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A study of Eurasian watermilfoil, macroinvertebrates and fish in a Washington lake

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ABSTRACT

This study was undertaken to gain experience propagating the milfoil weevil (*Euhrychiopsis lecontei* Dietz) and to monitor the macrophyte, macroinvertebrate, and fish communities at a milfoil weevil augmentation site between, 2002 to 2008, in a small lake in central Washington State. The milfoil weevil propagation was time consuming but not difficult. Over the course of the project, monitoring showed a significant decrease in the frequency and biomass of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and no change or a slight increase in frequency and biomass of other macrophytes at the augmentation site. The milfoil weevil took 5 years to establish in the lake, during which time a midge (Chironomidae) population started to control Eurasian milfoil growth. The fish community changed from one dominated by stunted pumpkinseed sunfish (*Lepomis gibbosus* L.) to a more balanced community of predator and prey fish. Fish diet analysis indicated that fish predation likely influenced herbivorous macroinvertebrate populations. This study supports the theory that fish and macroinvertebrate herbivores influence lake trophic interactions, affecting primary productivity as macrophyte growth.

Key words: biocontrol, *Euhrychiopsis lecontei*, milfoil midge, milfoil weevil, *Myriophyllum spicatum*, trophic cascade.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L., hereafter called Eurasian milfoil) is an invasive nonnative aquatic weed in many North American lakes (Smith and Barko 1990, Creed 1998). Where introduced outside its native range of Europe, Asia, and northern Africa (Cock et al. 2008), it often dominates the submersed plant community to the detriment of native plant diversity, fish and wildlife habitat, water quali-

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ty, flood control, recreation, and aesthetics (Nichols and Shaw 1986, Smith and Barko 1990, Madsen et al. 1991, Boylen et al. 1999, Valley and Bremigan 2002). In Washington State it is present in almost 150 lakes and several major rivers including the Columbia and Snake rivers (http://www.ecy.wa.gov/programs/eap/lakes/aquaticplants/index.htmlParsons 2009). Government agencies and waterfront property owners spend hundreds of thousands of dollars annually to control Eurasian milfoil in the state (K. Hamel, WA Dept. of Ecology, 2010, pers. comm.).

Because Eurasian milfoil is both prevalent and pervasive across temperate North America, biological control is an attractive management option. Biological control (biocontrol), which is the use of natural enemies to reduce pest populations (Cuda et al. 2008), can keep growth of the target species at acceptable levels over broad geographical areas when successful. Other control methods, such as chemical or hand removal, are often more expensive and generally provide only local control (Madsen 2000, Newman 2004).

Researchers began seeking natural enemies of Eurasian milfoil in parts of its native range in the 1960s; however, to date none have been proposed for introduction as a classical biological control agent (Cock et al. 2008). Meanwhile, several native or naturalized insect herbivores have been associated with declines of Eurasian milfoil in North America and have been studied to learn what conditions favor their proliferation (Kangasniemi 1983, Creed and Sheldon 1995, Creed 1998, Johnson et al. 2000, Newman and Biesboer 2000, Newman 2004).

The insect that has elicited the most interest and research as a biocontrol agent is the milfoil weevil (*Euhrychiopsis lecontei* Dietz; family Curculionidae), a beetle native to northern North America (Creed 1998, Tamayo et al. 1999). The native host is typically northern watermilfoil (*Myriophyllum sibiricum* Kom.) and possibly whorled watermilfoil (*M. verticillatum* L.; Newman 2004). It was first implicated in a Eurasian milfoil decline in Vermont in the late 1980s (Creed and Sheldon 1995) and subsequently in other areas of the Midwest (Lillie 2000, Newman and Biesboer 2000) and, speculatively, Washington (Creed 1998, Madsen et al. 2000).

The milfoil weevil is a milfoil specialist, living its whole life on its milfoil host. When water temperatures are above about 15 C, the female lays an average of about 2 eggs per day on the meristem. The larvae hatch and initially consume meristem tissue before tunneling into the stem where they continue consuming plant tissue. They pupate inside the stem and emerge as adults that feed on leaf tissue (Sheldon and O'Bryan 1996, Cofrancesco and Crosson 1999, Mazzei et al. 1999). In the Midwest where overwintering strategies have been studied, milfoil weevils develop flight muscles in the fall and overwinter in leaf litter or shallow soil along shore (Newman et al. 2001).

Another insect, the milfoil midge (*Cricotopus myriophylli* Oliver), has caused Eurasian milfoil declines in British Columbia and Canada (Kangasniemi and Oliver 1983, MacRae et al. 1990) and has been found associated with Eurasian milfoil in the Midwest and northeastern United States (Newman 2004). It is native to North America, its original host being northern watermilfoil (Kangasniemi et al. 1993, Newman and Maher 1995). The larvae live attached to the meristem in cases made of silk with Eurasian milfoil leaf fragments. It feeds on the meristem from both ends of the case, preventing further meristem growth. Results from lab studies showed that one milfoil midge larvae will consume one meristem in 2 to 3 days (MacRae et al. 1990). High populations of milfoil midge larvae prevent Eurasian milfoil from reaching the water surface and flowering (MacRae et al. 1990, Kangasniemi et al. 1993). The possibility of using this insect as a biocontrol agent was explored by the British Columbia government; however, research was abandoned when program funding was cut and difficulties culturing the milfoil midge were encountered (Kangasniemi et al. 1993).

Eurasian milfoil declines in British Columbia have also been attributed to larvae of the tardy caddisfly (*Triaenodes tardus*; Kangasniemi 1983). Caddisflies (Trichoptera) as a group are recognized consumers of aquatic vegetation (Jacobsen 1993). The genus *Triaenodes* is in the family Leptoceridae, a family with members associated with Eurasian milfoil in its native range (Cock et al. 2008) and shown to both consume milfoil vegetation and use it for case building (Wiggins 1977). When densities are high, they can strip all leaflets from milfoil. Although they show a preference for milfoils, they are known to also use other submersed macrophytes (Kangasniemi 1983, pers. observ.).

Another insect herbivore associated with Eurasian milfoil declines in the Midwest and northeastern parts of North America is the Lepidoptera (moth) larvae *Acentria ephemerella* Dennis & Schiffermuller (Johnson et al. 1998, Lord et al. 2003). This moth species has not yet been identified in Washington.

