Assessment of factors affecting the biological control of Eurasian watermilfoil

Final Report to the Minnesota Aquatic Invasive Species Research Center
ENRTF Phase I Project: Developing and evaluating new techniques to selectively control invasive plants: Activity 2 manipulating sunfish to enhance milfoil weevils

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Abstract:
Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most widespread and problematic invasive aquatic plants in Minnesota. Approaches to improve its management are needed to reduce economic and ecological costs of invasive control. We focused on assessing factors that limit biological control of Eurasian watermilfoil by the native milfoil weevil and other herbivores.

Enclosure experiments to assess the effect of sunfish predation on herbivore and milfoil abundance were largely unsuccessful. Weevil populations developed in the enclosures but there were no differences in weevil or milfoil abundance due to fish stocking. We failed to recover stocked fish from the enclosures and suspect that predation by herons removed the fish. Realistic enclosure experiments in natural lakes may not be feasible and experimental manipulations might be better conducted in small natural or artificial ponds or in large tanks.

We assessed herbivore abundance in metro lakes and found milfoil weevils in 12 of the 19 lakes surveyed. Herbivore abundance was higher in 2015 than 2016, but abundance during both years was lower than some prior years. Only 1 weevil was found in over 450 sunfish stomachs examined, in part due to low milfoil weevil density in many lakes. Milfoil weevil abundance was negatively correlated (r=-0.44) with sunfish abundance; lakes with high sunfish populations (> 50 sunfish/trapnet) will likely not support sufficient herbivore populations and biological control should not be considered in these lakes until sunfish are reduced.

However, some lakes with low sunfish populations also have low herbivore densities and factors other than sunfish are apparently limiting herbivores and biocontrol in these lakes. Possible limiting factors include lack of access to shoreline overwinter habitat, extensive mechanical harvesting or herbicidal control, and poor water or plant quality. Further work that also accounts for environmental variability is needed to identify factors limiting milfoil herbivores and biocontrol.

Introduction:

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most troublesome aquatic weeds in North America (Smith and Barko 1990). Chemical control of Eurasian watermilfoil with 2,4-D or triclopyr (Cason and Roost 2011, Netherland and Jones 2015) and fluridone (Wagner et al. 2007) can be effective at controlling the plant for several years, often with few negative effects on native plants. However, herbicide treatments are expensive, often need to be repeated every several years and can cause significant negative effects on native plant communities and systems (Wagner et al. 2007, Valley et al. 2006, Cason and Roost 2017. Furthermore, some stakeholders object to chemical treatments and desire different approaches. This led to an interest in biological control with herbivorous insects (Creed and Sheldon 1995, Sheldon and Creed 1995) and the most promising agent is the native milfoil weevil *Euhrychiopsis lecontei* (Newman 2004).

The milfoil weevil is native to North America (Creed 1998); its natural host plants were likely the native northern watermilfoil (*M. sibiricum*) and other native watermilfoils such as *M. verticillatum* (Solarz and Newman 2001). The milfoil weevil captured Eurasian watermilfoil as a new and preferred host when it was introduced to North America. Extensive host range testing indicated that the milfoil weevil is specialist on plants within the watermilfoil (*Myriophyllum*) genus (Solarz and Newman 2001, Sheldon and Creed 2003, Newman 2004) but that the insect performs best on the exotic Eurasian watermilfoil and poorest on the native northern watermilfoil; performance on a hybrid of the two species is better than on the native and may better (Borrowman et al. 2015) or worse than on the Eurasian variety (Roley and Newman 2006). The milfoil weevil spends the summer submersed on milfoil plants, completing all 4 life stages (egg, larva, pupa and adult) underwater and producing 3 to 4 generations before the adults move to shore to overwinter in leaf litter (Newman et al. 2001). In the spring, adults return to the lake and begin to lay eggs. Suitable overwinter habitat (dry sites with duff near shore) is required to sustain weevil populations (Thorstenson et al. 2013). In-lake densities have been related to amount of natural shoreline (Jester et al. 2000), but summer in-lake factors appear more important to weevil populations when shoreline habitat is available (Newman et al. 2001). The native milfoil weevil is widespread in Minnesota and North America (Creed 1998, Tamayo et al. 1999) and likely occurs naturally in most lakes that have Eurasian or northern watermilfoil (Borrowman et al. 2014).

The milfoil weevil has caused declines of Eurasian watermilfoil under controlled conditions (Creed and Sheldon 1995, Sheldon and Creed 1995, Newman et al. 1996) and in a number of lakes (Sheldon and Creed 1995, Newman and Biesboer 2000, Newman 2004), although there is considerable variability in effects across lakes (Reeves et al. 2008, Reeves and Lorch 2012). Summer-long densities of 0.25 to 0.5 weevils per stem may be sufficient to control the plant and densities > 1/stem have resulted in control (Newman 2004). In many lakes weevil populations do not reach sufficient density to control the plant (reviewed in Newman 2004). Identification and amelioration of factors limiting populations would enhance chances for successful control.

