Distribution of Eurasian and hybrid watermilfoil in Minnesota

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Abstract

Eurasian watermilfoil (*Myriophyllum spicatum* L.) hybridizes with the native northern watermilfoil (*M. sibiricum* Kom.), which raises new issues regarding management strategies to control infestations. To determine the distribution of hybrid (and coincidentally Eurasian and northern) watermilfoil in Minnesota, lakes across the state with a range of sizes and duration of invasion, were sampled during 2017-2018 for watermilfoil. A total of 62 lakes were sampled of which 43 contained Eurasian, 28 contained hybrid and 21 contained northern watermilfoil. Hybrid watermilfoil populations were found in lakes with or without either Eurasian or northern, indicating hybrids do not require parental taxa be present in order to populate a lake. There were clear significant geographic distinctions between northern and hybrid watermilfoil occurrences with 81% of hybrid populations occurring in lakes in the 7 county Twin Cities metro and 71% of northern populations occurring in lakes outside of the metro. Eurasian watermilfoil was evenly distributed across the state with about half of its occurrences within the metro and half outside. We found a total of 8 unique Eurasian genotypes, 57 hybrid genotypes, and 81 northern watermilfoil genotypes. We found that 93% of Eurasian watermilfoil infestations were the same widespread genotype. We found that 64% of hybrid watermilfoil infestations were unique hybrid genotypes, but we found four hybrid genotypes that were in multiple lakes. Assessment of environmental variables associated with milfoil infestations indicated that Eurasian, hybrid and northern watermilfoil are present in environmentally similar lakes and no lake specific variables significantly affected the probability of one taxon being present over another.

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum*) hybridizes with the native northern watermilfoil (*M. sibiricum* Kom.) (Moody and Les 2002). It is difficult to morphologically distinguish between Eurasian and hybrid watermilfoil with certainty (Moody and Les 2007) and thus both are typically treated as one species with similar management strategies. Recently concern has arisen for hybrid watermilfoil, which may respond differently to management or be more invasive than pure Eurasian. Although managers and aquatic botanists increasingly recognize Eurasian and hybrid watermilfoil as distinct taxa, they are not frequently distinguished when it comes to operational management strategies, control tactics, or evaluations of management actions. It is important to identify hybrid watermilfoil occurrences and determine its distribution to further our understanding of hybridity as it relates to invasiveness. Assessments of Eurasian and hybrid watermilfoil distribution can provide insights to distribution patterns of invasive aquatic plants and further our understanding of invasion biology.

Eurasian watermilfoil is native to Europe, Asia and Africa (Moody et al. 2016) and is now present in 48 states and three Canadian provinces (Pfingsten et al. 2019). Eurasian watermilfoil was first documented in Minnesota in Lake Minnetonka in 1987 and has since spread to over 300 waterbodies (Invasive Species Program 2017). Eurasian watermilfoil forms dense canopies that can reduce native species richness through the suppression of native vegetation (Madsen et al. 1991). As a result of the decrease in native plant populations, Eurasian watermilfoil can negatively affect animals that depend on the health of aquatic ecosystems (Eiswerth et al. 2000). Nuisance growth of Eurasian watermilfoil can also inhibit recreational use of waterbodies (Aiken et al. 1979). Millions are spent in the U.S. annually on the control of Eurasian watermilfoil and permits are issued for control on 80 to 100 lakes per year in Minnesota (Invasive Species Program 2017). Despite widespread control and prevention efforts, new cases of Eurasian watermilfoil invasions accumulate every year (http://www.dnr.state.mn.us/invasives/ais/infested.html).

The presence of hybrid watermilfoil has been confirmed in Minnesota, as well as in other parts of North America (Moody and Les 2007, Sturtevant et al. 2009, Borrowman et al. 2014). In Wisconsin ~150 hybrid watermilfoil infestations have been confirmed (Nault et al. 2018). Although hybrid watermilfoil populations have been documented, the spatial distribution and habitat characteristics associated with their presence have yet to be investigated. Determining the distribution of hybrid watermilfoil and identifying habitat characteristics associated with occurrences are important to understanding its spread and establishment as an invasive. By comparing Eurasian and hybrid watermilfoil distribution patterns we can determine what variables are associated with hybrid presence and further our understanding of hybrid spread.

Hybridization between introduced and native species can lead to novel recombinations with superior competitive phenotypes (Ellstrand and Schierenbeck 2000, Moody and Les 2002, 2007) increasing their likelihood of survival and establishment success in novel habitats. Through molecular genetic studies it has been determined that genetic diversity is quite high in hybrid watermilfoils as a result of sexual reproduction (Zuellig and Thum 2012, LaRue et al. 2013a). Hybrid watermilfoils may be capable of exhibiting traits that are not present in parental taxa (LaRue et al. 2013a), as well as combinations of parental traits creating novel phenotypes (Hovick and Whitney 2014, Thum and McNair 2018). As a result, hybrid watermilfoils have the ability of being more invasive.

Several studies have found that some hybrid watermilfoil genotypes are less affected by herbicides than Eurasian, including auxinic herbicides such as triclopyr and 2,4-D (2,4-dichlorophenoxy acetic acid) (LaRue et al. 2013c, Nault et al. 2018, Parks et al. 2016, Thum et al. 2017) as well as fluridone (Berger, Netherland and MacDonald 2012, 2015, 2017, Thum et al. 2012). Parks et al (2016) found a greater reduction in Eurasian watermilfoil in comparison to hybrid, following treatment with auxinic herbicides similar results were found by Nault et al (2018) following treatment with 2,4-D. A fluridone-tolerant population of hybrid watermilfoil has been confirmed in several studies (Berger 2012, Berger, Netherland and MacDonald 2012, Thum et al. 2012). The results of these studies indicate that the identification of hybrid watermilfoil occurrences is important to determine.

Previous studies in Minnesota have analyzed predictors of Eurasian watermilfoil invasions (Roley and Newman 2008, Kanankege et al. 2018). Roley and Newman (2008) determined that Eurasian watermilfoil occurrences were most accurately predicted by distance to the nearest invaded lake and duration of that invasion. Their study also identified other characteristics including lake size, alkalinity, Secchi depth, and lake depth as significant predictors of Eurasian watermilfoil occurrence (Roley and Newman 2008). Confirmed Eurasian watermilfoil infestations in Minnesota have also been determined to be confounded by human population densities as well as associated with interstate highways (Kanankege et al. 2018). Factors influencing the occurrence of hybrid watermilfoil have not been assessed; therefore we are unsure whether hybrid exhibits similar tendencies to Eurasian, or is much different from its exotic or native parents. By determining what habitats are susceptible to the invasion of hybrid watermilfoils, we can better provide information regarding which lakes are more likely to possess hybrids.

