MINNEHAHA CREEK WATERSHED DISTRICT

QUALITY OF WATER QUALITY OF LIFE

MEMORANDUM

DATE: January 26, 2017

TO: MCWD Board of Managers

FROM: Eric Fieldseth, MCWD AIS Program Manager

RE: Hybrid Milfoil Study Report

The invasive Eurasian Watermilfoil and the native Northern Watermilfoil have been known to be able to hybridize, and published literature has indicated that Hybrid Watermilfoil can be more resistant to herbicides. It's not well understood how herbicide treatments, or a history of little herbicide treatment, may influence the genetics of a population, and how that can influence management outcomes. It is also not well understood the current status of Hybrid Watermilfoil in lakes in the MCWD.

During the last 2 years, the MCWD has received AIS grant funding from Hennepin County to work with researchers from Montana State University and the University of Minnesota to better understand Hybrid Milfoil in Lake Minnetonka and Christmas Lake, and assess if hybrid populations are potentially influenced by herbicide treatments. The attached report is a culmination of work during those 2 years, and has several key takeaways that improved our understanding of hybrid milfoil, and has prompted several areas that warrant further research that could improve AIS prevention and management strategies for Eurasian Watermilfoil.

Key Takeways

- There are multiple genotypes of Eurasian, Northern and Hybrid Watermilfoils (meaning within each species, there are unique genetic strains).
- Some of these unique strains of hybrid appear to be more resistant to herbicides, and they may be even more aggressive spreaders. More research is needed on these specific strains that could impact prevention and management strategies for invasive milfoil.
- Hybrids generally appear more common in herbicide-treated lakes/bays.
- Northern watermilfoil (native species) was not present in herbicide-treated bays (the assumption is large-scale herbicide treatments may eliminate northern watermilfoil and push the population to more hybrids, which may have more resistance to herbicides in some cases).
- Only one sample of Hybrid was found in Christmas Lake, and there was a fairly even mix of native Northern watermilfoil and invasive Eurasian watermilfoil. This lake has had very limited management of Eurasian watermilfoil, especially in regards to herbicide treatments.

Conclusion

- There is a need to incorporate genetic testing into a more adaptive management framework for Eurasian Watermilfoil. Management actions can change the genetic makeup of a population, and there needs to be a series of monitoring and evaluation after treatment to inform future treatment options. Different formulations and rates of herbicides may be needed to provide better control some of these hybrid milfoil strains.

Next Steps

- This report has been shared with other researchers, state agency staff, and other local government agencies and lake associations in the District involved in milfoil management.

  There is also a grant proposal being developed by Montana State University to the Great Lakes Restoration Initiative (GLRI) to create a public and private regional partnership among industry professionals from Minnesota, Wisconsin and Michigan to take the research we performed, as well as the work of others across the region, and
  1.) Expand the study to more lakes
  2.) Do the necessary lab work to further evaluate these different hybrid milfoil strains and their response to different treatments
  3.) Evaluate the long-term value and economic feasibility of implementing a protocol to consider the impact of genetic variation on milfoil management outcomes as part of best practices for adaptive management of Eurasian watermilfoil.

  MCWD role in GLRI Proposal: The MCWD has been asked to participate in the role of providing some sampling on Lake Minnetonka and send plant samples to researchers at Montana State University, as well provide input on the evaluation of genetic sampling as part of an adaptive management strategy and to help disseminate the results. There is no financial ask of the District, and a limited amount of staff time would be provided.

Occurrence and Distribution of Eurasian, Northern and Hybrid Watermilfoil in Lake Minnetonka and Christmas Lake: Genetic Analysis Phase II

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Funding provided by Hennepin County, MN – AIS Grant
January 4, 2017

Summary:

Eurasian watermilfoil (*Myriophyllum spicatum*) can hybridize with the native northern watermilfoil (*M. sibiricum*) and all three taxa, Eurasian, northern and hybrid watermilfoil are present in Minnesota, but their occurrence and distribution is not well documented. Recent studies elsewhere indicate that some genotypes of hybrid watermilfoil can be tolerant of some auxin-mimic herbicides, leading to concern that treatments with these herbicides could select for genotypes that will be more difficult to control. Using microsatellite markers, we examined the genetic composition of watermilfoils in 2015 in three bays of Lake Minnetonka (Grays, North Arm and St. Albans) that are being managed with auxin-mimic herbicides to control Eurasian watermilfoil. In addition, we examined two bays (Smiths and Veterans) and one lake (Christmas Lake) in 2016 that have not been extensively managed with herbicides.

Eurasian, northern and hybrid watermilfoil genotypes were found throughout the study area. However, northern watermilfoil was only found in the untreated water bodies, and at relatively shallower depths compared to pure and hybrid Eurasian watermilfoil. Pure Eurasian watermilfoil was the dominant taxon in all three untreated water bodies. In contrast, hybrid watermilfoil was the dominant taxon in two of the treated water bodies (Grays and North Arm), and was the only watermilfoil found post-treatment in the third water body (St. Albans) despite pure Eurasian watermilfoil being dominant there in June before the herbicide treatment. The apparent association between taxonomic composition and treatment history suggests that intensively managed lakes may be more likely to become dominated by hybrid watermilfoil and less likely to harbor native northern watermilfoil, and this hypothesis warrants further investigation through laboratory and field study.