Work in Minnesota and elsewhere suggested that predation by sunfish (*Lepomis spp.* but, primarily bluegill, *L. macrochirus*, and its hybrids) can limit herbivore and milfoil weevil populations and thus its control of Eurasian watermilfoil. In experimental manipulations, weevil and other herbivore densities were reduced in the presence of sunfish (Ward and Newman 2006) and in a comparison across 11 lakes, milfoil weevil densities were negatively related to sunfish relative abundance (Ward and Newman 2006). Sunfish densities > 25-30 per trapnet can limit herbivore abundance and sunfish densities > 50 per trapnet allow few herbivores. In Minnesota and elsewhere, sunfish densities appear lower in lakes where herbivorous insects are controlling Eurasian watermilfoil (Newman 2004, Parsons et al. 2011, Parsons 2012). EnviroScience has stocked over 200 lakes in the US and Canada. Although they purport good success from stocking, the published evidence is equivocal (Reeves et al. 2008) and effective methods to reduce predation by fish would enhance the success of both natural and stocked (augmented) populations of milfoil weevils and other herbivores. If biological control with insects is to be operationally successful, management to reduce overabundant or stunted sunfish populations may be needed.

Overabundant and stunted sunfish are a major problem in Minnesota lakes (Drake et al. 1997, Shroyer et al. 2003, Jacobson 2005) and reducing sunfish density is not a trivial task. It is likely that a combination of predator enhancement and regulations to reduce harvest of large sunfish is required (Beard and Essington 2000, Aday et al. 2006), perhaps along with direct reduction by trapnetting or tournaments. However, if sunfish densities can be reduced and sunfish size-structure enhanced, this could create a quality sunfish fishery while also enhancing biological control of Eurasian watermilfoil.

To assess the potential to enhance biological control of Eurasian watermilfoil, enclosure experiments and field surveys were conducted. Enclosure experiments were conducted to determine if sunfish limit herbivore abundance and control of milfoil. To determine factors limiting herbivore abundance in lakes and the extent of sunfish consumption of herbivores, twenty lakes were surveyed for milfoil, herbivores and sunfish, most in both years. Sunfish stomach contents were assessed in ten of these lakes. These results were used to propose further study.

Methods

Enclosure experiments

Enclosure experiments were conducted in summer 2015 and 2016 in Cedar Lake (DOW 270039) and Peltier Lake (020004). Sites with Eurasian watermilfoil beds that also had some native plants in water depths between 1 and 2m were located in each lake.

In July 2015 three enclosures were installed in each lake. The vinyl impermeable enclosures (2.4m deep x 38m circumference) enclosed an area of approximately $100m^2$ with sides embedded in sediment with rebar "staples" and a lead line and held upright by floats along the top surface. The enclosures were allowed to equilibrate for a week before pre-treatment plant and water quality data were collected (see below). At the same time, two adjacent and similar areas were selected and marked to be used as open controls. Each of the three enclosures in a lake was randomly assigned a fish treatment level and then 0, 5 ($0.05/m^2$) or 20 ($0.2/m^2$) bluegill sunfish (collected from same lake) were stocked into the enclosures. Fish in Cedar Lake ranged from 100 to 160mm in length (22-80g) and in Peltier from 110 to 180 mm (30-150g) and each fish had a PIT tag implanted before stocking.

Prior to fish stocking weevil surveys were conducted and plant biomass and water quality measures were assessed within the enclosures and the control plots. Weevil surveys were conducted by collecting 8 milfoil stems (top 50 cm) from each of 6 locations (samples) within an enclosure or control area. Each sample of 8 stems was kept in a Ziploc bag until processing in the laboratory. Plant biomass was collected from 5 sites within each enclosure with the rotating rake method (Johnson and Newman 2011). Samples were kept in sealable bags in a cooler until they could be processed in the lab. Within each enclosure Secchi depth was measured as was transparency in a Secchi tube. Dissolved oxygen, temperature and light (PAR) profiles were measured with readings at the surface, 0.5m and 1m.

Weevils survey samples were counted for stems and meristerms and examined under 3x magnification for eggs, larvae, pupae and adult weevils, which were enumerated and preserved. Other herbivores such as the lepidopterans *Acentria* and *Parapoynx* were also enumerated. Results for each sample were expressed as numbers per stem and samples were averaged within an enclosure. Plant samples were kept in a cooler at 4 ˚C until processed, when they were sorted, identified to species, weighed, dried (65 ˚C for 2 days) and reweighed. Biomass (g dry/m^2) was determined for each species and for Eurasian watermilfoil and all native taxa combined.

