1.0 INTRODUCTION

Pigeon Lake is a deep headwater drainage lake in Manitowoc County consisting of 82 acres (Photograph 1.0-1). During the summer of 2015, Onterra conducted an acoustic-based bathymetric study of the lake. These data indicate that the lake has a maximum depth of 66 feet.

Photograph 1.0-1. Pigeon Lake, Manitowoc County, Wisconsin.

Eurasian watermilfoil (EWM) was first confirmed in Pigeon Lake in 1994. During the 2013 Onterra led surveys, two separate milfoil samples were sent into the Annis Water Resource Institute at Grand Valley State University in Michigan for DNA analysis. The analysis confirmed that the samples were both pure strain EWM and not a hybrid species. Hybrid watermilfoil, (*Myriophyllum sibiricum X M. spicatum*), a cross between Eurasian watermilfoil and the indigenous northern watermilfoil, is commonly mistaken for Eurasian watermilfoil or northern watermilfoil. Some strains of hybrid watermilfoil have been shown to be less susceptible to biological and certain herbicide control strategies (including 2,4-D). Nearby Manitowoc County lakes with hybrid watermilfoil include Silver Lake, Shoe Lake, Carstens Lake, and English Lake.

Pigeon Lake of Manitowoc County (PLMC) is the local citizen-based organization leading the management of Pigeon Lake. The group has worked for years to protect and enhance the lake and in 2013 received a Lake Management Planning Grant to complete a *Comprehensive Management Plan* for the lake with assistance from WDNR Grant Funds (AEPP-401-13). The plan was completed by Onterra, and accepted by the WDNR in July 2014. One of the goals outlined in the management plan is to "Develop monitoring and control strategy for EWM in Pigeon Lake".

In 2014, PLMC funds were utilized to have Onterra complete an EWM mapping survey during late-summer and found that the EWM population expanded in both area and density since 2013. The PLMC elected to move forward with the development of a control and monitoring strategy for a subsequent large-scale treatment. The PLMC applied for WDNR AIS-EPP Grants (<$10K sub-category) in December 2014 and 2015, both attempts being unsuccessful. As the EWM population continued to increase, the PLMC authorized a full suite of pretreatment surveys to occur in 2016 with a subsequent AIS-EPC Grant being applied for in February 2017. The PLMC also secured financial partnership from the Town of Liberty. Based on the 2016 late-summer EWM mapping survey, the EWM population was found to increasingly comprise a larger percent of the littoral zone and increase in density. With Onterra's assistance, the PLMC developed a three-year control and monitoring strategy in which a large-scale 2,4-D treatment would occur in year two (2017) of the project. A liquid 2,4-D herbicide treatment occurred in late-April 2017 with measured whole-lake epilimnetic concentrations near the target concentration of .375 ppm. Herbicide persistence was longer than anticipated, with concentrations exceeding the irrigation threshold (0.1 ppm ae) for almost 100 days.

The 2017 whole-lake 2,4-D treatment was highly effective on controlling EWM, with no EWM being located during late-summer surveys (point-intercept and meander-based) in the *year-of-treatment* (2017) or *year-after-treatment* (2018). *Two years-after-treatment* (2019) showed continued promising results with only a *single or few plants* being found which was subsequently removed by a contracted

professional hand harvesting firm. No EWM was located in Pigeon Lake during an August 2020 EWM mapping survey.

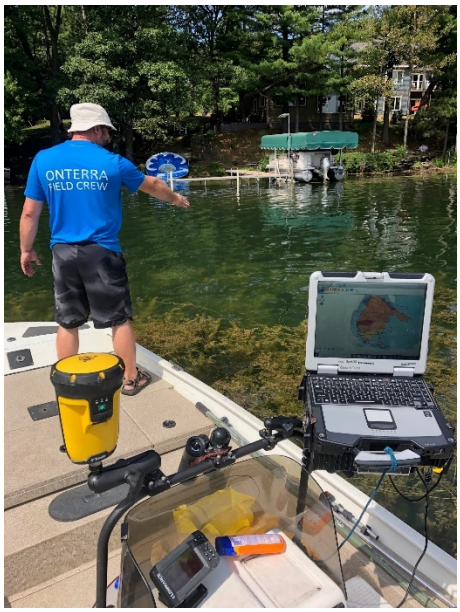
The PLMC contracted with Onterra to continue monitoring EWM in Pigeon Lake through the completion of an EWM mapping survey in August 2021. Upon the discovery of EWM, the PLMC would be prepared to solicit divers to harvest the EWM if applicable. Additionally, the PLMC contracted with Onterra to complete a whole-lake point-intercept survey in summer 2021 in order to track the overall aquatic plant community in Pigeon Lake. This report discusses the results of the EWM monitoring survey and presents the results of the 2021 whole-lake point-intercept survey with comparisons to previous surveys.