Genetic diversity for all three taxa indicates that sexual reproduction is common. Northern watermilfoil individuals were the most genetically diverse, followed by hybrid watermilfoil, and finally Eurasian watermilfoil. However, we also found clear evidence of extensive clonal reproduction, especially for a few hybrid and Eurasian watermilfoil genotypes that were found in numerous individuals from several water bodies. Water bodies also tended to harbor different genotypes, suggesting that sexual reproduction occurs independently in different water bodies, but that clonal reproduction can lead to extensive spread of specific genotypes within and among water bodies. This allows for the possibility that sexual reproduction generates a diversity of genotypes that may differ in their growth and herbicide response properties, which could facilitate evolutionary dynamics related to the evolution of increased competitive vegetative growth (invasiveness) or herbicide resistance.

Our temporal analysis of the three treated bays provides evidence of genetic dynamics that could indicate ongoing evolution of invasiveness and resistance. Of the three treated bays, Grays and St. Albans Bays had a relatively higher degree of herbicidal control compared to North Arm Bay. Although North Arm Bay was dominated by several hybrid genotypes even before treatment, we observed a rapid shift in the genetic composition post-treatment. In particular, we observed a large increase in the proportion of one genotype. It is unclear whether this rapid shift was due to invasive traits such as elevated growth rate or herbicide tolerance, or whether it was due to chance associated with a population bottleneck (reduction in amount of watermilfoil) from the herbicide treatments. Interestingly, the same genotype was the only genotype found in St. Albans Bay post-treatment, and this genotype was not found in pre- or post-treatment samples from Grays Bay, where the herbicide treatments were effective. Taken together, these results identify this genotype as a possible auxin-tolerant and/or faster spreading genotype, and further lab and field studies should explicitly test this hypothesis.

Overall, our study highlights some potential benefits of integrating genetic analysis into watermilfoil management projects. Specifically, genotype data can help inform adaptive management planning and evaluation by identifying associations between genetic composition and management history and control actions. In particular, temporal genetic monitoring may identify shifts in composition that may be associated with differences in management-relevant traits such as growth and herbicide response. Genetic analysis can identify specific genotypes of interest that can then be studied explicitly to inform control options, including trigger points for switching specific control techniques.

Background

Eurasian watermilfoil (*Myriophyllum spicatum*) can hybridize with the native northern watermilfoil (*M. sibiricum*) (Moody and Les 2007) and recent work has shown that hybrid milfoil can grow faster and may be more tolerant of herbicides (e.g., 2, 4-d) than Eurasian watermilfoil (LaRue et al. 2013a). Previous work (e.g., Moody and Les 2007) has shown that the native northern, Eurasian and hybrid watermilfoils are all present in Minnesota and Lake Minnetonka, but those data are old (early 2000's) and of limited scope, and used lower resolution methods.

During the past seven years, extensive, often baywide, herbicide treatments to control Eurasian watermilfoil have been conducted in bays of Lake Minnetonka (LMCD AIS Subcommittee 2012, Netherland and Jones 2015) and it is currently unclear what impact these treatments may have on the genetic structure of populations, including potentially selecting for more herbicide tolerant genotypes. Recent studies elsewhere indicate that some genotypes of hybrid watermilfoil may be more tolerant of some auxin-mimic herbicides (LaRue et al. 2013a; Parks et al. 2016; unpublished data), leading to concern that treatments with these herbicides could select for genotypes that will lead to diminishing control efficacy over time in lakes repeatedly treated with the same herbicides and use patterns. However, there are no data addressing this emerging issue.

While a comprehensive understanding of the potential for herbicide resistance in watermilfoil will require numerous and detailed studies of laboratory dose-response curves of different genotypes and careful field evaluation/confirmation of predicted efficacy, an important first step for any lake management project considering the potential for resistance evolution is to characterize the composition of managed water bodies. This characterization includes using existing genetic methods to distinguish between pure and hybrid Eurasian watermilfoil, as there is evidence indicating that these can exhibit different growth and herbicide response properties. In addition, genetic methods can be used to distinguish different genotypes of Eurasian and hybrid watermilfoil, as different genotypes can exhibit different growth and herbicide response properties (Glomski and Netherland 2010; Berger et al. 2012, 2015; Thum et al. 2012; Taylor et al. submitted).

Genotypic characterization of watermilfoil in water bodies could inform adaptive management of watermilfoil populations. Understanding whether a water body is genetically diverse versus dominated by a single genotype could provide valuable information. Water bodies that are genetically diverse may represent a diverse set of management-relevant phenotypes, such as growth rate, reproductive potential, and herbicide response. Diverse water bodies may hold high potential for rapid adaptation to local environmental conditions and management activities, and would warrant careful monitoring to determine whether effects of specific control techniques were proportionally or disproportionally effective on different genotypes. On the other hand, domination by a single genotype suggests there is little genetic variation for response to control activities. However, it is important to note that domination by a single genotype could be the result of previous selection for a specific genotype that exhibits tolerance to previous management efforts. In either case, dominant genotypes could be targeted for laboratory herbicide response spectrum studies to evaluate herbicide options. Finally, temporal monitoring of genotypes in populations could identify shifts in genetic composition over time that are possibly associated with control efforts. Genotypes that increase in relative abundance over time could be targeted for laboratory study of growth rate and herbicide response, which could identify trigger points for managers to alter their control techniques.

This project sought to characterize watermilfoil genotypes in three Lake Minnetonka Bays (Grays, St. Albans, and North Arm) that have had extensive herbicide treatment(s) to control Eurasian watermilfoil and two bays (Smiths and Veterans) and an adjacent lake (Christmas Lake) that have had little to no recent milfoil management with herbicides.

This project specifically asks the following questions:
1) What is the taxonomic composition of watermilfoils (Eurasian, northern, and hybrid) in Minnetonka Bays and Christmas Lake?
2) Does the composition differ in herbicide-treated versus untreated lakes?
3) Does the composition change within treated lakes over time or before and after treatment?
4) Are hybrid watermilfoil populations genetically distinct in different water bodies, and is there any relationship between genetic composition and management history?

