



# Eurasian and Hybrid Watermilfoil Genotype Distribution in the Minnesota Metro

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## Introduction

Some of the most severe plant infestations in North America involve several species in the watermilfoil genus *Myriophyllum* (Moody and Les 2002). Eurasian watermilfoil (*Myriophyllum spicatum*) has become a problematic aquatic weed largely due to its rampant spread. Eurasian watermilfoil was first found in Minnesota in Lake Minnetonka in 1987 and has since spread to 35 counties in over 300 waterbodies in 35 counties (Invasive Species Program 2015). Hundreds of millions are spent in the U.S. annually on its control (Homans and Newman 2011) with permits issued for control on 80 to 100 Minnesota lakes per year (Invasive Species Program 2015). It is currently the most widely managed aquatic weed in the United States (Bartodziej and Ludlow 1998).

Eurasian watermilfoil hybridizes with the native northern watermilfoil (*M. sibiricum*) (Moody and Les 2002), which raises new issues regarding management strategies for controlling infestations. It is quite difficult to distinguish between Eurasian watermilfoil and hybrids and thus are typically treated with similar management strategies. There is increasing concern that hybrid watermilfoil might be more invasive than Eurasian watermilfoil (Moody and Les 2002) or harder to control. A laboratory study by LaRue et al. (2013a) found that hybrid watermilfoils in Michigan had faster vegetative growth rates and other studies have shown increased tolerance to herbicides compared to Eurasian watermilfoil (Thum et al. 2012, Parks et al. 2016).

Through molecular genetic studies it has been determined that genetic diversity is quite high in hybrid watermilfoil as a result of sexual reproduction through the production of viable seeds (Zuellig and Thum 2012, LaRue et al. 2013b). This further increases the chance of genetic variation between Eurasian and hybrid watermilfoil and requires specific genotypes to be compared, as different genotypes may elicit different traits. The ability of hybrids to sexually reproduce also gives the advantage to recolonize a population following elimination of standing plants through herbicide application (Buhler et al., 1997).

## Methods

To determine the distribution of hybrid (and coincidentally Eurasian and northern) watermilfoil, 50 lakes in Minnesota were sampled with varying duration of invasion and size. We determined the number of lakes to sample per county based on their relative numbers of lakes with documented Eurasian watermilfoil infestations. At each lake, plants, when present, were collected at up to 100 randomly selected points, with an aim to collect  $\geq 25$  plants.

DNA was extracted from the plants and Eurasian, hybrid, and northern watermilfoil were identified to taxon using a genetic assay based on internal transcribed spacer (ITS) DNA sequence. The DNA was then further analyzed with microsatellites to identify genotypes (Fig. 1) and determine if specific genotypes display distribution patterns (Fig. 2-3). Currently, 31 lakes have been analyzed to genotype.

Lake	Number of Genotypes			Lake	Number of Genotypes		
	EWM	HWM	NWM		EWM	HWM	NWM
Auburn	1			Minnie Belle	1		3
Bald Eagle	1	1	2	Mitchell (Hennepin Co)	1		2
Big Carnelian			3	Mitchell (Sherburn Co)	1		3
Big Marine	2		5	Orchard			4
Bone		1		Otter		1	
Cedar	1			Phalen	1		
Coon	1	2		Rebecca	1	1	
Elmo	1	1		Riley	1		
Fish		1		Schmidt		1	
Gilbert Pit	1			South Lindstrom		1	4
Ham		1	1	Spectacle	2		4
Josephine		1		Steiger	1		
McCarron	1	1		Thomas		2	
Minnetonka Grays' Bay		6		Turtle	1	1	
Minnetonka North Arm Bay		7		Upper Prior	2	3	
				White Bear	1	1	

Figure 1. Total number of genotypes identified per taxa at individual lakes based on microsatellite data.