The pattern of hybrid watermilfoil distribution in Minnesota and North America has yet to be fully understood and requires further investigation. Lake management and human activities can accelerate the process of hybridization by bringing into contact previously isolated lineages (Ellstrand and Schierenbeck 2000) as well as by creating environments that are better suited for hybrid genotypes than parental species (Wu et al. 2015). Through the process of hybridization, novel genotypes can be generated that are better suited to withstand herbicidal treatments in comparison to parental taxa, which may lead to the displacement of the parental species (Ellstrand and Schierenbeck 2000, Schierenbeck and Ellstrand 2009).

Previous studies assessing Eurasian and hybrid watermilfoil distribution have found varying patterns of milfoil co-occurrence (Moody and Les 2007, Sturtevant et al. 2009, Borrowman et al. 2014).

This information can be used to assess whether hybrid watermilfoil coexists with Eurasian and northern or tends to displace its parental taxa over time. Moody and Les (2007) did not find that Eurasian and hybrid watermilfoil co-occurred in Minnesota lakes, although their coexistence within lakes was quite common in Michigan (Sturtevant et al. 2009). Hybrid watermilfoil populations in Ontario were only found in lakes where both parental species have been historically present (Borrowman et al. 2014). Novel hybrid watermilfoil genotypes may be able to thrive in habitats where parental species cannot, or grow earlier or faster, giving it the potential to have increased invasiveness; this relationship has not been assessed for hybrid watermilfoil populations.

The objective of our study was to describe the geographic distribution of hybrid watermilfoil among Minnesota lakes and relate this to human and environmental factors. We aim to determine if hybrid watermilfoil is geographically widespread across the state or more likely to be present in the metro region. We also assess if it is more likely to occur in lakes with native northern versus Eurasian watermilfoil and determine the influence of age of infestation on hybrid watermilfoil presence. Lastly, we determine if human interaction such as boat access and herbicidal management are significantly associated with the presence of hybrid watermilfoil. By conducting these analyses to assess factors associated with confirmed hybrid watermilfoil invasions we can better inform prevention and control efforts and gain some insight on hybridity as it relates to invasive spread and establishment.

Methods

Study sites

To determine the distribution of hybrid (and coincidentally Eurasian and northern) watermilfoil in Minnesota we sampled 62 lakes with varying duration of invasion and size in 24 counties across the state. We determined the number of lakes to sample per county based on the relative numbers of lakes with documented Eurasian watermilfoil infestations (includes hybrid) as of 2017 from the Minnesota Department of Natural Resources' (MNDNR) infested waters list:

https://www.dnr.state.mn.us/invasives/ais/infested.html. Lakes sampled ranged from 12.5 to 51,891 ha in size, 2.5 to 135 m in maximum depth, and the durations of infestation ranged from 1 to 31 years (Appendix A). Because the MNDNR does not differentiate between Eurasian and hybrid watermilfoil when identifying invasive milfoil infestations, the year first infested may be based on either Eurasian or hybrid. We also sampled and recorded presence of northern watermilfoil at each location, but our data does not fully reflect the distribution of northern watermilfoil in Minnesota because we sampled from only lakes listed as Eurasian/hybrid infested.

Field sampling and data collection

At each lake we navigated to ~100 pre-selected random points distributed within a predefined littoral zone (depth ≤ 4.6m). At each point, at least one individual stem was collected for each unique watermilfoil taxon found at that location and placed in a labeled sealable bag on ice in a cooler. Taxa were identified visually based on morphological features and leaflet counts. The following leaflet counts were used to identify each taxon: Eurasian 14-21 leaflet pairs, northern 5-9 pairs, and hybrid 10-13 pairs (Moody and Les 2007). At each surveyed point the depth and number of plant stems per taxon collected were recorded. It is important to note that if we did not detect a particular taxon at a lake, this does not mean it was not present. We sampled thoroughly within the littoral zone, but it is possible that we did not identify all present milfoil taxa present at surveyed lakes. Lakes may have contained a particular taxon of milfoil, but the relative abundance could have been below our detection limit and therefore was not found during our surveys.

Genetics

Total genomic DNA was extracted from a subset of collected plant samples using DNeasy Plant Mini Kits (Qiagen). When 20 or fewer plants were collected from a lake, all the samples collected were analyzed. We randomly subsampled at least 20 plants for analysis from lakes with more samples. To distinguish Eurasian, hybrid, and northern watermilfoil, plants were identified to taxon using a genetic assay based on internal transcribed spacer DNA sequence (ITS; Thum et al. 2006, Grafé et al. 2015). Genetic variation was quantified for sampled plants and specific clones were delineated using eight microsatellite markers developed by Wu et al. (2013) (Myrsp 1, Myrsp 5, Myrsp 9, Myrsp 12, Myrsp 13, Myrsp 14, Myrsp 15, and Myrsp 16). The protocols in Wu et al. (2013) were used to amplify each microsatellite locus. Fragment analysis was completed on fluorescently labeled microsatellite PCR products by the University of Illinois – Urbana-Champaign's Cor Sequencing Facility using an ABI 3730xl sequencer. GeneMapper, version 5.0 (Applied Biosystems) was used to score microsatellites. Microsatellites were used as dominant, binary data (i.e., presence or absence of each possible allele at each locus) using the R-package POLYSAT (Clark and Jasieniuk 2011). Distinct genotypes were delineated using Lynch distances and a threshold of 0 in POLYSAT (Clark and Jasieniuk 2011).

Data Analysis

Based on genetically determined taxon identifications, all surveyed lakes were mapped with ArcGIS 10.5 to indicate presence/absence of each milfoil taxon. The geographic distribution of all collected watermilfoil was determined, as well as relative distances from infestations. The distance from nearest infestation was determined by calculating the distance between our surveyed lake to the nearest Eurasian infestation (based on the 2017 MNDNR infested waters list). Infestations were assessed to determine relative occurrence in the seven county Twin Cities metro versus greater Minnesota. Milfoil genotype richness was calculated for each lake using rarefaction. The rarefaction score was calculated using the rarefy function of the VEGAN package in R. This method calculates an expected species richness based on sample size.

