

Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores

Basic Information

Title:	Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores
Project Number:	2014CT286B
Start Date:	3/1/2014
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	CT-4
Research Category:	Biological Sciences
Focus Category:	Invasive Species, Ecology, Water Quality
Descriptors:	None
Principal Investigators:	LaTina Steele, Michele Guidone

Publications

1. Steele LaTina, Michelle Guidone. 2015. Using Native Herbivores to Mitigate Eurasian Watermilfoil Invasion in New England. Benthic Ecology Meeting 2015. Quebec City, Canada. Oral Presentation.
2. *Ray, Courtney; Jessica Michaud; LaTina Steele; Michelle Guidone. 2015. The Use of Native Herbivores as a Mitigation Strategy for Eurasian Watermilfoil (*Myriophyllum spicatum*) in Connecticut Lakes. Connecticut Association of Wetland Scientists Annual Meeting. Southbury, CT. Poster Presentation.
3. Ray, Courtney; LaTina Steele; Michelle Guidone. [in preparation]. Use of Native Aquatic Herbivores as Biocontrol Agents for Eurasian Watermilfoil (*Myriophyllum spicatum*) in New England. *Oecologia*.
4. Steele LaTina, Michelle Guidone. 2015. Using Native Herbivores to Mitigate Eurasian Watermilfoil Invasion in New England. Benthic Ecology Meeting 2015. Quebec City, Canada. Oral Presentation.
5. *Ray, Courtney; Jessica Michaud; LaTina Steele; Michelle Guidone. 2015. The Use of Native Herbivores as a Mitigation Strategy for Eurasian Watermilfoil (*Myriophyllum spicatum*) in Connecticut Lakes. Connecticut Association of Wetland Scientists Annual Meeting. Southbury, CT. Poster Presentation.
6. Ray, Courtney; LaTina Steele; Michelle Guidone. [in preparation]. Use of Native Aquatic Herbivores as Biocontrol Agents for Eurasian Watermilfoil (*Myriophyllum spicatum*) in New England. *Oecologia*.

Proposal Title: *Mitigating Eurasian Watermilfoil Invasion Success and Ecosystem Impact Using Native Herbivores*

Final Report, FY 2014-2015

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Introduction & Research Objectives

Eurasian watermilfoil has invaded lakes across the state of Connecticut, often becoming dominant within these submerged aquatic vegetation communities. Factors contributing to milfoil's invasion success are poorly understood and are limited mainly to nutrient conditions and the broad assertion that invasion is less likely in lakes with an established submerged macrophyte community (Smith and Barko 1990, Madsen 1998). However, evidence suggests that allelopathic interactions between milfoil and epiphytic algae may contribute to milfoil's establishment (Gross et al. 1996). These allelopathic phenolic compounds produced by *M. spicatum* are also well-known feeding deterrents in terrestrial, aquatic, and marine plants (Constabel 1999). Thus, it seems reasonable that chemical interactions may reduce milfoil herbivory and play a role in its invasion success. Increased understanding of factors leading to successful milfoil invasions is critical for effective management and prevention of milfoil invasion, highlighting the importance of studies like the one proposed here. Managers, policy-makers, and those who use our state's lakes for recreational purposes will all benefit from this study.

Common techniques for eradicating nuisance milfoil involve costly and harmful chemical application and physical removal of milfoil. Such measures often need to be repeated in order to be effective and inherently affect other members of the lake community (e.g., DeLong and Mundahl 1996). Furthermore, physical removal of milfoil could increase its spread to other areas, since it is propagated via fragmentation (Maezo et al. 2010). Mitigation of *M. spicatum* using native herbivores is a much more palatable alternative to many common eradication measures.

Many studies have investigated the potential of a North American weevil to mitigate Eurasian watermilfoil impacts (e.g., Sheldon and Creed 1995). However, few have considered additional herbivores native to particular regions or the impacts of community composition (i.e., the identity and abundance of herbivores, predators, and algal species) that can also influence invasion success. For example, herbivorous snails may either directly or indirectly affect milfoil populations, as some gastropods feed on *M. spicatum* (Boland et al. 2008), while others positively impact milfoil growth by limiting the growth of algal competitors (Chase and Knight 2006). Predator identity and abundance is also vital to our understanding of milfoil success. In lakes where predators are abundant, herbivore populations may be suppressed to levels that inhibit their control of milfoil growth (Ward and Newman 2006). This last point is particularly important for making informed mitigation choices, as there are a number of predatory fish species that are commonly stocked for recreational fishing.