At the project outset, the milfoil weevil was known to occur naturally in many Washington lakes, especially in the eastern half of the state (Tamayo et al. 2000, Parsons 2009). During routine aquatic plant inventory work we noted that Eurasian milfoil dominance of the plant community varied between lakes. The reasons for these differences were unknown, but variations in herbivorous insect abundance were suspected. Elsewhere, several factors had been implicated as potentially limiting milfoil weevil abundance, including predation, lack of overwintering habitat, water temperature, sediment nutrient content, and overall health of the milfoil host plants (Cofrancesco 2000, Creed 2000). At the project beginning fish predation was of particular interest because other researchers had witnessed bluegill sunfish (*Lepomis macrochirus* Rafinesque) consuming milfoil weevils as they were released (Hanson et al. 1995), and other studies had demonstrated the ability of pumpkinseed (*Lepomis gibbosus* L.) and bluegill sunfish to suppress Eurasian milfoil herbivore populations (Sutter and Newman 1997). Both of these nonnative fish species are widespread in Washington State (Wydoski and Whitney 2003) and thus may be influencing milfoil herbivore populations.

Interest in biocontrol of invasive plants is strong in Washington, as is public interest in the milfoil weevil as an option to herbicide use. This study was conducted between spring 2002 and fall 2008 as an initial investigation into the efficacy of milfoil weevil population augmentation to control Eurasian milfoil. The 4 primary objectives of the study were to:

- gain local experience collecting, rearing, and releasing the milfoil weevil (milfoil weevil propagation);
- monitor the aquatic plant community at a milfoil weevil introduction site for several years;
- monitor macroinvertebrates associated with Eurasian milfoil at a milfoil weevil introduction site; and
- monitor fish community structure and diet in relation to Eurasian milfoil abundance at a milfoil weevil introduction site.

MATERIALS AND METHODS

Study site

Mattoon Lake, located near the town of Ellensburg in central Washington (Figure 1), was selected as the milfoil weevil introduction site. It is a 10.5 ha (26 ac) former gravel pit with a maximum depth of about 5 m (16 ft). Mattoon Lake is fed by subsurface flow from nearby Wilson Creek and has a seasonal outflow back to this creek. The lake is owned by the Washington Department of Fish and Wildlife (WDFW) and is managed and stocked for a year-round trout fishery. There is a boat launch and fishing dock on the west end. Mattoon is a clear-water lake that supports aquatic plant growth throughout (Secchi depth visibility was usually to the lake bottom during the study). At project inception, Eurasian milfoil dominated the submersed plant community in water 0.6 to 3.5 m deep, forming a broad band of surfacing vegetation around the lake. The dominant emergent vegetation during the project was yellow flag iris (*Iris pseudacorus* L.), with stands of willow (*Salix* sp L.) and cottonwood (*Populus* sp. L.) interspersed. Mattoon Lake was selected as the augmentation site due to abundant surfacing Eurasian milfoil, shallow depths with warm water, proximity to the home office (Yakima), and the lack of other plant management activities at the lake during the study period.

Milfoil weevil propagation

Adult milfoil weevils were collected from naturally occurring populations in Stan Coffin and Burke lakes in Grant County, central Washington (Figure 1). These lakes are part of the Quincy Wildlife Area, managed by WDFW for angling opportunities., Both lakes were identified in previous studies as supporting stable milfoil weevil populations (Tamayo et al. 1999), but neither has experienced active aquatic plant management programs. Both lakes were formed in the 1950s as a result of the Columbia Basin Irrigation Project. Burke Lake is fed by subsurface flow and seasonal drains; Stan Coffin Lake is fed by a small stream from an irrigation canal. Both lakes have small outflow channels (Dion et al. 1976). They both host Eurasian and northern watermilfoil, and late in the study a hybrid of the 2 species was confirmed (C. Anderson, University of Idaho, 2008, pers. comm.). None of the watermilfoil species was the dominant plant in either lake at the time of milfoil weevil collection; instead, a mixed community of pondweeds (*Potamogeton* L. and *Stuckenia* Börner spp.), macroalgae (*Chara* sp. Valliont) and northern watermilfoil prevailed. The lakes are undeveloped except for dirt access roads with concrete boat launches and pit toilets. They are surrounded by native and nonnative wetland and upland shrub-steppe vegetation.

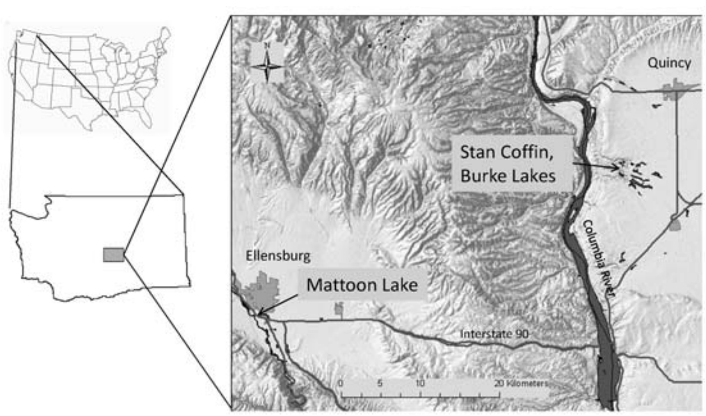

Figure 1. Washington State locations for milfoil weevil collection (Stan Coffin and Burke lakes) and milfoil weevil introduction (Mattoon Lake).

Adult milfoil weevils were gathered from the collection sites approximately weekly for up to 12 weeks throughout the summers of 2002 and 2003. Weevils were collected by snorkelers watching for movement on the milfoil plants, pinching off the plant portion containing the milfoil weevil, and placing it in a sealable plastic bag. The adult milfoil weevils were collected from northern watermilfoil because it was more plentiful than Eurasian milfoil during both years of collection. The peak collection period was the end of July through the end of August, when an experienced snorkeler could collect at a rate of about one adult milfoil weevil per minute. When weevils were not plentiful or water clarity was poor, the collection rate was lower, sometimes taking 5 or more minutes per weevil. During periods of peak milfoil weevil density we found 2 to 3 milfoil weevils per milfoil stem, a density thought to be sufficient to control Eurasian milfoil growth (Newman and Biesboer 2000).

In 2002 we collected 705 adult milfoil weevils from northern watermilfoil plants between mid-June through the end of September. Most of the milfoil weevils were collected from Stan Coffin Lake; a few were collected from Burke Lake. In 2003 Stan Coffin Lake experienced a prolonged cyanobacterial bloom and decreased milfoil weevil numbers; therefore, we searched for a new, productive collection site, choosing Burke Lake after some minimal collecting at other regional lakes. We collected 293 milfoil weevil adults between late June and early August.

