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

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Summary:

Eurasian watermilfoil (*Myriophyllum spicatum*) can hybridize with the native northern watermilfoil (*M. sibiricum*) and all three taxa, Eurasian, northern and hybrid watermilfoil are present in Minnesota, but their occurrence and distribution is not well documented. Recent studies elsewhere indicate that some genotypes of hybrid watermilfoil are more tolerant of some auxin-mimic herbicides and that extensive treatments may be selecting for these genotypes that will be more difficult to control. In addition, there is concern that hybrid watermilfoil may be more difficult for herbivores such as the milfoil weevil (*Euhrychiopsis lecontei*) to control. We examined the genetic composition (using AFLP markers) of watermilfoils in three bays of Lake Minnetonka (Grays, North Arm and St. Albans) that are being managed with herbicides to control Eurasian watermilfoil and two bays (Smiths and Veterans) and one lake (Christmas Lake) that have not been extensively managed with herbicides but were known to have populations of the milfoil weevil. The plant community was characterized in each bay/lake from point intercept surveys conducted in June (before herbicide treatment with triclopyr) and in August (after treatments) and samples of watermilfoil were collected for genetic analysis. Due to funding limitations about 1/3 of the collected samples were analyzed but the remainder are preserved for future analysis.

Eurasian, northern and hybrid watermilfoil genotypes were found, but northern watermilfoil was only found in the untreated bays; hybrid water milfoil was much more common in the treated bays whereas pure Eurasian and northern were more common in the untreated bays. Grays Bay hybrid appears distinct from Smiths Bay hybrid and both are distinct from Veterans, St. Albans and North Arm. The triclopyr treatments were effective in the three bays, with particularly good control in Grays and St. Albans Bay. In Grays Bay hybrid watermilfoil was dominant and the frequency of watermilfoil was reduced from about 50% occurrence to less than 6% occurrence after treatment. The hybrid watermilfoil in Grays Bay was effectively controlled. In St. Albans Bay, both hybrid and pure Eurasian were present and watermilfoil occurrence was reduced from 14% occurrence to below detection (although a few plants were spotted). In North Arm, the areas treated were smaller and some untreated areas resulted in little change in frequency from 12% in June to 10% in August. Only hybrid was detected in North Arm after treatment, but the low sample size analyzed makes it unclear if the hybrid was less susceptible in North Arm or if differential treatment or low sample size explain the lack of pure Eurasian.

In the untreated bays, northern watermilfoil was more common, particularly in water shallower than 2m. Hybrid watermilfoil appeared less common than the other taxa, although it appeared to be more abundant than northern at Veterans Bay. Weevil densities were low in 2015 but milfoil weevils were present in all three waterbodies and found on both Eurasian, hybrid and northern watermilfoil. There appeared to be little difference in abundance on northern and Eurasian and there was a suggestion that densities were higher on hybrid in Veterans Bay than on northern.

Overall, this analysis shows that all three watermilfoil taxa are present in Lake Minnetonka and Christmas Lake, hybrid watermilfoil appears more common and northern less common in bays that have had extensive herbicide treatments and there is a potential for intensive management to shift the frequency of the taxa. Additional analysis of the samples collected for this study should provide more insights and lead to better management recommendations.

Background

Eurasian watermilfoil (*Myriophyllum spicatum*) can hybridize with the native northern watermilfoil (*M. sibiricum*) (Moody and Les 2007) and recent work has shown that hybrid milfoil can grow faster and may be more tolerant of herbicides (e.g., 2, 4-d) than Eurasian watermilfoil (LaRue et al. 2013). Previous work (e.g., Moody and Les 2007) has shown that the native northern, Eurasian and hybrid watermilfoils are all present in Minnesota and Lake Minnetonka, but those data are old (early 2000's) and of limited scope, and used lower resolution methods. During the past seven years, extensive herbicide treatments to control Eurasian watermilfoil have been conducted in bays of Lake Minnetonka (LMCD AIS Subcommittee 2012) and it is currently unclear what impact these treatments may have on the genetic structure of populations, including potentially selecting for more herbicide tolerant genotypes. This project sought to characterize watermilfoil genotypes in Lake Minnetonka Bays (Grays, St. Albans, and North Arm) that have had extensive herbicide treatment(s) to control Eurasian watermilfoil.

In addition to herbicide management, it is currently unclear whether there are associations between genetic composition of watermilfoil populations and the performance of a potential biocontrol agent, the milfoil weevil (*Euhrychiopsis lecontei*; e.g., see Roley and Newman 2006, Borrowman et al. 2015). This project therefore also sought to characterize genetic composition of watermilfoils in two Lake Minnetonka bays (Smiths and Veterans), and nearby Christmas Lake, all of which historically have abundant milfoil weevil populations.

<u>This project specifically asks the following questions:</u>
1) What is the taxonomic composition of watermilfoils (Eurasian, northern, and hybrid) in Minnetonka Bays and Christmas Lake?
2) Does the composition differ in herbicide-treated versus untreated lakes?
3) Are hybrid watermilfoil populations genetically distinct in different water bodies, and is there any relationship between genetic composition and management history?
4) Are there any relationships between weevil occurrence and density and distinct watermilfoil taxa?