Most studies proposing herbivory as a milfoil control measure have been conducted in the Midwest or the southeast United States. Few have been conducted in New England, and none of those have considered the use of multiple native herbivores to mitigate milfoil impacts. Nor have those studies considered the role of chemical deterrents in determining when and where milfoil will invade, despite evidence that *M. spicatum* produces many allelopathic chemicals

(Gross et al. 1996, Spencer and Ksander 1999), which commonly contribute to plant invasion success (Callaway and Ridenour 2004). Connecticut lakes are home to many potential herbivores, including crustaceans (amphipods and crayfish), insect larvae, gastropods, and herbivorous fishes. Previous studies in other regions suggest that crayfish (Parker and Hay 2005, Maezo et al. 2010) and insect larvae (Johnson et al. 1998) are milfoil consumers, with some insects leading to shifts in community structure from milfoil-dominated systems to dominance by *Elodea canadensis* (Gross et al. 2001).

The objectives of the project were to 1) investigate the role of chemical interactions between plants and herbivores in determining milfoil invasion success, 2) identify native consumers with the potential to successfully mitigate milfoil invasions, and 3) measure the effects of milfoil invasion on community structure by comparing community composition and diversity between Eurasian watermilfoil and native aquatic plants.

Methods & Progress

Field Sampling

During summer 2014, five throw trap samples were collected in milfoil-dominated areas of Osbourndale Pond in Derby, Connecticut and another five throw trap samples were collected from *Elodea*-dominated areas of the same pond. All animals within each trap sample were identified to the lowest possible taxon and enumerated. Primer-E software was used to conduct Analysis of Similarity (ANOSIM) on a Bray-Curtis similarity matrix constructed using abundance of all taxa per m² to determine if community composition differed in milfoil and *Elodea* dominated areas. Results were considered significant at $p < 0.05$.

Upon identifying an appropriate reference pond without Eurasian watermilfoil (Colony Pond, Ansonia, CT), an additional five throw trap samples were collected from each of three areas: 1) milfoil-dominated areas of Osbourndale Pond, 2) *Elodea*-dominated areas of Osbourndale Pond, and 3) Colony Pond, where milfoil is not present. All plants within these traps were identified and the wet weight was recorded. Animals from these trap samples were preserved in 10% formalin for two weeks, then rinsed and stored in 70% isopropyl alcohol. Processing of these preserved samples is currently underway. Rose Bengal stain is being added to each sample prior to identifying and counting all animals within the sample.

Samples of Eurasian watermilfoil and three native aquatic plant species (*Elodea canadensis*, *Ceratophyllum demersum*, and *Potamogeton berchtoldii*) were collected during the summer in order to compare the chemical deterrent content of invasive milfoil and the native plant species. These samples were rinsed, placed in sample vials, flash frozen in liquid nitrogen, and stored at -80°C prior to freeze drying. Samples were ground to a fine powder in liquid nitrogen in their tubes and returned to the -80°C freezer until chemical analysis was performed.

Eurasian watermilfoil was sampled in the early morning hours (before sunrise) and in the late afternoon (just before sunset) to assess diurnal differences in chemical deterrent production; chemical analyses on these samples have not yet been conducted. The vacuum pump on the freeze dryer at Sacred Heart University is currently being replaced. When the pump has been replaced, samples will be dried, and chemical analyses will be performed.

Field Experiments

Diurnal differences in milfoil and *Elodea* consumption in the field were examined using tethering experiments. Five tether lines consisting of two feet of sisal rope with six pre-weighed milfoil fragments each and another five tether lines with six pre-weighed *Elodea* fragments each were deployed at approximately 08:00. Tethers were collected after 36 hours. The first 24 hours allowed time for animals to colonize the tethered fragments, and the following 12 hours allowed time for herbivores to feed on the plants during the day. These tethering methods were then repeated, deploying the tethers at approximately 19:00, with 24 hours to allow animals to colonize the tethered plants and another 12 hours to allow for additional feeding during the night. All plants were weighed a second time after tethers were collected from the field, and the change in weight (taking into account both consumption and growth) was calculated. Because the change in weight data were not normally distributed and did not meet the assumption of equal variance, a two-way ANOVA could not be used to determine if there were differences in consumption between the two plant species during the day and at night. Instead, two Mann-Whitney U tests were performed to determine if there were differences in consumption during the day and at night, with one test run on the milfoil data and a second test run on the *Elodea* data. Results were considered significant at $p < 0.025$ to account for multiple tests.