The bagged milfoil weevils were placed in a cooler or tub (without ice) and immediately transported to our rearing facility, a garage at the WDFW office in Yakima. Rearing techniques were established following the advice of Hanson et al. (1995) and Cofrancesco and Crosson (1999). The milfoil weevils were transferred to aquaria or translucent plastic tubs filled with untreated river water and fresh, bundled and weighted Eurasian milfoil stems brought from Mattoon Lake. Each Eurasian milfoil bundle contained about 5 stems with several meristems, no flowers, and of a length to keep most meristems below the water surface. Each aquarium or tub also had an aeration stone, a window-screen cover, and grow lights suspended overhead with a timer to achieve a 16 h day length. We had 6 containers ranging from 37.85 L to 75.7 L (10 to 20 gal) each. We placed adult milfoil weevils into aquaria or tubs at a maximum ratio of one adult per 3.765 L (1 gal) of water. The capacity of the 6 tanks was 376.5 L (100 gal), or 100 adult milfoil weevils. When the number of milfoil weevil adults collected exceeded the rearing tank's capacity, the extra adults were transferred directly to Mattoon Lake. In 2002 and 2003, 469 and 290 adult milfoil weevils, respectively, were stocked into rearing tanks.

The milfoil weevils were allowed to graze and lay eggs for 4 to 18 d. The tanks were checked every few days, and fresh Eurasian milfoil bundles were added if existing plants showed signs of damage. At the end of the rearing period we counted the number of eggs, larvae, and adults by observing each milfoil stem under a dissecting microscope or hand lens. Analysis of Variance was used to test for significant differences in milfoil weevil productivity by aquarium type. Correlation analysis was used to check for trends in productivity with rearing period. Additional details on our method are available from Parsons (2009).

After counting, the adult milfoil weevils and progeny were placed in sealable plastic bags at the rearing facility and taken directly to Mattoon Lake. From a raft or by snorkeling, we wound or tied the milfoil stems with the milfoil weevils and progeny around rooted surfacing milfoil. The cycle of collection, rearing, and release continued throughout the summers of 2002 and 2003 but was suspended at the end of 2003.

In 2002 we released the milfoil weevils at 2 approximately 30 m^2 release sites in Mattoon Lake, one at the northwest corner and one at the southwest corner (Figure 2). These sites were chosen for their extensive beds of Eurasian milfoil and because the west end of the lake is relatively protected from strong westerly winds that frequent the area. In 2002 we released 2832 milfoil weevils (622 adult, 1194 larvae, 912 eggs, and 104 pupae) in small weekly batches between 2 July and 24 September to the 2 release sites. Roughly equal numbers of milfoil weevils were placed at each site.

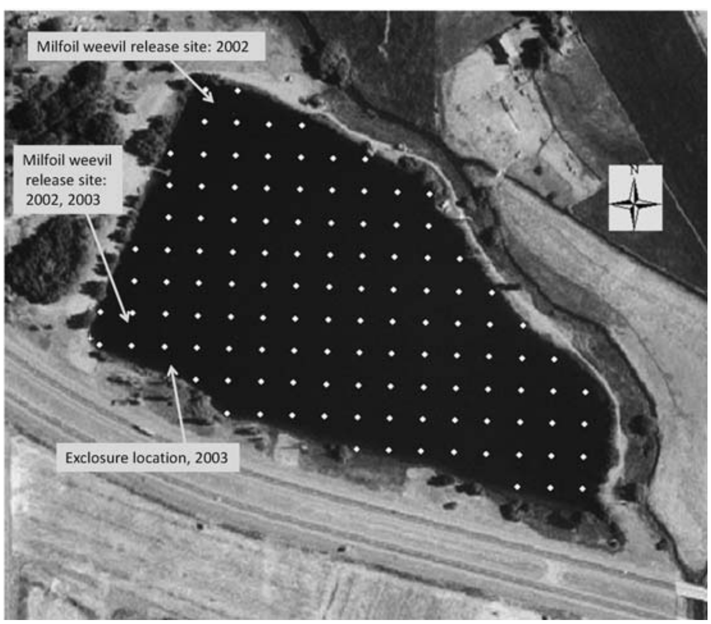

Figure 2. Mattoon Lake, WA, with locations of aquatic plant frequency sample points (white dots) and milfoil weevil release sites.

In 2003 we set up a fish exclosure similar to that used by Ward and Newman (2006) to protect the released milfoil weevils from fish predation. The exclosure consisted of a 3.05 by 3.05 m frame of PVC pipe surrounded with fish netting suspended by floats at the top and held in place with weights at the bottom. We placed the structure along the south shore in a dense Eurasian milfoil bed where public access was difficult except by boat (Figure 2). Once in place, fish that were inadvertently trapped inside were removed with traps and by angling. We then stocked milfoil weevils into the exclosure as well as at the southwest stocking site used in 2002. In 2003 we released 2485 milfoil weevils in approximately weekly batches between 9 July and 19 August. Of those, 1670 (181 adults, 1078 larvae, 302 eggs, and 109 pupae) were released in the fish exclosure and 815 (58 adults, 363 larvae, 374 eggs, and 20 pupae) in the southwest end of the lake.

Aquatic plant monitoring

A list of plant species observed in Mattoon Lake was created each summer from project inception in 2002 through 2008. In addition, we used species presence data obtained in 1994 prior to colonization of the lake by Eurasian milfoil (Parsons 2009). The aquatic plant community was quantified and monitored for changes between years with frequency of occurrence and biomass data.

Frequency of occurrence data were collected prior to the introduction of milfoil weevils in June 2002 and each subsequent June from 2003 through 2005. Data collection was suspended in 2006 then resumed in September 2007 and June 2008. Because frequency data simply measure species presence or absence, the data are robust to seasonal differences in plant abundance (Madsen 1999); therefore, we could justify including the September data. The point-intercept method was used to gather the presence–absence data as per Madsen (1999) at 115 to 119 points each year. We created a 30 m grid covering the whole lake using a Geographic Information System (GIS; Figure 2), with each grid intersection representing a sample point, provided as UTM (Universal Transverse Mercator) coordinates. A Geographical Positioning System (GPS) unit was used to locate the points in the field. At each point, macrophyte data were collected by deploying a sampling rake twice. All species observed were recorded. The data were analyzed using chi square 2-by-2 analysis between pretreatment (2002) and all posttreatment years for the species present in at least 10% of samples during at least one sampling event. The significance level was adjusted for multiple comparisons.

Biomass data were collected by SCUBA diver in June of 2002, 2003, 2004, and 2008 from 30 points randomly selected from the frequency data grid. At each point the diver placed a 0.1 m^2 frame on the sediment (Madsen 1993) and collected all above-ground plant matter and placed it in a mesh bag. The samples were sorted by species and dried in a forced-air oven at 70 C to a constant weight and weighed to 0.01 g accuracy. Analysis of variance was performed on log transformed data to check for significant differences before and after treatment. The resultant p-values were adjusted using a Bonferroni post-hoc test to adjust for multiple comparisons.

Macroinvertebrate monitoring

The milfoil weevil population at Mattoon Lake was monitored using 2 methods: a qualitative check for adult milfoil weevils and characteristic damage on Eurasian milfoil plants in the lake, and quantitative sampling where Eurasian milfoil stems were collected and examined in the lab.

