INTEGRATING DNA FINGERPRINTING OF INVASIVE WATERMILFOIL STRAINS INTO AQUATIC VEGETATION MONITORING AND ASSESSMENT

by

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of

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DEDICATION

This thesis is dedicated to my parents Susie McIntyre and Brandon Olds and to my grandparents Bob and Judy McIntyre for cultivating my love of science and the outdoors, for encouraging me to pursue my curiosity and sense of adventure, and for supporting me through every detour and bump in the road along the way.

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TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum* L.) and its hybrids with native northern watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum*) (invasive watermilfoils) are among the most heavily managed invasive aquatic plants in the United States. Within invasive watermilfoils there are distinct strains which are produced through sexual reproduction and can be maintained indefinitely by clonal propagation. Different strains can differ in their invasiveness (e.g., growth and potential for spread) and response to herbicides. However, strain identification is not routinely implemented as part of management planning or evaluation. In this thesis, we integrated molecular fingerprinting that can distinguish different Eurasian and hybrid watermilfoil strains into aquatic vegetation monitoring in eight Minnesota lakes over the course of three years. We provide proof of concept that by tracking strain incidence over time it is possible 1) to detect changes in strain composition of invasive watermilfoil populations, and 2) to identify priority strains for future growth and herbicide assays. This study demonstrates that integrating genetic fingerprinting into aquatic vegetation management and evaluation holds promise to inform management decisions by identifying those strains that have the most invasive potential. As far as we are aware, this is the first published study to examine the strain composition of invasive watermilfoil populations over time.

CHAPTER ONE

GENETIC MONITORING IN CLONAL INVASIVE SPECIES

Genetic Monitoring of Herbicide Resistance

Globally, invasive plant species pose a significant threat to biodiversity (Mack *et al.*, 2000; Simberloff and Rejmanek, 2019), and the control of invasive plants is becoming more difficult with the spread of herbicide resistance (Baucom, 2019; Heap I, 2021). Herbicide resistance is the result of adaptive evolution of weed populations to the strong selection pressure exerted by herbicides (Jasieniuk, Brûlé-Babel and Morrison, 1996; Neve, Vila-Aiub and Roux, 2009; Délye, Jasieniuk and Le Corre, 2013). However, changes in allele frequency in response to herbicide treatment are not routinely monitored in invasive populations.

The evolution of herbicide resistance can be understood through the integrative conceptual framework of evolutionary rescue (Délye, Jasieniuk and Le Corre, 2013; Kreiner, Stinchcombe and Wright, 2018). When a sudden environmental change causes population decline, evolutionary rescue occurs when that decline is reversed because adapted genotypes reproduce at a sufficient rate to avoid population extinction (Délye, Jasieniuk and Le Corre, 2013; Gonzalez *et al.*, 2013; Osmond and Coop, 2020). Although adaptation in a broad sense does not require population decline, evolutionary rescue in particular occurs after population decline in response to environmental change (Gonzalez *et al.*, 2013). When the environmental change in question is an herbicide treatment, individuals in the weed population that are less sensitive to the herbicide have a selective advantage. As a result, less sensitive individuals increase in frequency causing the population as a whole to shift towards herbicide resistant individuals (Jasieniuk, Brûlé-Babel and Morrison, 1996; Délye, Jasieniuk and Le Corre, 2013). Repeated treatments of the same populations with the same herbicide or with the same mode of action can rapidly lead to the evolution of predominantly resistant populations (Jasieniuk, Brûlé-Babel and Morrison, 1996).

The high fitness alleles that facilitate evolutionary rescue from herbicide treatment may arise from novel mutations or may be pre-existing in the population (Délye, Jasieniuk and Le Corre, 2013; Gonzalez *et al.*, 2013; Osmond and Coop, 2020). Compared to adaptation from novel mutations, adaptation from standing genetic variation has been reported to be faster and more predictable, because neutral (or even slightly negative) alleles that pre-exist in a population are likely to be at higher frequency than those newly arisen from mutation (Barrett and Schluter, 2008; Prentis *et al.*, 2008; Bock *et al.*, 2015). Herbicide resistance alleles can also be introduced into susceptible weed populations via gene flow from resistant populations (Ganie and Jhala, 2017; Sarangi *et al.*, 2017; Chorak and Thum, 2020).

Resistance evolution occurs through the increase in frequency and eventual fixation of resistance alleles (Broom, Tang and Waxman, 2003; Bell and Collins, 2008; Lachapelle and Bell, 2012). However, in asexually reproducing organisms, resistance alleles are linked to the rest of the genome. As a result, the increase and fixation of resistance alleles also increases the frequency of the clonal lineage (strain) carrying the resistance allele(s). For example, in clonal weed populations, the strain(s) with the herbicide resistance allele(s) increase in relative frequency following herbicide treatment (Baucom, 2019). However, this pattern may be obscured by clonal interference, the competition between lineages that carry alternative beneficial mutations (Gerrish and Lenski, 1998; Park and Krug, 2007).

In clonally reproducing organisms, multi-locus genotyping could allow researchers and managers to watch the evolution of herbicide resistance unfold. Multi-locus genotyping can identify and distinguish strains (Wu, Yu and Xu, 2013; Chorak and Thum, 2020; Thum *et al.*, 2020). Repeatedly sampling and genotyping would enable the detection of changes in relative frequency of different strains over time (Berg *et al.*, 2002), and changes that are associated with herbicide treatments could indicate variation in herbicide response (Parks *et al.*, 2016). Integrating genetic fingerprinting could facilitate identification and tracking of particularly invasive strains (Schwartz, Luikart and Waples, 2007; Stetz *et al.*, 2011), such as those that have a faster growth rate or are less susceptible to certain herbicides (LaRue *et al.*, 2013). It should be noted, however, that it is possible for members of the same strain to differ in their herbicide response because of somatic mutations (Michel *et al.*, 2004).

Despite calls from some geneticists (Rollins, Woolnough and Sherwin, 2006; Schwartz, Luikart and Waples, 2007; Thum *et al.*, 2020), longitudinal genetic analysis is not routinely integrated into standard management and evaluation protocols for invasive species (Kekkonen, 2016), although see Berg *et al.* (2002) and Ivkosic *et al.* (2014). Genetic monitoring and the concept of evolutionary rescue are widely used in conservation biology (Amos and Balmford, 2001; Carlson, Cunningham and Westley, 2014). However, in the study and management of invasive species, the use of molecular genetic techniques has primarily been restricted to identifying routes of invasion (Rollins *et al.*, 2009; Estoup and Guillemaud, 2010; Paulus *et al.*, 2014), to defining populations for management (Rollins, Woolnough and Sherwin, 2006; Rollins *et al.*, 2009), and to identifying gene flow (Rollins *et al.*, 2009). We are unaware of any studies that have used multi-locus genotyping over time to look for evidence of herbicide resistance in managed clonal invasive populations. The goal of this thesis is to identify any disproportionate changes in incidence between strains of an invasive aquatic plant in response to operational herbicide application. We do this by integrating genetic fingerprinting into existing aquatic plant surveys for invasive Eurasian and hybrid watermilfoil.

Eurasian Watermilfoil

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is one of the most widespread and heavily managed invasive aquatic plants in the United States (Bartodziej & Ludlow, 1997). Eurasian watermilfoil hybridizes with native northern watermilfoil (*Myriophyllum sibiricum* Komarov), and these hybrid lineages are also considered invasive to North America (Moody and Les, 2002, 2007; Zuellig and Thum, no date). Both pure Eurasian and hybrid watermilfoil (hereafter referred to as invasive watermilfoil) cause both environmental and economic damage by decreasing native plant and animal diversity and by inhibiting water recreation (Madsen *et al.*, 1991; Cheruvelil *et al.*, 2002). To minimize damage, invasive watermilfoil populations are heavily managed through herbicide application. However, the efficacy of herbicide treatments varies between invasive watermilfoil populations (Thum *et al.*, 2012; Berger, Netherland and MacDonald, 2015; Chorak and Thum, 2020). Some evidence suggests that hybrid watermilfoils are more invasive (faster growing and more resistant to herbicides) compared to their parental counterparts (LaRue *et al.*, 2013; Parks *et al.*, 2016), although see Poovey, Slade and Netherland (2007).

Invasive watermilfoils reproduce prolifically through vegetative reproduction, but, like most aquatic plant species (Barrett, Eckert and Husband, 1993; Philbrick and Les, 1996), they also maintain the capacity for sexual reproduction (Xiao *et al.*, 2010; Thum and Mcnair, 2018).

Within lakes, local reproduction is primarily clonal but sexual reproduction remains important at the landscape scale (Thum *et al.*, 2020). Within invasive watermilfoil, there are distinct genotypes or strains which are produced through sexual reproduction but can be maintained indefinitely by clonal propagation, and these strains can be distinguished using genetic markers (Wu, Yu and Xu, 2013; Taylor *et al.*, 2017). Strains can differ in their invasiveness (e.g., growth and potential for spread) and response to herbicides used to control them. For example, two strains have been documented as fluridone resistant, whereas many other strains are susceptible (Thum *et al.*, 2012; Berger, Netherland and MacDonald, 2015; Chorak and Thum, 2020). Variation among strains in their growth and response to other herbicides has also been documented (LaRue et al. 2013; Taylor et al. 2017; Netherland and Willey 2017; Guastello and Thum 2018).

It stands to reason that integrating genetic fingerprinting into invasive watermilfoil monitoring could help elucidate the evolution of herbicide resistance in invasive watermilfoil and could facilitate the identification of more resistant and/or invasive strains. Strains that carry resistance alleles (and are therefore less sensitive to the herbicide) should increase in relative frequency following herbicide treatment because they have a selective advantage. Similarly, strains with a relatively fast growth rate (more invasive) should increase in relative frequency over time because they can proliferate more quickly.