Methods

Point-Intercept Surveys

Treatment Bays - The plant community at each treated bay was assessed with point-intercept surveys before and after herbicide treatment. The treatments on the three bays occurred as part of ongoing management by the Lake Minnetonka Association; all three were treated with Triclopyr herbicide in June 2015. At each Bay, a grid was created across the bay, with increased spacing within treatment sites. Grays Bay had 70m spacing, with 35m spacing within the treatment sites. Grays was surveyed on June 15, 2015 and again on August 31, 2015. The survey resulted in 227 sample points within the 4.6m littoral zone, with 101 of those points coming from increased spacing in the treatment sites. St. Albans Bay had 50m spacing, with 25m spacing in the treatment sites. It was surveyed on June 8, 2015 and again on September 1, 2015. That resulted in 249 sample points in the littoral area, with 119 of those coming from increased spacing. North Arm Bay had 50m spacing, with 25m spacing in the treatment sites. It was surveyed on June 9, 2015 and again on September 3, 2015. That resulted in 341 sample points in the littoral area, with 80 of those coming from increased spacing.

At each sample point, depth was recorded and a weighted, double-headed rake was tossed, allowed to sink to the bottom, and retrieved to collect plants. Plants were given a relative density rating of 0 to 4 and each taxon present was recorded. At each point, if watermilfoil was found, a stem (top 50 cm) was cut off, rinsed off in water, and wrapped in a wet paper towel and placed in a sealable plastic bag and placed on ice in a cooler. Additional stems were also collected from each point watermilfoil was found, but they were from plants found at least 1m apart so as to not represent the same plant. All samples were immediately refrigerated back at the MCWD lab until they were shipped on ice to the Thum Lab at Montana State.

Untreated Bays/Lakes - The plant community at each untreated lake was assessed with point intercept surveys. At Christmas Lake, a 50m grid was created (109 sample points within the 4.6m littoral area) and sampled on July 5, 2016. On Smiths Bay, a 75m grid was created and sampled on July 12 (295 points in the 4.6m littoral area) and September 1, 2016 (292 points). On Veterans Bay, a 50m grid (90 points sampled within the 4.6m littoral area) was created and sampled on July 11 and September 2, 2016. We were only able to analyze the July samples for this project, but preserved the September samples for analysis in a future project.

Sampling methods and collection of plants for genetic analyses followed the same methods as for the treated lakes. However, only watermilfoil species were accounted for during the surveys of the untreated water bodies due to limited time and previous surveys conducted in 2015 that assessed the entire plant community.

Genetic analyses – Plant samples from early season (June) and late season (August) point intercept surveys from 2015 in Grays, St. Albans and North Arm were sent to Thum's lab at Montana State University for genetic analysis. Samples from untreated lakes were collected from

point intercept surveys in early July 2016 and also sent for analysis. The accompanying table describes the total number of plants and survey sites collected from each water body from these surveys. One to five plants were sampled per survey point. We extracted DNA from at least one plant from each survey point where plants were collected for genetic analysis. In some instances, the resulting genetic data did not pass quality control criteria, and those samples were omitted from the analysis. Nevertheless, our final dataset included one plant from nearly all sites in a given water body.

	Lake Type	"Early" No. collection sites (total no. plants collected)	"Late" No. collection sites (total no. plants collected)
Grays (2015)	Treated	117 (287)	4 (12)
St. Albans (2015)	Treated	58 (160)	3 (9)
North Arm (2015)	Treated	50 (107)	28 (77)
Veterans (2016)	Untreated	27 (63)	
Smiths (2016)	Untreated	96 (235)	
Christmas (2016)	Untreated	45 (109)	

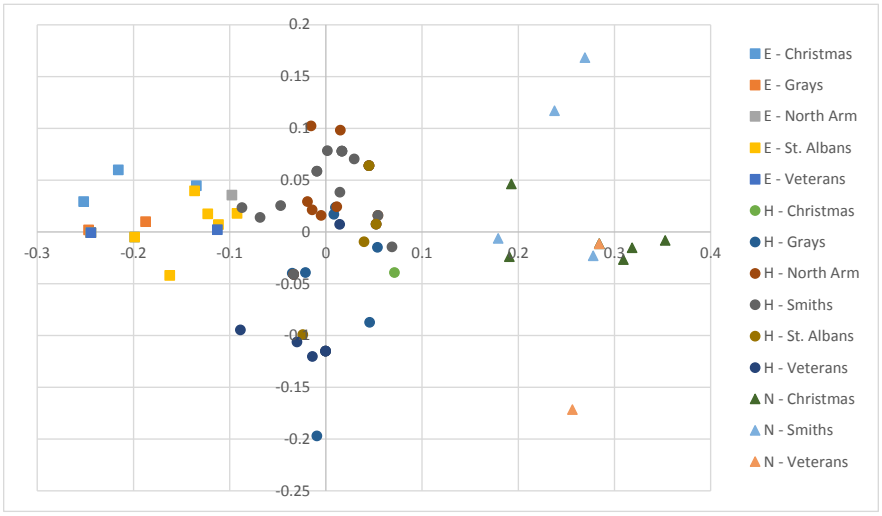
Genetic data from the first project (Thum et al. 2016) consisted of amplified fragment length polymorphisms (AFLPs), and were collected using standard methods employed in previous watermilfoil research in the Thum lab (Zuellig and Thum 2012, LaRue et al. 2013a and b). While these data clearly distinguished EWM, NWM, and HWM, and were useful in detecting patterns of overall genetic similarity and differentiation among populations, the AFLP data were difficult to distinguish specific genotypes. Therefore, in this 2016 project, we used seven microsatellite markers to genotype our samples (Myrsp1, Myrsp5, Myrsp9, Myrsp12, Myrsp13, Myrsp15, and Myrsp16 from Wu et al. 2013), because these markers are much easier to unambiguously score compared to AFLPs. We assumed that two individuals having the same microsatellite genotype across these seven microsatellite markers represent the same genetic individual (clone). We scored the microsatellite data using GeneMapper v 4.0 (Applied Biosystems). We used POLYSAT (Clark and Jasieniuk 2011) to distinguish unique clones based on the seven microsatellite loci and determine their frequencies of occurrence in each water body. We used a Principal Coordinates Analysis (PCoA) implemented in GenAlex v 6.5 (Peakall and Smouse 2006, 2012) to identify unique clones to their respective taxon (EWM, NWM, HWM), with reference to the AFLP identifications performed in the previous project in 2015 (see Thum et al. 2015, previous report).

