

# Fish predation on Eurasian watermilfoil (*Myriophyllum spicatum*) herbivores and indirect effects on macrophytes

Darren M. Ward and Raymond M. Newman

**Abstract:** We assessed the effect of fish predation on native and naturalized insect herbivores of the invasive aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum*) using manipulative field experiments within lakes and a field survey across lakes. For the field experiments, we manipulated sunfish (*Lepomis* spp.) density in cages in the littoral plant beds of two contrasting lakes: one with many sunfish, few watermilfoil herbivores, and abundant Eurasian watermilfoil; and one with few sunfish, many herbivores, and little Eurasian watermilfoil. Watermilfoil herbivores, including the milfoil weevil (*Euhrychiopsis lecontei*) and aquatic caterpillars, were suppressed by fish in both experiments. Herbivore density was also negatively related to sunfish abundance in the multiple-lake survey. We conclude that abundant sunfish can strongly suppress watermilfoil herbivores. Furthermore, in one of our experiments there was a marginally significant effect of fish exclusion on watermilfoil, suggesting that fish suppression of watermilfoil herbivores can have a positive, indirect effect on plant growth. Fish predation on macrophyte herbivores may be an important trophic interaction in freshwater lakes.

**Résumé :** Nous avons évalué les effets de la prédation par les poissons sur les insectes herbivores indigènes et naturalisés vivant sur la plante aquatique envahissante, le myriophylle à épis (*Myriophyllum spicatum*), par des expériences de manipulation en nature dans les lacs et par un inventaire de terrain dans une série de lacs. Dans les expériences de terrain, nous avons fait varier la densité des crapets (*Lepomis* spp.) dans des cages placées dans des herbiers littoraux de deux lacs très différents, l'un contenant beaucoup de crapets, peu d'herbivores du myriophylle et une forte densité de myriophylle à épis, l'autre avec peu de crapets, de nombreux herbivores et peu de myriophylle à épis. Les herbivores consommateurs de myriophylle, dont le charançon du myriophylle (*Euhrychiopsis lecontei*) et les chenilles aquatiques, ont été éliminés par les poissons dans les deux expériences. De plus, il existe une relation négative entre la densité des herbivores et l'abondance des crapets dans l'inventaire fait dans la série de lacs. Nous concluons que, lorsqu'ils sont nombreux, les crapets peuvent réduire fortement les populations d'herbivores consommateurs de myriophylle. De plus, dans l'une de nos expériences, il y avait un effet marginalement significatif de l'exclusion des poissons sur le myriophylle, ce qui laisse croire que la suppression des herbivores consommateurs de myriophylle peut avoir un effet positif indirect sur la croissance des plantes. La prédation par les poissons des herbivores consommateurs de macrophytes peut donc être une interaction trophique d'importance dans les lacs d'eau douce.

[Traduit par la Rédaction]

## Introduction

Changes in the aquatic macrophyte community, particularly those associated with exotic plant infestations, can drastically affect the functioning of lake ecosystems (Smith and Barko 1990; Madsen et al. 1991; Jeppesen et al. 1998). Despite historical opinion to the contrary, herbivores often play a key role in structuring aquatic macrophyte communities

(Lodge 1991; Newman 1991; Lodge et al. 1998). For example, herbivorous insects can suppress some invasive aquatic plants (Buckingham 1994; Creed and Sheldon 1995; Newman 2004). However, aquatic insects may be vulnerable to suppression by fish predators (Mittelbach 1988). If fish predators suppress key macrophyte herbivores, the interaction may have important cascading effects on lake ecosystems through a positive, indirect effect on macrophytes. In contrast, studies of indirect effects of fish on macrophytes to date have generally suggested negative effects of fish on macrophytes mediated by indirect, positive effects of fish on algae that shade macrophytes (Brönmark and Weisner 1992; Martin et al. 1992; Scheffer et al. 1993).

Here, we examine the impact of fish predation on herbivorous aquatic insects that are potential biological control agents for the invasive macrophyte Eurasian watermilfoil (*Myriophyllum spicatum*; hereafter referred to as milfoil); we also examine the implications of this interaction for milfoil growth. Milfoil is a rooted, submersed macrophyte that is one of the most widespread and problematic aquatic weeds

Received 7 June 2005. Accepted 24 November 2005.  
Published on the NRC Research Press Web site at  
<http://cjfas.nrc.ca> on 1 April 2006.  
J18732

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in North America. Milfoil often grows in dense, littoral plant beds, where its elongated stems form tangled surface mats that interfere with recreation and navigation and overwhelm native macrophytes (Smith and Barko 1990; Madsen et al. 1991). Milfoil infestations are also associated with overpopulation and stunting of sunfish (Engel 1995) and reduced abundance and diversity of littoral macroinvertebrates (Keast 1984; Cheruvilil et al. 2001). There have been some dramatic instances of successful control of milfoil by native and naturalized herbivorous insects, but herbivores do not reach sufficient density to suppress milfoil in many lakes (Newman 2004).

The primary milfoil herbivores and potential biological control agents in our Minnesota study lakes were the milfoil weevil (*Euhrychiopsis lecontei*) and the aquatic lepidopterans *Acentria ephemerella* and *Parapoynx* spp. The milfoil weevil is a native specialist herbivore of watermilfoils that has been shown to suppress milfoil growth in experiments (Creed and Sheldon 1995; Sheldon and Creed 1995; Newman et al. 1996) and has been associated with whole-lake milfoil declines (Creed and Sheldon 1995; Creed 1998; Newman and Biesboer 2000). Adult weevils live on the upper portion of the plant, laying individual eggs on milfoil meristems and feeding on stems and leaves. Milfoil weevil larvae cause most of the damage to milfoil by burrowing through the stem and consuming vascular tissue (Newman et al. 1996). Milfoil weevils produce three–five generations per summer (Mazzei et al. 1999). Aquatic caterpillars of the naturalized moth *A. ephemerella* are generalist herbivores that also show promise as milfoil control agents (Johnson et al. 1998, 2000; Gross et al. 2001). Caterpillars of the native moth genus *Parapoynx*, which have received some attention as potential control agents for milfoil (Habeck 1983), are also present in Minnesota.