To determine the influence of lake and environmental attributes associated with the presence of hybrid watermilfoil in Minnesota and to make comparisons between lakes, the following factors were assessed for each lake (or bay of Lake Minnetonka): age of infestation, number of vehicle/trailer parking spaces at water accesses, lake area, maximum depth and littoral area (water depth ≤ 4.6m) as obtained from the MNDNR's LakeFinder database <https://www.dnr.state.mn.us/lakefind/index.html>. Secchi depth and trophic state index data were obtained from the Minnesota Pollution Control Agency (MPCA) lake and stream water quality assessment database <https://cf.pca.state.mn.us/water/watershedweb/wdip/index.cfm>. Data were based on the ten-year average from state index data collected between June and September 2008 to 2017. Lakes were given milfoil management ratings on a scale of 0 to 3 to describe the extent of milfoil management, which include only herbicidal control, based on MNDNR permit approval data from 2009 to 2017. A zero indicates no management during this period, one indicates spot treatments (less than 2.5% of the littoral area), two indicates intermediate management (2.5-10% of littoral area) and three indicates lake wide treatments (greater than 10% of littoral area) targeting milfoil.

A total of four lakes were excluded from analyses of these lake attributes because sampling methods were inconsistent; however they were included in the taxa distribution map to indicate presence/absence. To calculate average values for assessed variables, lakes were grouped based on presence of each milfoil taxon. For example, EWM lakes include all lakes where Eurasian watermilfoil was found, and the same was done for HWM lakes and NWM lakes. This categorization makes it possible for the same lake to be present in more than one group, if multiple milfoil taxa were found at a lake. The averages were calculated for each environmental variable (Table 2) across lake groups at the statewide level. The averages were also calculated for each infestation associated variable (Table 4) at the statewide level, as well as by separating lakes by region (metro and greater MN). Variable averages were compared using a two-way analysis of variance (ANOVA) across taxa and region to assess for significant differences. A Tukey's honest significance test was used for analyzing significant variables following the ANOVA to determine significant differences within taxa analysis for each significant variable. This method determines if the relationship between the taxa group lakes and the significant variable to determine what specific variable and taxon are significantly associated.

Logistic regression analysis (LRA) was used to identify variables associated with the presence and absence of each milfoil taxon across surveyed lakes. This analysis was used to model the probability of the presence and absence of each milfoil taxon in relation to the assessed environmental (lake area, maximum lake depth, Secchi depth, and littoral area) and infestation-associated variables (age of infestation, region where lake is located, number of parking spaces at lake access, EWM management score, and distance from nearest infestation) variables. The LRA was performed with the software package R version 3.6.1 using the glm function. The results of LRA indicate which variables are associated with the increased probability of the presence of each milfoil taxon. A p-value of 0.05 was used to determine significance for all assessments.

Results

Milfoil distribution

Of the total 62 lakes sampled, 43 contained Eurasian, 28 contained hybrid, 21 contained northern watermilfoil, and no milfoil was found in two lakes. Eurasian watermilfoil was evenly distributed across the state (Figure 1). Hybrid watermilfoil was most commonly found in lakes in the metro (82%). Northern watermilfoil was most commonly found in lakes outside of the metro (68%). We found various taxa combinations in lakes where milfoil was found (Table 1). Eurasian watermilfoil was most commonly found with northern (60%) compared to hybrid (40%). Eurasian and northern watermilfoil were most commonly found together in lakes outside of the metro (83%); in part because northern was most common in greater MN. Of the 28 lakes that we found containing hybrid watermilfoil, 13 had only hybrid watermilfoil and no other milfoil taxa, and the remaining 15 had some combination with either Eurasian, northern, or both (Table 1). Hybrid watermilfoil-only infestations were mostly present in the metro (91%); only one hybrid exclusive infestation was found outside the metro. Hybrid watermilfoil was most commonly found in lakes with Eurasian (12 lakes) compared to northern (7 lakes). We found four lakes that contained all three taxa, half of which were in the metro and half in greater Minnesota (Table 1).