We used the genetic data to determine the locations and frequencies of occurrence of different taxa (Eurasian, northern, hybrid) and specific microsatellite genotypes for each taxon. For treated lakes, we note that intercept points had half the spacing in treated areas compared to untreated areas. Therefore, there may be some bias in our occurrence data if there are differences in taxonomic or genotypic composition between treated and untreated areas. However, we used the same intercept grids pre- and post-treatment, so the data from the two time points are comparable.

For a small proportion of individuals, microsatellite data for did not pass quality control. When this occurred, we used the previous AFLP data to assign these individuals to taxon for determining taxonomic composition.

Results & Discussion

Taxonomic composition of treated and untreated lakes – The figure below is a Principal Coordinates Analysis (PCoA) of the 59 unique microsatellite genotypes identified from a total of 378 individuals processed across the six water bodies (see also the table below). The PCoA analysis clearly distinguishes northern watermilfoil (NWM) on the right side of the figure (triangles; population labels preceded by an "N" in the legend). Hybrids occur in the middle portion of the figure (HWM; circles; populations preceded by an "H" in the legend). Finally, Eurasian watermilfoil occur on the left side of the figure (EWM; squares; populations preceded by an "E" in the legend). Note that while EWM and HWM are clearly distinct, they "blend together" more than HWM and NWM. This may indicate more extensive introgression of EWM genes into hybrids compared to HWM, but it isn't certain. The species identifications of genotypes were based on previous AFLP data (see previous report), which clearly distinguished all three taxa.



The table below shows the taxonomic composition of the six water bodies based on the microsatellite genotypes (see figure above). We collected microsatellite data from one individual per point-intercept sampling site. However, some samples did not pass quality control and were omitted from the analysis.

	PRE-TREATMENT			POST-TREATMENT		
	EWM	HWM	NWM	EWM	HWM	NWM
Grays (2015)	1	88		1	2	
North Arm (2015)	3	40			24	
St. Albans (2015)	39	12			3	
Christmas (2016)	26	1	18			
Smiths (2016)	59	29	4			
Veterans (2016)	19	7	2			

Herbicide-treated versus untreated water bodies had different taxonomic composition. We only found northern watermilfoil (NWM) in the three untreated water bodies, and pure Eurasian watermilfoil (EWM) was most common in these water bodies. In contrast, northern watermilfoil was not found in any of the three treated lakes, and hybrids (HWM) were most common in two of the treated lakes (Grays and North Arm) before treatment in June, and following treatment in late Summer. In St. Albans, EWM was most common in June, but we only found hybrids in late summer.

The causes for the apparent association between taxonomic composition and management history are unknown. LaRue et al. (2013a) found a similar pattern in the Upper Peninsula of Michigan and adjacent Wisconsin, where hybrid watermilfoils were more common in 2,4-D treated lakes compared to pure Eurasian and northern watermilfoil. It is possible that there are unidentified fitness trade-offs between hybrids and parental species in treated versus untreated lakes. It is also possible that hybrids will eventually take over untreated lakes, and there is some support for this hypothesis looking at Smiths Bay, where hybrids were common. Or, management activities such as herbicide treatments may accelerate a process of displacement by hybrids. This last hypothesis is supported by our finding that hybrid watermilfoil was the only taxon found post-treatment in St. Albans Bay, which was dominated by Eurasian watermilfoil pre-treatment (the other two treated Bays were already dominated by hybrids before treatment, but may have been dominated by pure Eurasian watermilfoil in the past). Unfortunately, without historical records of the distribution of these taxa, it is impossible to determine whether hybrids have displaced parental watermilfoils in managed lakes, or whether the pattern arose from some other mechanism. Routine surveys and monitoring of invaded and uninvaded lakes that clearly distinguishes Eurasian, northern, and hybrid watermilfoils would provide helpful information to determine whether there are predictable dynamics among these three taxa.

Genetic diversity and differentiation –

For the most part, the water bodies were genetically distinct from one another, although several genotypes were shared among at least some water bodies. Appendices 1-4 show the distributions and frequencies of different microsatellite genotypes.

We found nine distinct microsatellite genotypes across our 148 EWM individuals. Two EWM microsatellite genotypes were shared among water bodies. Genotype E4 was the most common EWM microsatellite genotype in Christmas, Smiths, Veterans, and St. Albans, and was also present in North Arm (1 out of 3 EWM samples pre-treatment). This suggests extensive clonal reproduction and spread of this genotype among water bodies. EWM genotype E8 was also shared among Christmas and St. Albans, but this genotype was not common in either water body. The remaining seven EWM microsatellite genotypes were only found in a single water body each. Thus, while clonal reproduction of genotype E8 appears extensive, the genetic diversity found among EWM samples suggests some degree of sexual reproduction of EWM in Lake Minnetonka. Alternatively, it is possible that some genetically distinct clones were not distinguished by our microsatellite analysis. Similarly, it is possible that the different genotypes result from scoring errors associated with microsatellite analysis, although we also find this unlikely.