2.0 2021 MONITORING RESULTS

It is important to note that two types of aquatic plant surveys are discussed in the subsequent materials: 1) whole lake point-intercept survey 2) Late-season EWM mapping survey.

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 whole-lake point-intercept survey has been conducted on the Pigeon Lake in 2005, 2012, 2016, 2017, 2018, and 2021. A whole-lake point-intercept survey was completed in 2021 consistent with the WDNR's recommended 5-years or less interval between surveys.

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 (Photograph 2.0-1). 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*.



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

Overall, each survey has its strengths and weaknesses, which is why both are utilized in different ways. A whole-lake point-intercept survey and a late-summer EWM mapping survey occurred in 2021 on Pigeon Lake which are discussed within the subsequent sections of this report.

2.1 Point-Intercept Survey

Onterra completed a whole-lake point-intercept survey in Pigeon Lake on July 8, 2021 as a part of maintaining periodic (3-4 year interval) quantitative vegetation monitoring. The point-intercept method as described in the WDNR publication (WDNR PUB-SS-1068 2010) was used to complete this study. This survey allows for a quantitative analysis of the aquatic plant community in the lake and is directly comparable to past or future surveys completed with the same methodology. Whole-lake point intercept surveys have been also been conducted on Pigeon Lake in 2005, 2012, and 2016-2018. The results of the 2021 point-intercept survey are highlighted below as well as a comparison of the previous surveys that have been completed to date.

Littoral frequency of occurrence is used to describe how often each species occurred in the points that are within the maximum depth of plant growth (littoral zone), and is displayed as a percentage. Figure 2.1-1 displays the littoral frequency of occurrence of aquatic plant species in Pigeon Lake in the 2021 point-intercept survey. A total of 20 native aquatic plant species were identified in Pigeon Lake during the 2021 survey with aquatic plants extending down to 24 feet deep in the lake.

