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The effects of predation on biological control of Eurasian watermilfoil

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ABSTRACT

Eurasian watermilfoil (EWM), *Myriophyllum spicatum* L., is an invasive aquatic macrophyte in North America. The aquatic milfoil weevil, *Euhrychiopsis lecontei,* is a native herbivore on milfoils that has been used as a biological control agent for EWM. The objective of this study was to determine if predation by sunfishes (*Lepomis* spp.) can suppress milfoil weevil populations below the density necessary to control EWM. In Lake Joanis, Wisconsin, where supplemental milfoil weevil stocking had not led to an increase in weevil density, 944 L mesh exclusion cages stocked with milfoil weevils were used to manipulate densities of small bluegill (*Lepomis machrochirus*) to 0, 2, and 4 per cage. Results indicated an inverse relationship between bluegill and milfoil weevil densities. Mean densities of milfoil weevils and mean percent of EWM stems showing milfoil weevil damage were significantly different among treatments (ANOVA, $P = 0.005$, $P = 0.0004$). The average density of milfoil weevils in cages with no bluegill was 0.31 w s^{-1} (weevils per stem). Cages with two bluegill averaged 0.02 w s^{-1}. Cages with four bluegill averaged 0.01 w s^{-1}. Stem damage ranged from 60.4% in control cages to 13.3% in cages with four bluegill. These results indicate that sunfish, even at relatively low densities, substantially reduce milfoil weevil densities and their ability to damage EWM. Protecting stocked milfoil weevils in cages could allow them to establish higher densities where they can serve as a control for milfoil in some lakes. Variable success of milfoil weevils in controlling EWM in different lakes reported in the literature could be attributable to variable densities of sunfish populations.

Key words: biocontrol, *Euhrychiopsis lecontei*, *Lepomis macrochirus*, *Myriophyllum spicatum*.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum*, EWM), since its introduction to the United States in the 1940s, is one of the most problematic species of invasive aquatic plant (Couch and Nelson 1985). Eurasian watermilfoil can have significant impacts on native plant species composition. It often outcompetes native species because it grows quickly and begins growing earlier in the spring than most native plants (Aiken et al. 1979). Because it grows in a tangled, crowded canopy, it can inhibit water circulation and alter fish community composition by providing a refuge for prey fish (Diehl 1988). Eurasian watermilfoil can spread quickly because it has adventitious roots, produces seeds that can establish new plants, and spreads through fragmentation. Fragments that remain on boats and boat trailers can spread EWM to other lakes (Kimbel 1982).

Euhrychiopsis lecontei (Coleoptera:Curculionidae) (hereafter milfoil weevil) is a specialist herbivore on milfoils (*Myriophyllum* spp.) (Solarz and Newman 1996). It is native to North America but prefers EWM where it is present (Newman et al. 1997). Adult milfoil weevils live on the upper portion of the plant and eat the stems and leaves. The females lay eggs on the tips of EWM. The larvae burrow into the stem and eat vascular tissue (Newman et al. 1996). They pupate inside the stem, further blocking nutrient transport to the tips. The damage caused by immature milfoil weevils often causes EWM to lose buoyancy (Creed and Sheldon 1992). Although adults are fully aquatic, they overwinter on shore under leaf litter (Newman et al. 2001) and can be affected by shoreline disturbance.

It is generally accepted that when milfoil weevils are present in sufficient densities, they will control the impact of EWM (Creed and Sheldon 1992, 1993, 1995; Newman et al. 1996). The minimum density necessary for significant declines in EWM varies from 0.25 to 0.5 milfoil weevils per stem (w s^{-1}) (Newman 2004). Complete collapses of EWM beds associated with natural milfoil weevil densities of 3 to 4 w s^{-1} have been observed in Vermont (Creed and Sheldon 1995). Newman and Biesboer (2000) documented a decline of EWM in Cenaiko Lake in Minnesota due to a natural milfoil weevil density of about 1.6 w s^{-1}. In both cases, native macrophytes regrew in open areas after the decline of EWM. Long-term studies suggest that milfoil weevil density and milfoil density can follow a predator-prey cycle as well as a seasonal cycle (Lillie 2000).