Methods

<u>Genetic analyses –</u> Plant samples from early season (June) and late season (August) point intercept surveys from 2015 were sent to Thum's lab at Montana State University for genetic analysis. The accompanying table describes the total number of plants and survey sites collected from each water body in 2015. Note that 1-5 plants were sampled per survey point. The original funds were sufficient to cover processing of ~60 plants per water body (~30 plants each for the "early/pre-treatment" and "late/post-treatment" surveys. For most of the surveys, watermilfoil plants were collected from more than 30 sites, and as such, a complete description of the genetic composition of watermilfoil populations in each water body requires the processing of at least one plant from the remaining collection sites on each water body.

	Herbicide treatment in 2015?	"Early" No. collection sites (total no. plants collected)	"Late" No. collection sites (total no. plants collected)
Grays	Yes	117 (287)	4 (12)
St. Albans	Yes	58 (160)	3 (9)
North Arm	Yes	50 (107)	28 (77)
Veterans	No	35 (103)	38 (117)
Smiths	No	56 (158)	36 (108)
Christmas	No	45 (141)	48 (145)

Genetic data consisted of amplified fragment length polymorphisms (AFLPs), and were collected using standard methods employed in previous watermilfoil research in the Thum lab (Zuellig and Thum 2012, LaRue et al. 2013). AFLP data were analyzed using Discriminant Analysis of Principal Components (DAPC) (Jombart 2008, Jombart and Ahmed 2011). We assigned each individual as Eurasian, northern, or hybrid using a DAPC without priors and a K value equal to three (number of groups that an individual can be assigned to). To examine the extent to which hybrid populations in different water bodies were genetically similar/distinct, we constructed DAPCs using combinations of priors for water body and sampling date (pre- versus post-treatment for treated water bodies; early versus late for untreated water bodies).

<u>Weevils –</u> Weevil surveys were conducted in June and August at Christmas Lake and Smith's Bay and early July and August at Veteran's Bay. Samples were collected from transects along each lake with three to five sampling stations extending from shore (0.5m depth) to deep (2.5-3m) water. Along steep shorelines only three stations were sampled and more stations were sampled when distance to deep water was greater. Plants had to reach to within 1m of the surface to be sampled and weevils rarely extend to deeper plants. At each station samples of 8 stems (top 50 cm) of a visible taxa were collected and placed in as sealable plastic bag. If both northern watermilfoil and Eurasian/Hybrid were present then two separate samples were collected. We generally can distinguish northern watermilfoil from Eurasian and hybrid watermilfoil, but not Eurasian from hybrid water milfoil. At each station we collected an additional 5 stems of the sampled taxa and placed in a separate bag for genetic analysis. For the genetic samples, collected stems were from at least 1m apart so as to not represent the same plant. Approximately 30 stations were sampled at each waterbody on each date.

Stems from the genetic samples were vigorously rinsed of periphyton and invertebrates and then wrapped in wet paper towel and placed on ice in a cooler until they were shipped within 24h on ice to the Thum lab in Montana. Procedures for sample processing and genetic analysis are given above.

Weevil samples were kept chilled and refrigerated until they were processed. Stems and meristems were counted and then were examined visually and under 3x magnification for eggs, larvae, pupae and adult weevils, which were enumerated. Abundance of each lifestage in a sample was estimated as the number per stem, and samples were then averaged to get an estimate (by milfoil taxon) for each lake.

Point-Intercept Surveys

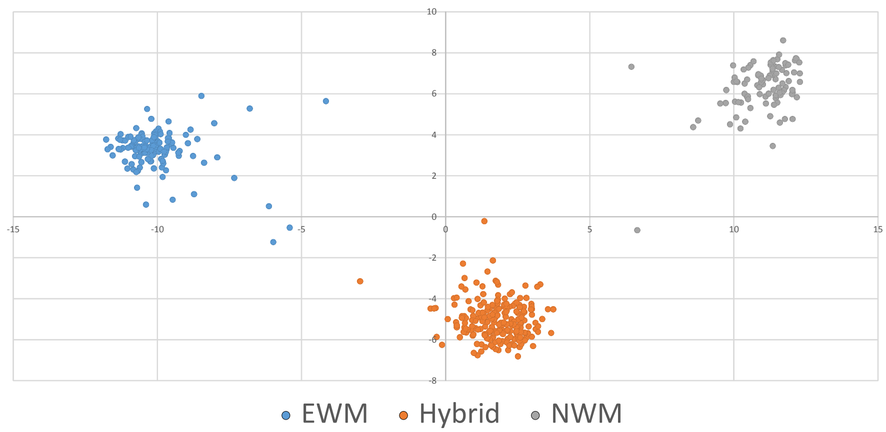
Untreated Bays/Lakes - The plant community at each untreated lake was assessed with point intercept surveys. At Christmas Lake, a 50m grid was sampled in June and a 70m grid was used in August resulting in 116 and 75 points within the 4.6m littoral sampled respectively. At Smith's Bay a 50m grid was sampled in July and a 70m grid was used in August resulting in 167 and 123 points within the 4.6m littoral sampled respectively. At Veteran's Bay a 50m grid was used in both July and August, resulting in 83 sampling points within the 4.6m littoral zone on each date. At each point depth was recorded and a weighted, 0.3-m wide 14 tined rake was tossed, allowed to sink to the bottom, and retrieved to collect plants. Plants were given a relative density rating of 0 to 5 and each taxa present was recorded. At a subset of plots biomass samples were collected but these data are not reported here. It should be noted that the weevil survey samples were collected from the shallower areas of the bay and thus do not include the deeper and further from shore areas included in the point intercept surveys (but the PI surveys do include all the weevil survey areas).