Biomass samples and water quality data were collected at the beginning, middle and end of the experiment and weevil surveys were conducted once per month. We attempted to retrieve fish at week three and thereafter using a combination of angling and trot lines as well as visual observation. The experiment ended in early October 2015.

We repeated these experiments in 2016 with an earlier start. Enclosures were installed in both lakes in June and randomly stocked the following week with 0, 5 or 20 sunfish. Fish were slightly bigger in 2016 with a range of 120-160 mm in Cedar and 120 to 200mm in Peltier. We spent more time securing the enclosures, using larger pins and a diver to check the seal. We also staked some of the enclosure to reduce escape of fish. Using the methods of 2015, we took plant biomass samples (5 per enclosure or control) at the beginning, middle and end of the experiment, measured water quality 4 times during the experiment and conducted weevil surveys once per month (6 samples per enclosure).

Field surveys

To further define the relationship between sunfish and herbivores, surveys of lakes for milfoil weevils and other herbivores were conducted and results compared to estimates of sunfish density. Point intercept surveys of aquatic macrophytes were conducted on a subset of lakes to quantify milfoil and native plant occurrence. Lakes were selected that had recent or planned fisheries surveys to get estimates of sunfish abundance and lakes that were known or recommended by contacts to have had abundance milfoil populations in the past.

In 2015 fourteen lakes were surveyed and in 2016 eighteen lakes were surveyed (Table 1). Over half the lakes were sampled two or more times each year. For each survey, approximately 30 sample stations were located at each lake, and stations were typically distributed around the lake on 10 transects with stations near shore (shallow, ≤1m), midway to edge of bed (1.5-2.0 m) and the outer edge of the bed (ca. 3m). At each station, 8 milfoil stems (top 50 cm of plant) were collected and placed into a sealable plastic bag. Samples were returned to the laboratory and kept refrigerated until they were processed (usually within 24h and always within 48h). For each sample, stems and meristems were counted as were eggs, larvae, pupae and adult weevils and lepidopteran larvae, which were preserved in 80% ETOH. Plants were examined under 3x magnification and if needed under a dissecting scope to verify eggs and larvae. Herbivore abundance is expressed as number per stem averaged over the number of samples collected.

Fish were collected for stomach samples from 6 lakes in 2015 and 10 lakes in 2016. In 2015 most fish were collected by electrofishing, whereas in 2016 fish were also collected by trapnet and angling. Stomach contents of each captured fish were obtained via gastric lavage and the contents were preserved in 80% ETOH. Stomach contents were later examined under a dissecting microscope (4-25X) and herbivores enumerated along with general groups of taxa (e.g., zooplankton, snails, chironomids, amphipods, etc.).

Plant communities were surveyed with point intercept sampling on 7 lakes to provide background for future study but those results are not presented here.

Results and Discussion

Enclosures

The enclosures stayed in place in all lakes but may have shifted slightly in 2015 after a large storm; the extra measures in 2016 appeared to eliminate any movement. Water clarity declined in both lakes throughout the summer in both 2015 and 2016 to 0.3-0.8m in July and August in Peltier and 1m in Cedar. Clarity was somewhat variable among enclosures in 2015 but in 2016 was very similar to in-lake clarity. Temperatures within the enclosures were slightly higher than outside on occasion but never exceeded 29 ˚C and dissolved oxygen was generally above 8 mg/L, although it was occasionally <4mg/L at the bottom of the Peltier enclosures. Environmental conditions did not appear limiting.

Plant biomass was variable among enclosures, lakes, and years even though we attempted to place the enclosures and controls in similar density beds each year (Table 2). Biomass of native plants and milfoil was generally higher in 2016 than 2015 and Cedar milfoil biomass was generally higher than Peltier in both years. In Cedar the native biomass was dominated by coontail. In Peltier, coontail was the most common native but Elodea was often nearly as abundant. Other taxa were present at low abundance and often sporadic but Peltier had greater diversity than Cedar.

There was no apparent effect of enclosure or fish treatment on milfoil or native plant biomass in either lake or either year (Table 2). Milfoil biomass generally declined over the season in all treatments, possibly along with decreases in clarity but there was no pattern or effect of treatment on the changes. Weevil densities were also highly variable although densities in Cedar in 2016 were extremely low in the lake and enclosures (only 1 weevil was found). In 2015 weevil densities increased in Cedar plots from <0.05 in July to > 0.27 in August and densities were highest in the no and low fish treatments and lowest in the high fish treatment and controls (Table 3). Density remained high in the low fish treatment but not in the no fish treatment. In contrast, weevil densities in Peltier decreased from a high of 0.2-0.6 in July to few in August and September. Similarly in 2016 densities in Peltier were highest in June and July with few weevils in August. There was no clear relationship to fish stocking density.