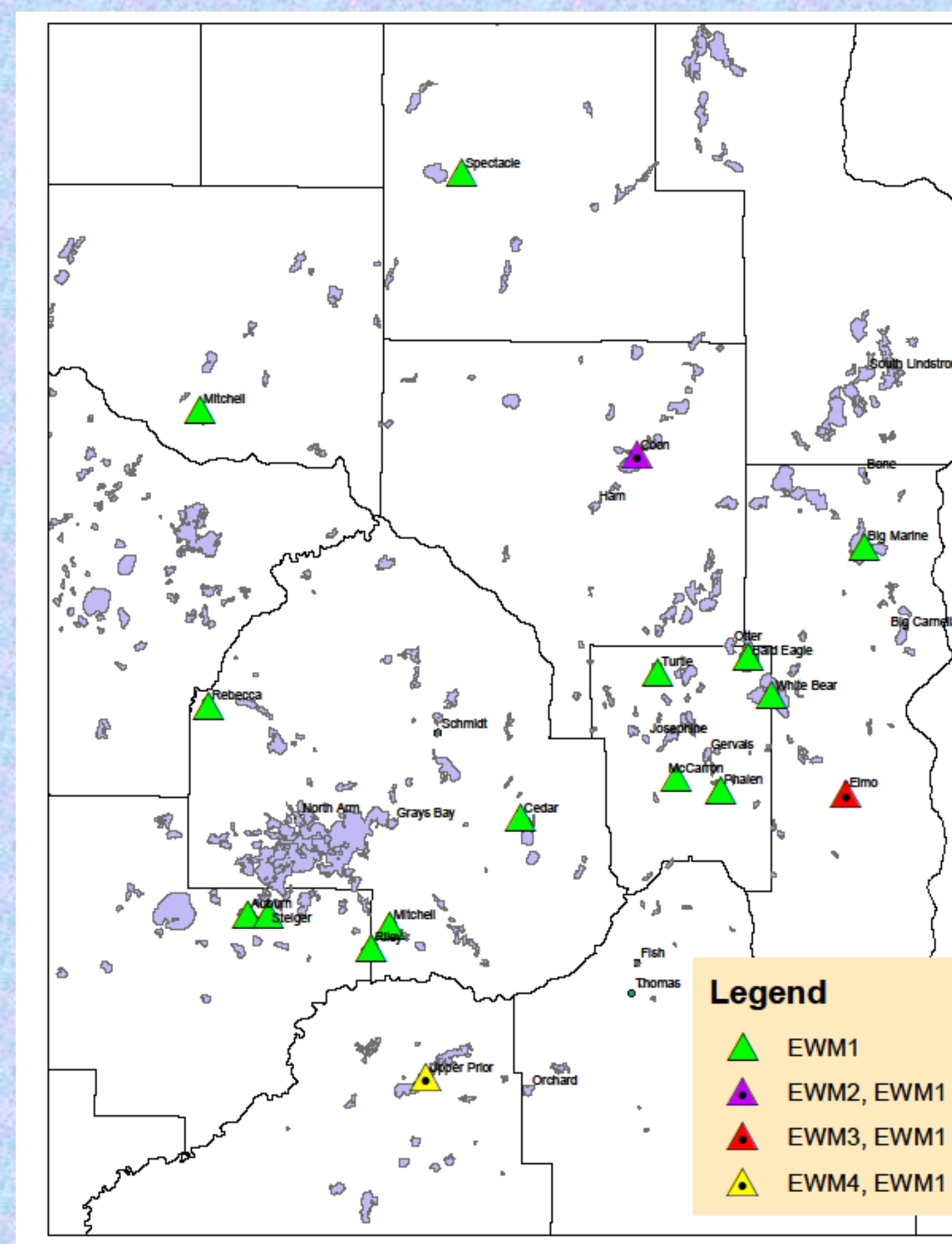


Figure 2. Eurasian watermilfoil genotype distribution of sampled lakes in Minnesota metro.