Isolating strains that are less susceptible to herbicides and/or are more invasive will allow researchers to prioritize strains for herbicide assays that assess herbicide response and to investigate the genetic basis of herbicide resistance in invasive watermilfoils. Further, this research will benefit management by enabling managers to tailor herbicide choices and control strategies to the susceptibility profile of the invasive milfoil population and to minimize unnecessary or ineffective herbicide treatments.

CHAPTER 2

INTEGRATING DNA FINGERPRINTING OF INVASIVE WATERMILFOIL STRAINS INTO AQUATIC VEGETATION MONITORING AND ASSESSMENT

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Kathryn A. Gannon

Contributions: Molecular processing, data analysis, spatial analysis, writing

Co-Author: Raymond Newman

Contributions: Sample design, sample collection, conceptual framework, writing feedback

Co-Author: Ryan Thum

Contributions: Technical assistance in strain identification, conceptual framework, writing feedback

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Title: **Integrating DNA fingerprinting of invasive watermilfoil strains into aquatic vegetation monitoring and assessment**

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ABSTRACT:

Eurasian watermilfoil (*Myriophyllum spicatum* L.) and its hybrids with native northern watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum*) (invasive watermilfoils) are among the most heavily managed invasive aquatic plants in the United States. Within invasive watermilfoils there are distinct strains which are produced through sexual reproduction and can be maintained indefinitely by clonal propagation. Different strains can differ in their invasiveness (e.g., growth and potential for spread) and response to herbicides. However, strain identification is not routinely implemented as part of management planning or evaluation. In this study, we integrated molecular fingerprinting that can distinguish different Eurasian and hybrid watermilfoil strains into aquatic vegetation monitoring in eight Minnesota lakes over the course of three years. We provide proof of concept that by tracking strain incidence over time it is possible 1) to detect changes in strain composition of invasive watermilfoil populations, and 2) to identify priority strains for future growth and herbicide assays. This study demonstrates that integrating genetic fingerprinting into aquatic vegetation management and evaluation holds promise to inform management decisions by identifying those strains that have the most invasive potential. As far as we are aware, this is the first published study to examine the strain composition of invasive watermilfoil populations over time.

Key Words: genetic monitoring, strain identification, herbicide resistance evolution, *Myriophyllum spicatum* L., northern watermilfoil, Myriophyllum sibiricum

INTRODUCTION:

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is one of the most heavily managed invasive aquatic plants in the United States (Bartodziej & Ludlow, 1997). Eurasian watermilfoil hybridizes with native northern watermilfoil (*Myriophyllum sibiricum* Komarov), and hybrids are also considered invasive to North America (Moody and Les, 2002, 2007; Zuellig and Thum, no date). Invasive watermilfoils cause environmental damage, by decreasing native plant and animal diversity, and economic damage, by inhibiting water recreation (Smith and Barko, 1990; Madsen, 1999; Cheruvelil *et al.*, 2002). Both taxa are heavily managed primarily through the use of herbicides.

Within invasive Eurasian and hybrid watermilfoil, there are distinct genotypes or strains which are produced through sexual reproduction, but can be maintained indefinitely by clonal propagation (Taylor *et al.*, 2017). Lakes are often dominated by a single strain, but some lakes can contain multiple strains (Thum *et al.*, 2020). Further, while different lakes often contain distinct strains, some strains are found in multiple lakes (Thum *et al.*, 2020).

The strain composition of invasive watermilfoil populations is relevant for management because different strains can differ in their invasiveness (e.g., growth and potential for spread) and response to herbicides used to control them (LaRue *et al.*, 2013). For example, two strains have been documented as fluridone resistant whereas many other strains are susceptible (Thum *et al.*, 2012; Berger, Netherland and MacDonald, 2015; Chorak and Thum, 2020). Variation among strains in their growth and response to other herbicides has also been documented (LaRue *et al.*, 2013; Berger, Netherland and MacDonald, 2015; Taylor *et al.*, 2017; Thum and Mcnair, 2018). Therefore, genetic composition of populations could change over time if faster-growing strains displace slower-growing strains, or if herbicide resistant strains replace susceptible ones following one or more herbicide applications. Changes in strain composition – if they could be observed – would have a range of important implications for management. For example, if a change in strain composition resulted in increased growth rate, then it would also increase the frequency and intensity of management necessary to control the population. Alternatively, changes in strain composition could change the population's susceptibility to certain herbicides and previously-used herbicides could be rendered ineffective.

Although the potential for changes in genetic composition clearly exists, strain identification is not routinely integrated into management planning or evaluation. To date, only two field study that we are aware of have integrated genetic identifications into evaluating management efficacy. Parks et al. (2016) observed a disproportionate decrease in hybrid watermilfoil compared to Eurasian watermilfoil following a treatment with two auxinic herbicides (2,4- Dicholorophenoxyacetic acid and 3,5,6-trichloro-2-pyridinyloxyacetic acid) in a large Michigan lake (Parks *et al.*, 2016). Nault et. al. observed that the efficacy of control efforts was significantly reduced in lakes harboring hybrid watermilfoil populations compared to lakes harboring only pure Eurasian watermilfoil populations (Nault *et al.*, 2018). However, both studies only distinguished pure Eurasian from hybrid watermilfoil, whereas no studies have distinguished different strains of invasive and compared their growth rate and herbicide response in the field. Integrating genetic fingerprinting into vegetation monitoring and herbicide evaluations offers the potential to observe changes in genetic composition that may identify specific strains as potentially problematic, either because they are relatively more invasive (e.g., faster growth and potential for spread) and/or because they have relatively lower sensitivity to a particular control tactic (e.g., a specific herbicide use pattern). Therefore, in this study, we integrated molecular fingerprinting that can distinguish different Eurasian and hybrid watermilfoil strains into aquatic vegetation monitoring in eight Minnesota lakes to determine if 1) the strain composition of invasive watermilfoil populations changed over time, and 2) if so, by monitoring those changes can we identify potentially problematic strains to prioritize for characterization?

MATERIALS AND METHODS

Field Sampling:

Between 2018 and 2020, field surveys and sample collection were carried out in eight waterbodies in Minnesota. Four of the waterbodies surveyed were separate bays in Lake Minnetonka. However, because Lake Minnetonka is large (>22 square miles) and each of these bays are managed individually, for the purposes of our analysis each bay is considered to be an independent population (*Table 1*). Of the eight waterbodies surveyed, five received herbicide treatments aimed at controlling milfoil in at least one of the three years surveyed. Some bays received spot treatments meaning that the herbicide was applied in priority areas rather than to the lake as a whole. Spot treatments are a common practice in invasive watermilfoil management especially in the state of Minnesota. In four of those waterbodies, the herbicides 2,4-D, florpyrauxifen-benzyl, and diquat were applied as spot treatments. In one waterbody (North Arm Bay of Lake Minnetonka) fluridone was applied as a whole-bay treatment (*see Table 1*).

Sampling sites in each waterbody were pre-determined as a point intercept grid over the littoral zone (defined as a depth of less than 4.5 meters) (Mikulyuk *et al.*, 2010; Eltawely, Newman and Thum, 2020), with grid spacing set to yield approximately 150 points in the littoral zone of each lake. At each sampling point, a rake was thrown over each side of the boat and drawn back in to collect plant material (rake toss) (Mikulyuk *et al.*, 2010; Thum *et al.*, 2012; Parks *et al.*, 2016). At each point where milfoil was observed, a representative meristem was collected for genetic analysis. Each sample was given a number and character code corresponding to the lake in which it was found and the point in that lake (Eltawely, Newman and Thum, 2020). Each meristem was placed in a sealed bag and placed on ice. Upon return to the laboratory each sample was placed in a labeled paper envelop which was then placed in a sealable plastic bag with silica beads to dry the samples. Samples were then transported to Montana State University.

Six waterbodies were sampled twice per year, at the beginning of the growing season between June 1st and June 15th and the end of the growing season between August 15th and August 30th : Bald Eagle, Christmas, Grays Bay (Lake Minnetonka), Ham, Independence, Phelps Bay (Lake Minnetonka). Smith's Bay in Lake Minnetonka was only sampled once per year in June as a reference (untreated lake), and although the North Arm of Lake Minnetonka was visited twice in 2018, the late season survey in August following fluridone treatment did not locate any watermilfoil (see *Table 1*). Christmas Lake was only sampled in early summer (June 5th) in 2020 due to logistical constraints.

Molecular Fingerprinting:

Total genomic DNA was extracted from the collected meristems using DNeasy Plant Mini Kits (Qaigen) according to the manufacturer's instructions. As in Thum et al. (2020), we determined the taxon and strain for each sample by using eight microsatellite loci (Myrsp 1, Myrsp 5, Myrsp 9, Myrsp 12, Myrsp 13, Myrsp 14, Myrsp 15, and Myrsp 16) from Wu et al., (2013). Fragment analysis of the fluorescently labeled microsatellite PCR products was carried out by the University of Illinois Urbana-Champaign Core Sequencing Facility using an ABI 3730xl sequencer. Scoring of microsatellites was carried out in GeneMapper, version 5.0 (Applied Biosystems, Waltham, MA, USA). Microsatellites were treated as dominant, binary data based on the presence or absence of each possible allele at each locus. Distinct strains were delineated using Lynch distances and a threshold of zero in POLYSAT (Clark & Jasieniuk, 2011).