Milfoil weevils are widely distributed across Wisconsin (Jester et al. 2000), but only reach densities above 0.5 w s^{-1} in a few lakes. Jester et al. stocked as many as 10,000 milfoil weevils into study plots to try to achieve densities of 1, 2, and 4 milfoil w s^{-1}. They found that 100% of plots stocked with 4 w s^{-1} and 60% with 1 w s^{-1} exhibited significant declines in EWM. The density of milfoil weevils stocked declined within 5 wk. Their study did not make use of enclosures, so the milfoil weevils might have simply moved out of the plots. The authors also speculated that sunfish predation could have been a factor, reducing weevil densities.

Several attempts at milfoil weevil stocking to control EWM in North America have been made. Reeves et al. (2008)

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looked at data from 30 lakes stocked with milfoil weevils in Michigan and Wisconsin. The results were highly variable and several lakes did not have detectable milfoil weevils at the end of the season. Thorstenson (2011), reared and then stocked 13,000 milfoil weevils in August 2008 and 9,000 in August 2009 into Lake Joanis, a 9.3 ha lake on the University of Wisconsin–Stevens Point campus that harbored nuisance levels of EWM. This stocking was estimated to be sufficient to bring the natural density of milfoil weevils from 0.01 to 0.03 w s^{-1} up to over 2 w s^{-1} and represented the highest stocking rate achieved per unit area of a whole-lake treatment reported in the literature. The post-stocking density in September, 2008 was 0.03 w s^{-1}. Milfoil weevils migrate to the shore in September and October (Newman et al. 2001), so it is possible that they had already left the lake. The density of milfoil weevils the following June, however, was 0.06 w s^{-1} and continued to fall over the summer. Because Lake Joanis is part of a protected reserve, its shoreline is almost 100% natural (Thorstenson 2011) and should provide excellent overwintering habitat for milfoil weevils (Newman et al. 2001). The density 23 d after stocking in August 2009 was 0.03 w s^{-1}. Subsequent surveys conducted through August 2015 indicated that milfoil weevils were present in the lake, but only at very low (< 0.03 w s^{-1}) densities.

Previous experiments with exclusion cages have concluded that fish predation can have a significant effect on milfoil weevil populations. Ward and Newman (2006) constructed PVC cages with plastic mesh to exclude or include sunfish in two lakes in Minnesota. One had naturally high milfoil weevil density and low sunfish density, the other high sunfish density and low weevil density. They stocked milfoil weevils and sunfish into the cages where necessary. There were initially five sunfish in half of the cages and none in the other half, but fluctuating water levels allowed fish in and out. The cages in this study were specifically designed to prevent this. It appears that sunfish suppressed milfoil weevil density in both situations. Parsons et al. (2011) used a similar exclosure to stock milfoil weevils in a Washington state lake. They also stocked milfoil weevils outside the exclosure. The only location to retain milfoil weevils after stocking was in an exclosure.

Skawinski (2014) reported on the relationship among a large number of water quality, geographic, and land use variables in relation to weevil density on 14 Wisconsin lakes over 2 yr. None of the water quality, geographic, or land use variables were found to be significantly correlated with milfoil weevil density. Coarseness of in-lake substrate was negatively correlated to milfoil weevil density and the only variable to show significant correlation.

The question as to why milfoil weevil populations remained at low densities in a lake with suitable shoreline habitat and dense beds of EWM was addressed in this study. The answer to this question is paramount for assessing the viability of milfoil weevils as a biocontrol agent of EWM. The objective of this study was to determine if bluegill (*Lepomis macrochirus*) predation had a significant impact on milfoil weevil abundance in predator exclusion cages in Lake Joanis, a 9.3 ha lake in central Wisconsin.

MATERIALS AND METHODS

Lake Joanis is a human-made lake built in 1976 on the University of Wisconsin–Stevens Point campus. It has a maximum depth of 7.6 m (25 ft) and a surface area of 9.3 ha (23 ac). It is a seepage lake with inputs from groundwater and precipitation (Portage County Lake Study 2005). The average secchi depth is 4.27 m (14 ft). It has a very large population of small sunfish that is almost entirely bluegill (*Lepomis macrochirus*). A single pass with a 2.6-m (8-ft) seine through one of the milfoil beds typically collect 60 to 80 bluegill. There are also small numbers of pumpkinseed (*Lepomis gibbosus*) and green sunfish (*Lepomis cyanellus*). The lake is surrounded by a nature reserve, thus its shoreline is mostly natural with no development. The EWM beds on Lake Joanis occur in 0.3 to 4.6 m (1 to 15 ft) of water. Because of its shallow depth and clear water, EWM occurred in approximately 90% of the lake at the time of the study.