For the qualitative inventory, experienced milfoil weevil-collecting snorkelers conducted 20-minute visual searches in selected areas of the lake. Snorkelers kept a mental tally of observed milfoil weevils and recorded the data when the inventory time was complete. All observations were made from the surface in milfoil beds growing close to the surface. In 2002, prior to milfoil weevil introduction, the sites included one of the future augmentation sites as well as 2 sites at the opposite (east) end of the lake. At the end of summer 2002 and during subsequent surveys through 2008, sites included the 2 augmentation areas at the west end of the lake as well as a site along the northeast shore. During 2003 and 2004 we also checked the area of the fish exclosure. Inventories continued at least once per summer from 2002 through 2008, except 2006.

The quantitative macroinvertebrate data were obtained prior to milfoil weevil release in June 2002 and again in September 2002, August 2003, September 2005, October 2007, and July 2008. The month of sample collection varied because this time-consuming task had to be scheduled around other duties. Samples were collected by snorkelers who pinched off the upper approximately 0.5 m of stems (with several meristems) and gently placed them in sealable plastic bags. In 2002, two stems were collected from 25 points randomly selected from the plant frequency sampling grid covering the whole lake. From 2003 on, the shore was divided into 20 equal segments, and between 5 and 10 stems were collected in each segment from Eurasian milfoil close to the surface. This eased sample collection for the snorkelers because no deep water areas were included. It also increased the chances of finding the milfoil weevil because they tend to be more prevalent in shallower water (Lillie 2000, and pers. observ.). In the lab, each plant was inspected with a dissecting microscope. We counted all milfoil weevil life stages and noted any characteristic weevil damage to milfoil stems. In addition, starting in 2003, we quantified all macroinvertebrates that were collected with the Eurasian milfoil stems to the lowest taxonomic group practical. Analysis of variance was performed on log-transformed data to check for significant changes between 2003 data and subsequent years. The resultant p-values were adjusted using a Bonferroni post-hoc test to adjust for multiple comparisons.

Fish community and diet monitoring

The fish community was sampled by WDFW at the end of May 2002 and again in June 2008. The methods used are detailed in Divens (2003, 2008), and portions pertinent to data reported in this paper are summarized below.

Fish were captured using standardized population assessment techniques (Bonar et al. 2000), including boat electrofishing, gill netting, and fyke netting, during the night to maximize the type and number of fish captured. Sampling locations were selected by dividing the shoreline into 3 sections of approximately 400 m each. All 3 sections were sampled individually by boat electrofishing, maneuvering slowly through shallow water (approximately 1 to 3 m deep) for 600 sec in each section, for a total of 1800 sec.

Gill nets and fyke nets were set in the evening and retrieved the following morning in 2 sections each, totaling 2 net-nights of effort for each net type. The gill nets were 45.7 m long by 2.4 m deep, constructed of 4 sinking panels (2 each at 7.6 m and 15.2 m long) of variable-size monofilament mesh (1.3, 1.9, 2.5, and 5.1 cm stretch). The gill nets were set perpendicular to the shoreline with the small-mesh end tied off on shore and the large end anchored off shore. Fyke (modified hoop) nets were constructed of five 1.2 m diameter hoops with 2 funnels, and a 2.4 m cod end (6 mm nylon delta mesh). Attached to the mouth of the net were two 7.2 m wings and a 30.5 m lead. The fyke nets were also set perpendicular to the shore. The lead was tied on shore, and the cod end was anchored off shore with the wings anchored at approximately a 45° angle from the net lead.

Each fish captured was identified to species. Most fish were measured to total length (mm) and weighed (g), though fish <70 mm long were not weighed due to inadequate scale precision. When large numbers of obviously similar-sized fish were collected simultaneously, a subsample was measured and weighed. The remaining fish were counted, and the subsample data expanded. Weights were then assigned using a length–weight regression formula.

Species composition by number and weight were calculated from all data collected using boat electrofishing, gill netting, and fyke netting. All fish, including young-of-the-year, were used in the calculations. Relative weight was used to evaluate the condition of the prevalent fish species. It was calculated for fish that were directly measured and weighed by taking the percent of the actual weight of a fish at a given length divided by the national 75th percentile weight for that species and length (Anderson and Neumann 1996).

Stomach samples were collected from pumpkinseed sunfish, largemouth bass (*Micropterus salmoides* Lacepede), yellow perch (*Perca flavescens* Mitchill), rainbow trout (*Oncorhynchus mykiss* Walbaum), and brown trout (*Salmo trutta* L.). Our sample objective was 30 samples from each species; however, a variable number of fish stomach samples were actually collected from each fish species due to loss of some samples and difficulty collecting the desired number of some fish species. Stomach samples were collected by flushing gut contents from individual fish using gastric lavage. Samples were preserved in ethanol and analyzed in the lab by a contracted macroinvertebrate specialist (2002) or by the authors (2008). All taxa were counted and identified to the lowest group practical. For the beetles in the Curculionidae family (weevils) and, in 2008, Trichoptera with cases characteristic of *Triaenodes* sp., individuals were identified to species.

RESULTS AND DISCUSSION

Milfoil weevil propagation

The number of progeny produced per adult milfoil weevil was variable. In 2002, average productivity was 5.5 milfoil weevils of all life stages per adult weevil stocked into the tank or 0.5 progeny (eggs, larvae, pupae) per adult milfoil weevil (male and female not differentiated) per day. Higher productivity was attained in 2003 with an average of 9.9 milfoil weevils per adult weevil stocked, or 0.8 progeny per adult per day. Hanson et al. (1995) attained slightly lower numbers with mean productivity of 2.75 and 5.9 progeny per adult over 2 years of rearing. Sheldon and O'Bryan (1996) found female weevils laid an average of 1.9 eggs day^{-1}. If half our adult weevils were females, our productivity was 1 to 1.6 progeny $female^{-1}$ day^{-1}. Considering there is likely some natural mortality between egg and pupae, we thought our productivity results were reasonable.

Productivity was not significantly affected by container type (glass aquaria vs. plastic tub; $p = 0.4$). The highest productivity was achieved with a rearing period between 8 and 14 d, although there was no significant trend between rearing period and productivity ($r^2 = 0.1$). Although rearing period varied, other methods did not vary during the time periods, so variability in productivity could have been due to ambient temperature differences in the unairconditioned rearing shed. Another possibility is the fecundity of the milfoil weevils themselves because larger milfoil weevils produce greater numbers of progeny (Newman 2004). We did not measure these variables.