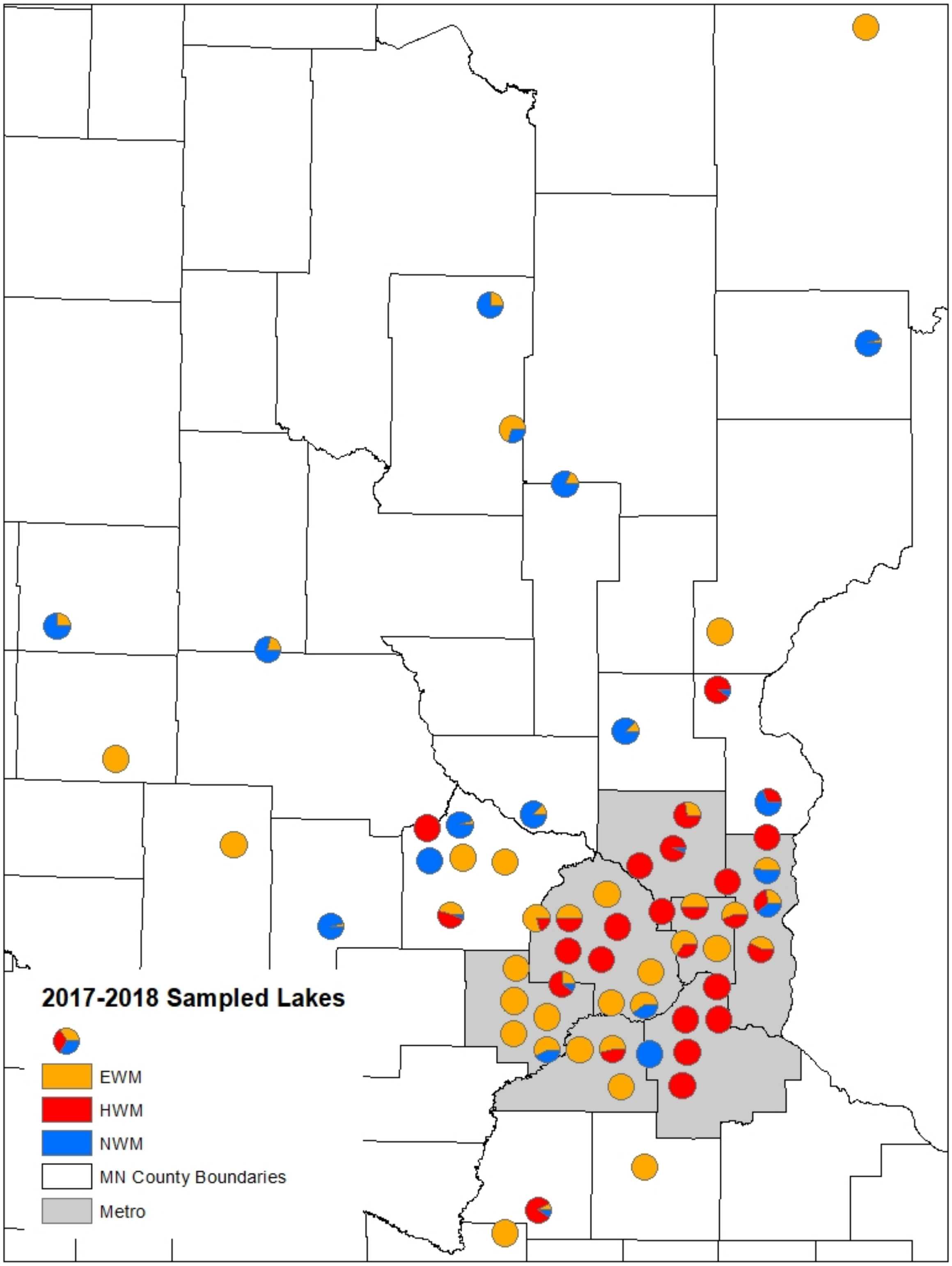

Figure 1. Statewide occurrence of Eurasian (EWM, orange), hybrid (HWM, red), and northern (NWM, blue) watermilfoil proportions in lakes sampled in 2017 and 2018. Metro counties are shaded gray.

Table 1. Number of lakes in the seven county metro, greater Minnesota, and total statewide for combinations of taxa (EWM = Eurasian watermilfoil, HWM = hybrid watermilfoil, NWM = northern watermilfoil) present in all surveyed lakes.

	EWM only	HWM only	NWM only	EWM & HWM	NWM & HWM	EWM & NWM	All three taxa	Total
Greater Minnesota	8	1	1	0	2	10	2	24
Metro	10	12	0	8	1	3	2	36
Total	18	13	1	8	3	13	4	60

Milfoil genotyping

Based on number of unique genotypes, Eurasian watermilfoil was the least diverse (8 genotypes), hybrid was intermediate (57) and northern was the most diverse (81; Table 2). We found that northern was significantly more diverse than Eurasian and hybrid watermilfoil (p = 0.01) within lakes based on the average milfoil genotype richness per lake. Lakes where we found northern typically (18 of 21) had multiple northern genotypes (3 to 8) whereas 93% of lakes with Eurasian had only one Eurasian watermilfoil genotype. Lakes with hybrid watermilfoil typically had a single hybrid genotype (16 lakes), although we found twelve lakes that had two or more hybrid genotypes (Appendix B).

Table 2. Number of genotypes found for each taxon from assessed milfoil samples. Average milfoil genotype richness per lake was calculated using rarefaction (EWM = Eurasian watermilfoil, HWM = hybrid watermilfoil, NWM = northern watermilfoil).

Taxon	Total number of unique genotypes	Average milfoil genotype richness/lake
EWM	8	2.5 ± 0.3
HWM	57	2.7 ± 0.3
NWM	81	3.5 ± 0.3

There was one widespread Eurasian genotype that was found in 93% of lakes where we found Eurasian watermilfoil. This indicates that most lakes containing Eurasian watermilfoil have the same genotype. Hybrid genotypes were not typically repeated between lakes, although we found four hybrid watermilfoil genotypes that were in more than one lake. Lakes Elmo and Coon shared a hybrid watermilfoil genotype (Figure 2). Lac Lavon shared a hybrid watermilfoil genotype with Cobblestone (Figure 2). Lac Lavon also shared a different hybrid watermilfoil genotype with Alimagnet (Figure 2).

Lastly we found one hybrid watermilfoil genotype in seven different lakes: Bald Eagle, Bone, Fish, Josephine, Otter, South Lindstrom, and White Bear (Figure 2). No northern watermilfoil genotypes were in more than one lake.