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

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

Results & Discussion

Taxonomic composition of treated and untreated lakes – The figure below is a DAPC of 216 AFLP loci collected from the subsample of plants from the 2015 collections (n=281 plants). The DAPC analysis clearly distinguishes Eurasian (EWM; blue circles) from northern watermilfoil (NWM; gray circles) and hybrid watermilfoil (Hybrid; orange circles). This analysis was used to classify each plant as EWM, NWM, or HYBRID (=HWM) in the accompanying table below, which summarizes the taxonomic composition of each water body.

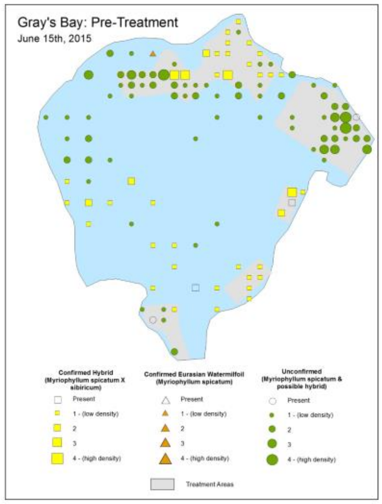


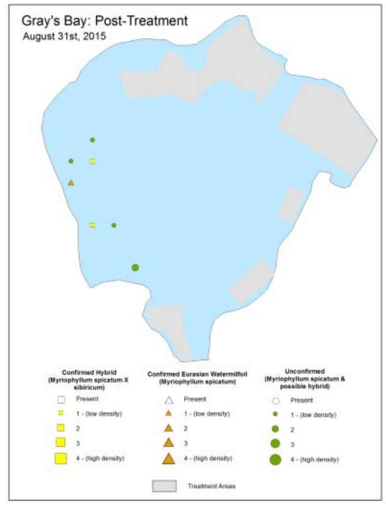
	EARLY/PRE			LATE/POST		
	EWM	NWM	HWM	EWM	NWM	HWM
Grays	1		42	1		2
St. Albans	20		8			3
North Arm	2		25			17
Smiths	1	14	6	2	26	
Veterans	16	5	8	15	4	4
Christmas	14	15		16	7	1

Although these data are preliminary, and do not represent sampling from all of the survey sites, it is interesting to note the stark contrast in composition between herbicide-treated versus untreated lakes. Specifically, we only found northern watermilfoil (NWM) in untreated lakes, whereas hybrids were common to dominant in treated lakes.

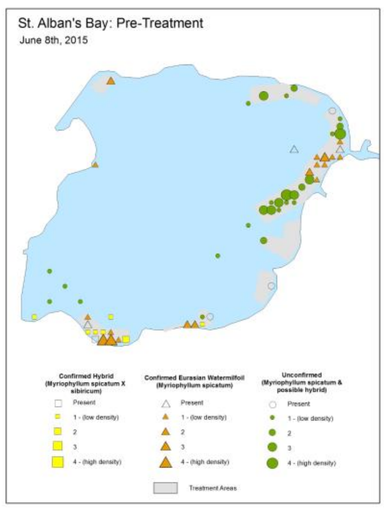
Comparison of pre- and post-treatment composition –

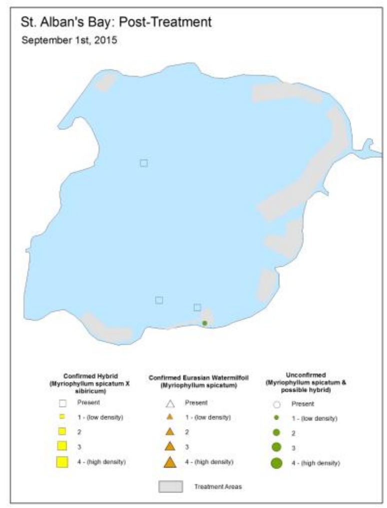
Grays Bay was dominated by hybrid watermilfoil before treatment. Results from our study demonstrate that the herbicide treatment was very effective. The only watermilfoil detected during the post-treatment survey was located in the western end of the Bay, which was not treated. It appears that the hybrid watermilfoil strain(s) present in Gray's Bay at the time of treatment are susceptible to the herbicide treatment.