Aquatic plant monitoring

Prior to invasion by Eurasian milfoil, Mattoon Lake had a submersed plant community composed mainly of native waterweeds (*Elodea canadensis* Michx. and *E. nuttallii* [Planch.] St. John) and thin-leaf pondweeds (a combination of *Potamogeton pusillus* L., *P. foliosus* Raf., *Stuckenia pectinata* [L.] Börner, and *S. filiformis* [Pers.] Börner) (Table 1). By 2002, when Eurasian milfoil dominated the lake, several of the native species had declined in abundance, and 3 native plants (three-stamen waterwort, Richardson's pondweed, and water-buttercup) were not found (Table 1). In the year immediately following initial milfoil weevil introductions, northern watermilfoil also became so rare that it was not found during surveys. By the end of the study, northern watermilfoil and Richardson's pondweed were recovering, but three-stamen waterwort, and water-buttercup still had not been found.

Eurasian milfoil was the dominant plant in Mattoon Lake in 2002, forming a band of surfacing vegetation around the lake. It was present in 87% of frequency sample points (Figure 3), and mean dry biomass was 304 g m^{-2} (Table 2). Eurasian milfoil frequency of occurrence was significantly reduced from 2002 levels in each year of data collection after 2003 (Figure 3). In the final year of data collection, Eurasian milfoil was present at 50% of sample points, representing a decrease in frequency of occurrence of 37% over the study period. Eurasian milfoil biomass also declined significantly in all years compared with 2002 (Table 2). In 2008, the final year of the project, the mean dry biomass was 32 g m^{-2}, close to a 10-fold reduction from the project's start.

Of the native species, frequency of pondweeds in the genus *Stuckenia* were significantly higher in 2007 and 2008 than in 2002 (Figure 3), although they were still a fairly minor component of the plant community. *Stuckenia* spp. were not collected in biomass samples in 2002 or 2003, but a small number were collected in 2004 and 2008, though not significantly greater than 2002. The frequency and biomass of elodea and coontail varied from year to year but was never significantly different from 2002. Note that coontail is non-rooted and has a sprawling growth form, making biomass samples difficult to collect using a frame placed on the lake sediment; therefore, coontail biomass numbers may be high relative to the other plant species. Additional species were collected in small numbers, but neither their frequency of occurrence nor biomass changed significantly during the study period. Total plant biomass also did not change significantly during the study.

Based on these data and personal observations in the field, by the study's conclusion in 2008, Eurasian milfoil was still common in the lake but no longer dominated the plant community. It did not form a surfacing mat in 2007 or 2008, and other plant species could be seen growing among it. The native species were expanding and filling in areas opened up by the declining Eurasian milfoil. These results are consistent with other studies where declines of Eurasian milfoil have been caused by herbivorous insects (Creed and Sheldon 1995, Johnson et al. 1998, Lillie 2000, Newman 2004).

TABLE 1. SUBMERSED AQUATIC PLANTS FOUND IN MATTOON LAKE AND THE MONTH/YEAR OF OBSERVATION.

Scientific name	Common name	8/94	6/02	6/03	6/04	6/05	6/06	9/07	6/08
Ceratophyllum demersum L.	Coontail; hornwort	X	X	X	X	X	X	X	X
Chara sp. Valliant	muskwort	X	X	X	X	X	X	X	X
Elatine triandra Schkur	three-stamen waterwort	X							
Elodea spp. Rich in Michx.	waterweed	X	X	X	X	X	X	X	X
Myriophyllum sibiricum Kom.	northern watermilfoil	X	X			X	X	X	X
Myriophyllum spicatum L.	Eurasian water-milfoil		X	X	X	X	X	X	X
Potamogeton crispus L.	curly leaf pondweed	X	X	X	X	X	X	X	X
Potamogeton richardsonii (Bennett) Rydb.	Richardson's pondweed	X		X	X	X	X	X	X
Potamogeton sp (thin leaved) L.	thin leaf pondweed	X	X	X	X	X	X	X	X
Ranunculus aquatilis L.	water-buttercup	X							
Stuckenia spp. Borner	pondweed	X	X	X	X	X	X	X	X

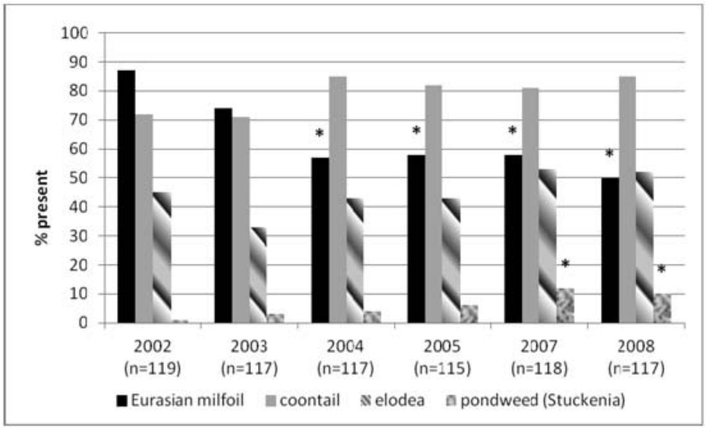

Figure 3. Mattoon Lake aquatic plant frequency of occurrence chi-square analysis results for species present in at least 10% of samples during at least one sampling event. A star indicates values significantly different from 2002. Significance level adjusted to p ≤ 0.01 for multiple comparisons.

Macroinvertebrate monitoring

In early summer 2002, prior to milfoil weevil augmentation, no milfoil weevils were found in Mattoon Lake. In fact, milfoil weevil establishment at a level detectable by our sampling methods took several years. At the end of the first stocking year (2002) we found no sign of the milfoil weevils at either stocking location or other areas in the lake (Table 3 and 4). For that reason and the presence of high numbers of fish with the potential to prey on milfoil weevils (see fish community and diet monitoring section below), we stocked most of the milfoil weevils in the fish exclosure in 2003. From our qualitative monitoring results we found establishment of the milfoil weevils in the exclosure through 2003 and in that vicinity after the exclosure was removed through midsummer 2004 (Table 3). After that time, however, the qualitative inventory methods did not reveal milfoil weevil presence again until late summer 2007 and midsummer 2008 (no inventory took place in 2006).

The quantitative macroinvertebrate monitoring provided more details about the macroinvertebrate community associated with the snorkeler-collected Eurasian milfoil in Mattoon Lake (Table 4). Milfoil weevil results were similar to the qualitative monitoring. The milfoil weevil was not observed until inventories at the end of summer 2007 and in 2008 (again, no inventory took place in 2006). The 2007 inventory was prompted by obvious signs of milfoil weevil damage to the Eurasian milfoil noticed while at the lake for a different purpose. Milfoil weevils could have been present in the lake since 2002 but at densities too low to be detected by our survey methods, except in the area of the exclosure in 2004.