Table 1. Lakes surveyed for herbivores in 2015 and 2016 with lake Division of Waters ID number, area (ha), year of most recent DNR fisheries survey, mean number of sunfish (all *Lepomis spp.*) per trapnet found in the survey and years of weevil surveys.

Lake	DOW ID	Area (ha)	Fish Survey	Sunfish/net	Weevils Sampled
Auburn	10004400	114	2012	78	2015-2016
Cedar	27003900	66	2009	58	2015-2016
Cenaiko	02065400	12	2009	16	2015-2016
Centerville	02000600	192	2013	40	2015-2016
Christmas	27013700	108	2013	34	2015-2016
Firemen's	10022600	3	2010	38	2016
Minnetonka	27013300	5751			
Smiths Bay					2015-2016
Veterans Bay					2015-2016
Mitchell	27007000	46	2015	71	2015-2016
Otter	02000400	122	2013	26	2015-2016
Peltier	02000300	123	2013	5	2015-2016
Pierson	10005300	120	2013	23	2016
Rebecca	27019200	106	2011	271	2015
Riley	10000200	120	2015	12	2015-2016
Round	27007100	12	2015	17	2016
Schmidt	27010200	15	1990	22	2016
Steiger	10004500	67	2014	86	2015-2016
Susan	10001300	35	2014	19	2015-2016
Zumbra	10004100	94	2015	31	2016

Table 2. Plant biomass (g dry/m^2) of Eurasian watermilfoil (MSPI), native plants and all plants and number of taxa in enclosures by lake, date and fish treatment (C= open control).

Peltier	Treat	MSPI	Native	Total Biomass	N/sample
7/23/15	No Fish	9.7	333.7	343.4	3
9/2/15	No Fish	.	.	.	.
10/2/15	No Fish	26.7	74.9	101.7	3
7/23/15	Low Fish	21.3	598.5	619.9	3
9/2/15	Low Fish	21.1	180.7	201.8	3
10/2/15	Low Fish	12.6	307.7	320.3	3
7/23/15	High Fish	41.1	472.0	513.1	3
9/2/15	High Fish	22.0	624.1	646.1	3
10/2/15	High Fish	33.6	282.4	316.1	3
7/23/15	C1	71.2	598.5	669.7	3
9/2/15	C1	30.9	180.7	211.6	3
10/2/15	C1	36.6	307.7	344.3	3
7/23/15	C2	33.2	598.5	631.7	3
9/2/15	C2	3.5	180.7	184.2	3
10/2/15	C2	16.2	307.7	323.9	3

Cedar	Treat	MSPI	Native	Total Biomass	Taxa
7/30/15	No fish	1132.4	507.8	1640.2	3
9/4/15	No fish	141.4	125.4	266.8	3
10/2/15	No fish	161.9	106.1	267.9	2
7/30/15	Low Fish	989.3	582.7	1572.0	2
9/4/15	Low Fish	217.4	148.7	366.1	2
10/2/15	Low Fish	111.9	110.4	222.3	3
7/30/15	High Fish	695.3	632.6	1327.9	2
9/4/15	High Fish	90.7	280.4	371.1	3
10/2/15	High Fish	200.4	233.4	433.8	2
7/30/15	Control 1	1765.7	3580.9	5346.6	4.0
9/4/15	Control 1	190.8	486.8	677.6	3
10/2/15	Control 1	143.3	174.8	318.1	3
7/30/15	Control 2	928.2	643.8	1572.0	3
9/4/15	Control 2	372.4	216.8	589.3	3
10/2/15	Control 2	251.0	255.2	506.2	3

Table 2. continued.