Statistical Analysis

Changes in milfoil populations were analyzed at three distinct time scales: within a single growing season (June to August), over one year (June one year to June of the next), and over a three year period (June 2018 to June or August 2020). The incidence for a given strain in a waterbody was interpreted as the total number of points at which that strain was collected in each year. For each strain we also calculated the percent of sampled sites where that strain was found. We refer to identified strains by a four-character code: the first two letters indicate the waterbody in which the strain was identified; the middle letter indicates whether the strain is pure Eurasian watermilfoil (E), native northern watermilfoil (N), or a hybrid (H); and the final number distinguishes strains of the same taxa within the same waterbody. The abbreviation MC stands for Minnesota Clones and is used to distinguish strains that are found in multiple waterbodies included in this study. We excluded from analysis any strain that was observed only once, because, without multiple occurrences, it was unclear whether singletons represent distinct strains or if they were the result of sequencing error. To determine whether the strain composition of each milfoil population changed over time, we used a simulated Chi Squared test for homogeneity based on 2,000 permutations to compare each set of timepoints. Because each of our data points represents a site on the lake that was visited repeatedly, they violate the assumption of independence necessary to have full confidence in the results of a Chi Squared test. However, we believe that the simulation based Chi Squared test is still useful to provide an indication of strain dynamics until more robust sampling methods are implemented. Further, Chi Squared analysis has been used previously in similar studies of aquatic vegetation using point intercept surveys (Mikulyuk *et al.*, 2010; Nault *et al.*, 2018). All statistical analysis was carried out in R version 3.6.3 (RStudio Team, 2018).

RESULTS AND DISCUSSION

TREATED LAKES

Bald Eagle

In Bald Eagle Lake in June 2018, the most abundant strain was hybrid BE-H-3 followed closely by a Eurasian strain (MC-E-1) and a northern strain (BE-N-2). Following 2,4, D treatment in July 2018, all three strains (taxa) decreased (*Figure 2.1. A*) (*Supplemental Map 1)*. Then, as the population recovered, there was a shift in its genetic composition: BE-N-2 and BE-H-3 increased disproportionately relative to MC-E-1, which was not found after 2018 (*Table 2.2*). Interestingly, the BE-N-2 increased the most, which is counter to the general expectation that pure Eurasian and hybrid strains will outcompete native northern strains.

We found strong evidence for a change in the strain composition of the milfoil population in Bald Eagle from June of 2018 to June of 2019 and from June of 2018 to June of 2020 (Simulated Chi Squared p-values <.001) , but we did not find any strong evidence of a change within any of the three separate growing seasons (p-values > .10) (Table 2. 2).

Both BE-H-3 and MC-E-1 are also found in other lakes around the Minneapolis metropolitan area. MC-E-1 is widespread across Minnesota, and BE-H-3 is the most commonly-encountered hybrid strain in Minnesota thus far (Eltawely, Newman and Thum, 2020; Thum *et al.*, 2020). The disproportionate increase of BE-N-2 and BE-H-3 compared to MC-E-1 in Bald Eagle Lake may indicate that they are relatively more invasive strains (*Figure 2.1. A*). All three strains would therefore be of interest for laboratory study to investigate and compare their relative growth rate and 2,4-D response.

Grays Bay

Grays Bay was spot-treated with florpyrauxifen-benzyl in all three years and was also spot-treated with diquat in 2019 and 2020. Despite these repeated herbicide treatments, the overall milfoil incidence continued to increase and MC-H-12 remained the most abundant strain (*Figure 2.1. C*). We did not detect a statistically significant change in genetic composition (p-value > .10) (*Table 2.2*). However, it is notable that MC-H-12 disproportionately increased compared to the other strains present in both 2018 and 2019 even with herbicide treatment (*Figure 2.1.C*). Further, MC-H-12 does not appear to occur disproportionately in untreated areas, and therefore its persistence is unlikely to be an artifact of where herbicide applications occurred

(*Supplemental Map 2*). MC-H-12 may be either relatively fast to recolonize or less sensitive to the florpyrauxifen-benzyl, and therefore further laboratory study of MC-H-12 is warranted.

Ham

In Ham Lake, hybrid strain HM-H-14 was by far the most abundant strain in 2018, whereas northern watermilfoil strain HM-N-15 was comparatively rare (*Figure 2.1.D*). HM-H-14 decreased disproportionately more than HM-N-15 following spot-treatments with florpyrauxifen-benzyl in 2018, and there was therefore a statistically significant change in genetic composition between 2018 and 2019 (p-value = 0.006) (*Table 2.2*). It is important to note that the disproportionate decrease of HM-H-14 may be because it was selectively targeted for herbicide treatment, whereas areas with native northern watermilfoil were not treated. However, in the absence of treatments in 2019 and 2020, HM-H-14 rebounded to pre-treatment abundance by the end of 2020 (*Figure 2.1.D*), and we did not find any statistical evidence for a change in composition between June 2018 and August 2020 (p-value > .10) (*Table 2.2*). A visual comparison of HM-H-14 incidence in treated versus untreated areas indicates that the florpyrauxifen-benzyl treatments appeared to be effective, and that the persistence and increase in HM-H-14 over time reflects recolonization of treated areas from untreated areas (*Supplemental Map 3*). Therefore, there is no immediate concern regarding the efficacy of florpyrauxifen-benzyl on this strain, but additional monitoring, and careful evaluation of the size of treated areas relative to recolonization ability of this strain, is warranted.

Phelps Bay

Phelps Bay was treated with florpyrauxifen-benzyl in 2020, and we observed a concomitant, significant change in genetic composition between June and August of 2020 (p-value < .001) (*Table 2.2*). Specifically, the incidence of several hybrid strains was zero (or nearly so), and none of the hybrid strains in this bay raise any immediate concern about resistance to florpyrauxifen. In contrast, northern watermilfoil strain (PB-N-10) persisted (*Figure 2.1.G*). Based on visual inspection of treated areas, it is unclear whether or not hybrid strains in Phelps Bay were disproportionately treated compared to northern strains (*Supplemental Map 4).* The persistence of PB-N-10 (and the concomitant change in genetic composition) raise the possibility that PB-N-10 may be less susceptible to florpyrauxifen compared to the hybrid strains present in 2018. This is surprising because observations of herbicide resistance are commonly associated with strains of invasive hybrid watermilfoil rather than native northern watermilfoil. Further genetic monitoring in Phelps Bay is warranted to determine whether the PB-N-10 will continue to outcompete and displace hybrid strains in the absence of herbicide.

North Arm

In North Arm, a bay-wide ("whole lake") fluridone treatment in 2018 virtually eliminated invasive watermilfoil, and there was no evidence for fluridone resistance by any of the strains present (*Figure 1.F*). Further, there was no evidence for a change in genetic composition between any time points during the study period (p-value > .10) (*Table 2.2*). However, MC-H-7, which was the dominant strain in June 2018 before treatment, was also the dominant strain in 2020 when a small number of plants were found (8 out of the 14 milfoil plants found in August of 2020 where MC-H-7) (*Figure 2.1.F*) (*Supplemental Map 5)*. It is unclear whether the recolonization of MC-H-7 in 2020 reflects regrowth of a small number of plants that survived the 2018 treatment, or recolonization from other Minnetonka bays (MC-H-7is also found in Smith's and Grays Bays). While there are no immediate concerns that MC-H-7 is fluridone resistant, further monitoring of North Arm is warranted to determine if it is fast growing (more invasive), which could explain its rapid appearance in North Arm Bay following treatment.

UNTREATED WATERBODIES

Smiths

In Smith's Bay, hybrid strain MC-H-7 was the most abundant strain in all three years (2018, 2019, and 2020), although overall milfoil increased between 2018 and 2019 and then decreased between 2019 and 2020 (*Figure 1.H*) (*Supplemental Map 6)*. These changes appear to be driven primarily by the dominant strain (MC-H-7), which increased disproportionately from 2018-2019, but then decreased disproportionately from 2019-2020 (although it remained the dominant strain). As a result of these disproportionate changes, we observed marginally significant changes in genetic composition in Smith's Bay from 2018 to 2019 (p-value = 0.054) and from 2019 to 2020 (p-value = 0.051) (*Table 2.2*).

Independence

In Independence Lake in 2018, Eurasian strain IN-E-1 was slightly more abundant than hybrid strain IN-H-99. However, IN-H-99 increased over the next three years and became the more dominant strain (*Figure 2.1.E*) (*Supplemental Map 7*), although this change was not statistically significant (p-value > .10) (*Table 2.2*). Nevertheless, the switch in rank between IN-H-99 and IN-E-1 is interesting and is worth additional monitoring to determine whether the hybrid strain will outcompete IN-E-1 and displace it.

Christmas

In Christmas Lake, the milfoil population was dominated by Eurasian strain CH-E-2 in 2018 (*Figure 2.1.B*). However, over the course of our study, the northern watermilfoil strain CH-N-1 increased in incidence, while the Eurasian watermilfoil strain CH-E-2 decreased (*Supplemental Map 8)*. By the end of 2019, CH-N-1 displaced CH-E-2 as the most common strain in the population which created strong evidence for a change in strain composition from June to August of 2019 (p-value = 0.036) and between June of 2019 and June of 2010 (p-value = 0.017).This pattern is surprising, because Eurasian watermilfoil is typically assumed to outcompete native northern watermilfoil, and because MC-E-1 in particular is widespread.

OVERALL

As far as we are aware, this is the first published study to examine the strain composition of invasive watermilfoil populations over time. Two previous studies have documented changes in the relative frequency of pure Eurasian vs. hybrid watermilfoil (Parks *et al.*, 2016; Thum *et al.*, 2017; Nault *et al.*, 2018). However, none of these studies examined the invasive water miloil population composition beyond distinguishing pure Eurasian from hybrid watermilfoil. To our knowledge this is the first study to document changes in strain composition in invasive watermilfoil strains.

In six of the eight waterbodies, we found evidence for a change in strain composition (meaning that strains increased or decreased disproportionately to one another) in a single year (June of one year to June of the following year): Bald Eagle Lake, Christmas Lake, Ham Lake, the North Arm of Lake Minnetonka, Smith's Bay, and Phelps Bay in Lake Minnetonka (*Table 2: One Year*). Additionally, in two waterbodies we detected strong evidence of a change in strain composition within a single growing season: Christmas Lake in 2019 and Phelps Bay (Lake Minnetonka) in 2020 (*Table 2: Growing Season*). The fact that changes were observed indicates that the genetic composition of invasive watermilfoil populations can be dynamic over time, and the number of lakes in which changes were observed (6 out of 8 in a three year period) suggests that such changes are common when multiple strains co-occur (*Table 2.2, One Year*).