We found 11 distinct microsatellite genotypes among the 24 NWM individuals in our analysis. Only one of these genotypes, N1, was shared among two water bodies (Christmas and Veterans). The remaining 10 NWM genotypes were found only once each. This suggests that sexual reproduction is a more common reproductive mode for NWM compared to EWM.

We found 34 distinct microsatellite genotypes among the 206 HWM individuals in our analysis. Five of these microsatellite genotypes were shared among two or more water bodies (H4, H8, H10, H12, and H18). Of the shared genotypes, H8 is of the most interest. This genotype was found in North Arm, St. Albans, and Smiths Bays. This genotype was dominant in both North Arm and St. Albans following treatment. It was also common in the untreated Smiths Bay (20% relative frequency of HWM) where 12 other HWM microsatellite genotypes were found. H4 was shared among North Arm and Smiths Bays, but this genotype was rare in both bays. The H10 genotype was shared among Grays, North Arm, and Smiths Bays. It was common in Grays, but rare in the other two water bodies. H12 was shared among Grays, North Arm, St. Albans, and

Veterans Bays, but was not found in any post-treatment samples of the treated bays, suggesting this genotype is susceptible to the herbicide treatments used in these water bodies. Finally, H18 was shared among Smiths and Veterans Bays. It was the most common genotype in Veterans Bay.

Hybrid genetic diversity was surprisingly high in the five water bodies where they were found. However, genetic diversity of hybrids was markedly decreased in the three treated water bodies following treatment. This decreased diversity is expected in populations undergoing population bottlenecks. However, it may also indicate differential survival and subsequent reproduction of certain genotypes over others. This is of special interest for genotype H8. The high genetic diversity of hybrids in untreated water bodies, and treated water bodies pre-treatment, suggests extensive sexual reproduction. This may occur either due to extensive sexual reproduction of different EWM and NWM genotypes (e.g., different F_1 genotypes through numerous hybridization events in different water bodies) and/or through subsequent sexual reproduction by hybrid genotypes (F_2 and later, backcrosses; see LaRue et al. 2013b), and greater study of the reproduction of EWM, NWM, and HWM is warranted.

A broader implication of extensive sexual reproduction is that the diversity of genotypes may represent a diversity of ecologically relevant traits such as growth and herbicide response characters. It is possible that hybridization plays an important role in generating diverse genotypes that may fuel adaptation to local environmental conditions, including adaptation to control techniques such as herbicide use patterns. For example, extensive sexual reproduction may create a diverse hybrid population, and local factors (e.g., herbicide treatment) may exert selection pressures on this diverse population, leading to dominance by one or a few genotypes that are well-suited to the local environment and subsequently spread via extensive asexual propagation. This may lead to the reduced genetic diversity and dominance by a single clone in treated water bodies like St. Albans and North Arm. This hypothesis can be tested using a combination of laboratory study of growth and response by specific genotypes of interest, and continued genetic monitoring to identify significant increases in relative abundance of specific genotypes. This information would be valuable to managers making decisions about when and how to manage specific water bodies with specific genotypes.

Comparison of pre- and post-treatment genetic composition –

The triclopyr treatments were effective in the three bays, with particularly good control in Grays and St. Albans Bay. In Grays Bay hybrid watermilfoil was dominant and the frequency of watermilfoil (any type) was reduced from 48% occurrence to 3% occurrence after treatment. In St. Albans Bay, both hybrid and pure Eurasian were present and watermilfoil occurrence was reduced from 19% occurrence to <1%.. In North Arm, the areas treated were smaller. Both hybrid and pure Eurasian were present and watermilfoil occurrence in the bay showed little overall reduction with treatment, from 13% to 8%. The two treated areas in the north end of the bay received greater control, being reduced from 13% and 18% respectively to 0% post-treatment. Two treated areas in the southern portion of the lake had watermilfoil remaining in or directly adjacent to the treated area. Some reduction occurred directly in the treated areas, from 30% and 24% respectively, to 15% and 5% post-treatment.

Grays Bay was dominated by hybrid watermilfoil before treatment (Appendix 1). The only watermilfoil detected during the post-treatment survey was located in the western end of the Bay, which was not treated. Three post-treatment samples were processed; two of these were HWM and one was EWM. The two HWM samples found post-treatment (H10 and H28) were the two most common genotypes pre-treatment. H28 was the most common genotype, and was only

found in Grays Bay. H10 was the second most common, and was also found in North Arm and Smiths Bays. These genotypes appear to be susceptible to the herbicide treatment applied in Grays Bay, although laboratory study of growth and herbicide response and/or continued monitoring of this genotype would be needed to confirm this hypothesis.

As with Grays Bay, the herbicide treatment appears to have been very effective in St. Albans Bay, as watermilfoil frequency of occurrence was very low post-treatment (Appendix 2). St. Albans Bay had higher relative abundance of pure EWM compared to HWM pre-treatment. However, while we found only three sites with watermilfoil post-treatment, all of them were hybrid and found outside the treatment areas. Furthermore, all three individuals were hybrid genotype H8, which is the same genotype that appears to have survived treatment in North Arm, and rapidly increased relative abundance compared to other hybrid genotypes in North Arm following treatment. Thus, it is possible that this genotype is more tolerant of the specific herbicide treatment method used compared to pure EWM and the other hybrid genotypes present pre-treatment. We recommend growth and herbicide response study of this specific genotype, as well as continued genetic monitoring of this population to determine whether this specific genotype increases over time in St. Albans Bay.