Peltier	Treatment	MSPI	NATIVE	TOTAL BIOMASS	TAXA/SAMPLE
6/29/16	No Fish	13.6	16.2	37.8	4
7/27/16	No Fish	2.1	1288.7	1327.5	4.8
8/24/16	No Fish	10.4	561.9	589.0	4.8
6/29/16	Low Fish	47.5	259.2	336.9	5.2
7/27/16	Low Fish	45.8	395.5	456.7	5.6
8/24/16	Low Fish	58.2	306.8	366.3	4.8
6/29/16	High Fish	2.8	220.3	244.9	5.4
7/27/16	High Fish	8.2	490.2	523.5	4.8
8/24/16	High Fish	3.0	273.2	279.5	3.2
6/29/16	Control 1	40.2	75.9	120.5	5
7/27/16	Control 1	2.4	205.5	211.5	3.2
8/24/16	Control 1	13.1	505.8	420.2	4.25
6/29/16	Control 2	0.0	75.9	94.4	4.2
7/27/16	Control 2	0.0	203.9	209.3	3.2
8/24/16	Control2	0.0	185.5	188.0	1.8

Cedar	Treatment	MSPI	Natives	Total Biomass	Taxa/Sample
6/30/16	No Fish	120.4	299.0	419.9	2.6
7/26/16	No Fish	42.9	196.9	240.1	2.8
8/26/16	No Fish	53.9	652.3	706.2	3
6/30/16	Low Fish	411.7	281.7	698.2	4.4
7/26/16	Low Fish	258.8	220.0	480.0	4
8/26/16	Low Fish	289.3	363.0	652.3	3.8
6/30/16	High Fish	214.0	235.0	453.6	2.8
7/26/16	High Fish	5.9	182.3	188.9	1.8
8/26/16	High Fish	11.3	471.0	482.4	2.6
6/30/16	Control 1	132.4	139.1	272.8	4
7/26/16	Control 1	34.5	66.9	101.9	2.4
8/26/16	Control 1	207.3	429.4	637.1	2.8
6/30/16	Control2	585.2	323.6	917.9	3.4
7/26/16	Control2	53.4	111.7	165.7	1.6
8/26/16	Control2	88.1	436.4	524.7	2.4

Table 3. Milfoil weevil densities (total of all life stages/stem) in enclosures (fish density, none, low or high) and control plots (C1 and C2) in 2015 and 2016 at Peltier and Cedar Lakes.

	Weevils (total/stem)			
Peltier	7/17/15	8/24/15	9/14/15	10/3/15
None	0.606	0.043	0.068	0.063
Low	0.194	0.048	0.043	0.000
High	0.533	0.086	0.000	0.000
C1	0.421	0.083	0.000	0.000
C2	0.265	0.042	0.109	0.000
Cedar	7/30/15	8/26/15	9/15/15	10/3/15
None	0.091	0.596	0.000	0.000
Low	0.000	0.674	0.426	0.103
High	0.000	0.271	0.128	0.143
C1	0.043	0.022	0.022	0.000
C2	0.040	0.167	0.000	0.024
Peltier	6/29/16	7/20/16	8/24/16	
None	0.146	0.417	0.000	
Low	0.208	1.039	0.339	
High	0.033	0.224	0.000	
C1	0.361	0.707	0.000	
C2	0.049	.	0.000	
Cedar	6/30/16	7/19/16	8/26/16	
None	0	0	0	
Low	0	0	0.021	
High	0	0	0	
C1	0	0	0	
C2	0	0	0	

Despite multiple efforts with traps, angling and trot lines, starting at the midpoint of each experiment as well as the end, we were not able to retrieve any of the stocked fish from the enclosures. Snorkeling observations (though limited by the poor clarity) also failed to reveal fish large enough to have been stocked. Observations in 2016 lead us to suspect that herons, which would perch on the floating rims of the enclosures, consumed many if not all of the stocked fish. Thus it is likely that we did not sustain a differential fish density and predation pressure which would also explain the lack of differences in weevil density or milfoil or plant biomass. The declining and low milfoil biomass in Peltier enclosures in 2016 could be due to the high abundance of weevils in July but the disappearance of weevils in August is puzzling. Similarly, the general decline of milfoil in Cedar enclosures in 2015 could be related to the high density of weevils found at mid-experiment, but differences among enclosures do not appear related to weevil density.

Conducting good enclosure experiments is a challenge; it is difficult to find sites with high milfoil biomass that include native plants and that are similar across locations. For example, in Peltier the sites we used in 2015 had almost no milfoil in 2016 so sites on the other side of the lake needed to be used. Year to year differences in water clarity and changes in clarity can also be important and the poor clarity in Peltier and in 2016 in Cedar likely affected plants as well as inhibited our ability to monitor the fish populations. If heron predation is a factor, ways to prevent predation need to be devised. Mesh covers pose their own problems. For future experiments, sites in lakes with better clarity may be more suitable and an even earlier start of the experiment may be good. Alternatively, it may be more effective to conduct these experiments in artificial or natural ponds or in very large (>25m^2) deep (≥1.5m) tanks.