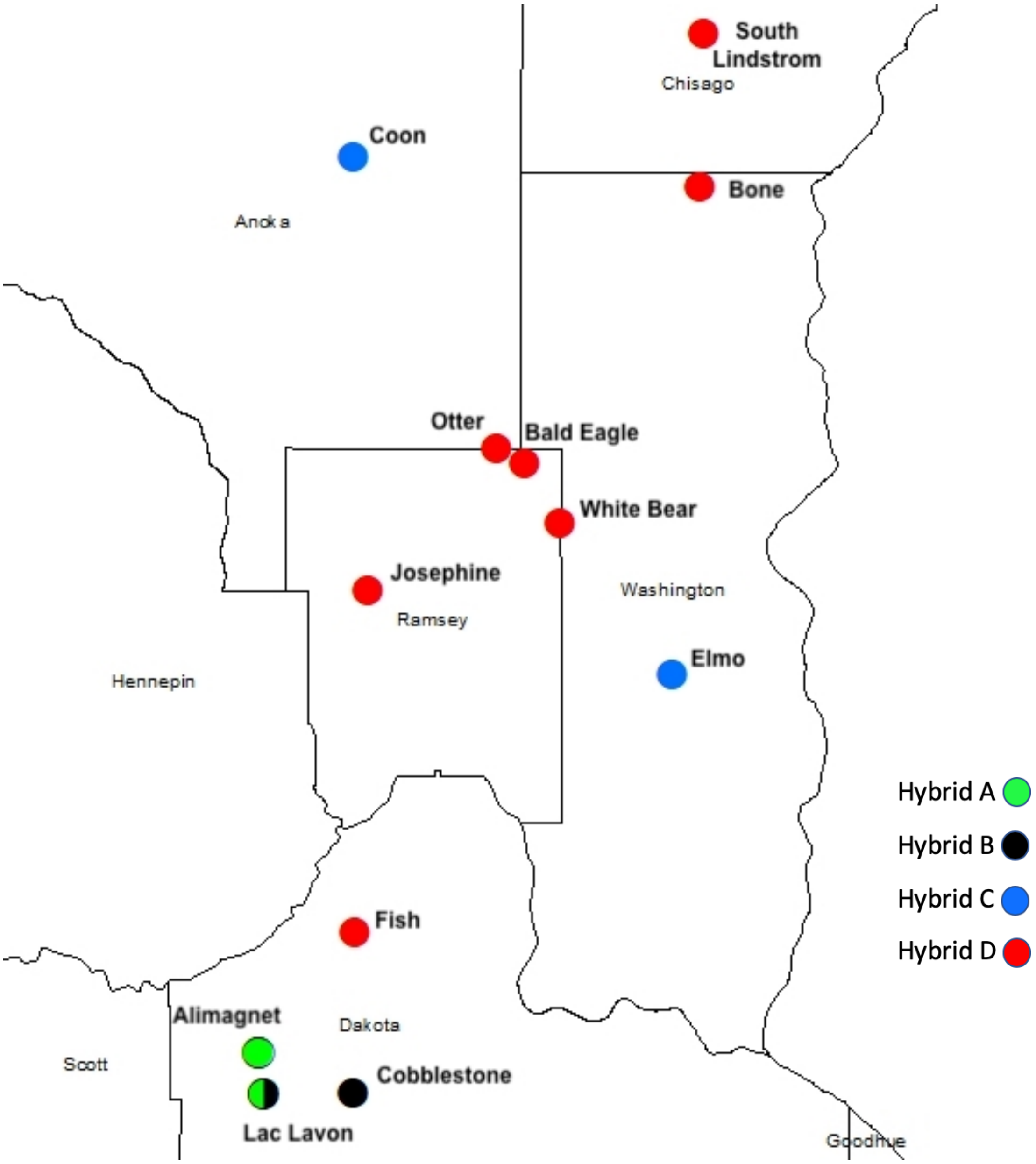

Figure 2. Distribution of shared hybrid watermilfoil genotypes in 2017-2018 surveyed lakes.

Environmental attributes analysis

There were no significant differences in environmental attributes such as area, depth, clarity, littoral area or trophic state among lakes containing the three taxa (Table 3). The LRA indicated that northern watermilfoil presence was positively associated with littoral area (p = 0.04; Table 4) and is marginally more likely to be found in lakes with shallower maximum depth (p = 0.07) and smaller lake area (p = 0.09). No significant relationships were found to explain Eurasian and hybrid watermilfoil presence (Table 4).

Table 3. Mean values and standard errors for environmental attributes of lakes classified as containing either Eurasian (EWM), hybrid (HWM), or northern (NWM) watermilfoil.

Lake type[a]	**Lake area (ha)**	**Max depth (m)**	**Secchi depth – water clarity of a lake (m)**	**Littoral Area (ha)**	**Trophic State Index**
EWM Lakes	299 ± 62	17.5 ± 3.2	2.5 ± 0.3	159 ± 28	53 ± 2
HWM Lakes	202 ± 45	12.3 ± 1.5	2.4 ± 0.2	122 ± 29	53 ± 1
NWM Lakes	314 ± 52	14.3 ± 1.7	2.8 ± 0.3	177 ± 31	51 ± 2
ANOVA p-value	0.26	0.39	0.58	0.64	0.55

[a] Lake types include all lakes with the taxon present and therefore a lake may be represented in more than one category.

Table 4. Estimates for y-intercepts of environmental variables based on logistic regression models along with the associated standard error, z-value, and p-value. Lakes assessed by Eurasian (EWM), hybrid (HWM), and northern (NWM) watermilfoil presence/absence.

	Y-intercept	**Standard error**	**Z-value**	**P-value**
EWM	-0.75	5.94	-0.13	0.90
Lake area	0.02	0.01	1.55	0.12
Littoral area	-0.02	0.01	-1.18	0.24
Maximum depth	0.05	0.06	0.92	0.36
Secchi depth	-0.23	0.61	-0.38	0.71
Trophic state index	0.02	0.09	0.20	0.84
HWM	0.62	4.73	0.13	0.90
Lake area	-0.004	0.004	-1.00	0.32
Littoral area	0.007	0.007	0.99	0.32
Maximum depth	-0.04	0.04	-1.04	0.30
Secchi depth	0.18	0.49	0.37	0.72
Trophic state index	-0.01	0.07	-0.19	0.85
NWM	-2.72	5.27	-0.52	0.61
Lake area	-0.005	0.003	-1.67	0.09*
Littoral area	0.01	0.006	2.01	0.04**
Maximum depth	-0.05	0.03	-1.83	0.07*
Secchi depth	0.92	0.58	1.59	0.11
Trophic state index	0.002	0.077	0.030	0.976

**significant ($p < 0.05$)
*marginally significant ($p < 0.10$)

Infestation-associated variables analysis

There was a statistically significant interaction between the effects of region and taxon on distance to the nearest infestation ($p = 7.51 \times 10^{-06}$; ANOVA: Table 5). Lakes containing hybrid watermilfoil in greater MN were found to be further from infestations when compared to all other taxon and region combinations. There were no significant differences in the remaining infestation-associated variables among lakes (ANOVA: Table 5). The LRA revealed that the presence of hybrid ($p = 0.01$) and northern ($p = 0.001$) watermilfoil was associated with region (Table 6). The probability of hybrid watermilfoil being present in a lake is higher in the MN metro (Table 5 and Table 6). The probability of northern watermilfoil being present in a lake is higher in greater MN (Table 5 and Table 6). The importance of region made the overall northern watermilfoil LRA significant as well (p = 0.04). The LRA of hybrid watermilfoil indicated that age of infestation (p = 0.73), parking spaces (p = 0.44), and management scores (p = 0.34) were not significantly associated with hybrid presence. Although hybrid watermilfoil presence had greater probability in the metro, this did not directly translate to hybrid infestations being significantly closer to other invasive milfoil infestations (p = 0.43).

Table 5. Mean values and standard errors for infestation associated variables of lakes classified as containing either Eurasian (EWM), hybrid (HWM), or northern (NWM) watermilfoil and p-values for taxon, region, and taxon by region.