Throughout the course of this study, we identified three strains of invasive watermilfoil that increased disproportionately compared to other co-occurring strains : BE-H-3 found in Bald Eagle Lake, MC-E-1 present in Bald Eagle and Smith's Bay, and MC-H-12 found in Gray's Bay, North Arm, and Smith's Bay. We therefore recommend that the growth rate and herbicide response of these strains should be investigated via vegetative growth and herbicide assays Two of these strains, BE-H-3 and MC-E-1 (both identified in Bald Eagle Lake) are widespread across Minnesota (Thum *et al.*, 2020). However, in Bald Eagle Lake following a treatment with 2,4-D, BE-H-3 increased disproportionately compared to MC-E-1 which indicates that BE-H-3 could be more invasive (faster growing and more resistant) compared to MC-E-1. As both of these strains are found in a number of other lakes in the region (Thum *et al.*, 2020), including them in laboratory assays could reveal insights about their relative invasiveness that could be applied to the management multiple lakes. Additionally, MC-H-12 identified from Grays Bay warrants further investigation because it increased in both 2018 and 2019 despite repeated exposure to florpyrauxifen-benzyl, which indicates that it either has a fast growth rate and/or is less sensitive to herbicide treatment.

Interestingly, in three lakes we observed native northern watermilfoil strains that appeared to be outcompeting their invasive counterparts. In Bald Eagle Lake, native strain BE-N-2 decreased less than its invasive hybrid and pure Eurasian counterparts following 2,4-D treatment in 2018. Additionally, over the next two years BE-N-2 continued to increase and remained the most common strain. In Phelps Bay, native strain PB-N-10 persisted despite florpyrauxifen-benzyl treatment while its hybrid counterparts perished. In both Bald Eagle and Phelps Bay, visual inspections of the maps are inconclusive as to whether or not the invasive pure Eurasian and hybrid watermilfoil strains were disproportionately treated compared to native northern strains . Additionally, in Christmas Lake, over the course of this study native northern watermilfoil strain CH-N-1 displaced pure Eurasian strain CH-E-2 as the most common strain in the population. This pattern is surprising, because long term field observation suggests that both hybrid and pure Eurasian watermilfoil are generally more invasive and outcompete native northern watermilfoil (Aiken, 1979; Nichols, 1994), although see (Valley and Newman, 1998). One possible explanation for the disproportionate increase of native northern watermilfoil over pure Eurasian watermilfoil may be the presence of native milfoil weevil (*Euhrychiopsis lecontei*) because weevils prefer Eurasian watermilfoil over native northern (Newman, 2014). Our observations suggest that the interactions between native and invasive watermilfoils may warrant further investigation.

The herbicide response of the rest of the strains detected in this study did not raise immediate concerns. However, where feasible, we recommend continued genetic monitoring to identify potentially problematic strains before they become management concerns.

RECOMMENDATIONS:

Genetic monitoring can and should be used as a diagnostic tool to assist managers with identifying potentially problematic strains. However, integrating genetic fingerprinting into aquatic vegetation surveys is not a sufficient method of confirming resistance, and laboratory assays are still necessary. Here we identify potentially confounding factors that should be accounted for when interpreting the results of genetic monitoring efforts, and we offer suggestions for further research to address these challenges.

First, when interpreting lake-wide dynamics in spot treated lakes, it is important to consider the spatial structure of milfoil strains and whether there is any relationship between where each strain is found and where spot-treatments where applied. For example, if one strain happens to disproportionately occur inside or outside of treated areas, then it may disproportionately decrease or increase, which could confound inferences about its relative sensitivity to the herbicide application. We therefore recommend further investigation into the spatial structure of milfoil strains within lakes, and that genetic monitoring efforts focus on full lake treatments to avoid some of the confounding factors associate with spot treatments.

Secondly, it is important to recognize that genetic monitoring cannot necessarily distinguish between plants that recolonized treated areas from untreated areas, versus plants that survived herbicide treatment because they exhibit some level of resistance, per se. For example, fast-growing strains may not exhibit resistance, but may effectively recolonize treated areas over short periods of time, giving the appearance of resistance. Nevertheless, we argue that strains that occur within treated areas relatively quickly after treatment should be prioritized for laboratory study to specifically test whether they are resistant and/or fast growing (i.e., relatively more invasive).

Finally, it is important to distinguish between statistical significance and biological significance when interpreting genetic monitoring data. Restrictions in sample size present a challenge in acquiring adequate statistical power to detect biologically significant changes. For example, rare strains will have low counts, and concomitantly low statistical power to detect changes. Similarly, when herbicide treatments are efficacious overall, the number of survey points with milfoil post-treatment will be low, and therefore the power to detect changes in composition may be low unless and until milfoil occurrence increases to pre-treatment levels. Specific to spot treatments, the number of intercept points that fall within treated areas may be low, which will limit the power to detect changes in treated areas. To address the challenge of statistical power, we recommend in silica power analyses and simulations to determine the amount of sampling necessary to detect changes of different magnitudes under different initial frequency scenarios.

CONCLUSION

This study demonstrates that integrating genetic fingerprinting into aquatic vegetation management and evaluation holds promise to inform management decisions by identifying potentially problematic strains. We provide proof of concept that it is possible 1) to detect changes over time in strain composition of invasive watermilfoil populations, and 2) to identify high priority strains for further testing based on the pattern of those changes. We also provide sampling and interpretation recommendations for genetic monitoring strategies in the future. We argue that genetic monitoring holds the potential to be an important tool for invasive watermilfoil control and encourage lake management plans to include it where feasible.

SOURCES OF MATERIALS

[1]DNeasy Plant Mini Kit. Qiagen Corp. 27220 Turnberry Lane, Suite 200, Valencia, CA 91355.

ACKKNOWLEDGEMENTS

We would like to thank R. Newman and J. Feely for their help with the collection of the Minnesota samples used in this study. We would also like to thank the Minnesota Aquatic Invasive Species Research Center, and Montana State University for providing the funding of this project. We thank Greg Chorak, Hannah Hoff, Jeff Pashnick, Ashley Wolf, and Zach Kuzniar for providing friendly review of this manuscript.

Table 2.1: The location of all waterbodies included in this study and the number of sites samples in each waterbody.

LAKES SURVEYED

Lake	*State*	*County*	*Latitude*	*Longitude*	*Sites Sampled*
Christmas	MN	Henepin	44.8968	-93.5445	113
Bald Eagle	MN	Ramsey	45.1159	-93.0165	151
Grays Bay	MN	Henepin	44.9539	-93.4945	125
Ham	MN	Anoka	45.2571	-93.2219	147
Independence	MN	Henepin	45.0293	-93.645	198
North Arm	MN	Henepin	44.9587	-93.62	229
Phelps Bay	MN	Henepin	44.9156	-93.649	148
Smith' Bay	MN	Henepin	44.9526	-93.5672	127

SURVEY & TREATMENT HISTORY

	2018		2019		2020	
Lake	*Sampling*	*Treatment*	*Sampling*	*Treatment*	*Sampling*	*Treatment*
Christmas	Jun &Aug	None	Jun &Aug	None	June	None
Bald Eagle	Jun &Aug	2,4-D	Jun &Aug	None	Jun &Aug	None
Grays Bay	Jun &Aug	florpyrauxifen-benzyl	Jun &Aug	florpyrauxifen-benzyl	Jun &Aug	Diquat & florpyrauxifen-benzyl
Ham	Jun &Aug	florpyrauxifen-benzyl	Jun &Aug	None	Jun &Aug	None
Independence	Jun &Aug	None	Jun &Aug	None	Jun &Aug	None
North Arm	Jun &Aug	Fluridone	August	None	August	None
Phelps Bay	None	None	August	None	Jun &Aug	Diquat & florpyrauxifen-benzyl
Smith' Bay	July	None	July	None	July	None

Table 2.2: The sampling times and herbicide treatments for each waterbody for each year of the study

CHANGE IN COMPOSITION

		Growing Season			**One Year**		**Three Years**
Lake	*Treatment*	*2018*	*2019*	*2020*	2018-2019	*2019-2020*	
Bald Eagle	2018, 2020	0.136	0.347	0.276	<.001	1	<.001
Grays Bay (Minnetonka)	2018, 2019, 2020	0.793	0.94	0.222	0.584	0.969	0.18
Ham	2018	0.304	0.256	0.754	0.006	0.143	0.33
North Arm (Minnetonka)	2018	*NA*	*NA*	*NA*	0.009	0.642	0.28
Phelps Bay (Minnetonka)	2020	*NA*	*NA*	0.008	*NA*	<.001	<.001
Smith' Bay (Minnetonka)	--	*NA*	*NA*	*NA*	0.061	0.054	0.36
Christmas	--	0.199	0.036	*NA*	0.096	0.017	<.001
Independence	--	0.678	1	0.302	0.335	0.279	0.35

Table 2.3: The amount of evidence for a change in strain composition in each waterbody at each of three timescales: within the growing season of each year, between years sampled, and over the three year window. P-values are based on a simulated Chi squared test for homogeneity with 2,000 permutations. The treatment column indicates the years that each waterbody received an herbicide treatment (for the specific herbicide used at each timepoint see Table 1).