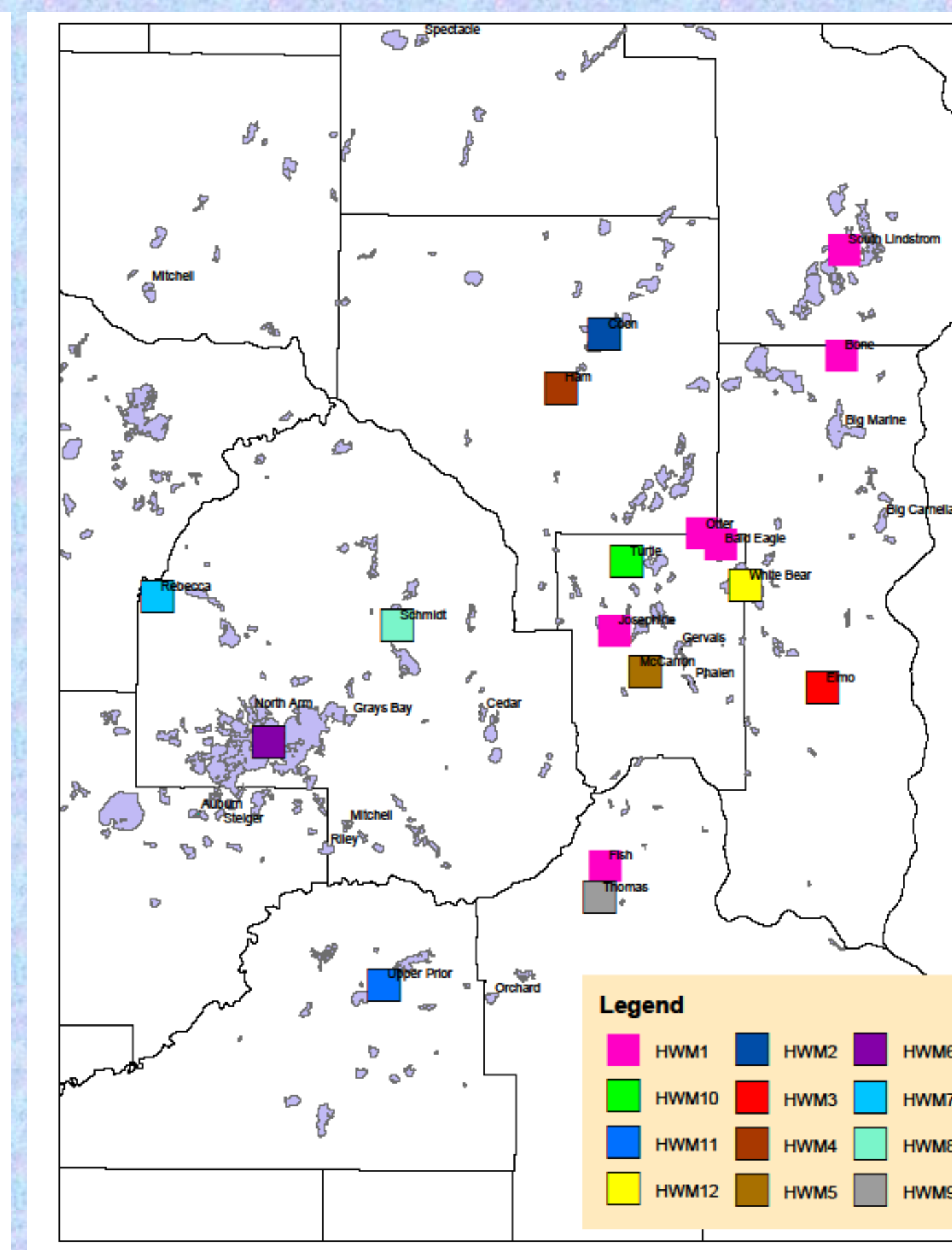
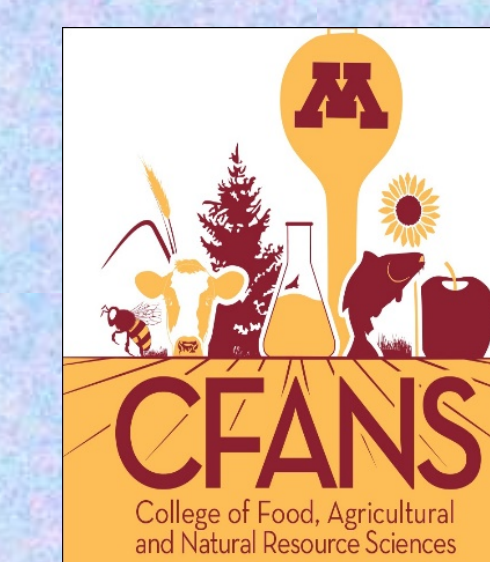


Figure 3. Hybrid watermilfoil genotype distribution of sampled lakes in Minnesota metro.

## Acknowledgements

We thank TJ Ostendorf, Alex Franzen, and Jeff Korff for lab assistance and the following funding sources:



## Observations

Genetic analysis of ITS and microsatellites were completed for 31 lakes for a subset of plant samples collected. Some lakes were found to have watermilfoil populations of just hybrid whereas others had all Eurasian. Lakes do not necessarily need to have northern or Eurasian in order to have hybrid. We also found that a few lakes had all three taxa (northern, Eurasian, and hybrid).

Amongst the lakes with Eurasian there was found to be one widespread genotype, but different genotypes (3 additional) were found in lakes Coon, Elmo, and Upper Prior (Fig. 2). In terms of hybrids, 12 genotypes were found. Five lakes shared the same genotype (Bald Eagle, Bone, Fish, Josephine, Otter, and South Lindstrom; Fig 3). All remaining lakes had different hybrids, although within a lake there was usually just a single genotype present (Fig. 1). Northern had the greatest number of genotypes across lakes, with each lake having a different genotype present, and lakes typically had multiple genotypes. Our data is not a true representation of the distribution of Northern because we were sampling Eurasian infested lakes.

## Future Work

Based on the data collected so far, we have found that Eurasian and hybrid watermilfoil are genetically diverse in the Minnesota metro. It is also apparent that there is a widespread common genotype of both taxa. Determining which lakes have this common genotype will provide further our understanding of the spread of the infestation.

We will examine the spatial occurrence of hybrid watermilfoil using spatial tools such as logistic regression to determine whether hybrid occurrence is related to distance from Lake Minnetonka (initial infestation in state), and spatial autocorrelation statistics to determine whether the geographic distribution of hybrids is random versus over or under-dispersed around the state.

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