		Number of lakes	**Average age of infestation (years)**	**Average number of parking spaces at water access**	**Average management score**	**Average distance from nearest infestation (km)**
EWM	Statewide	41	16.6 ± 1.3	22.0 ± 4.1	1.0 ± 0.1	20.8 ± 3.5
	Metro	21	19.7 ± 1.8	31.9 ± 7.1	0.9 ± 0.2	7.9 ± 1.3
	Greater MN	20	13.2 ± 1.7	11.5 ± 2.6	1.0 ± 0.2	34.1 ± 5.6
HWM	Statewide	26	19.2 ± 1.8	27.7 ± 6.4	1.2 ± 0.2	11.3 ± 2.2
	Metro	21	20.2 ± 2.1	29.5 ± 7.5	1.3 ± 0.2	7.2 ± 0.8
	Greater MN	5	15.0 ± 2.9	21.6 ± 10.9	1.2 ± 0.6	30.7 ± 7.1
NWM	Statewide	21	17.8 ± 1.9	23.0 ± 4.4	1.1 ± 0.2	29.4 ± 5.3
	Metro	6	21.2 ± 4.1	35.8 ± 8.5	1.0 ± 0.4	12.9 ± 3.0
	Greater MN	15	16.4 ± 2.1	17.8 ± 4.6	1.1 ± 0.3	36.0 ± 6.6
p-values						
Taxon by Region			0.907	0.703	0.193	<0.001
Taxon			0.474	0.671	0.518	<0.001
Region			0.003	0.006	0.299	<0.001

Table 6. Estimates for y-intercept of infestation associated variables based on logistic regression models as well as associated standard error, z-value, and p-value. Lakes assessed by Eurasian (EWM), hybrid (HWM), and northern (NWM) watermilfoil presence/absence.

	Y-intercept	**Standard error**	**Z-value**	**P-value**
EWM	0.43	0.93	0.46	0.65
Region	1.21	0.76	1.59	0.11
Age of infestation	0.01	0.04	0.37	0.71
Parking spaces	0.01	0.02	0.76	0.45
Management score	-0.51	0.34	-1.53	0.13
Distance to nearest infestation	0.06	0.10	0.60	0.55
HWM	0.04	0.87	0.04	0.97
Region	-1.71	0.69	-2.46	0.01*
Age of infestation	0.01	0.04	0.35	0.73
Parking spaces	0.01	0.02	0.77	0.44
Management score	0.31	0.33	0.95	0.34
Distance to nearest infestation	-0.07	0.09	-0.78	0.43
NWM	-2.10	1.01	-2.08	0.04*
Region	2.482	0.772	3.214	0.001*
Age of infestation	0.01	0.04	0.28	0.78
Parking spaces	0.02	0.01	1.31	0.19
Management score	-0.08	0.34	-0.25	0.81
Distance to nearest infestation	-0.02	0.05	0.48	0.63

*significant ($p < 0.05$)

Discussion

Milfoil distribution and associated factors

We found clear distinctions in the distribution of Eurasian, hybrid and northern watermilfoil in Minnesota. Eurasian was evenly distributed across the state, hybrid was most commonly found in the metro and northern watermilfoil was most commonly found in greater Minnesota. The concentration of hybrid watermilfoil in the metro may be explained by the process by which hybridization occurs. Hybridization occurs within a lake as a result of sexual reproduction between Eurasian and northern watermilfoil, which requires that both taxa are established within a lake. Hybrid watermilfoil may also be present as a result of sexual reproduction between hybrids (LaRue et al. 2013a). These processes may take more time, thus preventing hybrid watermilfoil from having more widespread distributions (Schierenbeck and Ellstrand 2000). Based on this rationale we initially predicted that hybrid watermilfoil would most likely be present in lakes with older ages of infestation, but our analysis did not find this variable to be significant. Hybrid watermilfoil presence was not associated with older invasions.

We predicted that lakes with intensive management histories would be more likely to have hybrid watermilfoil present as a result of direct pressures affecting lake wide milfoil populations that may favor herbicide tolerant hybrids. Milfoil populations containing herbicide tolerant hybrids are more likely to be dominated by hybrids following herbicide treatment as a result of hybrids outcompeting northern and Eurasian. However, our analysis did not find that management score was not significantly associated with hybrid watermilfoil presence. This suggests that the hybrid watermilfoil populations in Minnesota are not directly developing in response to management pressures. It is important to note that hybrid watermilfoil encompasses many different strains and they are not equal in terms of herbicide response (Taylor et al. 2017). Herbicide tolerant hybrid watermilfoil genotypes have been identified in Michigan (Berger et al. 2012, Thum et al. 2012, Berger et al. 2015). Therefore, lakes should be managed alongside active genetic characterization to assess population changes that arise. Frequent monitoring of hybrid watermilfoil populations is important to verify the efficacy of milfoil management and detect tolerant genotypes.

We did not find hybrid watermilfoil presence associated with closer infestations. Rather hybrid watermilfoil presence was associated with metro lakes specifically. This suggests that human mediated interactions in the metro are linked to hybrid watermilfoil spread and/or formation. People visiting lakes in the metro are more likely to come in contact with hybrid watermilfoil, increasing the likelihood of spreading it from one lake to another. We found common hybrid watermilfoil genotypes were present in multiple lakes only in the metro. We did not see this in lakes of greater MN where we found hybrid watermilfoil. This finding suggests that hybrid watermilfoil is being clonally spread more often in the metro. This spread could be as a result of waterfowl movement, lake connectivity, or boater traffic. The metro is also more densely populated than greater Minnesota, therefore lakes tend to be closer to interstate highways and major cities. Hybrid watermilfoil was more commonly found in the metro which may be associated with human population densities, similar to previous findings of Eurasian infestations (Kanankege et al. 2018). Increased sampling within the metro could provide more information regarding the factors associated with hybrid watermilfoil infestations in the metro.

The types of lakes that hybrid watermilfoil inhabited were very similar to those of Eurasian and northern in regards to lake attributes. Wu et al (2015) quantified and compared the climate niches of Eurasian and northern watermilfoil in two co-occurring regions in their native range. Their study found that hybrid watermilfoil was more commonly found in the region where Eurasian and northern watermilfoil occupied similar environments, because of increased contact between the two taxa (Wu et al 2015). Hybridization was less likely to occur where Eurasian and northern watermilfoil occupied areas unique to their respective niche. This finding may describe the relationship among hybrid watermilfoil populations in MN. We did not find significant differences in the environments occupied by each milfoil taxon because hybridization is likely to occur in lakes that are within the common niche of Eurasian and northern watermilfoil. As a result, hybrid watermilfoil occupies lakes within this same climate niche. This suggests that hybrids are more commonly found in lakes where both Eurasian and northern watermilfoil have historically been found or currently are present.