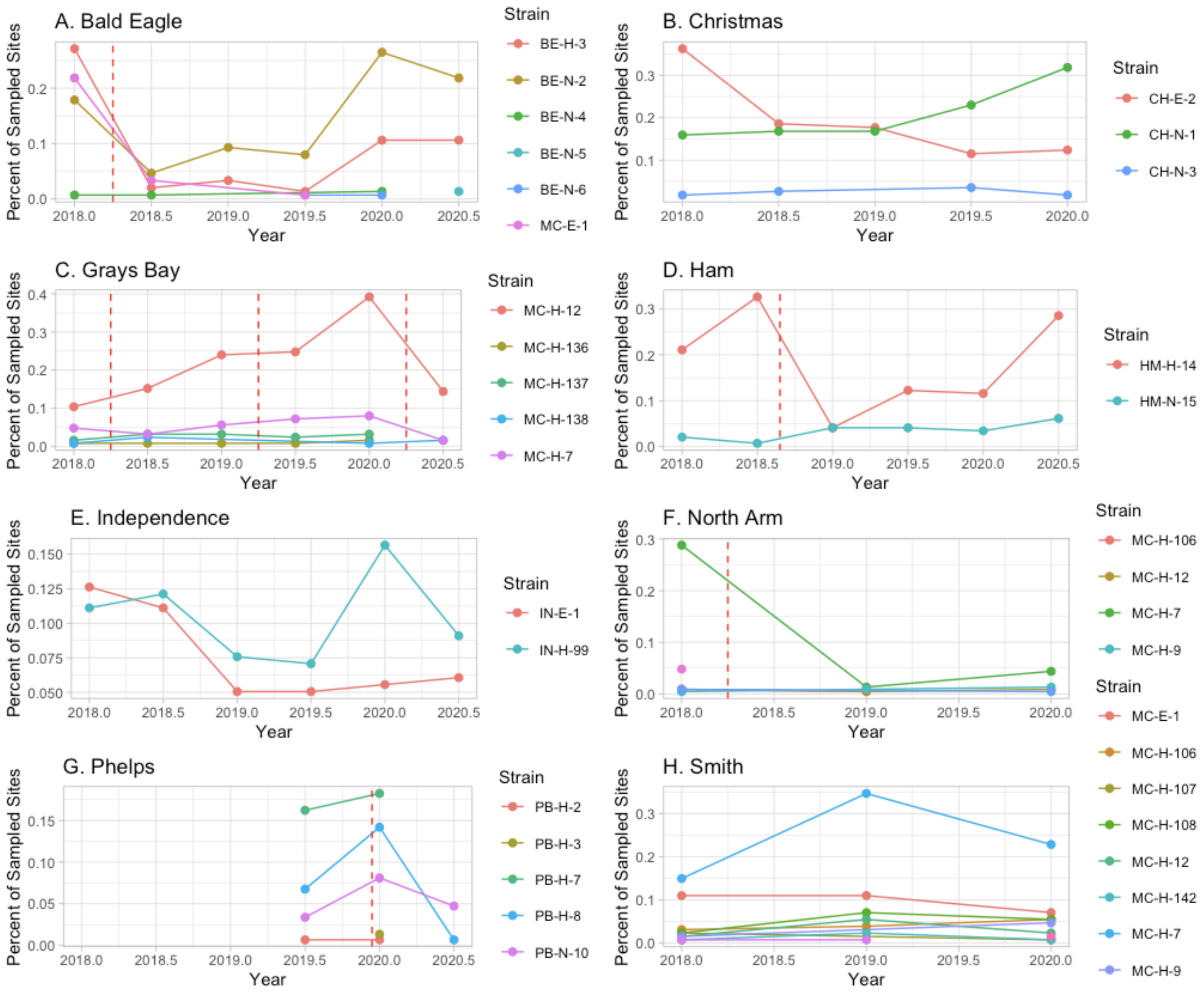


Figure 2.1: The strain composition of the milfoil population in each waterbody over time. Each panel represents a different waterbody. Point shapes and line types indicate different strains within the waterbody. The x axis represents the sampling timepoint, the y-axis represents the percent of sampled sites where each strain was found (number of sites were the strain was collected divided by the total number of sites where milfoil was collected). Strains are identified by a four-character code: the first two letters indicate the waterbody in which the strain was identified, the middle letter indicates whether the strain is pure Eurasian watermilfoil (E), native northern watermilfoil (N), or a hybrid (H), and the final number distinguishes strains of the same taxon within the same waterbody. The abbreviation “MC” stands for “Minnesota Clones” and is used to distinguish strains that are found in multiple waterbodies included in this study.

SUPPLEMENTAL MAPS

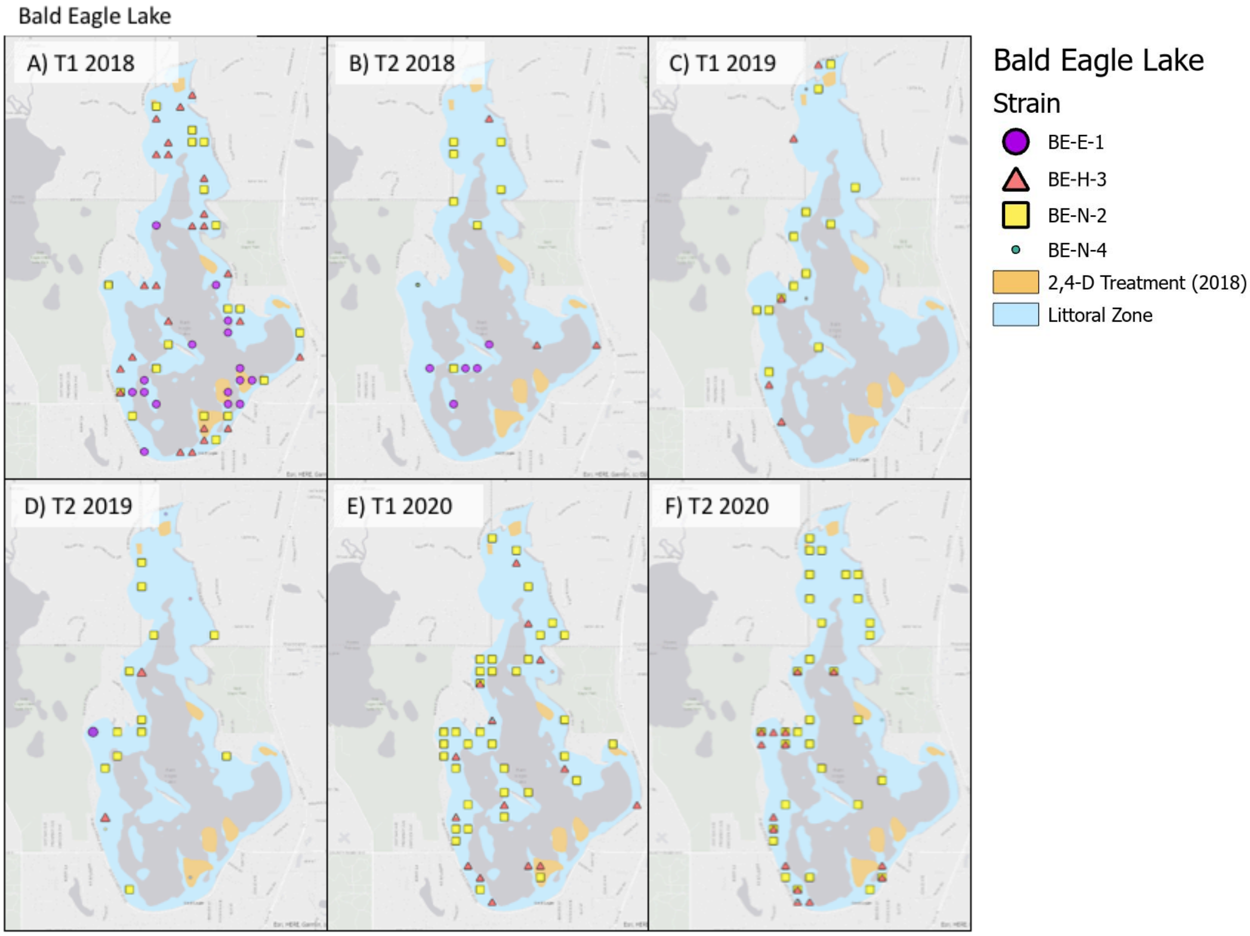


Figure 2.2: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in Bald Eagle Lake. Eurasian strains are represented by circles, hybrid strains are represented by triangles, and northern strains are represented by squares. Strains within each taxa are distinguished by color. The littoral zone is shaded in light blue and the areas spot treated with the herbicide 2,4-D are shaded orange. Panel (A) shows the strain distribution in June 2018, (B) August 2018, (C) June 2019, (D) August 2019, (E) June 2020, and (F) August 2020.