In this study, conducted in 2014, the exclusion cages used consisted of perforated, galvanized angle iron bolted together to form a 0.91 m (3 ft) by 0.91 m by 1.22 m (4 ft) tall cube, with an open bottom. The approximate volume of water in the cages was 944 L (250 gal). At the bottom of the cage, additional pieces of angle iron were bolted upside down to secure the cages in the sediment over the EWM. A 3 mm (1/8 in) plastic aquaculture mesh was attached to the outside walls of the cage with zip ties. The mesh was also sewn on all sides with 13.6 kg (30 lb) test-braided fishing line. The lids of the cages were created by folding mesh and zip-tying it at the corners. They were fastened to the cages with custom-made bungee cords. Twenty-four exclusion cages were placed in Lake Joanis on 3 July. There were eight replications of three treatments. The number of bluegill in each treatment was 0, 2, or 4. Placement of the cages was dependent on water levels and accessibility. Because the number of milfoil weevils present at the time of placement was unknown, the cages were placed randomly within blocks composed of one of each replication to avoid a situation in which all replications of a treatment were grouped in the highest or lowest density of starting milfoil weevils. As the cages were placed on the milfoil beds, they were pushed firmly into the sediment. Cages were searched to remove any fish accidentally captured during placement.

The milfoil weevils stocked into the cages were collected from Springville Pond, a local impoundment with an abundance of milfoil weevils, on 4 June 2014 and bred in tanks to achieve the numbers required for stocking. From a kayak, EWM was hand-pulled and placed in 3.8-L (1-gal) freezer bags for transport to the lab in an uncooled cooler. The milfoil was searched immediately and all milfoil weevils at every life stage were retained on the stems where they were found. Any macroinvertabrate predators on the milfoil were removed. Milfoil weevils were raised in 37.8-L (10-gal) tanks in the University of Wisconsin–Stevens Point greenhouse for stocking into the cages. Eight adult milfoil weevils were stocked into each of 10 tanks on 5 June. Additional EWM stems 30 cm (11.8 in) from Lake Joanis were added to the tanks to bring the total number of stems per tank to 15. All stems were affixed to a small rock with rubber bands. Each week, an additional bunch of 15 weevil-free stems from Lake Joanis was added as food and egg-laying sites for the

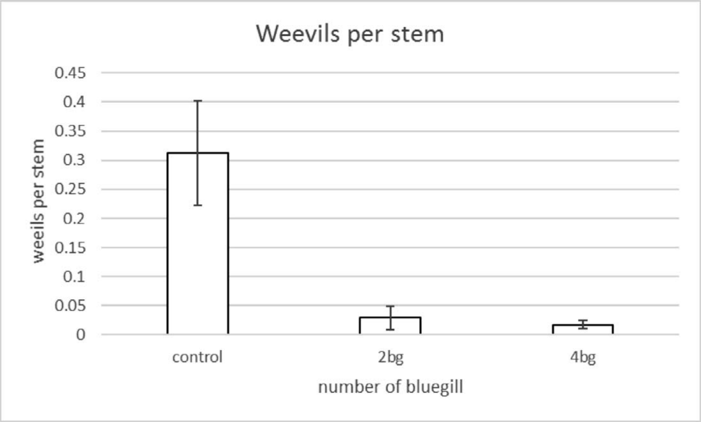

Figure 1. Mean number of *E. lecontei* weevils per Eurasian watermilfoil stem from in-situ exclosure cages with 0 (control), 2, and 4 bluegill placed in Lake Joanis, Wisconsin. Error bars represent standard error.

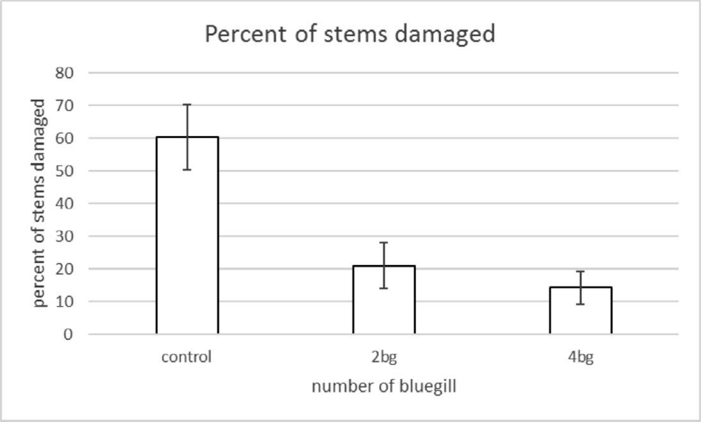

Figure 2. Mean percent of damaged Eurasian watermilfoil stems from in-situ exclosure cages with 0 (control), 2, and 4 bluegill placed in Lake Joanis, Wisconsin. Error bars represent standard error.

milfoil weevils. During periods of temperatures above 32.2 C (90 F), bamboo screens were placed on top of the tanks as shade to control water temperature. After 30 d, approximately 300 milfoil weevil adults and eggs were removed from the tanks and placed in 24 plastic bags with EWM to be stocked in the cages.