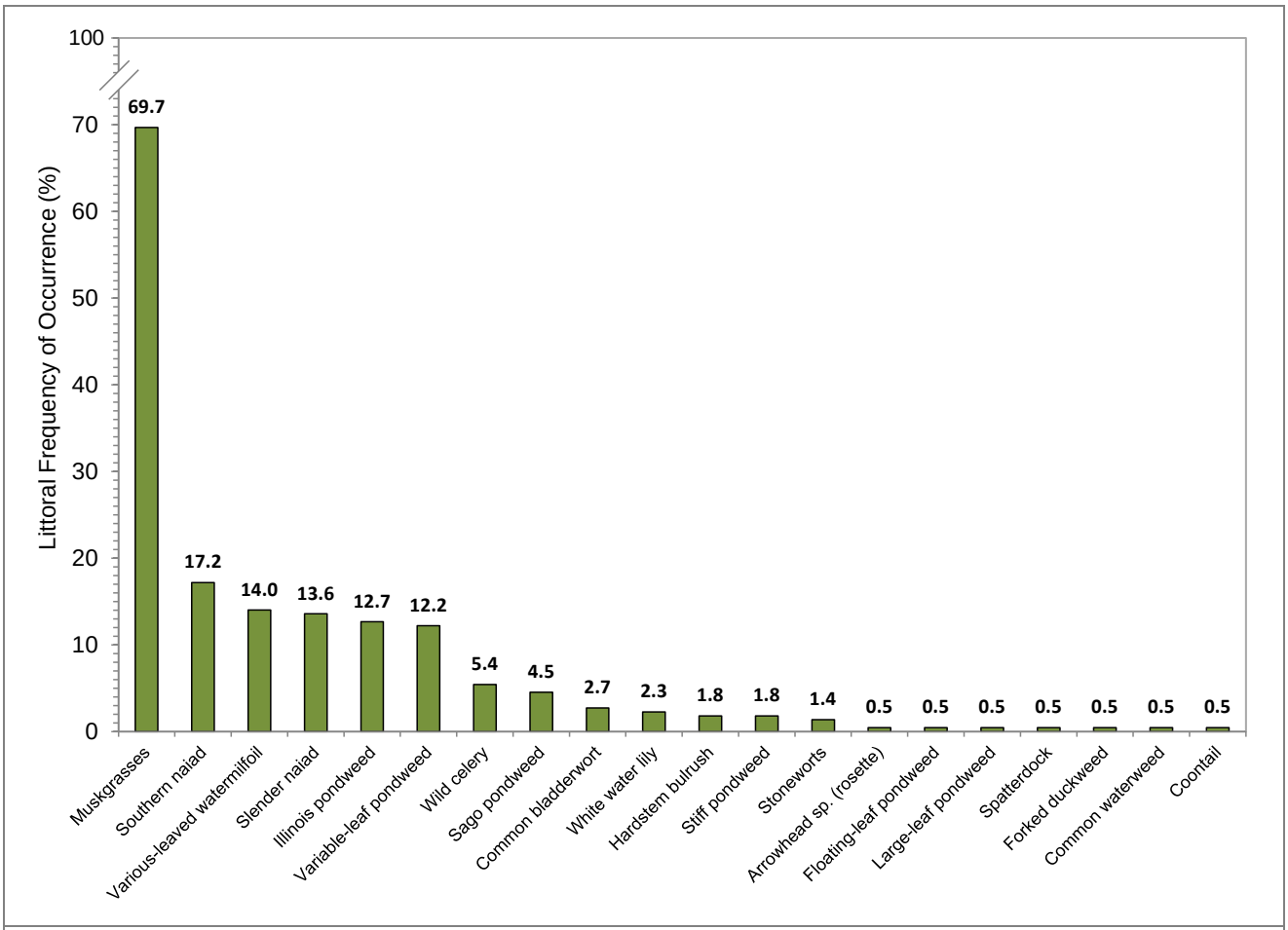


Figure 2.1-1. Pigeon Lake littoral frequency of occurrence of aquatic plant species from a 2021 point-intercept survey.

A comparison of the point-intercept surveys allows for detecting changes in the aquatic plant community over time. The average number of native species per sampling location within the littoral zone of the lake has been relatively stable between 1.57 and 1.84 species/site from 2012-2021 (Figure 2.1-2). The lowest recorded value for this metric occurred in the first survey conducted during 2005 with 0.97 species per site. The 2021 survey found 1.62 species per site which was slightly lower than the 1.84 species documented in the previous survey completed in 2018.

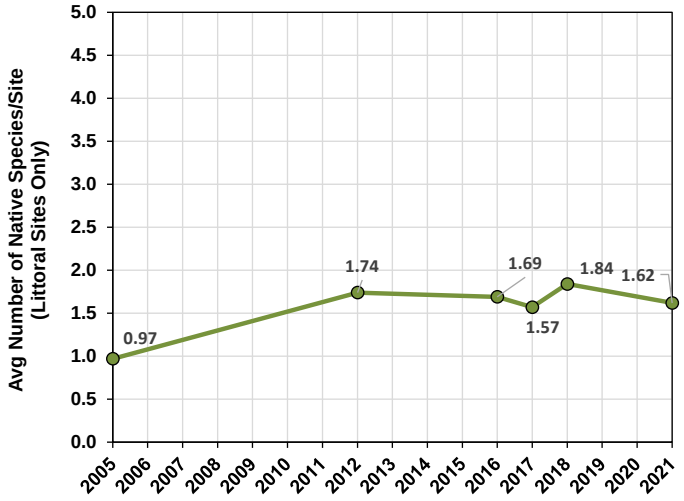


Figure 2.1-2. Average number of native aquatic plant species in Pigeon Lake from point-intercept surveys.