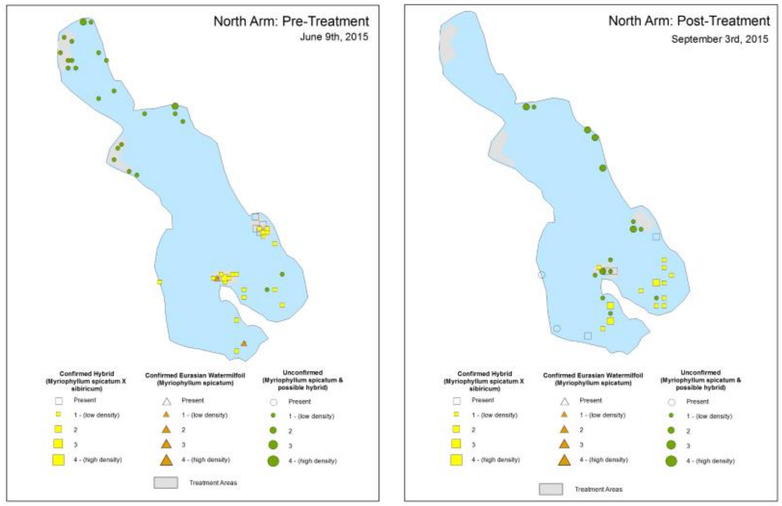


St. Albans Bay had higher relative abundance of pure EWM compared to HWM pre-treatment. As with Grays Bay, the herbicide treatment appears to have been very effective in St. Albans Bay, as watermilfoil frequency of occurrence was very low post-treatment, and the only watermilfoil detected was located outside of herbicide treatment areas. However, hybrid watermilfoil was the only watermilfoil detected post-treatment.



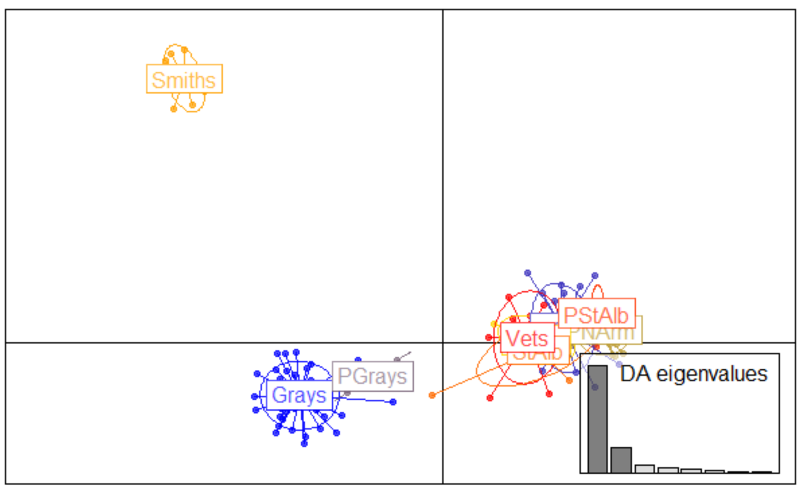


Like Grays Bay, North Arm was dominated by hybrid watermilfoil pre-treatment. However, control in North Arm was not as good as it was for Grays Bay. The northern portion of the lake exhibited a large reduction in watermilfoil following treatment. In contrast, the reduction of watermilfoil in the southern part of the lake was not as great as for the northern portion. These areas were dominated by hybrid watermilfoil pre-treatment, and only hybrids were detected in the post-treatment survey of these areas. Thus, it is possible that the hybrid genotype(s) present in North Arm were less susceptible to herbicide treatment compared to Grays Bay. However, it is also possible that the effective exposure of herbicide (concentration and/or exposure time) was different between North Arm and Grays Bay.



Genetic diversity and differentiation of hybrids –

Are the populations with qualitatively different patterns of herbicide control genetically different? We performed a DAPC using only hybrid individuals in the six lakes (because the treated water bodies were dominated by hybrids), and using both water body and time (pre/early versus post/late) as priors. Interestingly, Grays Bay, which was dominated by hybrid watermilfoil but exhibited high herbicide control, was genetically distinct from hybrid watermilfoils in the other water bodies; of particular interest is North Arm, which exhibited relatively lower herbicide control compared to Grays Bay. This suggests that the difference in the extent of control between these two Bays may be related to the different genotype(s) of hybrid watermilfoil present in the two different bays. A comparison of dose-response curves among these genetically distinct populations of hybrid watermilfoil could be useful for testing this hypothesis.



Smiths Bay hybrids were also genetically distinct from other hybrids. This is interesting because we did not detect hybrids in our late season survey of Smith's Bay but not all the samples have been processed.

Weevil and plant surveys in untreated lakes – The plant community at all three sites was fairly diverse, with 15 to 19 native taxa at Smith's Bay and Veteran's Bay and 24-25 native taxa at Christmas Lake (Table 1). Curlyleaf pondweed and Eurasian watermilfoil were present at all sites. Although the exotics were typically present at more than 50% of the sites, native plants were typically present at more than 90% of the sites. Christmas Lake had the greatest diversity per point with 4 native taxa per point in June and 4.5 per point in August. In Smith's Bay, native taxa increased from 2.3 per point in July to 3.4 per point in August whereas native taxa in Veterans decreased from 2.7 per point in July to 2.2 per point in August (Table 1).