The slow establishment of milfoil weevils could have been affected by our initial milfoil weevil stocking densities. In their augmentation work, Jester et al. (2000) found milfoil weevil augmentation had variable results, sometimes without any increase in weevil presence in the short term. They also found higher stocking rates provided more consistent declines in Eurasian milfoil. Commercial milfoil weevil stocking for Eurasian milfoil control employs high stocking rates in a concentrated time to try to obtain fast milfoil weevil population establishment (M. Hilovsky, Enviroscience, 2008, pers. comm.). While we had a fairly high overall stocking rate, particularly in the milfoil exclosure, the weevils were added gradually over a period of several weeks. Thus, a higher stocking rate over a shorter time period may have accelerated milfoil weevil establishment at Mattoon Lake.

In 2007, the mean milfoil weevil density (including all life stages) was 0.1 per stem (Table 4). This is at the low end of the range thought capable of causing a Eurasian milfoil decline (Newman 2004). However, the samples were collected in October, and milfoil weevils start migrating to overwintering sites by mid-September in Minnesota where their life history has been carefully studied (Newman et al. 2001). No work has been done on this aspect of milfoil weevil life history in Washington, but if the timing is similar, milfoil weevil densities may have started to decline before samples were collected. At the time of sampling, most examined milfoil stems had holes and tunnels characteristic of milfoil weevil damage. The 2008 inventory took place in midsummer, and at that time a mean of 0.29 milfoil weevils per stem was found. This was within the range thought capable of causing milfoil declines (Newman 2004). In a survey of 17 other

TABLE 2. MATTOON LAKE AQUATIC PLANT MEAN DRY BIOMASS BY YEAR OF COLLECTION FOR SUBMERSED SPECIES. STANDARD DEVIATION IN PARENTHESES WITH BONFERRONI ADJUSTED ANOVA RESULTS.

	Biomass (g m^{-2})			
Plant	2002	2003	2004	2008
Eurasian milfoil	304 (313)	123 (179)*	91 (107)*	32 (54)*
coontail	273 (503)	259 (356)	389 (797)	699 (983)
elodea	19 (51)	9 (21)	6 (22)	24 (49)
muskwort	0	7 (40)	0	0
curly leaf pondweed	0.5 (14)	0	0.1 (0.5)	0.1 (0.3)
thin leaf pondweed	0.3 (0.7)	0.1 (0.4)	0	0.1 (0.5)
pondweed (*Stuckenia* sp.)	0	0	0.5 (2)	0.5 (2)
Total	597 (459)	399 (326)	487 (765)	756 (961)

*Significantly different from June 2002 biomass at p ≤ 0.05.

TABLE 3. QUALITATIVE MILFOIL WEEVIL INVENTORY RESULTS. NUMBERS OF ADULT MILFOIL WEEVILS OBSERVED IN 20 MIN WHILE SNORKELING IN AREAS OF MATTOON LAKE DESIGNATED: SW = SOUTHWEST CORNER, NW = NORTHWEST CORNER, N = NORTH SHORE AT EAST END, SE = SOUTH SHORE AT EAST END, EXCLOSURE = VICINITY OF FISH EXCLOSURE. DATES WHERE NO DATA WERE OBTAINED ARE INDICATED BY A DASH (—).

	Number of Adult milfoil weevils observed in 20 min					
Date	SW	NW	N	SE	exclosure	Total
6/24/2002	—	0	0	0	—	0
9/3/2002	0	0	0	—	—	0
7/1/2003	0	0	0	—	—	0
8/20/2003	0	0	0	—	—	0
9/9/2003	—	—	—	—	3	3
9/24/2003	—	—	—	—	7	7
7/22/2004	0	0	—	—	3	3
8/16/2004	—	—	—	—	0	0
9/7/2005	0	0	0	—	—	0
9/11/2007	6	5	—	—	—	11
7/24/2008	4	3	2	—	—	9
9/12/2008	1	0	1	—	—	2

TABLE 4. MEAN NUMBER OF MACROINVERTEBRATES PER MILFOIL STEM BY DATE WITH TOTAL AT THE BOTTOM (ONLY MILFOIL WEEVILS COUNTED IN 2002). DATA WITH A MEAN OF <0.1 STEM^{-1} FOR ALL DATES WERE EXCLUDED. LOWEST TAXONOMIC DIVISION IS GIVEN WITH THE COMMON NAME. N IS THE NUMBER OF MILFOIL STEMS EXAMINED. S = PRESENCE SUSPECTED, P = PRESENCE CONFIRMED BY TAXONOMIST.

	2002 Jun and Sep (N = 100)	Aug-03 (N = 200)	Sep-05 (N = 100)	Oct-07 (N = 100)	Jul-08 (N = 100)
Cause damage to Eurasian milfoil					
Milfoil weevil (*Euhrychiopsis lecontei*)	0	0	0	0.11	0.29*
Tardy caddisfly (*Triaenodes tardus*)		0	0	0.11*	0.07
Milfoil midge (*Cricotopus myriophylli*)	s	s	s	s	p
Other macroinvertebrates					
Midge** (Family Chironomidae)		1.4	1	1.6	2.75*
Micro-caddisfly (Family Hydroptilidae)		0.6	0.1*	0.22	2.71*
Seed shrimp (Class Ostracoda)		0.19	0.17	0.52	3.94*
Amphipods (Order Amphipoda)		0	0.01	0.24*	0.93*
Mites (Family Hydrachnidae)		0.84	0.75	0.11*	0.99
Snails (Class Gastropoda)		0.01	0.07	1.12*	0.15
Worms (Order Haplotaxida)		1.79	0.49*	0.21*	1.53
Hydra sp.		0.01	1.08	4.13*	0.89
Flatworms (Phylum Platyhelminthes)		0.12	8.03*	2.01*	1.53*
Total Macroinvertebrates		5.06	11.87*	11*	16.58*

*significantly different from 2003 data (log transformed data, $P \leq 0.05$ with Bonferroni adjustment for multiple comparisons).
**includes milfoil midges.

Washington lakes, Tamayo et al. (2004) found milfoil weevil densities ranged from 0 to 0.6 weevils per stem. These numbers are low compared with results from some studies in the Midwest and eastern United States where milfoil weevil densities have reached one or more per stem during Eurasian milfoil declines (Creed and Sheldon 1995, Jester et al. 2000, Newman and Biesboer 2000, Newman 2004). However, in a review of milfoil weevil augmentation projects in 29 Midwestern lakes, Reeves et al. (2008) found a wide range of milfoil weevil densities that overlapped our numbers.