Control in North Arm was not as good as it was in St. Albans or Grays Bays. The northern portion of the lake exhibited a large reduction in watermilfoil following treatment. In contrast, the reduction of watermilfoil in the southern part of the lake was not as great as for the northern portion. Like Grays Bay, North Arm was dominated by hybrid watermilfoil pre-treatment (Appendix 3). However, we observed a large shift in the composition of hybrid genotypes from before to after treatment. Specifically, microsatellite genotype H8 was common in the southern portion of the lake pre-treatment, but rarely occurred in the northern portion of the lake where control was comparatively better. Microsatellite genotype H8 was the dominant genotype in North Arm Bay following treatment. This suggests that this specific genotype may be more tolerant to the specific treatment method compared to other genotypes that were present pre-treatment. It is also possible that the increase in relative abundance of H8 was due to chance associated with the demographic bottleneck (i.e., reduced population size) imposed by the herbicide treatments. However, H8 is the same microsatellite genotype remaining after treatment in St. Albans. We recommend laboratory study of growth and herbicide response of hybrid genotype H8 to directly test for any evidence of herbicide tolerance in this genotype. In addition, we recommend continued genetic monitoring of these water bodies to determine if this specific genotype continues to increase in relative abundance, which would further indicate its relatively higher invasiveness and tolerance.

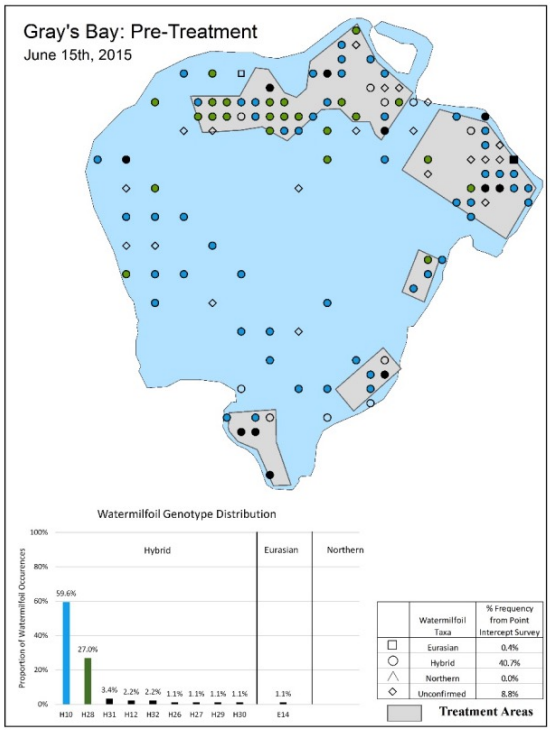
Conclusions and recommendations –

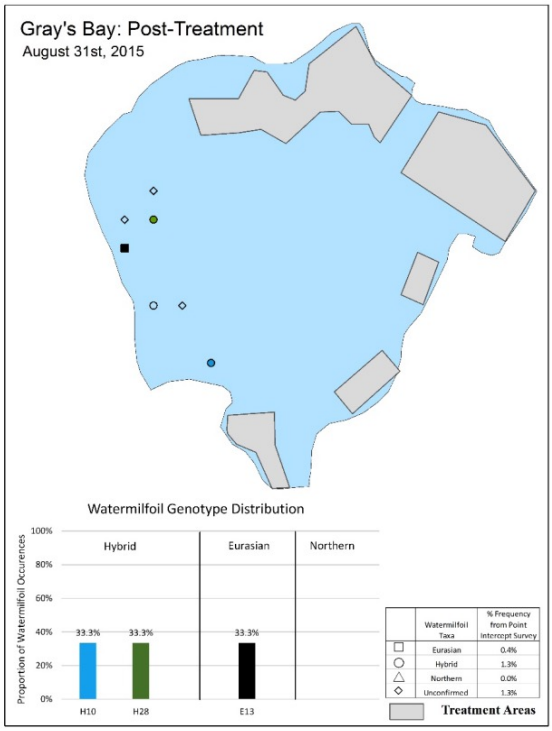
Overall, our study highlights some potential benefits of integrating genetic analysis into watermilfoil management projects. Specifically, genotype data can help inform adaptive management planning and evaluation by identifying associations between taxonomic and genetic composition and management history or specific control actions. In particular, temporal genetic monitoring may identify shifts in composition that may be associated with differences in management-relevant traits such as growth and herbicide response. Genetic analysis can identify specific genotypes of interest that can then be studied explicitly to inform control options, including trigger points for switching specific control techniques.

We recommend the following next steps: 1) conduct genetic surveys and monitoring on these study lakes, 2) conduct genetic surveys and monitoring on a larger number of lakes in Minnesota, 3) conduct laboratory growth and herbicide response studies on a select number of

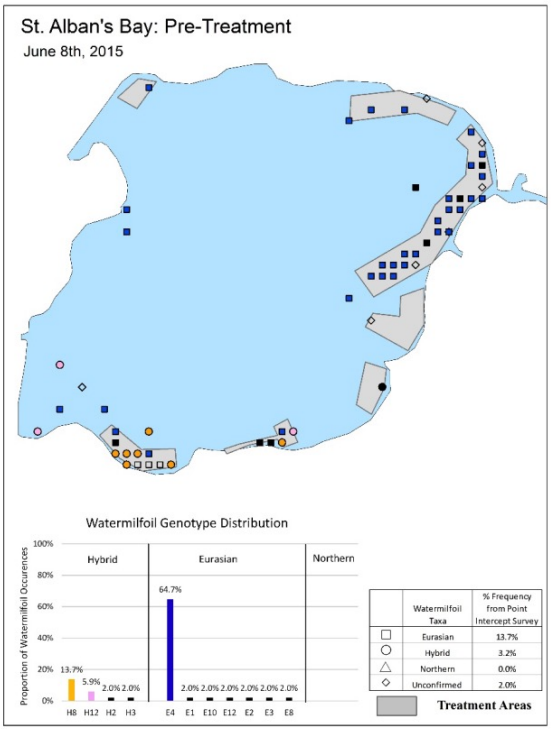
judiciously chosen genotypes identified in this study (e.g., hybrid genotype H8, which exhibited rapid relative increases in two treated bays following treatment).