Overall, Eurasian watermilfoil and coontail (*Ceratophyllum demersum*) were the most common taxa, often found at 50% or more of sites, but flatstem pondweed (*P. zosteriformis*) was also common in all lakes, being found at 40-50% of sites in Christmas and Veteran's and 60-70% of sites at Smith's Bay (Table 2). Canada waterweed (*Elodea canadensis*), wild celery (*Vallisneria americana*), Richardsons pondweed (*P. richardsonii*) and water stargrass (*Zosterella dubia*) were common at all sites. Northern watermilfoil was most common at Christmas Lake (17-23% of sites) but was also found at Smith's and Veteran's Bays at lower frequency.

Northern watermilfoil was more common in shallower sites (<2m) and infrequent at deeper sites (Table 3). In contrast, Eurasian watermilfoil was often more frequent in deeper sites (>2m) than in shallower sites, but was always found in shallow sites at frequencies between 20-40% (Table 3). These observations suggest that northern watermilfoil, at least in the presence of Eurasian watermilfoil, is restricted to shallower water and Eurasian does well in water ≥ 2m deep.

For the weevil surveys, northern watermilfoil was typically found at the shallower stations nearer shore, although in Christmas Lake it was occasionally found at deeper sites. There was no clear difference in abundance of milfoil weevils on northern or Eurasian watermilfoil (Table 4), but densities were low and variable at all sites. There was some suggestion in Veterans that July density on northern was lower than on hybrid and Eurasian and that possibly hybrid had more weevils than either northern or Eurasian. However, because the sampling scheme was unable to clearly distinguish the hybrid and Eurasian watermilfoils and the presence of both at the sites, no conclusions should yet be drawn.

Genetic analysis of the plants from the weevil survey sites indicate a mix of Eurasian, northern and hybrid watermilfoil genotypes (Table 5). At Christmas Lake both northern and Eurasian were common and so far only one hybrid has been identified. However, the number of samples analyzed was low and it should be considered that the samples are biased toward waters shallower than 3m. At Smiths Bay, northern watermilfoil genotypes were much more common than either Eurasian or hybrid, although again, these samples are biased to shallower water and stations closer to shore (<120m from shore). In contrast, at Veterans Bay, hybrid watermilfoil was more common than northern watermilfoil, and Eurasian composed more than half the samples analyzed (Table 5). The results so far indicate no clear differences in weevil abundance between watermilfoil taxa.

Table 1. Frequency of occurrence of native taxa, exotics (*M. spicatum* and *P. crispus*), and any plants (all), along with total number of taxa and native taxa and number of taxa per sampling point at Christmas Lake, and Smith's Bay and Veteran's Bay of Lake Minnetonka.

Lake	Date	Natives	Exotics	All	# Taxa	Native Taxa	Native/pt	Taxa/pt
Christmas	6/22/15	92.20%	56.00%	94.80%	27	25	4	4.1
Christmas	8/12/15	98.70%	42.70%	98.70%	26	24	4.5	4.6
Smith's	7/1/15	98.80%	86.80%	100.00%	17	15	2.3	3.5
Smith's	8/21/15	100.00%	52.80%	100.00%	21	19	3.4	4
Veteran's	7/23/15	98.80%	66.30%	100.00%	19	17	2.7	3.4
Veteran's	8/26/15	88.00%	55.40%	96.40%	21	19	2.2	2.7

Table 2. Frequency of occurrence of the most abundant plants, found in Christmas Lake and Smith's Bay and Veteran's Bay of Lake Minnetonka. Plant abbreviations are Cdem = *Ceratophyllum demersum*, Char = *Chara spp.*, Ecan = *Elodea canadensis*, Msib = *Myriophyllum sibiricum*, Mspi = *M. spicatum*, Pcri = *Potamogeton crispus*, Pric = *P. richardsonii*, Pzos = *P. zosteralla*, Rlon = *Ranunculus longirostris*, Spec = *Stuckenia pectinata*, Vame = *Vallisneria americana*, Zdub = *Zosterella dubia*.

Lake	Date	Cdem	Char	Ecan	Msib	Mspi	Pcri	Pric	Pzos	Rlon	Spec	Vame	Zdub
Christmas	6/22/15	0.53	0.27	0.14	0.23	0.37	0.38	0.1	0.53	0.51	0.09	0.16	0.15
Christmas	8/12/15	0.49	0.35	0.12	0.17	0.47	0.01	0.35	0.43	0.39	0.24	0.49	0.27
Smiths	7/1/15	0.83	0.02	0.28	0.03	0.56	0.81	0.28	0.59	0.16	0.01	0.02	0.08
Smiths	8/21/15	0.69	0.03	0.23	0.13	0.53	0.07	0.26	0.72	0.32	0.03	0.29	0.5
Veterans	7/23/15	1	0.11	0.28	0.06	0.74	0.2	0.2	0.47	0.06	0.06	0.24	0.27
Veterans	8/26/15	0.59	0.05	0.24	0	0.61	0.01	0.11	0.41	0.04	0.02	0.34	0.37

Table 3. Percentage occurrence of northern watermilfoil (Msib) and Eurasian watermilfoil (Mspi) in shallow (<2m) and deeper water at Christmas Lake, and Smith's Bay and Veteran's Bay of Lake Minnetonka.