Laboratory Experiments

A series of four separate laboratory experiments were conducted to quantify milfoil consumption by the following native herbivores: amphipods (*Hyaella azteca*), snails (*Physella* sp.), mayfly larvae (*Caenis* sp.), and milfoil weevils (*Euhrychiopsis lecontei*). Choice feeding experiments were used to test the palatability of invasive milfoil and native *E. canadensis* to *H. azteca*, *E. lecontei* and *Physella* sp. T-tests and Mann-Whitney U tests were used, as appropriate, to determine if there were differences between control treatments (milfoil only) and herbivore treatments (milfoil + one herbivore species). Paired t-tests were used to determine if amphipods, weevils, and snails consumed different quantities of milfoil and *Elodea* in choice feeding experiments, since the data from all choice experiments were normally distributed and had homogenous variances. Results were considered significant at $p < 0.05$ in all cases.

Chemical Analyses

A simple colorimetric assay, the Folin-Denis assay (cf. Steele et al. 2005), was used to quantify total reactive phenolics in freeze-dried and ground samples of milfoil, *E. canadensis*, and two additional native plant species, *Potamogeton berchtoldii* and *Ceratophyllum demersum*. A one-way ANOVA was used to determine if there were differences in phenolic concentrations among plant species, since the phenolic data were normally distributed and variances among groups were equal. A post-hoc Tukey test was used to identify which plants had significantly different phenolic concentrations from each other. Results were considered significant at $p < 0.05$.

Results and Discussion

Results from the throw trap sampling suggest that in Osbourndale Pond, invasive milfoil has not had a detrimental impact on the lake community (Figure 1). A similar suite of animals seems to take up shelter in both milfoil and the native *Elodea*, which are the two dominant aquatic plants in Osbourndale Pond. In areas such as this where milfoil has not caused a noticeable effect on the consumer community, costly and ecologically harmful removal methods for the invasive plant may not be necessary. Likewise, the particular herbivore community found at this site may be acting to prevent milfoil from overgrowing the area. Additional experiments will be performed during summer 2015 (Year 2 of the project) to help address this question. However, the information gained at this one site may still be useful in determining lake characteristics that, when absent, may lead to greater effects of milfoil invasion (e.g., lack of plant competitors, lack of amphipods and snails).

The field tethering experiment suggests that herbivores may be more active during the night than during the day, though the difference in biomass reduction at night compared to during the day was only significant in *Elodea* and not milfoil (Figure 2). Plant samples were collected to determine if chemical deterrent production in milfoil and *Elodea* changes during the night and during the day to match times of greatest herbivore activity. These samples have not yet been analyzed.

Data obtained from the feeding experiments suggest that locally abundant native herbivores like the amphipod *Hyaella azteca* and the snail *Physella* sp. may be effective in controlling milfoil biomass and in mitigating its effects (Figure 3). Data from feeding preference tests are consistent with those results, since they showed that amphipods will still consume milfoil, even in the presence of other, less chemically defended plant species (Figures 4 & 5). Likewise, snails also consumed milfoil in the presence of alternative plant prey, showing no preference for either milfoil or native *Elodea canadensis* (paired t-test, $t = 0.29$, $p = 0.78$, $n=10$).

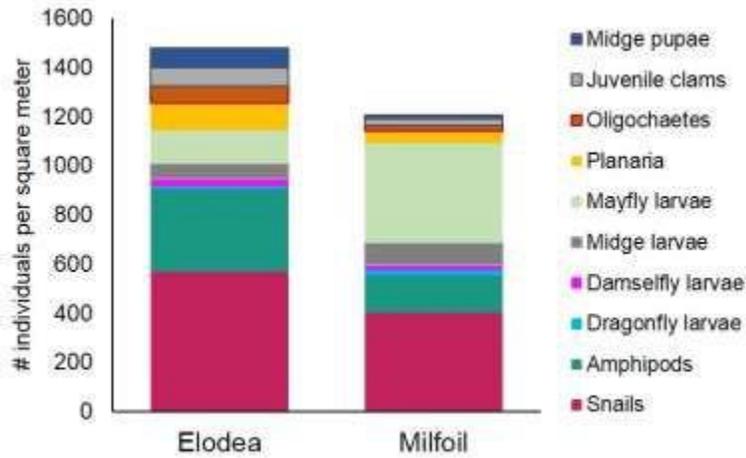


Figure 1. Most abundant taxa in throw trap samples (# individuals/m²) collected in *Elodea*- and milfoil-dominated areas of Osbourndale Pond (Derby, CT). Analysis of similarity (ANOSIM) detected no significant differences in community composition between habitats (Global R = 0.036, p = 0.333).