Muskgrasses were the most frequently encountered aquatic plant species in Pigeon Lake in the 2021 survey with an occurrence of 69.7%. Muskgrasses are a genus of macroalgae of which there are seven species in Wisconsin (Photo 2.1-1). Dominance of the aquatic plant community by muskgrasses is common in hardwater lakes like Pigeon Lake, and these macroalgae have been found to be more competitive against vascular plants (e.g. pondweeds, milfoils, etc.) in lakes with higher concentrations of calcium carbonate in the sediment (Kufel and Kufel 2002; Wetzel 2001). Muskgrasses require lakes with good water clarity, and their large beds stabilize bottom sediments. Studies have also shown that muskgrasses sequester phosphorus in the calcium carbonate incrustations which from on these plants, aiding in improving water quality by making the phosphorus unavailable to phytoplankton (Coops 2002). Due to their lack of vascular tissue, muskgrasses are unable to translocate herbicides; therefore, they are typically unaffected by herbicide use. As shown on Figure 2.1-3, muskgrasses were largely unimpacted by the 2017 control action and actually exhibited non-statistically valid population increases between 2016, 2017, and 2018.

Photo 2.1-1. The aquatic macroalgae muskgrasses (*Chara* spp.). Photo credit Onterra.

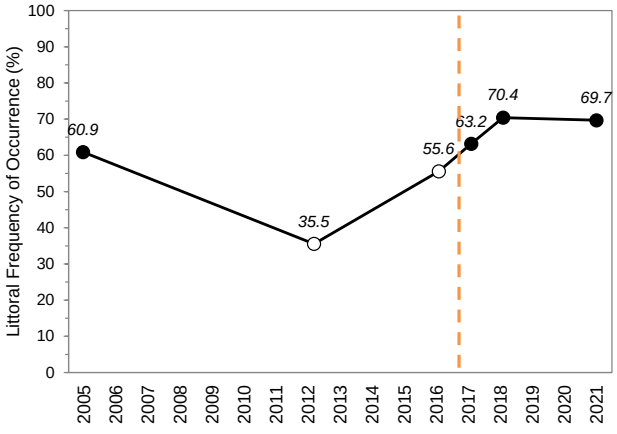


Figure 2.1-3. Littoral occurrence of muskgrasses in Pigeon Lake. Open circle indicates that occurrence is statistically different from previous survey (Chi-Square α = 0.05).

Together, naiad species are some of the most frequently encountered true-plants within Pigeon Lake. Slender naiad is a submersed annual plant that produces numerous seeds. Slender naiad is considered to be one of the most important sources of food for a number of migratory waterfowl species (Borman et al. 2014). In addition, slender naiad's small, condensed network of leaves provide excellent habitat for aquatic invertebrates. Southern naiad is similar to slender naiad, and they are often difficult to separate in a field setting. While southern naiad is native to North America, observations have been indicating that populations of this plant have been expanding and behaving invasively, particularly in northern Wisconsin lakes. It is not known if this behavior represents recent introductions of these plants to waterbodies where it was not found naturally, or if certain environmental conditions are favoring the expansion of southern naiad. Due to the uncertainty in consistent identification of these species over the years, the occurrences of slender and southern naiad are combined when comparing the 2021 data to previous point-intercept surveys.

Slender naiad has been shown to be particularly susceptible to large-scale 2,4-D treatments during the year of treatment (Nault et al. 2018). Onterra's experience is that slender naiad populations often rebound as early as the year after treatment, sometimes exceeding pretreatment levels. After exhibiting a statistically valid decrease in occurrence during the year of the whole-lake 2,4-D treatment, slender and southern naiad showed this rebound in 2018 returning to slightly above the pre-treatment occurrence documented in 2016. The population of naiads exhibited a statistically valid reduction in occurrence between 2018 and 2021 but an occurrence of 30.8% in the 2021 survey indicates a significant population remains present in the lake (Figure 2.1-4).