Milfoil co-occurrence

We have found that hybrid watermilfoil-only infestations occur quite commonly (13 lakes), especially in the metro. This contrasts the findings of Sturtevant et al. (2009) which found the only two of fifteen lakes surveyed in MI and IN contained only hybrid watermilfoil and no other taxon. In lakes where hybrid watermilfoil was present with a parental taxon, hybrids were more often present with

Eurasian rather than northern. Similar to the results of Sturtevant et al. (2009) we found that Eurasian and hybrid watermilfoil were commonly found to co-occur in lakes, which also contrasts the findings of Moody and Les (2007) that their coexistence was not found in Minnesota. Northern watermilfoil was infrequently found in MI and IN (Sturtevant et al. 2009) and was present in only two lakes, whereas we found northern in 21 lakes (34%). It is important to note that we selected lakes to sample based on documented Eurasian/hybrid watermilfoil infestations, so this may account for why northern would be found in fewer lakes, because our data do not truly describe northern watermilfoil's distribution.

In lakes where two milfoil taxa were present, hybrid and northern watermilfoil were more commonly found with Eurasian rather than each other. Lakes containing Eurasian and northern watermilfoil were more often in greater Minnesota as a result of northern being most common there. A similar relationship was found with lakes containing Eurasian and hybrid watermilfoil; these were most commonly found in the metro, which is where hybrid was also most commonly found. Very few (seven lakes) contained northern and hybrid watermilfoil, as a result of their occurrences being largely in different regions of the state. Hybrid watermilfoil may be less commonly occurring with northern because northern may be outcompeted by invasive Eurasian (or hybrid) over time (Nichols 1994) or because northern may be eliminated by repeated herbicide treatments to control invasives. Reduced presence of northern watermilfoil has been found in previous studies (Moody and Les 2007, Sturtevant et al. 2009) and warrants further investigation as the spread of hybrids increase. Northern watermilfoil likely had a greater presence in the metro than it currently does, but with increasing prevalence of hybridization, northern watermilfoil populations are decreasing through either competition, genomic contamination (Moody and Les 2007), or use of herbicides (Roshon et al. 1999).

We found all three milfoil taxa present in four lakes (Bald Eagle, German, Howard, and Smith's Bay of Lake Minnetonka). We found that two lakes (German and Howard) predominately consisted of Eurasian and hybrid watermilfoil and only a single northern sample was collected. In contrast Bald Eagle and Smith's Bay had relatively similar abundances of all three milfoil taxa. Co-occurrence of milfoil taxa may be influenced by spatial distribution of taxa within the lake, as suggested by Sturtevant et al. (2009). Lower abundances of northern watermilfoil in these lakes may be as a result of species overlap in which milfoil taxa inhabit common areas and are thereby directly impacted by competitive interactions for resources. This form of distribution increases the likelihood that northern watermilfoil abundance will decrease over time, because northern may be outcompeted by established invasive milfoil populations (Valley and Newman 1998). Lakes with equal abundances of all three milfoil taxa may have clearer distinctions in the locations of each taxon population within a lake, thereby lessening the impact of competition because populations are spread apart rather than clustered. Milfoil populations that were previously separated may be coming into contact through human mediated processes such as management and recreational water use. If Eurasian and northern watermilfoil co-occur in a lake, northern may be more likely to hybridize with Eurasian than breed with northern. These interactions may catalyze the speed in which the native northern watermilfoil decreases in abundance as it continues to come in contact with Eurasian, leading to hybridization (Ellstrand and Schierenbeck 2000). Analysis of within lake composition and distribution of milfoil taxa is needed to better clarify these relationships.

Conclusion

Our study has located 28 confirmed occurrences of hybrid watermilfoil that can be assessed in terms of responses to herbicide management. In order to predict where hybrid watermilfoil will infest next, it is important to look at where it is currently present. On average, the metro lakes we surveyed had more hybrid watermilfoil occurrences and more parking spaces at lake accesses on average in comparison to greater Minnesota, indicating that metro lakes have more traffic which can create more opportunities to spread hybrid and Eurasian watermilfoil. Although hybrid watermilfoil was more commonly found in the metro, it is important to note that they were present in five lakes in greater Minnesota and because these were unique genotypes, likely arose in these lakes. This finding indicates that hybridization is not restricted to the metro and readily occurs in areas where hybrid watermilfoil is less commonly found, such as greater MN. Management efforts should focus on containing known Eurasian and hybrid watermilfoil infestations. These efforts should specifically target lakes with high recreational traffic, so as to reduce the potential of Eurasian and hybrid watermilfoil introductions to other lakes.

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Appendix A. Lakes sampled in 2017-2018 including lake area, maximum depth and year of Eurasian infestation for each lake sampled.

Lake name	County	Lake ID	Lake area (hectares)	Maximum depth (m)	Year of Eurasian infestation
Alimagnet	Dakota	19-0021	41.9	3.51	2012
Auburn	Carver	10-004401	117.6	25.6	1989
Bald Eagle	Ramsey	62-0002	423.6	11.0	1989
Ballantyne	Blue Earth	07-0054	150.0	17.7	2012
Bay	Crow Wing	18-0034	938.8	22.6	1992
Big Marine	Washington	82-0052	728.1	18.9	2004
Bone	Washington	82-0054	89.6	9.1	2006
Cedar	Hennepin	27-0039	66.3	15.5	1990
Cedar	Wright	86-0227	319.8	32.9	2010
Christmas	Hennepin	27-0137	108.1	26.5	1992
Chub	Carlton	09-0008	126.8	8.5	2009
Cobblestone	Dakota	19-0456	14.1	5.5	2011
Constance	Wright	86-0051	70.7	7.0	2016
Coon	Anoka	02-0042	599.4	8.2	2003
Crooked	Anoka	02-0084	46.5	7.9	1990
East Rush	Chisago	13-006901	599.2	7.3	1992
Elmo	Washington	82-0106	103.9	42.7	2005
Emily	Crow Wing	18-0203	291.7	4.0	2014
Fish	Dakota	19-0057	12.4	10.2	2009
Fox	Rice	66-0029	126.1	14.3	2009
German	Le Seuer	40-0063	320.4	15.5	2002
Gervais	Ramsey	62-0007	95.1	12.5	1995
Gilbert Pit	St. Louis	69-1306	102.8	135.0	1999
Gilchrist	Pope	61-0072	136.0	7.3	1996
Green	Kandiyohi	34-0079	2250.3	33.5	2000
Ham	Anoka	02-0053	77.1	6.7	2013
Harriet	Hennepin	27-0016	138.1	26.5	1991
Howard	Wright	86-0199	301.5	11.9	2003
Independence	Hennepin	27-0176	342.7	17.7	1989
Indian	Wright	86-0223	56.4	9.5	2003

