountered native aquatic plant species (Figure 2.1-2). White water lily was relative common in the lake with an occurrence of 22.0%. Eurasian watermilfoil exhibited an occurrence of 22.0%.



The occurrence of HWM in Round Lake from all point-intercept surveys is displayed on Figure 2.1-3. In the first point-intercept survey completed in 2010, the occurrence of HWM was 18.8%. HWM showed a statistically valid increase from 2013-2014 and reached 48.2% in 2017. The 2018 treatment resulted fluridone in the occurrence declining to 3.5%. From 2019-2022 the occurrence of HWM increased each year reaching 31.1% in 2022. The 2023 survey found HWM to have an occurrence of 22.0%, which was down slightly from the previous year.

Figure 2.1-4 displays the number of pointintercept survey sampling locations that contained either native plants only, HWM





plants only, or native plants and HWM plants from surveys completed in 2010-2023 in Round Lake. The data demonstrate the varying HWM population over time with fewer sampling locations containing HWM after the 2018 fluridone treatment. The number of sampling locations containing native aquatic plants has been relatively consistent in recent years.





Grass Lake

The most frequently encountered aquatic plant species in the 2023 point-intercept survey in Grass Lake were wild celery (47.2%), slender naiad (28.5%), and muskgrasses (27.8%) (Figure 2.1-5). Three non-native species were sampled during the survey including Eurasian watermilfoil (34.0%), starry stonewort (0.7%), and curly-leaf pondweed (0.7%). Other commonly encountered native species include several pondweeds, as well as common waterweed. Floating-leaf species including spatterdock and white water lily were both well represented in the survey with 7.6% and 6.9% occurrences respectively.



Intercept Survey.



The occurrence of HWM in Grass Lake from all point-intercept surveys is displayed on Figure 2.1-6. These data show large fluctuations for HWM over time in response to this species being the target of herbicide management strategies. Within 2-3 years after herbicide management, HWM has shown to rebound to relatively high levels with an occurrence above 30%. Most recently, the occurrence of HWM was lowered from 30.4% in 2020 to 1.4% in 2021 which corresponded to the year of two ProcellaCOR spot treatments in the lake that impacts expanding beyond had the application areas. The occurrence of HWM showed statistically valid increases from 2021-2022 and again from 2022-2023.



occurrence. Open circles on represent a statistically valid change from previous survey.

Figure 2.1-7 displays number of sampling locations that contained native plants, HWM and native plants, or HWM only from the point-intercept surveys in Grass Lake. These data capture the loss of HWM between 2015-2017 which corresponds with a 2016 whole-lake fluridone treatment. The figure indicates a gradually increasing HWM population from 2017-2020, followed by a loss of much of the HWM in 2021, and then another increasing population trend from 2021-2023. The number of sampling locations with native aquatic plants present has been relatively stable from 2017-2023.

Littoral Frequency of Occurrence (%)





Pine Lake

In the 2023 point-intercept survey for Pine Lake, muskgrasses (21.3%) and wild celery (20.4%) were the most frequently encountered native aquatic plant species (Figure 2.1-8). Seven native aquatic plant species were present on the survey rake during the survey as well as two non-native species. Eurasian watermilfoil was the third most frequently encountered species in the survey with an occurrence of 17.2%. Starry stonewort was present at nine sampling locations resulting in an occurrence of 4.1%





The occurrence of HWM in Pine Lake from all point-intercept surveys is displayed on Figure 2.1-9. The first survey in this dataset indicated an occurrence of HWM of 14.5% in 2010. The occurrence was reduced to 1.9% in 2013 following a whole-lake 2,4-D treatment. By 2015 the occurrence of HWM had reached 19.4% which led to the 2016 fluridone treatment strategy. Since the 2016 treatment. HWM has increased incrementally during every year, reaching 17.2% in 2023 which is approaching the levels last documented in 2015.

Figure 2.1-10 displays number of sampling locations that contained native plants, HWM and native plants, or HWM only from the point-intercept surveys in Pine Lake. These



data show the loss of HWM following the 2016 fluridone treatment along with a drop in the number of sampling locations containing native species as well. The number of sampling locations with native species and with HWM has increased every year from 2017-2021. In 2022, the number of points with

native species decreased by 27 in absence of herbicide management and the 2023 survey showed a similar proportion of natives and HWM as 2022.