Five adult milfoil weevils and 10 eggs attached to EWM were stocked into each of the 24 cages on 6 July. On 7 July, a seine was used to collect bluegills from an area adjacent to the cages. The bluegills were selected for a size range of 5 to 8 cm (2 to 3 in) and placed in the cages at the designated densities. The lids were fixed in place to prevent escape or intrusion by jumping and protect against temporarily elevated water levels. The cages were visually inspected weekly using an underwater camera to verify the number of bluegill present.

On 18 August, after an exposure period of 42 d, all EWM (1,604 stems) to a depth of 50.8 cm (20 in) from the surface found in the cages were collected, sealed in gallon-sized freezer bags, and refrigerated. A small seine was used to extract all fish. Fish were euthanized by placing them on ice and stored in a freezer. All milfoil stems were counted and searched under magnification on a light table to count the number of all life stages of milfoil weevils (eggs, larvae, and adults) present. The presence of damage caused by larval milfoil weevils was also recorded. To count as a damaged stem in the absence of larvae or pupae, a small entry hole and a larger exit hole with a hollow tunnel between them needed to be present. This requirement removed the possibility of mistakenly including nonweevil damage in the total. Every attempt was made to sort the samples when fresh. Because of the large number of samples, some were preserved in 80% isopropyl alcohol before sorting.

Analysis of variance including Tukey's test were performed on the number of milfoil weevils per stem and percent damaged stems in each density treatment to assess the effect of the presence of bluegills with the null hypothesis of no difference between treatments. Because the data from damaged stems was expressed as a percentage, it was arcsine square root transformed before analysis. Two sets of replications (six cages) were excluded from analyses because of a lack of evidence of milfoil weevils or weevil activity. All statistical processes were performed in R (www.r-project.org), using the R Commander (RCMDR) module.

RESULTS AND DISCUSSION

After 42 d, density of *E. lecontei* per Eurasian watermilfoil stem (w s^{-1}) in cage enclosures with two and four bluegill, respectively was reduced 15- to 30-fold compared to controls (Figure 1). The difference in mean density values between treatments was significant (ANOVA: df = 2,15, F = 7.6, $P = 0.005$). The mean number of w s^{-1} ranged from 0.31 in the controls to 0.01 in the cages with four bluegill. The highest number of milfoil weevils was 58 in a single control cage. Nine of 12 cages with two or more bluegill had two or fewer milfoil weevils. Five cages total had no milfoil weevils; three of those had two bluegill, and two had four bluegill.

There were 31.9% of stems found to have been damaged by milfoil weevil larvae. Damage in density treatments was significantly different from the control (ANOVA: df = 2,15, F = 13.5, $P = 0.0004$). The mean percentage of damaged stems ranged from 60.4% in the control treatment without bluegill to 13.3% in the treatment replicates with four bluegill (Figure 2). The only single treatment replicate with no stem damage had four bluegill. The highest percentage of stem damage was found in a control cage replicate with no bluegill (84%), and the least damage in a replicate cage with four bluegill (no damage).

Bluegill predation had a significant effect on milfoil weevil abundance in predator exclusion cages in Lake Joanis. Although the density of bluegill in the EWM beds in Lake Joanis was not directly assessed prior to cage deployment, it likely exceeded the density of bluegill placed in the exclosures. For this reason, we do not believe that the weevils migrated out of the cages due to predator pressure because it was higher outside the cages. Studies of bluegill diets have shown that they consume milfoil weevils (Newbrough 1993, Sutter and Newman 1997, Maxson 2016). Both adult and larval milfoil weevils were found in the stomachs of bluegill retrieved from the cages in the present study. Bluegill in the littoral zone feed on benthic invertebrates, mollusks, cladocerans, and miscellaneous insects (Gerry et al. 2013). As bluegill grow from juveniles to adults, they select larger prey (Walton et al. 1992). In the presence of predators, they often seek refuge in dense vegetation (Dewey et al. 1997). Bluegill in EWM stands often become stunted (Ward and Newman 2006) and remain at a size where small insects such as milfoil weevils are selected for prey. Stands of EWM support less diversity of invertebrates than native milfoils (Wilson and Ricciardi 2009). If bluegill, piscivorous predators, milfoil weevils, and EWM are present in a lake, all of these factors can lead to a situation in which bluegill can feed on milfoil weevils for an extended portion of their lives.