			<2m	≥2m
Christmas	6/22/2015	Msib	27%	14%
		Mspi	31%	68%
Christmas	8/14/2015	Msib	15%	11%
		Mspi	37%	37%
Veteran's	7/23/2015	Msib	16%	0%
		Mspi	39%	58%
Veteran's	8/26/2015	Msib	0%	0%
		Mspi	26%	48%
Smith's	7/1/2015	Msib	19%	1%
		Mspi	20%	50%
Smith's	8/21/2015	Msib	20%	8%
		Mspi	31%	64%

Table 4. Number (and 2 SE) of milfoil weevil eggs, larvae, pupae and adults per stem found on Eurasian (EWM) and northern (NWM) watermilfoils at Christmas Lake and Smith's Bay and Veteran's Bay of Lake Minnetonka. N= number of samples of 8 stems each.

		Eggs/Stm	Larvae/Stm	Pupae/Stm	Adults/Stm	Total/Stm	N
Christmas	EWM	0.027	0.015	0	0.006	0.049	25
June	2se	0.03	0.017	0	0.012	0.033	
	NWM	0	0	0	0.006	0.006	21
	2se	0	0	0	0.012	0.012	
	EWM	0.014	0	0.005	0.014	0.032	28
	2se	0.02	0	0.009	0.015	0.025	
	NWM	0.035	0	0	0.093	0.128	23
	2se	0.041	0	0	0.061	0.081	
Smiths	EWM/HWM	0	0.02	0	0.01	0.03	11
June	2se	0	0.04	0	0.02	0.061	
	NWM	0	0.008	0	0.012	0.02	28
	2se	0	0.013	0	0.024	0.026	
	EWM	0	0	0	0.031	0.031	4
	2se	0	0	0	0.063	0.063	
	NWM	0.028	0.004	0.01	0.034	0.077	26
	2se	0.029	0.009	0.015	0.023	0.053	
Veterans	EWM/HWM	0.207	0.044	0.008	0.033	0.292	27
July	2se	0.126	0.047	0.015	0.028	0.148	
	NWM	0	0	0	0.031	0.031	7
	2se	0	0	0	0.041	0.041	
	EWM/HWM	0.047	0	0	0.047	0.094	26
	2se	0.054	0	0	0.039	0.078	
	NWM	0.086	0	0	0	0.086	10
	2se	0.171	0	0	0	0.171	

Table 5. Number of samples identified as pure *Myriophyllum spicatum* (EWM), *M. sibiricum* (NWM) and hybrid of these two taxa (HWM) from weevil survey samples at Christmas Lake and Smith's Bay and Veteran's Bay of Lake Minnetonka. The proportions (Prop) of each taxa identified are given as percentages.

		EWM	NWM	HWM	PropEWM	PropNWM	PropHWM
Christmas	June	14	14	0	50%	50%	0%
	Aug	15	7	1	65%	30%	4%
Smiths	June	0	12	3	0%	80%	20%
	Aug	2	26	0	7%	93%	0%
Vets	June	16	5	8	55%	17%	28%
	Aug	15	3	4	68%	14%	18%

<u>Plant Surveys in Treated Bays</u>

Bay-wide analysis (pre and post surveys) –

Overall plant diversity and abundance did not show much change before and after treatment in all three bays. Distribution and abundance of watermilfoil varied across bays with genetic analysis revealing the presence of Eurasian watermilfoil and Hybrid watermilfoil. Northern watermilfoil has not been identified in any of the genetic samples thus far.

Grays Bay went from 19 taxa in June to 18 in August. The percentage of littoral points vegetated decreased slightly, going from 94% in June to 87% in August. Hydroacoustic surveys were also passively performed during the surveys using CiBiobase, which showed percent area covered in June as 95%, and 91.5% in August. The average biovolume from CiBiobase showed 62% in June, and 51.3% in August post-treatment. The frequency of occurrence of native plants in the littoral zone decreased similarly, going from 92% to 87%. Eurasian watermilfoil saw a large decrease, going from 47.6% occurrence, to only 5.5% post-treatment. Of that 47.6% occurrence of Eurasian watermilfoil, Hybrid milfoil occurred at 17.5% of the sites, Eurasian watermilfoil at 0.8%, and the remaining has not been genetically confirmed yet. Of the 5.5% found post-treatment, Eurasian watermilfoil was confirmed at 0.8% of sites, Hybrid at 1.6% and the rest have yet to be genetically confirmed. Curlyleaf pondweed was also abundant in June, showing up at 22% of littoral sample sites. Native plants showed some shift in composition and abundance between surveys. Coontail (*Ceratophyllum demersum*) remained abundant in both surveys, occurring at 30% of sites in June and 37% in August. Flat-stem pondweed (*Potamogeton zostiformis*) was the second most abundant plant in June occurring at 33% of sites, but was found less frequently in August, occurring at only 9% of sites. Fries' pondweed (*Potamogeton friesii*) was found at 16% of sites in June, but was not found at all in August. Wild celery (*Valisneria Americana*) and water stargrass (*Heteranthera dubia*) both were found more frequently in the August survey, going from 10 to 41% and 6 to 37% respectively.