Photo 2.1-2. Slender naiad (*Najas flexilis*; left) and southern naiad (*N. guadalupensis*; right). Photo credit Onterra.

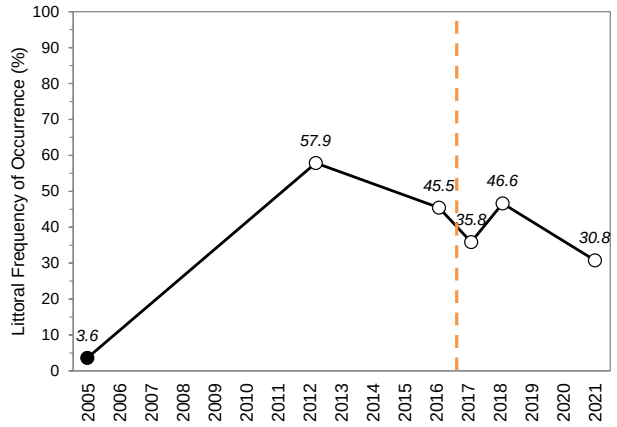


Figure 2.1-4. Littoral occurrence of slender and southern naiad in Pigeon Lake. Open circle indicates that occurrence is statistically different from previous survey (Chi-Square α = 0.05).

The 2021 point-intercept survey yielded an EWM littoral frequency of occurrence of 0% and EWM has not been present on any of the three point-intercept surveys that have occurred since the 2017 herbicide treatment (Figure 2.1-5). Prior to treatment, EWM had an occurrence of 18.2% in 2005, 7.6% in 2012, and 23.2% in 2016.

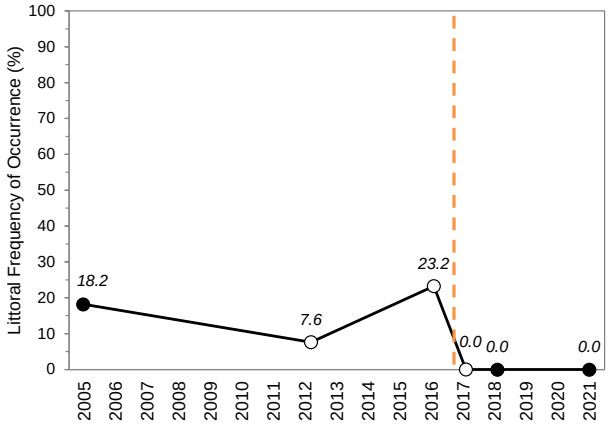


Figure 2.1-5. Pigeon Lake Eurasian watermilfoil occurrence from 2005-2021. Open circle indicates that occurrence is statistically different from previous survey (Chi-Square α = 0.05). Dashed line indicates large-scale herbicide treatment.

Various-leaved watermilfoil exhibited a statistically valid increase in occurrence from 6.7% in 2018 to 14.0% in 2021 (Figure 2.1-6). This species can be confused with EWM due to morphological similarities between the two species. In 2021, various-leaved milfoil was common in Pigeon Lake with flowering stalks protruding above the waters' surface in many locations of the lake (Photo 2.1-3). Following the 2017 herbicide treatment, reductions in both various-leaved watermilfoil and northern watermilfoil were documented, although various-leaved watermilfoil populations have recovered while the northern watermilfoil (*Myriophyllum sibiricum*) has not been detected in the lake since.

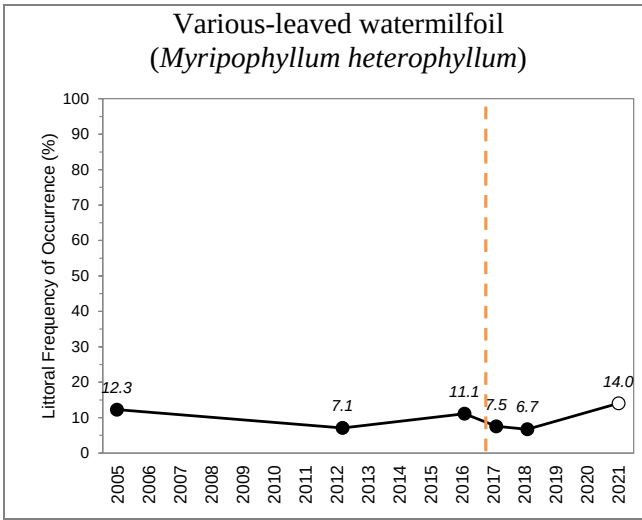


Figure 2.1-6. Littoral occurrence of various-leaved watermilfoil in Pigeon Lake. Open circle indicates that occurrence is statistically different from previous survey (Chi-Square α = 0.05). Orange dashed line indicates the 2017 large-scale herbicide treatment.