Josephine	Ramsey	62-0057	47.0	13.4	2012
Lac Lavon	Dakota	19-0446	26.7	9.8	1988
Little Birch	Todd	77-0089	339.7	27.1	2003
Locke	Wright	86-0168	56.7	14.9	2011
McCarron	Ramsey	62-0054	29.7	17.4	2000
McMahon	Scott	70-0050	65.7	4.3	2007
Mille Lacs	Mille Lacs	48-0002	51891.3	12.8	1998
Minnetonka Grays'	Hennepin	27-013301	74.6	11.0	1987
Minnetonka North Arm	Hennepin	27-013313	127.1	17.7	1987
Minnetonka Smiths'	Hennepin	27-013302	184.1	9.1	1987
Minnie-Belle	Meeker	47-0119	240.2	14.9	2010
Mitchell	Hennepin	27-0070	46.1	5.8	2002
Mitchell	Sherburne	71-0081	68.6	10.1	2007
Oscar	Douglas	21-0257	471.7	7.6	1992
Otter	Anoka	02-0003	127.0	6.4	1989
Phalen	Ramsey	62-0013	80.0	27.7	1997
Piersons	Carver	10-0053	108.0	12.2	1991
Pokegama	Pine	58-0142	601.5	7.6	2005
Rebecca	Hennepin	27-0192	106.5	9.1	1989
Riley	Carver	10-0002	119.9	14.9	1990
Schmidt	Hennepin	27-0102	18.1	7.6	1990
Somers	Wright	86-0230	61.3	6.4	2013
South Lindstrom	Chisago	13-0028	184.0	10.4	2010
Spectacle	Isanti	30-0135	98.2	15.7	2007
Staring	Hennepin	27-0078	67.6	4.9	2015
Steiger	Carver	10-0045	67.1	11.3	2001
Sugar	Wright	86-0233	406.2	21.0	1990
Swede	Carver	10-0095	175.2	3.7	2008
Thomas	Dakota	19-0067	16.8	2.4	2011
Turtle	Ramsey	62-0061	182.1	8.5	2000
Upper Prior	Scott	70-0072	157.9	15.2	2000
White Bear	Washington	82-0167	982.5	25.3	1988

Appendix B. Summary of genetic analyses of lakes sampled surveyed in 2017-2018. The number of each taxon identified from samples collected in each lake is presented and the number of distinct genotypes is indicated for each taxon in each lake.

		COUNTS PER TAXON			**GENOTYPE COUNT PER LAKE**		
LAKE	County	EWM	HWM	NWM	EWM	HWM	NWM
COON	Anoka	11	29		1	2	
CROOKED	Anoka		20			3	
HAM	Anoka		97	6		1	1
OTTER	Anoka		64			2	
BALLANTYNE	Blue Earth	20			1		
CHUB	Carlton	1		19	1		1
AUBURN	Carver	24			1		
PIERSONS	Carver	19			1		
RILEY	Carver	21			1		
STEIGER	Carver	20			1		
SWEDE	Carver	13			1		
EAST RUSH	Chisago		18	2		1	1
SOUTH LINDSTROM	Chisago		9	19		1	4
BAY	Crow Wing	14		6	1		3
EMILY	Crow Wing	2		6	1		6
ALIMAGNET	Dakota		20			1	
COBBLESTONE	Dakota		2			1	
FISH	Dakota		20			1	
LAC LAVON	Dakota		20			5	
THOMAS	Dakota		5			2	
OSCAR	Douglas	5		15	1		5
CEDAR	Hennepin	5			1		
CHRISTMAS	Hennepin	48		33	1		5
HARRIET	Hennepin	20			1		
INDEPENDENCE	Hennepin	43	44		1	1	
MINNETONKA-GRAYS	Hennepin		54			5	
MINNETONKA-NORTH ARM	Hennepin		20			7	
MINNETONKA-SMITHS	Hennepin	14	37	6	2	10	4
MITCHELL	Hennepin	24		16	1		3
REBECCA	Hennepin	21	8		1	1	
SCHMIDT	Hennepin		62			2	
STARING	Hennepin	8			1		
SPECTACLE	Isanti	3		22	1		4
GREEN	Kandiyohi	2			1		
GERMAN	Le Seuer	1	9	1	1	5	1
MINNIE-BELLE	Meeker	1		25	1		5
MILLE LACS	Mille Lacs	2		10	1		2
POKEGAMA	Pine	5			1		
GILCHRIST	Pope	20			1		
BALD EAGLE	Ramsey	35	43	50	1	1	3
GERVAIS	Ramsey						
JOSEPHINE	Ramsey		19			1	
MCCARRON	Ramsey	21	11		1	1	
PHALEN	Ramsey	4			1		
TURTLE	Ramsey	6	6		1	1	
FOX	Rice	20			2		
MCMAHON	Scott	4			1		
UPPER PRIOR	Scott	14	10		2	2	
MITCHELL	Sherburne	5		34	1		3

GILBERT PIT	St. Louis	9			1		
LITTLE BIRCH	Todd	4		15	1		6
BIG MARINE	Washington	12		13	1		8
BONE	Washington		19			1	
ELMO	Washington	16	23		1	1	
WHITE BEAR	Washington	24	12		1	1	
CEDAR	Wright			20			6
CONSTANCE	Wright	17			1		
HOWARD	Wright	9	10	1	1	6	1
INDIAN	Wright		1			1	
LOCKE	Wright						
SOMERS	Wright	2			1		
SUGAR	Wright	1		19	1		5