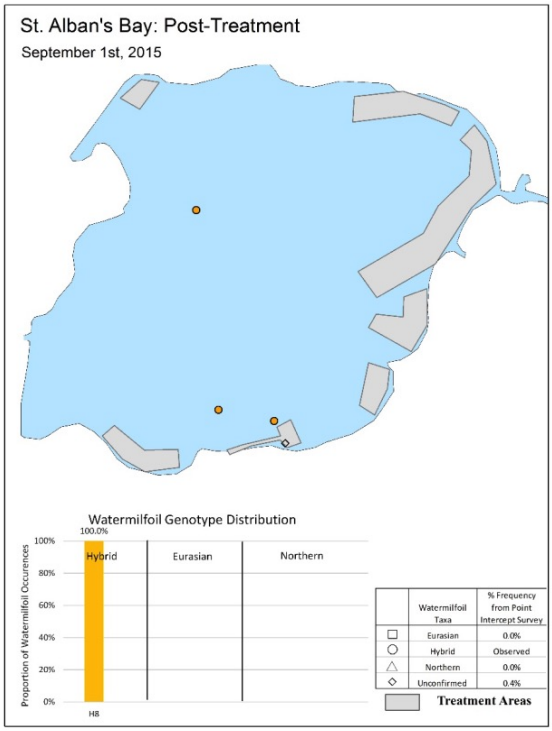
Appendix 1. Pre- and post-treatment point-intercept results for Gray's Bay. The specific microsatellite genotypes found are shown in each figure (circles – hybrids; squares – Eurasian; triangles – northern), and their proportion relative to all watermilfoil collected is shown at lower left. The percent occurrence for each taxon (Eurasian, northern, hybrid) across all intercept points is shown at lower right of each panel. In some cases, watermilfoil was collected, but the molecular data did not pass quality control and was omitted ("unconfirmed"). Note that the density of intercept points was twice in treated areas (gray polygons) relative to untreated areas.



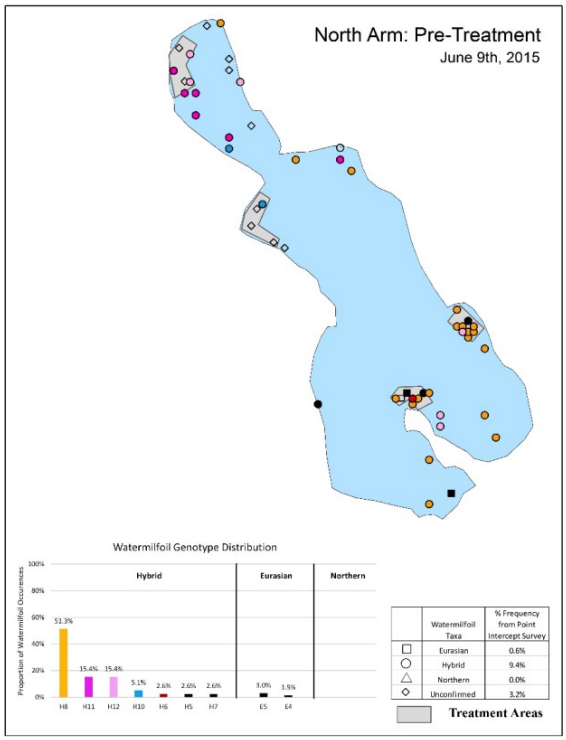


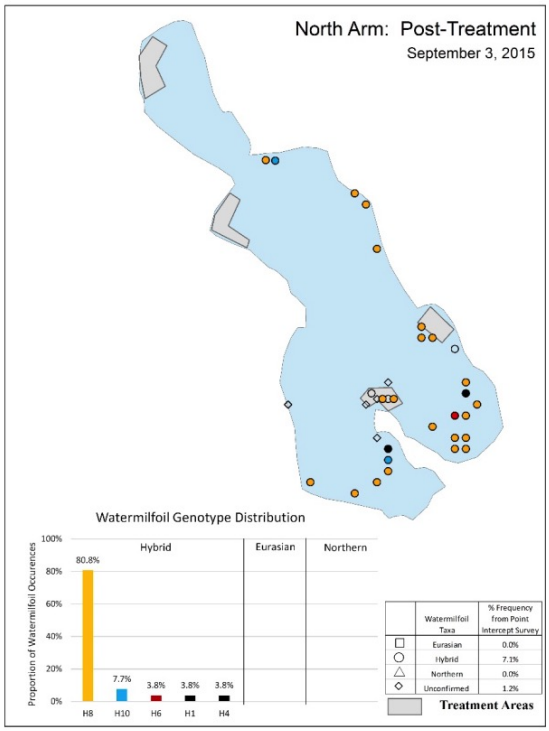
Appendix 2. Pre- and post-treatment point-intercept results for St. Alban's Bay. The specific microsatellite genotypes found are shown in each figure (circles – hybrids; squares – Eurasian; triangles – northern), and their proportion relative to all watermilfoil collected is shown at lower left. The percent occurrence for each taxon (Eurasian, northern, hybrid) across all intercept points is shown at lower right of each panel. In some cases, watermilfoil was collected, but the molecular data did not pass quality control and was omitted ("unconfirmed"). Note that the density of intercept points was twice in treated areas (gray polygons) relative to untreated areas.