Photo 2.1-3. Various-leaved watermilfoil in Pigeon Lake. Photo credit Onterra.

The littoral frequency of occurrence of four additional species that are common in Pigeon Lake are highlighted in Figure 2.1-7. The occurrence of Illinois pondweed exhibited a statistically valid increase in occurrence from 2018 to 2021. The occurrence of this species has been variable over the period of study with occurrences ranging as low as 2.2% in 2018 and 29.9% in 2012. The occurrence of variable-leaved pondweed has been relatively stable since 2012 with and exhibited an occurrence of 12.2% in the

2021 survey. Sago pondweed and wild celery each showed statistically valid decreases in occurrence between 2018 and 2021.

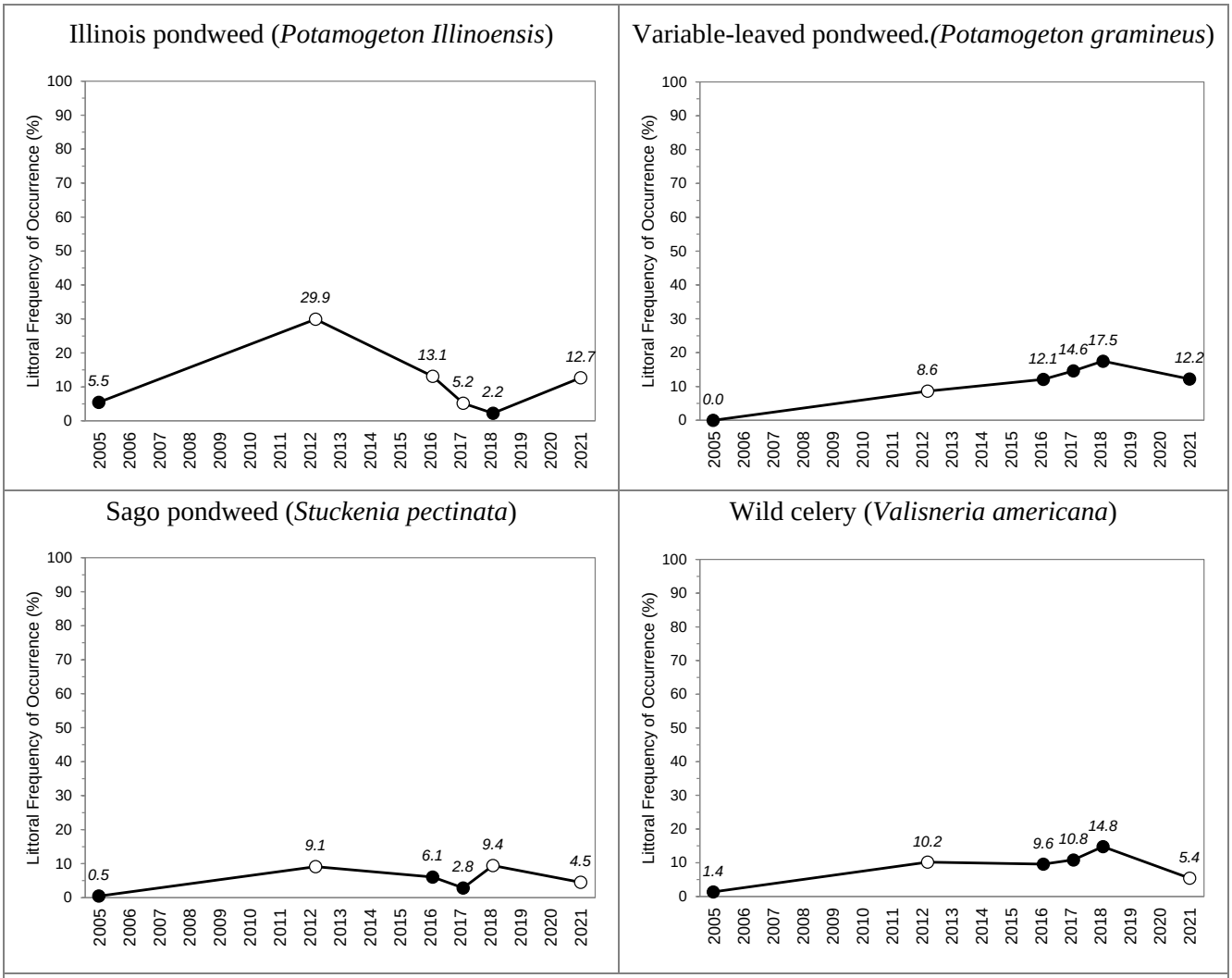


Figure 2.1-7. Littoral frequency of occurrence of select native aquatic plant species from 2005-2021 point-intercept surveys in Pigeon Lake. Open circle represents statistically valid change in occurrence from previous survey.

The data that continues to be collected from Wisconsin lake's is revealing that aquatic plant communities are highly dynamic, and populations of individual species have the capacity to fluctuate, sometimes greatly, in their occurrence from year to year and over longer periods of time. These fluctuations can be driven by a combination of natural factors including variations in temperature, ice and snow cover (winter light availability), nutrient availability, water levels and flow, water clarity, length of the growing season, herbivory, disease, and competition (Lacoul and Freedman 2006). Adding to the complexity of factors which affect aquatic plant community dynamics, human-related disturbances such as the application of herbicides for non-native plant management, mechanical harvesting, watercraft use, and pollution runoff also affect aquatic plant community composition (Asplund and Cook 1997; Lacoul and Freedman 2006).

2.2 Late-Summer EWM Peak-biomass Survey