In North Arm Bay, 21 taxa were found in June, and 22 in September. The percentage of littoral points vegetated remained relatively unchanged, ranging from 82-83% between the two surveys. The frequency of occurrence of native plants increased from 68% in June to 78% in September. Eurasian watermilfoil was not overly abundant in June, only being found at 12% of sites. Of that 12%, genetic analysis confirmed Eurasian at 0.4% and Hybrid at 4.6%, the rest has not been genetically confirmed yet. Bay-wide, Eurasian watermilfoil did not decrease much by the September survey, still being found at 9.6% of all sites. Of that 9.6%, genetic analysis confirmed Hybrid milfoil at 4.6% of sites, and the rest has not been genetically confirmed yet. Curlyleaf pondweed was the most dominant plant in June, being found at 66% of littoral sites. There were other small changes in percent occurrence of native plants between surveys; Coontail increased from 51% to 62%, flat-stem Pondweed decreased from 19.5% to 7%, clasping-leaf pondweed (*Potamogeton richardsonii*) increased from 4% to 13%, wild celery increased from 3% to 16.5% and Water Stargrass increased from 1% to 11%.

In St. Albans Bay, 18 taxa were found in June and 20 in September. The percentage of littoral points vegetated decreased slightly from 97% in June, to 91% in September. The frequency of occurrence of native plants increased from 84% to 91%. Eurasian watermilfoil was not overly abundant in June, only being found at 13.5% of littoral sites. Of that 13.5%, genetic analysis confirmed Eurasian watermilfoil at 2%, Hybrids at 2% and the remaining has not been confirmed yet. Post-treatment, Eurasian watermilfoil was not detected on the sample rake, but a few sparse plants were observed by the surveyors. Genetic analysis of those samples revealed they were Hybrid milfoil. Curlyleaf pondweed was also fairly abundant in June, being found at 45% of littoral sites. The composition and abundance of native plants also changed some between the two surveys. Flat-stem pondweed was the most abundant plant in June, being found at 49% of sites, however, it was only found at 17% of sites in September. Coontail remained abundant during both surveys, ranging from 34.5%-41% between the two surveys. White-stem pondweeed (*Potamogeton praelongus*) decreased, going from 20% to 3%. Robbin's pondweeed (*Potamogeton robbinsii*) increased, going from 19% in June to 35% in September. Wild celery also showed a large increase, going from 8% to 51% by September.

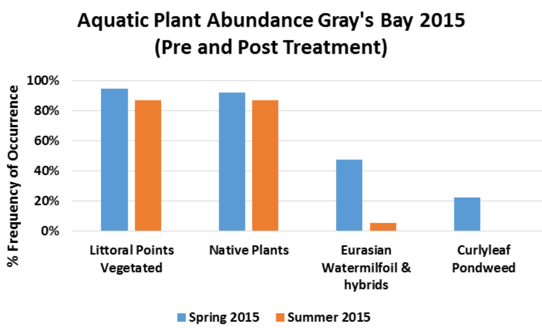
Treatment Site Analysis (Pre and Post Surveys)

Further analysis of the sample points within the treatment sites found a complete reduction of Eurasian watermilfoil in Grays Bay. Prior to treatment, the survey in June found Eurasian watermilfoil to be present at 46% of sample sites in the multiple treatment zones, 11.5% was confirmed Hybrid, and the rest has not been analyzed. Post-treatment, no Eurasian watermilfoil was observed in the treatment sites. Diversity remained relatively unchanged, 18 taxa were found during the June survey, and 19 were found in August. Native taxa composition and abundance had some change, with several species showing increased frequency. Wild celery showed an increase between surveys from 1.3% in June, to 42% in August. Water stargrass also increased from 5% to 36%. Other native species showed a decline between surveys. Flat-stem pondweed was found at 38% of sample sites in June, but only 0.7% in August. Fries' pondweed found at 14% of sites in June, but was not detected in August. White-water crowfoot (*Ranunculus aquatilis*) decreased from 10% in June, to 1.5% in August. Coontail was abundant in both surveys, ranging from 34-41% between the two surveys.

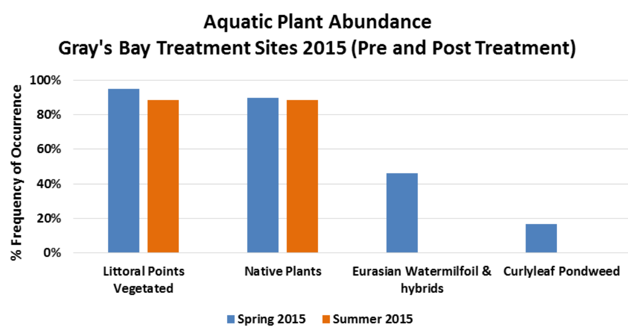
In North Arm Bay, Eurasian watermilfoil was reduced from 20.5% in June to 4.5% in September. Of the 20.5% from June, 10.7% of sites were Hybrid, 0.9% was Eurasian, and the rest has not been genetically confirmed yet. Of the 4.5% that was remaining post-treatment, 0.9% of sites was Hybrid, and the rest has yet to be confirmed. Diversity of taxa remain relatively unchanged, with 14 taxa being found in June, and 15 found in September. Native taxa and abundance showed relatively minor changes, with no large increases or decreases in species abundance.