Note, however, that because the Eurasian milfoil decline in Mattoon Lake was evident prior to widespread milfoil weevil establishment in high enough numbers to be found by our survey methods, other insect herbivores likely played an important role in the Eurasian milfoil decline. From the start of the study we observed meristem damage that seemed to be caused by associated midge larvae living in silk cases on those meristems. (From our notes: Jun 2002, 44% of stems had midge-damaged meristems; Sep 2002, 76% had midge-damaged meristems; Aug 2003, 100% of stems had at least some midge-damaged meristems.) The samples of midges identified from collections early in the study were a combination of midges, with the *Cricotopus sylvestris* Fabricius group most common. These were a species other than the milfoil midge, *Cricotopus myriophylli*, but positive identification to species could not be made from larval samples. Other identified Chironomids included *Psectrocladius* Kieffer sp., *Tanytarsus* Van Der Wulp sp., *Pseudochironomus* sp., *Ablabesmyia* Johannsen sp., the *Psectrocladius sordidellus* group, *Parakiefferiella* sp., and *Glyptotendipes* sp. (W. Bollman, Rhithron Associates Inc., 2011, pers. comm.). The milfoil midge was positively identified in Mattoon Lake from samples collected in 2008 (D. Langill, EcoAnalysts Inc., 2009, pers. comm.). To our knowledge, this is the first confirmed population of the milfoil midge in Washington; however, it was assumed to be in the state because the work done on the milfoil midge in southern British Columbia bordered Washington (Kangasniemi et al. 1993).

Thus, due to difficulty identifying midge larvae to species and our focus on the milfoil weevil in this study, we do not know what percentage of the midge larvae found on Eurasian milfoil were the milfoil midge or what other species were causing the damage to the Eurasian milfoil meristems. Other studies have found several midge species associated with Eurasian milfoil (Kangasniemi and Oliver 1983, Chilton 1990, Balci and Kennedy 2003). However, from those studies, only one other midge species, *Endochironomus subtendens* Townes, was noted to damage the milfoil, and the injury it caused did not impact plant growth (Kangasniemi and Oliver 1983). In Mattoon Lake the midge-caused damage to the meristem was extensive enough in many cases to cause blackened tissue, decay, and apparent death of the meristem as well as twisting of the flower stems and other leaf deformities characteristic of milfoil midge damage (Kangasniemi 1983). Because overall midge density was consistently high (1 per stem or greater; Table 4), and observed midge-caused meristem damage was also high, we concluded that midges were likely playing a key role in reducing Eurasian milfoil growth, indicating a need for additional research into midge herbivory on Eurasian milfoil in Washington.

The tardy caddisfly, also found in Mattoon Lake starting in 2007 (Table 4), has the ability to strip Eurasian milfoil of leaf material (Kangasniemi 1983). We have seen similar results at other lakes in Washington where tardy caddisfly densities have been noticeably high. The tardy caddisfly did not reach sufficient densities to cause this dramatic impact in Mattoon Lake; however, they may have contributed to the Eurasian milfoil decline by increasing plant stress.

Competition between the milfoil herbivores may have been a confounding factor in milfoil weevil establishment. The milfoil midge (and likely other herbivorous midges) and milfoil weevil depend on the apical meristem during larval development. Competition between herbivores on milfoil has been postulated by Johnson et al. (2000) for interactions between the moth *Acentria* and the milfoil weevil, and by Creed (1998) for the moth, milfoil weevil, and milfoil midge, all of which depend on the meristem. Thus, presence of meristem-dependent midges in Mattoon Lake could have slowed establishment of the milfoil weevil.

The overall macroinvertebrate community was similar to that found by Tamayo (2003) in eastern Washington lakes. The community was variable year to year, which may have been complicated by variations in the time of year samples were collected because abundance of some taxa fluctuate seasonally (Sloey et al. 1997, Balci and Kennedy 2003). Total macroinvertebrate abundance per stem was lowest in 2003 and then increased over the study period as Eurasian milfoil frequency and biomass declined (Table 4). This is consistent with the findings of Cheruvelil et al. (2002) who found increased macroinvertebrate density and biomass with decreasing cover of Eurasian milfoil. Also, Sloey et al. (1997) found that macroinvertebrate density was higher along Eurasian milfoil bed edges than in the bed centers. The decreasing Eurasian milfoil frequency and biomass in Mattoon Lake would have increased the bed edges, allowing macroinvertebrates to proliferate. Previous studies have also shown that diverse plant communities support a more diverse macroinvertebrate community than monocultures (Chilton 1990, Balci and Kennedy 2003). Thus, as other plant species recolonized the declining Eurasian milfoil beds, an increase in macroinvertebrate types and numbers would result.

Fish community and diet monitoring

Eight fish species were collected from Mattoon Lake each year in 2002 and 2008, 7 of which were the same each year: pumpkinseed sunfish, largemouth bass, yellow perch, rainbow trout, largescale sucker (*Catostomus macrocheilus* Girard), bridgelip sucker (*Catostomus columbianus* Eigenmann and Eigenmann), and brown trout. In 2002 one channel catfish (*Ictalurus punctatus* Rafinesque) was caught and in 2008 one goldfish (*Carassius auratus* L.) was caught.

The 2002 data are indicative of a fish community out of balance, with high numbers of small pumpkinseed sunfish and relatively few bass or other picivorous fish (Table 5). At that time, the plant community was dominated by topped out stands of Eurasian milfoil. Dense plant beds growing to the water surface are often associated with stunted fish communities (Engle 1995, Dibble et al. 1997). The dense plants, especially dense stands of Eurasian milfoil, support fewer macroinvertebrates to feed the fish and are also more difficult for fish to forage through (Dibble et al. 1997, Sloey et al. 1997, Valley and Bremigan 2002, Theel and Dibble 2008).

In 2008 the number of pumpkinseed sunfish had decreased whereas the weight increased, indicating fewer, larger fish than in 2002. In 2008 the largemouth bass population had increased by both number and weight compared with 2002 (Table 5). Yellow perch numbers decreased while the weight stayed the same, also indicating fewer, larger fish. Rainbow trout were the same by number and weight between the study years; however, this species is routinely stocked in the lake, so numbers may be independent of lake condition.

Table 5. Species composition by number and weight for common (≥1% by weight and number) fish captured at Mattoon Lake in 2002 and 2008.

	Species composition			
	number		weight (kg)	
Species	2002	2008	2002	2008
Pumpkinseed sunfish	1156	1070	13	34
largemouth bass	35	229	7	34
yellow perch	41	25	2	2
rainbow trout	14	14	2	2
largescale sucker	26	13	24	19

Relative weight data for the common species showed an improvement in condition in 2008 compared with 2002 for pumpkinseed sunfish and yellow perch (Divens 2008). This corroborates the species composition data that showed fewer but larger fish. The change in fish community structure from one of stunted forage fish and few picivores to one of fewer and better condition forage fish and more numerous picivores is common when dense invasive plant growth is managed (Savino and Stein 1982, Engle 1995, Dibble et al. 1997, Valley and Bremigan 2002, Theel and Dibble 2008). Similarly, a study by Lord et al. (2003) found that bluegill and pumpkinseed sunfish numbers positively correlated with Eurasian milfoil growth and negatively correlated with populations of the herbivorous moth *Acentria ephemerella* and the picivorous fish walleye (*Sander vitreus*). While other predators such as predacious invertebrates, birds, and bats could also have contributed to limiting the herbivore populations (Lord et al. 2003), this study found high sunfish numbers associated with high invasive plant growth and low macroinvertebrate populations.