Lake Joanis was previously stocked at one of the highest rates of milfoil weevils per acre without a long-term rise in weevil densities (Thorstenson 2011). Another study without exclosures saw milfoil weevil densities drop within weeks after stocking (Jester et al. 2000). In the present study, the presence of bluegill reduced the number of milfoil weevils found in cages. The presence of bluegill also reduced the damage done to EWM by remaining milfoil weevils. Because damage to EWM is the intended result of weevil stocking, it is perhaps more important than the number of milfoil weevils found in assessing the impact of bluegill (Havel et al. 2017). The damage done by milfoil weevils in some control cages was almost complete, with collapse of most stems in the cage. In this study, the very high rates of damage inside the cages where bluegill were excluded suggest that the use of milfoil weevils as a biocontrol agent for EWM should consider the potential impact of bluegill and other insectivorous fish populations on weevil densities.

At this time there is no commercial source of milfoil weevils to be used in stocking in Wisconsin. Milfoil weevils are widely distributed throughout the state at varying densities (Skawinski 2014). Directly stocking milfoil weevils from other lakes is not permitted in Wisconsin because of potential transfer of invasive species. Volunteers have successfully captured and reared sufficient numbers of milfoil weevils to use in stocking programs within lakes in predator-free tanks (Thorstenson 2015). Given the effectiveness of the control cages in this study at protecting milfoil weevils from predation, similar in-situ exclosures could be used as part of future stocking efforts to increase the chances of milfoil weevils establishing densities necessary for EWM control. In cases where there is a native population of milfoil weevils and a moderate density of bluegills, exclusion cages could be placed on EWM beds as a refuge for milfoil weevils. A. L. Thorstenson (unpub. data) at Golden Sands Resource Conservation and Development used the 24 cages from this study to assess this possibility in Lake Joanis. She found that 68% of the stems in cages had weevil damage and the density of milfoil weevils in a point intercept survey of the entire lake rose to 0.25 w s^{-1}, the highest recorded milfoil weevil density in lake history.

Mechanical harvesting of EWM negatively affects milfoil weevil populations by removing the apical meristems they use for egg laying, larval feeding, and pupation (Sheldon and O'Bryan 1996). It might be possible to integrate mechanical harvesting and biocontrol using milfoil weevils by limiting the harvesting to less than 15% of the total area (Newman and Inglis 2009). Harvesting in strips while leaving most of the shallow EWM beds intact with exclosures placed adjacent to the strips could potentially protect and concentrate milfoil weevils. The creation of more edge habitat in the beds caused by mowing in strips could also provide more opportunities for predation on bluegill by predators such as largemouth bass (*Micropterus salmoides*) (Trebitz et al. 1997).

Several other factors have been identified as necessary for maintaining sustainable weevil populations. Thorstenson et al. (2013) collected milfoil weevils from the shore of two lakes in central Wisconsin to assess overwintering habitat requirements. They found that milfoil weevils prefer dry sites close to shore. They also found that milfoil weevils likely require sites at a minimum of 50 cm above the water line. At least some duff is thought to be necessary for successful overwintering. They likely use only the top 5 cm of the soil, so cover such as leaf litter is essential (Newman et al 2001). Skawinski (2014) found that weevil densities were negatively correlated with coarseness of in-lake substrate. Water quality and local geographic factors were not found to be a factor in weevil density.

The results of this experiment, along with similar studies, indicate the clear effects of sunfish predation on milfoil weevil densities. We cannot yet quantify the minimum density of sunfish necessary for these effects to inhibit biocontrol of EWM. Larger cages with lower densities could provide more insight into this threshold. Future studies should make use of fully enclosed exclusion cages capable of anchoring under the sediment to hold sunfish numbers at precisely prescribed levels as applied in this study. An investigation into protecting milfoil weevils already present in a lake from predation rather than stocking them would be helpful to guide future biocontrol management of EWM. More research is needed into management approaches integrating biocontrol and mechanical removal.

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