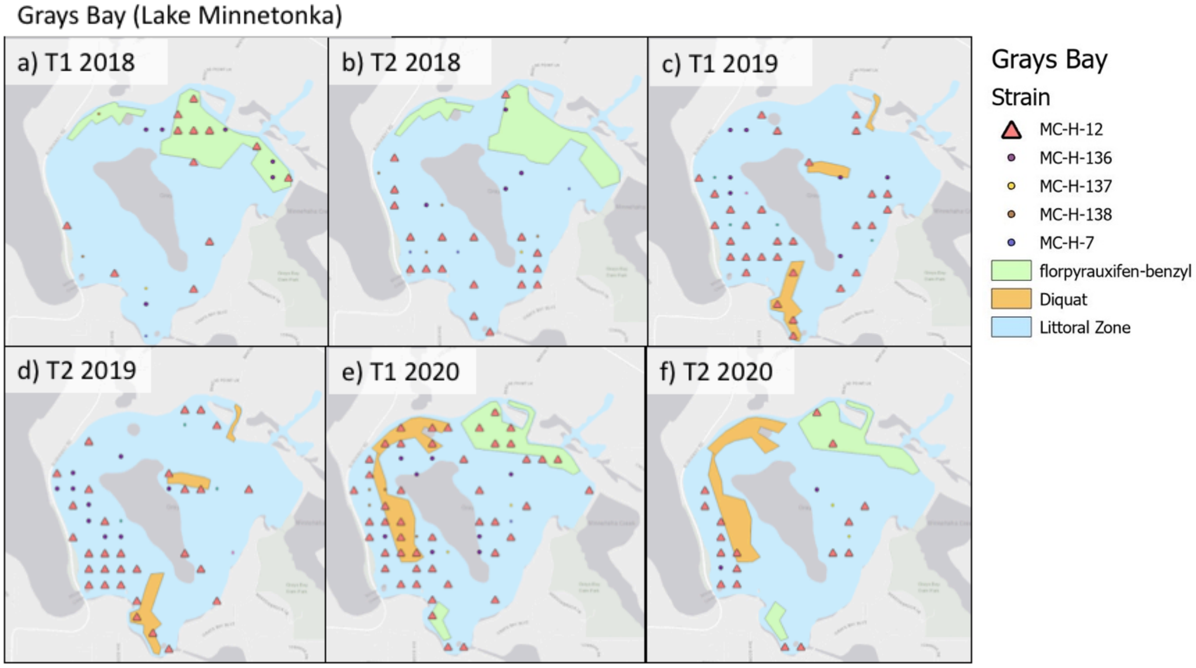


Figure 2.3: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in Grays Bay in Lake Minnetonka. Sites where hybrid strain MC-H-12, the most abundant strain in the bay, was found are represented by pink triangles. Sites with all other strains of milfoil are represented by dots and the color of those dots indicates the strain present . The littoral zone is shaded in light blue, the areas spot treated with the herbicide florpyrauxifen-benzyl are shaded green, and areas spot treated with the herbicide Diquat are shaded orange. Panel (A) shows the strain distribution in June 2018, (B) August 2018, (C) June 2019, (D) August 2019, (E) June 2020, and (F) August 2020.

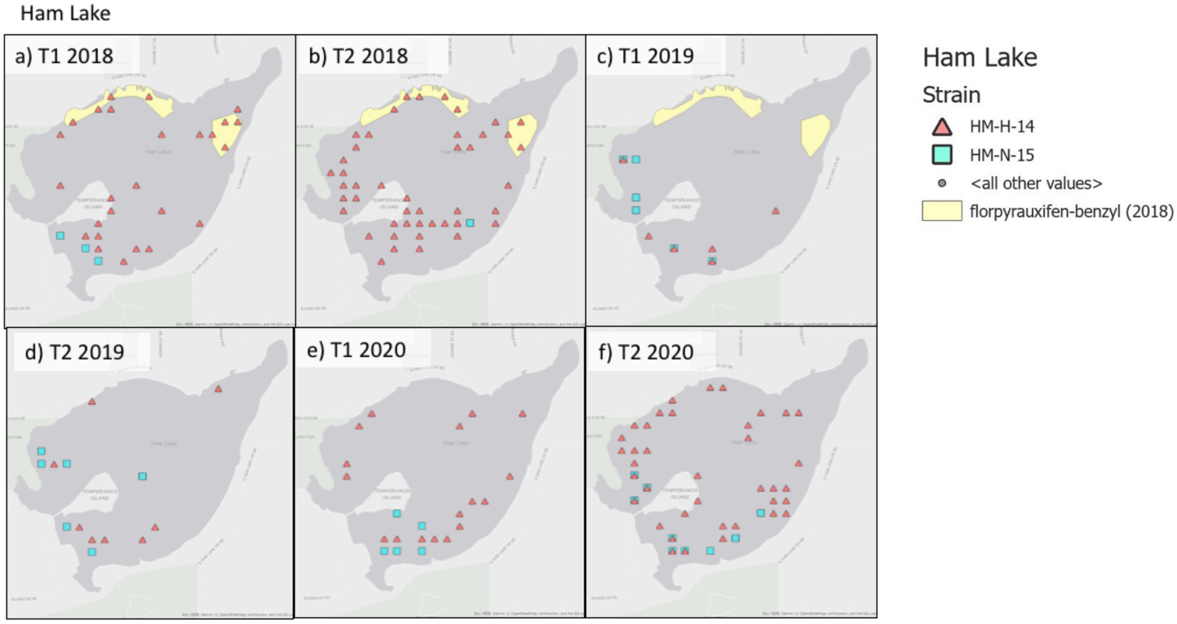


Figure 2.4: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in Ham Lake. Sites where hybrid strain HM-H-14 was found are represented by pink triangles and sites where northern strain HM-N-15 was found are represented by blue squares. Areas that were spot treated with flurpyrauxifen-benzyl in July of 2018 are shaded in yellow. Panel (A) shows the strain distribution in June 2018, (B) August 2018, (C) June 2019, (D) August 2019, (E) June 2020, and (F) August 2020.

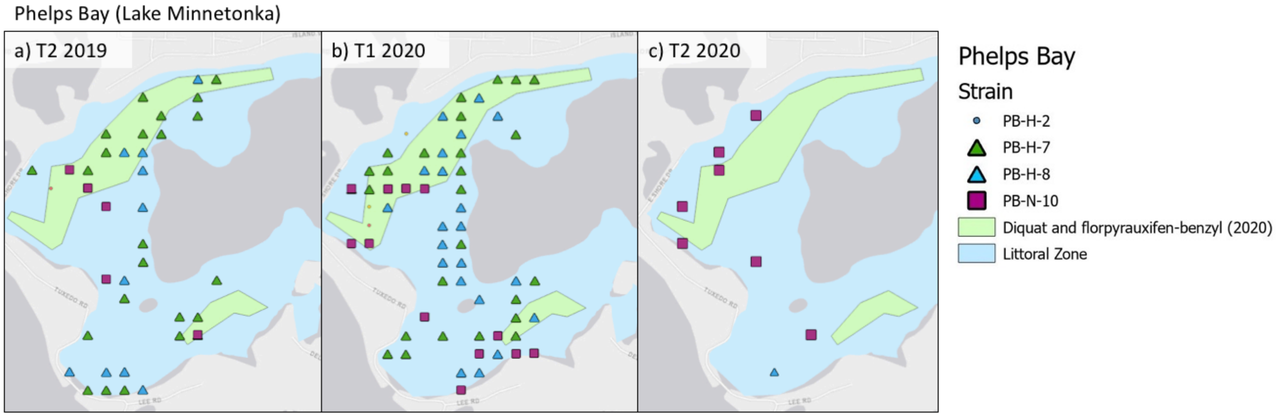


Figure 2.5: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in Phelps Bay in Lake Minnetonka. Strains are distinguished by shape and color.. The littoral zone is shaded in light blue and the areas spot treated with the herbicides diquat and florpyrauxifen-benzyl in June of 2020 are shaded green. Panel (A) shows the strain distribution in August of 2019, (B) June 2020, (C) August 2020.

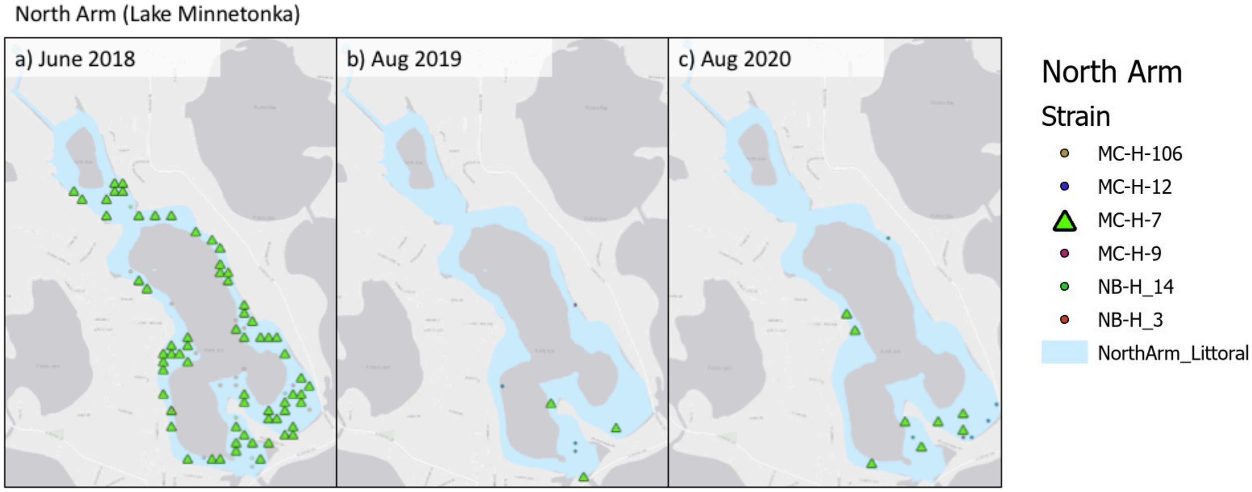


Figure 2.6: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in the North Arm of Lake Minnetonka. Sites where hybrid strain MC-H-7, the most abundant strain in the bay, was found are represented by green triangles. Sites with all other strains of milfoil are represented by dots and the color of those dots indicates the strain present . The littoral zone is shaded in light blue. In July of 2018 North Arm received a full bay bay treatment with the herbicide fluridone. Panel (A) shows the strain distribution in June 2018 before the fluridone treatment, (B) August 2019 one year after the fluridone treatment, (C) August of 2020 two years after treatment.