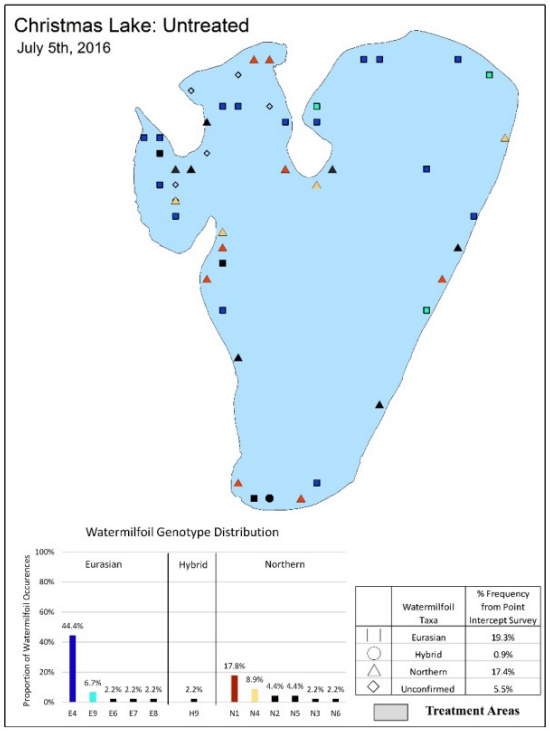


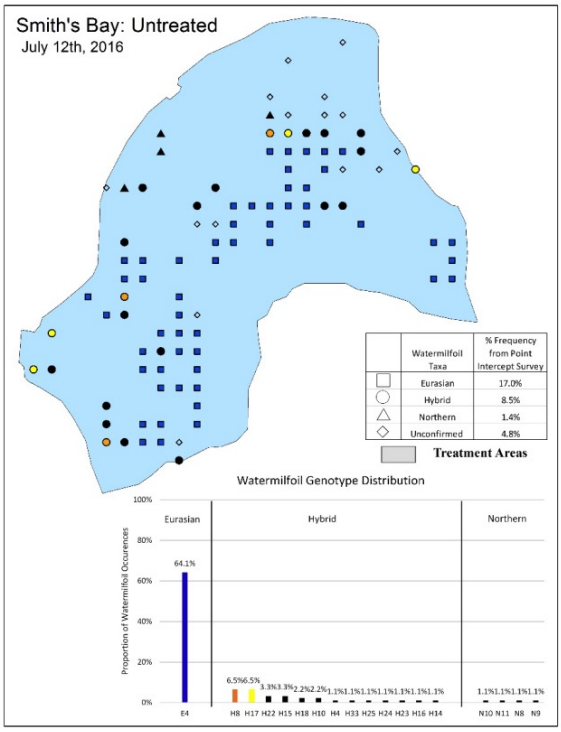
Appendix 3. Pre- and post-treatment point-intercept results for North Arm Bay. The specific microsatellite genotypes found are shown in each figure (circles – hybrids; squares – Eurasian; triangles – northern), and their proportion relative to all watermilfoil collected is shown at lower left. The percent occurrence for each taxon (Eurasian, northern, hybrid) across all intercept points is shown at lower right of each panel. In some cases, watermilfoil was collected, but the molecular data did not pass quality control and was omitted ("unconfirmed"). Note that the density of intercept points was twice in treated areas (gray polygons) relative to untreated areas.

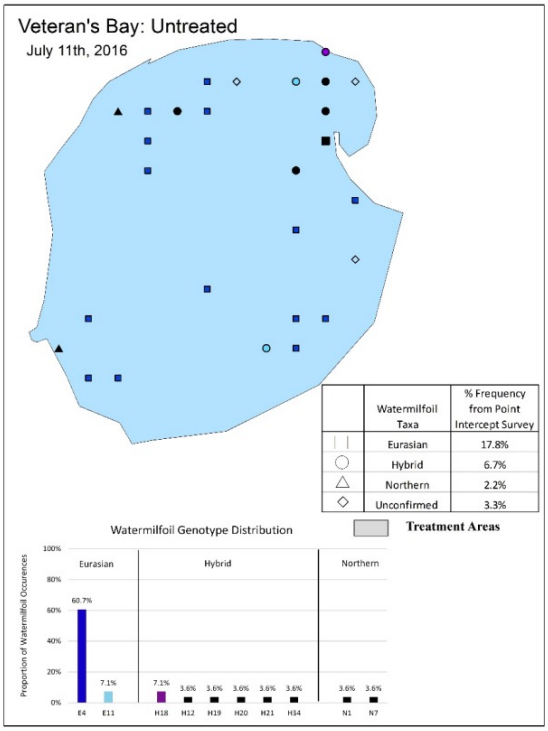




Appendix 4. Point-intercept results for Christmas Lake and untreated bays. The specific microsatellite genotypes found are shown in each figure (circles – hybrids; squares – Eurasian; triangles – northern), and their proportion relative to all watermilfoil collected is shown at lower left. The percent occurrence for each taxon (Eurasian, northern, hybrid) across all intercept points is shown at lower right of each panel. In some cases, watermilfoil was collected, but the molecular data did not pass quality control and was omitted ("unconfirmed").







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