Fish stomach content results showed that the diet of fish in Mattoon Lake varied both by year and by fish species (Table 6). Only one fish, a yellow perch in 2002, had nothing in its stomach. In the remaining fish, the overall dominant taxon in stomach samples was water fleas, planktonic animals that may or may not frequent vegetated areas, depending on species. They are well known as an important food source for fish (Smith 2001). The next most commonly consumed group was the Diptera-other, which included midge pupae and adults as well as other fly taxa. Other commonly consumed groups were midge larvae, mites, caddisflies, and copepods. Sutter and Newman (1997) also found these groups to be common in fish stomach samples.

Stomach content samples showed that the fish consumed some milfoil herbivores; one milfoil weevil adult was found in a pumpkinseed sunfish in 2008, representing a minor percent of the overall pumpkinseed diet (0.1%). As a caveat, these data were collected at the start of the milfoil weevil breeding season, at a time when weevil numbers were likely relatively low because all would have been survivors from the previous winter.

In 2002 we only identified caddisflies to order, but in 2008 we identified the tardy caddisfly separate from other caddisflies. Trout especially seems to target this species, which is a relatively large invertebrate that swims from plant to plant, a habit that makes them vulnerable to predation (Kangasniemi 1983). We found relatively few (0.07) of these caddisflies per milfoil stem in 2008, but a mean of nearly 5 per trout stomach were recovered from stomach samples, with 30 in one individual fish. This suggests that predation by trout could reduce population density of this potential milfoil biocontrol agent.

We can speculate on the impact fish predation had on the herbivorous midges. Midge larvae were common in the diet of all fish studied. The midges damaging Eurasian milfoil meristems in this study were generally in a case on the milfoil meristem. Evidence that fish were picking invertebrates off plants was found in some samples: in 2008 we noted Eurasian milfoil meristems in 2 rainbow trout stomachs, Eurasian milfoil and a chrionomid silk case in one yellow perch, and occasional plant pieces in pumpkinseed sunfish and largemouth bass stomachs. Therefore, there is circumstantial evidence that fish predation could exert pressure on the herbivorous midge community.

In conclusion, we accomplished our project objectives over the study duration. The first, that of collecting and rearing the milfoil weevil, was straight-forward. Once collected and placed in aquaria, the milfoil weevils propagated well and voraciously consumed the milfoil in their tanks. The greater challenge was collecting the brood stock; keen eyesight and a good attention span are a must.

TABLE 6. STOMACH CONTENT ANALYSIS RESULTS FROM FISH COLLECTED AT MATTOON LAKE IN MAY 2002 AND JUNE 2008. VALUES ARE THE SUM OF EACH TAXONOMIC CATEGORY FOR THAT FISH SPECIES. COMMON AQUATIC TAXA ARE INCLUDED (THOSE WITH ≥1% OF DIET IN AT LEAST ONE COLUMN, PLUS MILFOIL WEEVILS). FISH CODES ARE PS = PUMPKINSEED SUNFISH, YP = YELLOW PERCH, LMB = LARGEMOUTH BASS, TROUT = BROWN AND RAINBOW TROUT COMBINED. N IS THE SAMPLE SIZE.

	2002				2008			
Taxa	PS (N = 30)	YP (N = 35)	LMB (N = 18)	Trout (N = 19)	PS (N = 56)	YP (N = 18)	LMB (N = 21)	Trout (N = 14)
milfoil weevil	0	0	0	0	1	0	0	0
Amphipods	26	7	0	4	16	13	14	5
Water flea	1423	3829	979	55	1629	1715	295	2083
Copepod	192	954	108	30	5	2	5	1
midge larvae	379	231	27	71	58	94	4	158
Diptera - other	93	87	44	1341	48	40	111	347
mayfly	2	10	9	29	0	0	0	0
snails	0	2	0	2	9	55	0	5
leeches	0	0	0	0	2	6	0	134
mites	21	75	2	187	14	47	3	175
Dragonfly, damselfly	9	100	13	13	1	3	17	13
seed shrimp	8	7	0	3	18	9	5	4
fish	0	0	2	2	0	0	11	0
Tardy caddisfly (2008)	—	—	—	—	11	0	3	68
Trichoptera - other (2008)	—	—	—	—	33	6	20	1
Trichoptera - all (2002)	64	20	23	280	—	—	—	—

Results of the other 3 objectives were interdependent. During the 7 years spanning this study, Eurasian milfoil declined significantly in Mattoon Lake, and evidence points to a combination of insects causing the decline. In the first few years, herbivorous midges seemed to be a key impact on Eurasian milfoil growth. The milfoil weevil was likely present in low numbers between the start of augmentation and obvious establishment and may have contributed to the initial milfoil decline. In the last 2 years of the study, the milfoil weevil was present in numbers sufficient to cause milfoil declines, the tardy caddisfly was present, and the milfoil midge was present at least during the final year, so all likely contributed to the milfoil decline during that time.

Early in the study, Mattoon Lake had dense invasive plant growth, few macroinvertebrates, stunted forage fish competing for the limited and difficult to find food, and few large picivorous fish. By June 2008, however, the lake seemed more "in balance," with reduced Eurasian milfoil growth providing more diverse and lower growing aquatic vegetation, higher macroinvertebrate abundance associated with the Eurasian milfoil including a greater presence of herbivorous insects, and lower density and better condition pumpkinseed sunfish and yellow perch, accompanied by a higher density of largemouth bass. Difficult to determine is whether the milfoil decline preceded the shift away from the overpopulation of stunted pumpkinseed sunfish, or if another factor caused the pumpkinseed sunfish population to decline, thus allowing the milfoil herbivores and other macroinvertebrates to flourish. At the end of the study we found evidence of fish consuming the milfoil herbivores while the Eurasian milfoil was in decline, so limited predation pressure is likely not incompatible with maintaining populations of herbivorous insects. However, overpopulation of stunted pumpkinseed sunfish early in the study may have been facilitating the Eurasian milfoil dominance. Thus, this study supports the idea proposed by Ward and Newman (2006) that fish and macroinvertebrate herbivores can be important in lake trophic interactions, influencing primary production not only as algal growth, but also as macrophyte growth.

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