2.2 Late-Season HWM Mapping Survey

Onterra field crews visited Cloverleaf Lakes on September 26, 2023 to conduct the Late-Season HWM Mapping Survey. The purpose of the survey was to search for and map all occurrences of HWM while it is expected to be near its peak growth stage. The results of the survey are displayed on Map 1. The survey found HWM present throughout many littoral areas of the lakes consistent with its historical footprint within the lakes. A few areas within Grass Lake were mapped as *surface matted* in density during the survey while much of the colonized areas around the entire system were comprised of various densities spanning from *highly scattered*, *scattered*, *dominant*, or *highly dominant*. A submersible camera was deployed in deeper waters within Round Lake in order to search for populations that were not visible from surface viewing. A total of 24.4 acres of colonized HWM were mapped during the 2023 survey including 17.5 acres in Pine Lake, 6.4 acres in Grass Lake, and 0.4 acres in Round Lake. Most of the colonized areas of HWM were growing off shore, often just out from many pier-heads in water depths of approximately 6-16'.

3.0 CONCLUSIONS & DISCUSSION

Aquatic plant monitoring completed on the Cloverleaf Lakes during 2023 showed the native species occurrence to be comparable to past surveys. The lakes have a moderate quality aquatic plant community that is dominated by muskgrasses, wild celery, and a handful of other native species, with hybrid watermilfoil also comprising a substantial population in 2023. The late-summer 2023 HWM mapping survey found HWM to be widespread in the lakes with some particularly dense areas clearly impacting recreational use of the lake.

Starry stonewort populations are showing some signs of population expansion, although at a relatively slow rate. Additional reporting and discussion specific to starry stonewort is available within reporting associated with a separate project for the CLPA.

4.0 EWM MONITORING & MANAGEMENT STRATEGY DEVELOPMENT

Action # 2 within the CLPA's 2022 Comprehensive Management Plan is to "*actively manage HWM to keep system wide population low*" as a step towards meeting Goal #3 to "*Manage AIS and Prevent Establishment of New AIS*." The action outlines the CLPA's integrated pest management strategy which utilizes herbicide application as the primary tool for HWM management, with hand harvesting employed as follow-up to herbicide treatments and for targeting HWM in strategic scale-appropriate locations. The Plan sets a threshold for when to consider herbicide spot treatments or whole-lake treatments. Whole lake treatment would be considered when the whole lake point-intercept survey is approaching 20% littoral frequency of occurrence of EWM. The 2023 point-intercept surveys confirmed the occurrence of HWM was approaching or had exceeded the trigger set in the management plan for considering herbicide treatments within each of the three lakes comprising the Cloverleaf Lakes.

The CLPA opted to move forward with a fall 2023 WDNR grant application to fund EWM herbicide treatments in spring 2024. This plan included targeting all HWM colonies in Pine & Grass Lakes would with direct application of ProcellaCOR, reaching meaningful concentrations when uniformly mixed within the entire lake (epilimnetic). These whole-lake treatments were to be conducted following Best Management Practices and result in a lake-wide concentration of approximately 1.0 ppb within Pine and Grass Lakes. For reference, the 2021 trial ProcellaCOR on Grass Lake only had a lake-wide potential of 0.26 ppb. Round Lake was not originally considered for herbicide treatment in 2024 despite meeting the trigger set forth within the management plan for consideration. Much of the HWM population in Round Lake is present in deeper waters that were not interfering with recreational uses of the lake and therefore are of lower priority for the CLPA to manage in 2024 as compared to populations in Grass Lake and Pine Lake.

Unfortunately, the AIS Control Grant was not successful. The grant cycle was very competitive, with only 4 projects receiving funding in this category statewide. After much discussion, the CLPA opted to postpone the large-scale HWM management plan potentially until spring 2025 following another grant application opportunity in fall of 2024. The CLPA will instead focus their 2024 efforts on manual removal of HWM with Diver Assisted Suction Harvesting (DASH) in high-use areas, as well as conducting financial fund raising activities in the event that future grants continue to be difficult to obtain.