Onterra ecologists visited Pigeon Lake on August 17, 2021 to conduct a Late-Summer EWM Mapping Survey to search the lake for signs of any resurgent population (Photograph 2.2-1). The survey crew noted great visibility with favorable weather conditions and a Secchi disk measurement of 8.5 feet. The entire littoral area of the lake was searched during the visit with some extra focus given to all areas where EWM has been located previously. The field crew supplemented the visual survey with the select use of a submersible camera in areas of the lake where EWM had been located in the past. No EWM was located anywhere in Pigeon Lake during the course of the survey. It was noted that various-leaved watermilfoil dominated many areas of the lake where EWM had once been present.

Photograph 2.2-1. Pigeon Lake during an August 2021 EWM mapping survey.

3.0 CONCLUSION AND DISCUSSION

The 2021 whole-lake point-intercept survey shows Pigeon Lake continues to harbor a diverse population of native aquatic plant species. Some species showed statistically valid changes in occurrence since the previous point-intercept survey and these changes are likely due to natural year-to-year population variability driven by environmental factors.

The EWM population has remained very low in Pigeon Lake since the 2017 whole-lake herbicide treatment with none being located in 2021. The 2017 treatment was highly effective on controlling EWM, with very little to no EWM being located during late-summer surveys (point-intercept and meander-based) since 2017. It is possible that EWM may be still present in Pigeon Lake; however, the population is at a density that is below detection limits with the recent monitoring methodologies.

The PLMC should give consideration for continued monitoring for any remnant or resurgent EWM in 2022. It is encouraged that volunteers from the PLMC periodically search Pigeon Lake for suspected EWM plants during 2022. If volunteers detect suspected EWM, the location should be marked with GPS coordinates and then the PLMC may consider contracting for a professional EWM mapping survey or for a follow-up hand harvesting effort.

The PLMC may give consideration for a continued professional EWM monitoring effort in 2022 through the completion of a late-season EWM mapping survey. Similar to 2021, the PLMC may consider timing the survey in mid to late-summer to allow sufficient time to enact a hand harvesting effort in the event EWM is found.