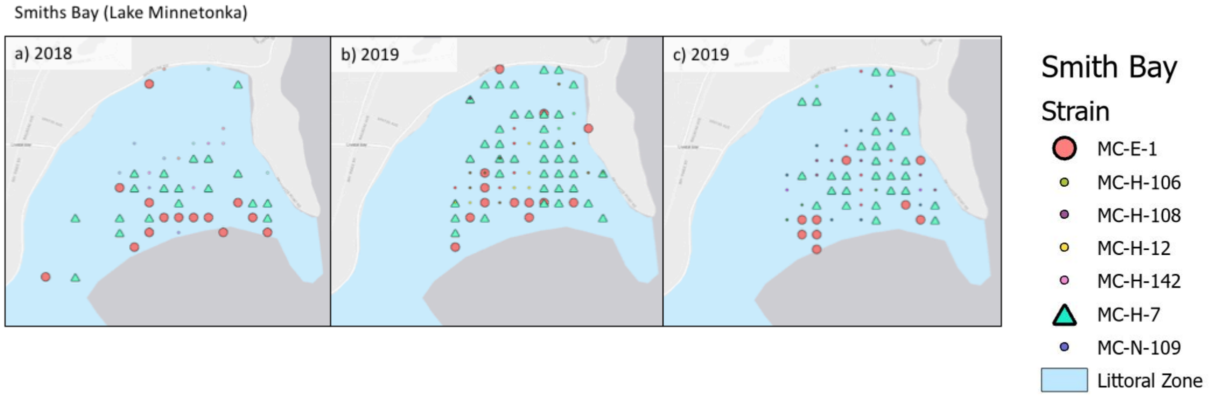


Figure 2.7: Maps of the distribution of strains of northern, Eurasian, and hybrid watermilfoil in the Smith Bay of Lake Minnetonka. Sites where hybrid the widespread strain MC-E-1was found are represented by large pink circles and sites where the widespread strain MC-H-7 was found are represented by turquoise triangles. Sites with all other strains of milfoil are represented by dots and the color of those dots indicates the strain present. The littoral zone is shaded in light blue. Panel (A) shows the strain distribution in June of 2018, (B) in June of 2019, and (C) in June of 2020.

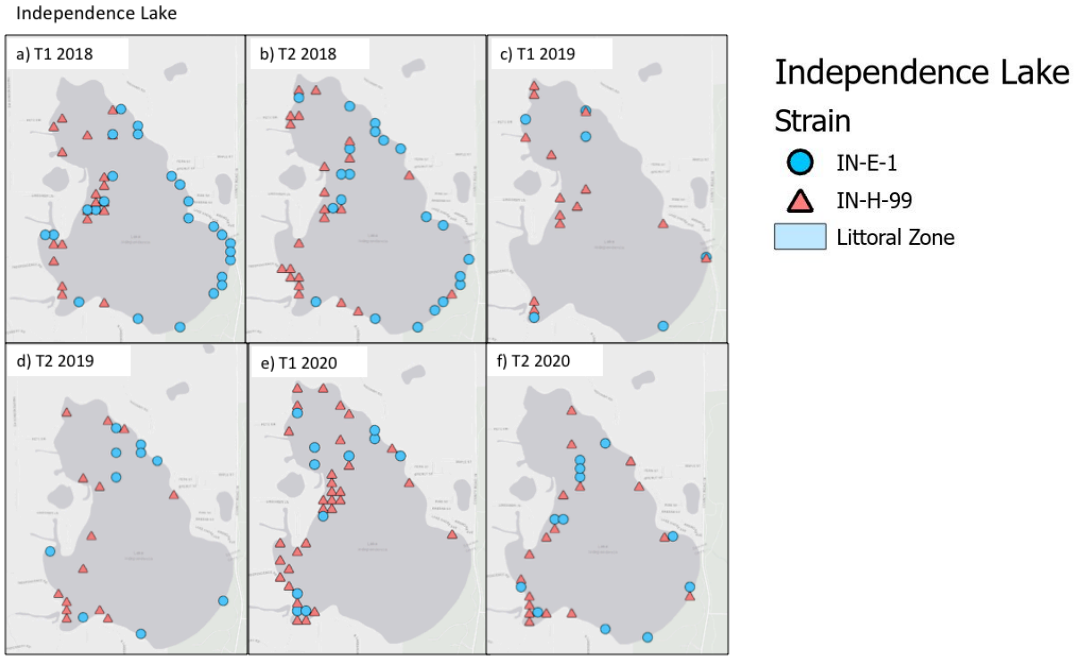


Figure 2.8: Maps of the distribution of strains of Eurasian and hybrid watermilfoil in Independence Lake. Sites where Eurasian strain IN-E-1was found are represented by turquoise circles and sites where hybrid strain IN-H-99 where found are represented by pink triangles. The littoral zone is shaded in light blue. Panel (A) shows the strain distribution in June 2018, (B) August 2018, (C) June 2019, (D) August 2019, (E) June 2020, and (F) August 2020.

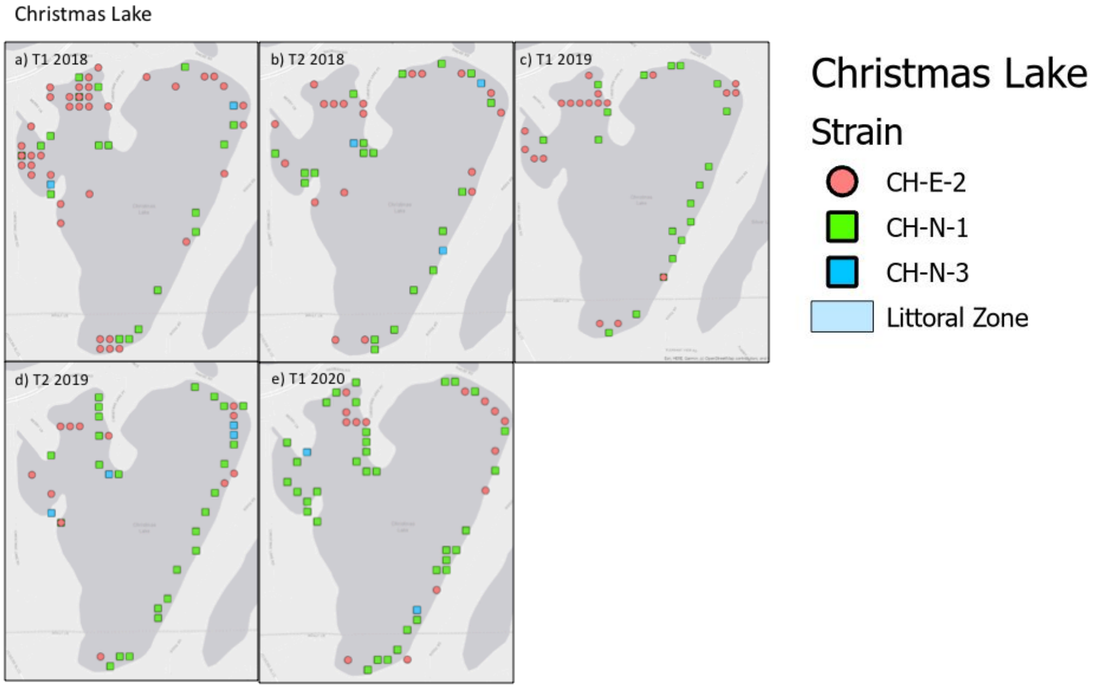


Figure 2.9: Map of the distribution of Eurasian and northern strains of watermilfoil in Christmas Lake. Sites where Eurasian strain CH-E-2 was found are represented with pink circles, sites where northern strain CH-N-1 was found are represented by green squares, and sites where northern strain CH-N-3 was found are represented by blue squares.

CHAPTER THREE

IMPROVING GENETIC MONITORING OF INVASIVE WATERMILFOIL IN MANAGED LAKES

Genetic surveys can help inform management decisions if the strains present have previously been characterized/screened. Further, large differences between strains in growth rate or herbicide response can be detected by integrating genetic identification into point intercept surveys conducted before and after treatment. However, while large differences between strains can create large changes in strain relative frequency, less extreme differences are likely to create only subtle changes in strain relative frequency, which may be difficult or impossible to distinguish from the effects of confounding variables. These less extreme differences between strains may still be important for long term management. Ensuring adequate sample size will help to increase the power to detect more subtle changes in strain frequency.

Sample Size

In our study, we implemented a systematic grid-based point intercept sampling methodology for aquatic plants that was designed and tested by Mikulyuk et. al. in 2010 (Mikulyuk *et al.*, 2010). Similar sampling methods have been used in other studies investigating invasive Eaurasian watermilfoil, for example see (Parks *et al.*, 2016; Nault *et al.*, 2018; Eltawely, Newman and Thum, 2020). However, this point intercept based approach was designed with the goals of accurately defining species richness and detecting changes in the frequency of each species (Mikulyuk *et al.*, 2010). It was not designed with the intention of comparing these changes in frequency between species, and it therefore does it necessarily produce adequate sample size to test for differences between species (or strains) in how they change overtime (Mikulyuk *et al.*, 2010).

Many of waterbodies surveyed in this study yielded a small sample size even though more than 110 sites were visited in each lake based on the pre-determined point intercept grid. For example, in Ham Lake 147 sites were visited twice per year, but on average milfoil was only present at 32 sites, and therefore the sample size available for detecting changes in strain composition was always well below 147.

It was especially difficult to collect sufficient plant material following a successful herbicide treatment. For example, in the North Arm of Lake Minnetonka following a full bay fluridone treatment in July 2018, milfoil was not present at any of the sites visited in August of 2018. Similarly, one year later in August 2019, milfoil was found at only six sites. While the paucity of milfoil is a positive indication for the efficacy of the herbicide treatment, such effective treatments pose difficulties in sample size and analysis.

Spot treatments introduced further complications. Because spot treatments fundamentally impact only a portion of the waterbody, evaluation of their impact on the milfoil population must be confined to analyzing treated areas rather than analyzing the waterbody as a whole. The results in treated areas can be compared to those from untreated areas. However, observed changes in untreated areas can not necessarily be attributed to the treatment nor can they be considered independent from the treatment. Therefore, the sample size available for investigating the impact of herbicides in spot treated lakes was restricted to the number of sites on the grid that fell within treated areas which severely limited our analysis. For example, when Grays Bay in Lake Minnetonka received a spot treatment in 2018, only 22 sites on the PI grid fell within the treated areas. Of those sites, milfoil was present at only 13 before treatment and only 3 sites after treatment.