Field Surveys

Milfoil weevils were found in 12 of the 19 lakes surveyed (Tables 4 and 5). Aquatic lepidopterans were found in 8 lakes though never as abundant as milfoil weevils. As is typical, weevil eggs were most common, followed by larvae and adults. Weevil abundance was generally higher in 2016 than 2015 and weevils were not found in several lakes in 2016 where they had been present in 2015. Highest densities (0.3-0.8/stem) were found in Centerville, Peltier, and the bays of Lake Minnetonka. Weevils were relatively abundant in Auburn and Susan in early 2015 but were not found in surveys in later 2016. Densities both years, but particularly in 2016, were lower than in years past and many previous studies (Newman 2004) and no lakes attained a density of 0.5/stem or sustained a density ≥ 0.25/stem throughout the summer.

Total weevil density was negatively related to sunfish density (sunfish per trapnet set; Fig. 1) with a correlation of -0.44, a marginally significant correlation ($p = 0.066$ for 1 tailed test). It is clear that few weevils are found in lakes with sunfish densities greater than 70 sunfish per trapnet but there are also lakes with no or few weevils despite a low sunfish catch per trapnet (<20/net). At high sunfish densities, weevils may be limited by sunfish predation if other factors are not limiting but other factors may be limiting weevils in some lakes that have low sunfish densities. Currently, it is not clear what those factors may be, but they could include overwinter habitat, water temperature, harvesting or herbicidal control. Both mechanical harvesting (Newman and Inglis 2009) and herbicidal control (Knight and Havel 2016) have been shown to limit weevil populations.

To determine the degree of predation on milfoil weevils by sunfish we examined the stomachs of over 450 sunfish from 10 lakes (Table 6). We found 1 adult milfoil weevil in these samples (Peltier 2016). Although some samples were from open water and contained primarily zooplankton (Table 6) many stomachs contained snails, amphipods and chironomids that are typically associated with plants. This is a much lower occurrence of milfoil weevils than found by Sutter and Newman (1997), but may in part be explained by the relatively low densities of weevils we encountered during our weevil surveys. If weevils are rare they will not likely be found in the diet. It is possible that sampling earlier in the season would reveal more predation but Sutter and Newman found equally high rates in August compared to June and July.

Table 4. Weevil and lepidopteran density (N/stem and 2SE) of all life stages in surveys in 2015. Number of samples is given beneath the lake name.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Lepidopt
Auburn	6/2/15	0.048	0.012	0	0.011	0.071	0
27	2SE	0.040	0.013	0	0.013	0.055	0
Auburn	8/31/15	0	0	0	0	0	0
27	2SE	0	0	0	0	0	0
Cedar	6/11/15	0	0	0	0	0	0
30	2SE	0	0	0	0	0	0
Cenaiko	6/25/15	0	0	0	0	0	0
26	2SE	0	0	0	0	0	0
Cenaiko	8/20/15	0	0	0	0	0	0
26	2SE	0	0	0	0	0	0
Centerville	7/15/15	0.150	0.030	0.008	0.119	0.307	0
24	2SE	0.213	0.029	0.017	0.071	0.225	0
Christmas	6/15/15	0.015	0.008	0	0.006	0.029	0
46	2SE	0.017	0.009	0	0.009	0.020	0
Christmas	8/11/15	0.024	0	0.003	0.050	0.076	0.003
50	2SE	0.022	0	0.005	0.031	0.041	0.005
Mitchell	6/8/15	0	0	0	0	0	0
31	2SE	0	0	0	0	0	0
Mitchell	7/20/15	0	0	0	0	0	0
28	2SE	0	0	0	0	0	0
Mitchell	8/21/15	0	0	0	0	0	0
28	2SE	0	0	0	0	0	0
Otter	7/20/15	0.179	0.004	0	0.031	0.213	0.016
27	2SE	0.123	0.007	0	0.033	0.135	0.015
Peltier	6/23/15	0.060	0.004	0	0.087	0.151	0.004
30	2SE	0.064	0.008	0	0.058	0.078	0.008
Rebecca	6/19/15	0	0	0	0	0	0
30	2SE	0	0	0	0	0	0
Riley	6/1/15	0.061	0.018	0	0.009	0.088	0.076
36	2SE	0.055	0.017	0	0.012	0.062	0.146
Riley	7/29/15	0.079	0.004	0	0.031	0.115	0
28	2SE	0.074	0.009	0	0.024	0.094	0
Riley	8/31/15	0.149	0.093	0.005	0.026	0.273	0.003
30	2SE	0.148	0.069	0.012	0.031	0.222	0.007
Smith's Bay	6/29/15	0	0.011	0	0.011	0.022	0
39	2SE	0	0.013	0	0.018	0.024	0
Smith's Bay	8/17/15	0.025	0.004	0.009	0.034	0.071	0
32	2SE	0.025	0.008	0.013	0.021	0.047	0
Steiger	6/9/15	0	0	0	0	0	0
27	2SE	0	0	0	0	0	0
Susan	6/3/15	0.003	0.004	0	0	0.007	0
27	2SE	0.005	0.008	0	0	0.010	0
Susan	7/30/15	0.102	0	0	0.004	0.106	0
29	2SE	0.091	0	0	0.009	0.091	0
Susan	9/2/15	0	0.005	0	0.010	0.010	0
26	2SE	0	0.010	0	0.013	0.019	0
Vet's Bay	7/21/15	0.154	0.033	0.006	0.032	0.224	0
35	2SE	0.098	0.035	0.011	0.022	0.116	0
Vet's Bay	8/25/15	0.058	0	0	0.033	0.091	0
35	2SE	0.061	0	0	0.028	0.073	0