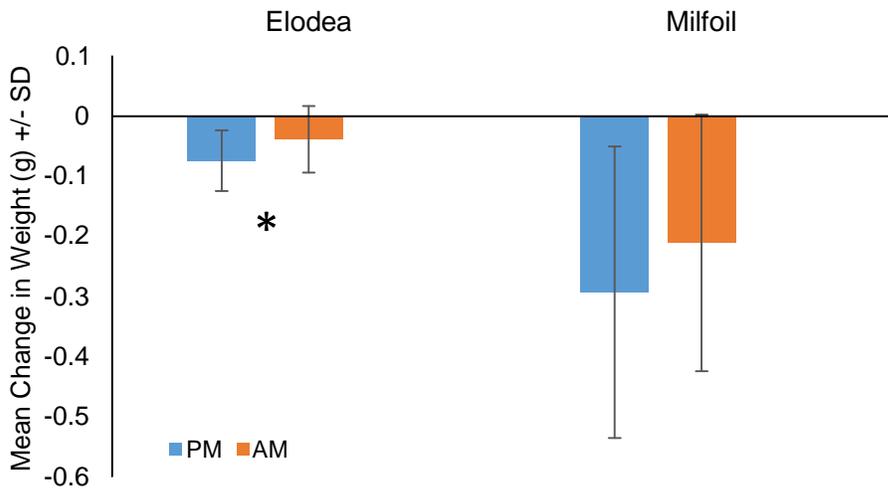


Figure 2. Mean change in weight of *Elodea canadensis* and Eurasian watermilfoil (*Myriophyllum spicatum*) after being deployed in the field in the morning (AM) or evening (PM) and remaining in the field for 36 hours. Mann-Whitney U tests indicated that significantly more *E. canadensis* was lost during the night than during the day (U = 159, p = 0.001), while there was not a significant difference in milfoil loss during the night and day (U = 315, p = 0.146). Asterisk indicates a significant difference in weight change between AM and PM.