Further, in diverse lakes, the number of samples for a given strain, particularly rare strains, was insufficient to make comparisons at the strain level. For example, Smith's Bay in Lake Minnetonka contains 10 different strains (not including singletons), and five of those strains were collected fewer than ten times total across all three years. Three strains were found fewer than five times.

Causality: Gene Flow and Spatial Structure

Based on survey data, it is challenging to establish whether observed changes in strain composition were caused by differential response to herbicide treatments or by other confounding factors. Gene flow between populations/waterbodies and the spatial structure of strains within waterbodies both complicate the interpretation of results.

Gene Flow

It is widely established that, in at least some cases, regional processes can impact the distribution of freshwater plants (Husband and Barrett, 1996; Dahlgren and Ehrlén, 2005). In the case of invasive watermilfoil milfoil, previous studies have documented strains that are shared between multiple lakes (Thum *et al.*, 2020) and between different bays of large lakes (Eltawely, Newman and Thum, 2020), indicating that strains can spread both between and within waterbodies. For example plant fragments of different strains can be introduced from adjacent lakes or from other regions within the same lake via boat movements or hydrologic connectivity (Eltawely, Newman and Thum, 2020). For example, in different bays in Lake Minnetonka observed shifts in strain composition may be due in part to introductions from adjacent bays. The movement of plant fragments could increase/inflate the relative abundance of an already present strain or could introduce and support the establishment of new strains in the area. Such patterns of strain movement are of particular importance when evaluating response to herbicide treatment because (re)colonization from connected waterbodies and/or untreated areas in the same waterbody could wash away any dynamics that are the result of different herbicide response.

Spatial Structure

The spatial structure of milfoil populations within waterbodies may also affect the relative impact of herbicide application on different taxa or strains. Field observations indicate that strains of the same taxa tend to grow together and in some cases at different depths (Newman, 2014, 2020). For example, in Phelps Bay in Lake Minnetonka in 2019 before herbicide treatment, the northern shore and southern inlet were inhabited primarily by interspecific hybrid watermilfoil, while the central region of the bay contained a combination of northern and hybrid milfoil (Fig 3.2). When Phelps Bay was treated with florypyrauxifen-benzyl and diquat in July 2020, the spot treatments were applied along the northern shore and near the southern inlet rather than in the central region of the bay. Subsequently, the incidence of hybrid watermilfoil disproportionately decreased compared to the incidence of northern watermilfoil (Figure 3.1). It is possible that the change in relative frequency was in part the result of a biological difference between northern watermilfoil and interspecific hybrids in response to florypyrauxifen-benzyl and diquat. However, the areas of the bay that were treated in relation to the areas of the bay that contained each taxon may also have contributed to the observed change in relative frequency (Fig 3.2).

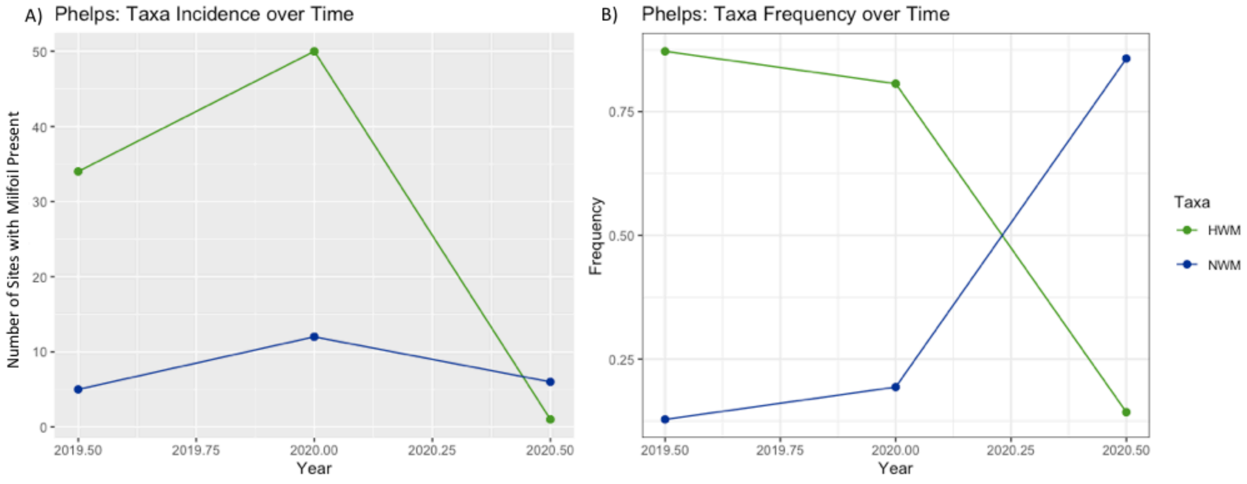
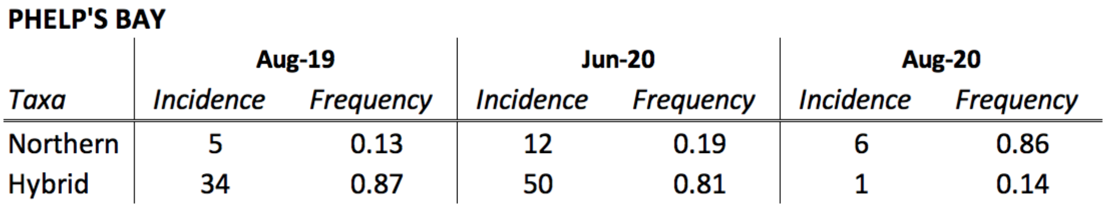


Figure 3.1: The incidence (A) and frequency (B) of northern watermilfoil and hybrid watermilfoil in Phelps Bay (Lake Minnetonka) in August 2019, June 2020, and August 2020. Incidence is equal to the number of sites at which each taxon (northern or hybrid) was collected at each timepoint. Frequency is equal to incidence divided by total milfoil abundance. The green line represents hybrid watermilfoil and the blue line represents northern watermilfoil.



PHELP'S BAY

	Aug-19		Jun-20		Aug-20	
Taxa	*Incidence*	*Frequency*	*Incidence*	*Frequency*	*Incidence*	*Frequency*
Northern	5	0.13	12	0.19	6	0.86
Hybrid	34	0.87	50	0.81	1	0.14

Table 3.1: Incidence and frequency for northern watermilfoil and hybrid watermilfoil in Phelps Bay (Lake Minnetonka) in August 2019, June 2020, and August 2020. Incidence is equal to the number of sites at which each taxon (northern or hybrid) was collected at each timepoint. Frequency is equal to incidence divided by the total number of sites were milfoil was present. A total of 148 sites were sampled in Phelps Bay at each timepoint.

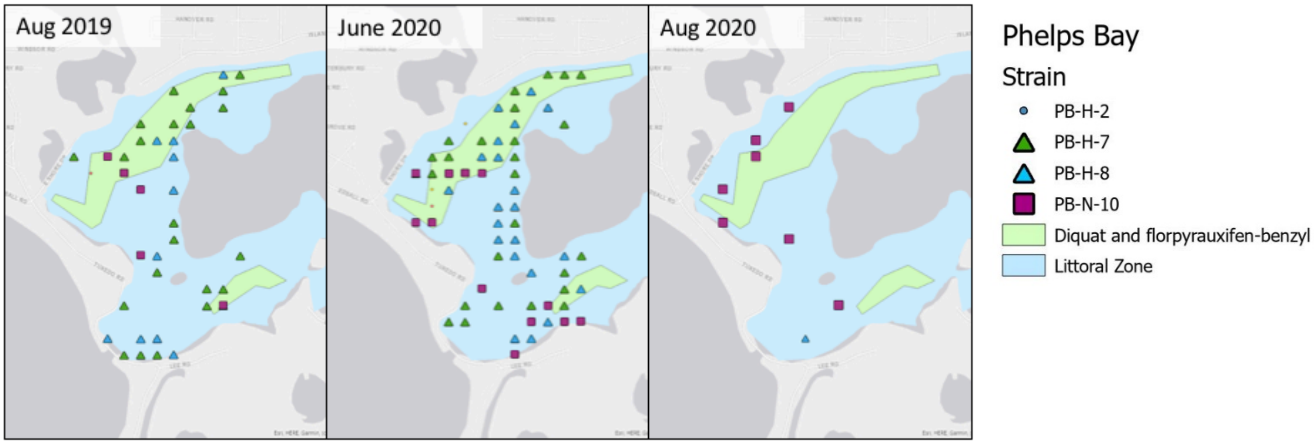


Fig 3.2: Map of the littoral area of Phelps Bay (Lake Minnetonka) at each time point. Light blue shading indicates the littoral zone, green triangles represent sites where strain PB-H-7 was collected, and blue triangles represent sites where hybrid strain PB-H-8 was collected, and purple squares indicate sites were northern strain PB-N-10 was collected. Green shaded polygons represent areas that were treated with diquat and florpyrauxifen-benzyl in July 2020.

Future Research

We recommend power analyses and simulations be used to determine the amount of sampling necessary to detect changes of different magnitudes under different initial frequency scenarios (Larsson *et al.*, 2008; Tallmon *et al.*, 2010; Hoban *et al.*, 2014). In the absence of quantitative guidance on sample size, we offer the below recommendations based on our study. We recommend that:

- A predetermined number of samples (>60) be randomly collected before and after treatment rather than using a pre-determined point intercept grid (Tallmon *et al.*, 2010).
- If research is carried out in spot treated lakes, then the same predetermined number of samples be randomly collected within treated areas and in untreated areas for comparison.

- Lakes with a previous history of poor control/ineffective treatment should be prioritized for monitoring because of the increased probability of observing a more invasive strain.
- Lakes receiving full lake treatments be prioritized over spot treated lakes in order to minimize confounding variables.
- Lakes be prioritized that harbor only 2-3 strains to ensure that an adequate number of samples can be collected for each strain. However, we recognize that this requires previous genetic survey which is not always feasible.

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