Table 5. Weevil and lepidopteran density (N/stem and 2SE) of all life stages in surveys in 2016. Number of samples is given beneath the lake name.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Lepidopt
Auburn	6/7/16	0	0	0	0	0	0
30	2 SE	0	0	0	0	0	0
Auburn	7/18/16	0	0	0	0	0	0
33	2 SE	0	0	0	0	0	0
Cedar	6/1/16	0	0	0	0	0	0
32	2 SE	0	0	0	0	0	0
Cedar	8/16/16	0	0	0	0	0	0
31	2 SE	0	0	0	0	0	0
Cenaiko	6/7/16	0	0	0	0	0	0
26	2 SE	0	0	0	0	0	0
Cenaiko	7/25/16	0	0	0	0	0	0
26	2 SE	0	0	0	0	0	0
Centerville	6/8/16	0.006	0.005	0	0	0.011	0
25	2 SE	0.011	0.010	0	0	0.015	0
Centerville	7/21/16	0.074	0	0	0.004	0.078	0.010
25	2 SE	0.082	0	0	0.008	0.083	0.014
Christmas	7/6/16	0.003	0.016	0.006	0.013	0.038	0
47	2 SE	0.006	0.014	0.008	0.011	0.023	0
Christmas	7/28/16	0.024	0	0	0.003	0.027	0
53	2 SE	0.025	0	0	0.005	0.027	0
Christmas	8/22/16	0.020	0	0	0.035	0.055	0
48	2 SE	0.022	0	0	0.045	0.055	0
Firemen's	8/24/16	0	0	0	0	0	0
28	2 SE	0	0	0	0	0	0
Mitchell	6/14/16	0	0	0	0	0	0
21	2 SE	0	0	0	0	0	0
Mitchell	7/13/16	0	0	0	0	0	0
22	2 SE	0	0	0	0	0	0
Mitchell	8/17/16	0	0	0	0	0	0
8	2 SE	0	0	0	0	0	0
Otter	6/2/16	0.024	0.004	0.004	0	0.032	0
33	2 SE	0.021	0.008	0.009	0	0.023	0
Otter	7/12/16	0.008	0.013	0	0	0.021	0
32	2 SE	0.016	0.015	0	0	0.021	0
Otter	8/15/16	0.004	0.022	0	0.005	0.031	0
31	2 SE	0.008	0.037	0	0.009	0.039	0

Table 5
Continued

Peltier	5/26/16	0.101	0.150	0.021	0	0.273	0
30	2 SE	0.076	0.074	0.024	0	0.105	0
Peltier	6/27/16	0.042	0.031	0.013	0.043	0.128	0
30	2 SE	0.083	0.036	0.018	0.038	0.123	0
Peltier	8/18/16	0.099	0	0	0.004	0.104	0
28	2 SE	0.122	0	0	0.009	0.124	0
Piersons	8/2/16	0.025	0	0	0	0.025	0
32	2 SE	0.025	0	0	0	0.025	0
Riley	6/1/16	0.051	0	0	0	0.051	0
36	2 SE	0.102	0	0	0	0.102	0
Riley	7/26/16	0.063	0.034	0	0.011	0.107	0
30	2 SE	0.058	0.027	0	0.015	0.069	0
Riley	8/22/16	0.020	0	0	0	0.020	0
25	2 SE	0.024	0	0	0	0.024	0
Round	7/28/16	0.051	0.005	0	0.017	0.073	0.004
31	2 SE	0.056	0.011	0	0.020	0.061	0.008
Schmidt	8/15/16	0	0	0	0	0	0
30	2 SE	0	0	0	0	0	0
Smith Bay	7/14/16	0.102	0.006	0	0.035	0.143	0
44	2 SE	0.096	0.008	0	0.046	0.108	0
Steiger	7/25/16	0	0	0	0	0	0.005
27	2 SE	0	0	0	0	0	0.009
Susan	6/1/16	0.003	0.005	0	0	0.008	0
23	2 SE	0.006	0.010	0	0	0.011	0
Vet's Bay	7/21/16	0.185	0.012	0.002	0.009	0.209	0.003
42	2 SE	0.099	0.019	0.005	0.010	0.103	0.006
Zumbra	8/4/16	0	0	0	0	0	0
32	2 SE	0	0	0	0	0	0

Table 6. Fish sampled for stomach contents in 2015 and 2016 and dominant prey taxa for each sampling session. Only 1 milfoil weevil was found; an adult weevil in Lake Peltier in 2016.