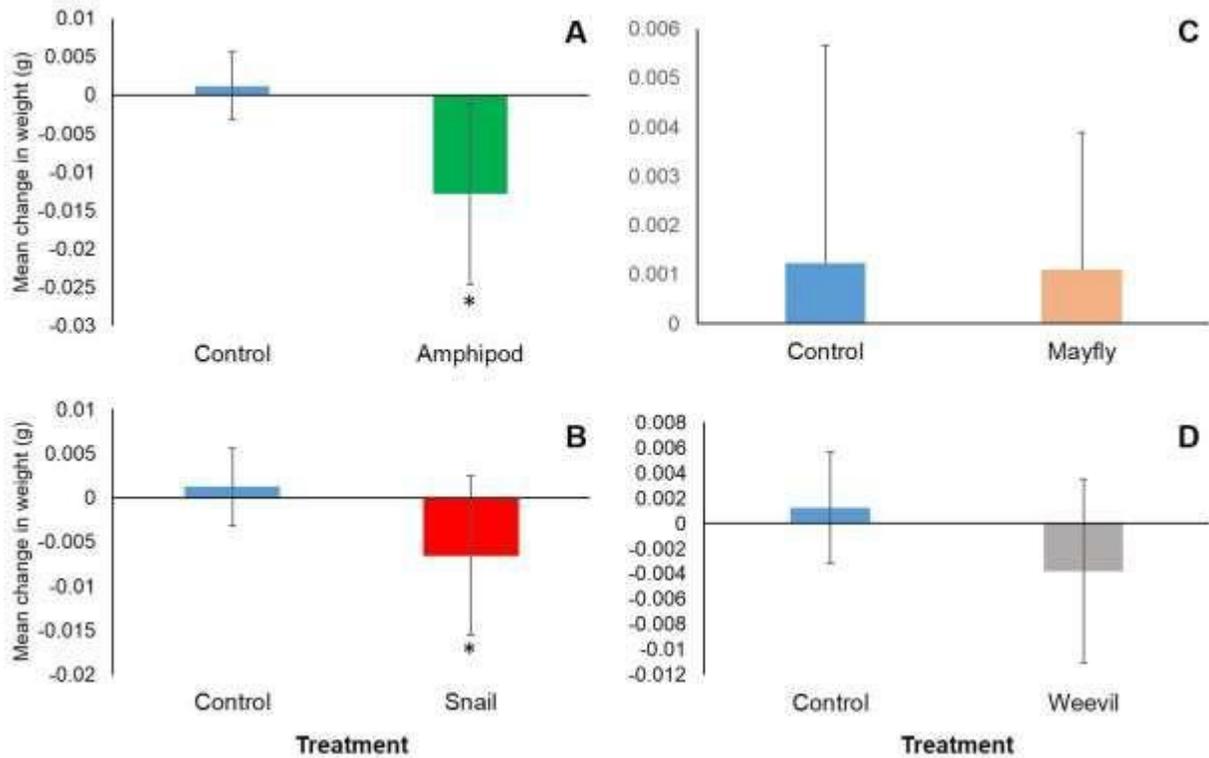


Figure 3. Change in *Myriophyllum spicatum* weight (g) after one week alone (controls) and following feeding by A) 10 individuals of the amphipod *Hyaella azteca* (Mann-Whitney test $W = 61.0$, $df = 13$, $p = 0.0010$, $n = 10$), B) 4 Physidae snail individuals (t-test $t = 2.45$, $df = 13$, $p = 0.029$, $n = 10$), C) 4 mayfly larvae (t-test $t = 0.08$, $df = 15$, $p = 0.938$, $n = 10$), and D) one milfoil weevil individual (t-test $t = 1.72$, $df = 11$, $p = 0.114$, $n = 8$). Each panel represents one experiment. Asterisk next to the error bar indicates a significant difference from the control.

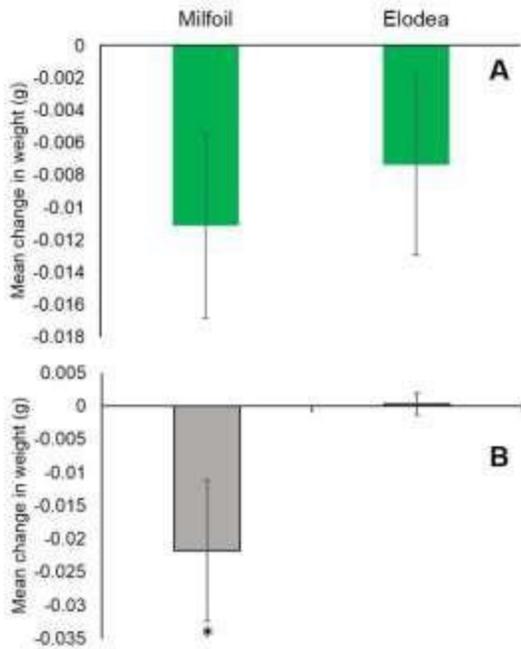


Figure 4. Mean change in weight of *Myriophyllum spicatum* and *Elodea canadensis* following one week of feeding by A) the amphipod *Hyalella azteca* (paired t-test $t = -1.93$, $p = 0.086$, $n = 10$) and B) the weevil *Euhyrchiopsis lecontei* (paired t-test $t = -6.485$, $p < 0.001$, $n = 10$) in choice experiments. Asterisk next to error bar indicates a significant difference between treatments.

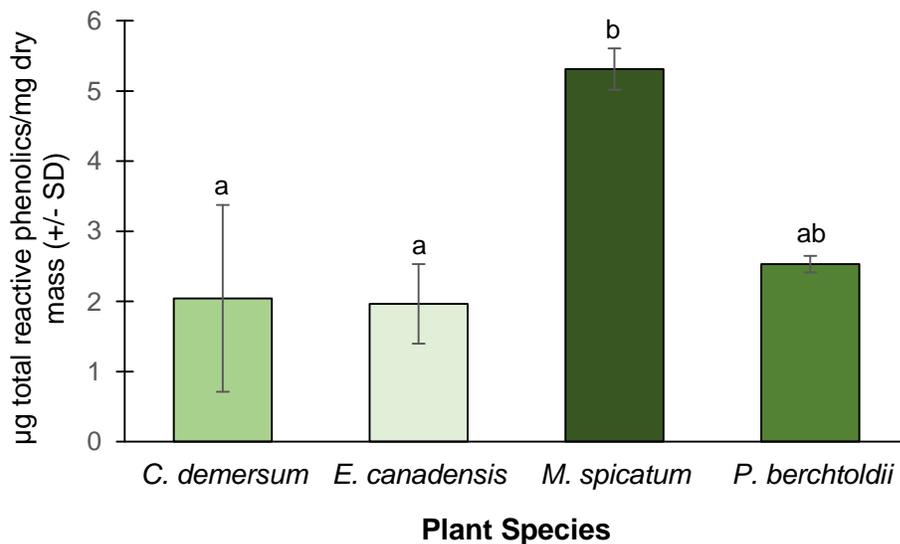


Figure 5. Total reactive phenolic content (µg phenolics/mg dry mass ± standard deviation) of four aquatic plant species: *Ceratophyllum demersum*, *Elodea canadensis*, *Myriophyllum spicatum*, and *Potamogeton berchtoldii*. Different letters over the error bars indicate significant differences among species (ANOVA $F_{1,17} = 4.953$, $p = 0.012$).

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