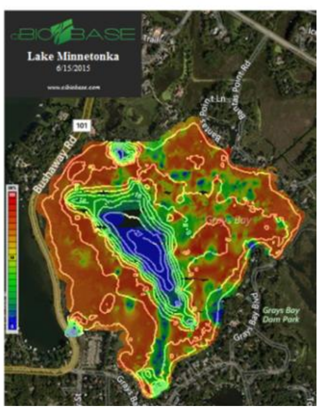
In St. Albans Bay, Eurasian watermilfoil was reduced from 30.1% in June, to 0.8% in September. Of the 30.1% from June, 12% of sites had confirmed Eurasian, 3.8% Hybrid and the rest has not been genetically confirmed yet. No post-treatment samples from the treatment sites have been genetically analyzed yet. Native taxa composition and abundance had some change between surveys. Wild celery increased from 4.5% occurrence in June, to 43% in September. Several other species showed larger declines in frequency, Flat-stem pondweed was found at 54.1% of sites in June, but only 6.3% of sites in September. White-water crowfoot decreased form 40% to 17% post-treatment. Elodea also decreased from 16% to 4%. Coontail remained abundant between both surveys, ranging from 46-52%. There were no changes in species diversity, 18 species were found in each survey.

Grays Bay - Whole-Bay

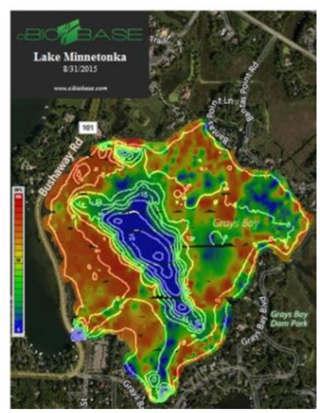


Grays Bay – Treatment Sites

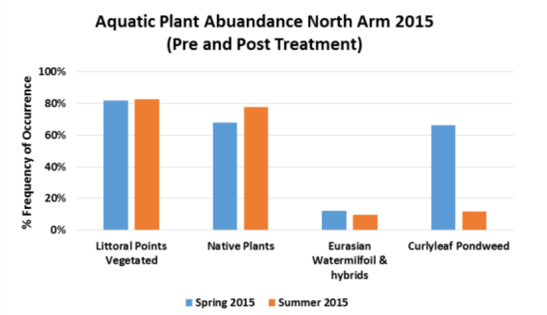




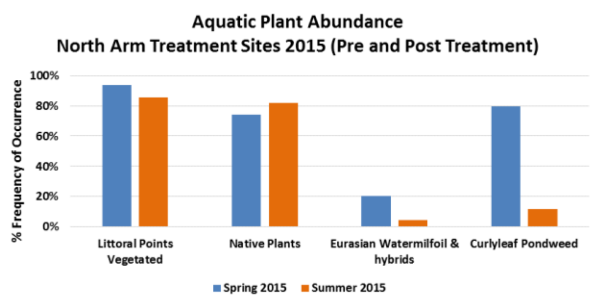
Pre and Post-Treatment Biovolume Map. Generated by CiBiobase.



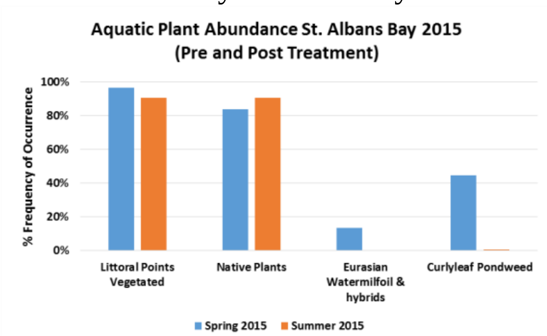
North Arm Bay – Whole Bay



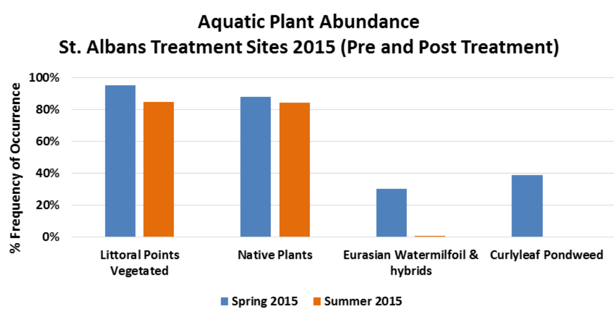
North Arm Bay – Treatment Sites



St. Albans Bay – Whole Bay



St. Albans Bay – Treatment Site



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