Lake	Date	Bluegill	Pumpkinseed	DominantTaxa
Auburn	8/31/15	25	0	Zooplankton
	9/15/15	19	0	Zooplankton
	8/4/16	50	0	Amphipods
Cedar	8/4/15	29	0	Aquatic Diptera
	8/10/15	26	0	Aquatic Diptera
	7/11/16	2	3	Snails
	7/12/16	25	0	Snails
Centerville	8/11/15	26	1	Aquatic Diptera
	9/1/15	13	12	Aquatic Diptera
	8/1/16	9	4	Chironomids
Christmas	8/23/15	3	9	Snails and insects
	9/14/15	7	5	Snails and insects
	8/18/16	7	4	Chironomids
Otter	8/12/15	2	25	Snails and insects
	9/3/15	0	27	Snails and insects
	8/9/16	0	4	Chironomids
	8/17/16	5	7	Chironomids
Peltier	8/3/15	23	3	Zooplankton
	8/5/15	27	3	Zooplankton
	7/5/16	1	4	Chironomids
	7/8/16	24	0	Chironomids
Piersons	8/2/16	45	4	Amphipods
Round	8/9/16	20	0	Zooplankton
Steiger	8/3/16	49	1	Chironomids
Zumbra	8/3/16	44	6	Amphipods

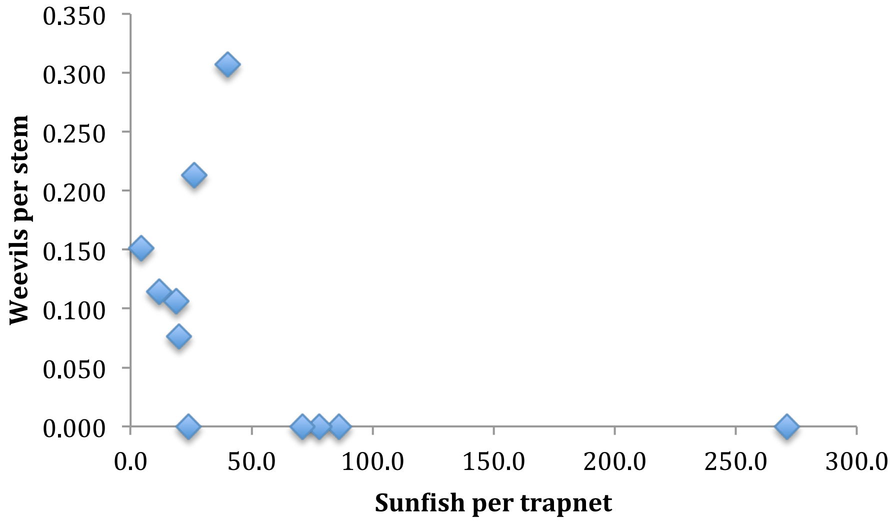

Figure 1. Relationship between number of weevils per stem (total of all life stages) and sunfish catch in survey lakes. R = -0.44

Conclusions

Lakes with high sunfish populations will likely not support sufficient herbivore populations to control milfoil and biological control should not be promoted in these lakes until sunfish are reduced. However, some lakes with low sunfish populations also have low herbivore densities and factors other than sunfish are apparently limiting herbivores and biocontrol in these lakes. Possible limiting factors include lack of access to shoreline overwinter habitat (Jester et al. 2000, Thorstenson et al. 2013), extensive mechanical harvesting (Newman and Inglis 2009) or herbicidal control (Havel et al. 2017, *in review*), and poor water or plant quality (Miller et al. 2011, Marko and Newman *in press*). These results indicate that more work is needed to assess factors limiting milfoil weevil populations. The relative importance of these factors is unknown and work that also accounts for year to environmental variability is needed to determine the importance of factors limiting milfoil herbivores and biocontrol.

Longer term data sets will be needed to help identify these factors. We will conduct a broader analysis of the data from this project in combination with previous data from 2011-2014 and a series from 1994-2004 to see if we can detect a climate or environmental signal or identify other factors that might explain variation in milfoil weevil abundance.

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