Effects of fish predators on aquatic invertebrates are highly variable (Pierce and Hinrichs 1997), and macrophytes may interfere with fish predators (Crowder and Cooper 1982), but fish predation can dramatically suppress littoral aquatic insect populations (Mittelbach 1988). Previous investigations of fish predation on milfoil herbivores showed that yellow perch (*Perca flavescens*) do not generally consume milfoil weevils or affect weevil density (Creed 2000b). However, bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) sunfish do consume milfoil weevils and might limit weevil populations (Newbrough 1993; Sutter and Newman 1997). Sunfish rarely consume milfoil weevil larvae and pupae, as they live inside the milfoil stem, but adults are regularly consumed (Sutter and Newman 1997). Milfoil weevils do not appear vulnerable to most invertebrate predators (Newbrough 1993; Creed 2000b). Fish predation on lepidopterans has not been addressed in terms of milfoil control, but some studies suggest that aquatic caterpillars are vulnerable to fish predation (Mueller and Dearing 1994; Vromant et al. 1998; Dorn et al. 2001).

We performed field experiments to determine whether sunfish suppress milfoil herbivores and have a positive, indirect effect on milfoil. We used cages to manipulate sunfish density in littoral milfoil beds of two contrasting Minnesota lakes: Cedar Lake with abundant milfoil, low density of milfoil herbivores, and many sunfish; and Otter Lake with less milfoil, many herbivores, and few sunfish. Additionally,

we conducted surveys of milfoil herbivore density across lakes to determine if herbivore density was related to sunfish abundance at the whole-lake scale.

## Materials and methods

### Field experiments

#### *Cedar Lake*

Cedar Lake is in an urban park and residential area in Minneapolis, Hennepin County, Minnesota. Milfoil was first reported in Cedar Lake in 1990 and now dominates the macrophyte community (>60% of plant biomass; Newman and Maher 1995). Milfoil weevils and lepidopteran herbivores have been found only sporadically at Cedar Lake in summer surveys since 1994, and herbivores have had no apparent effect on milfoil density in Cedar Lake (Newman 2004). In a July 2000 fishery survey, the trapnet catch per unit effort of sunfish at Cedar Lake (Table 1) was more than four times the median catch for similar lakes statewide (MN DNR 2004).

We placed 20 cages at sites <2.2 m deep in a milfoil bed in the northeast bay of Cedar Lake in July 1998. Results of preliminary 1998–1999 experiments were similar and are in Ward (2002); results here are for 2000 and 2001. Cages consisted of 1.25 cm bar nylon mesh netting attached to a 3 m × 3 m × 1 m polyvinyl chloride (PVC) pipe frame (enclosed bottom area = 9 m<sup>2</sup>). Frames were anchored in the substrate with bricks; netting enclosed the frame sides and extended to floats at the water surface. Cages were open on the top and bottom. Ten cages were closed on all sides (fish enclosures) and 10 had two 1.5 m wide openings on opposite sides (open cages) that allowed fish to enter. Weevils were rare in Cedar Lake (0–0.04 total weevils-stem<sup>-1</sup> in 1998–2002 summer surveys), so half of the cages (five closed and five open cages) were stocked with adult milfoil weevils collected from other lakes (methods below). Treatments were assigned to cages in a randomized complete block design, with five blocks indicating position along the shoreline. The cages were folded down and left in place over the winter, but five cages had to be relocated in spring because of water level fluctuations or because repairs were required.

Adult milfoil weevils for stocking in the cages were collected from area lakes via snorkeling. Weevils were transported in sealable plastic bags (25–50 weevils-bag<sup>-1</sup>, each on a 6–10 cm long milfoil meristem) and were generally stocked within 48 h (72 h maximum). Stocking entailed tying the milfoil meristems, with associated weevils, to milfoil in the cages with biodegradable twine. In 2000, we stocked 125 adult weevils-cage<sup>-1</sup> between 7 and 19 July; in 2001, we stocked 175 adult weevils-cage<sup>-1</sup> between 5 June and 15 July (13.8–19.4 weevils-m<sup>-2</sup>, <0.2 weevils-stem<sup>-1</sup>). Stocking densities were within the range of densities observed in Minnesota lakes and below stem densities associated with milfoil suppression (>0.5-stem<sup>-1</sup>; Newman 2004).

We removed fish from enclosure cages prior to stocking weevils by angling and trapping with wire minnow traps. Removal of all fish was impossible and fish occasionally invaded cages, especially during a high water period that lifted cages off the sediment in 2001. Therefore, angling and trapping to remove fish were continued throughout the

**Table 1.** Characteristics of lakes where experiments and surveys were performed and experimental treatments used.

Lake	Lake area (ha)	% Littoral	Maximum depth (m)	Average Secchi (m)	Sunfish catch rate	Weevil density	Lepidoptera
<b>Experiments and surveys</b>							
Cedar*	68	37	15.5	1.7	101	Low	Absent
Otter†	134	99	6.4	2.7	2, 6‡	High, high‡	Present, present‡
<b>Surveys only</b>							
Auburn	106	61	11.3	2.4	113	Low	Present
Bald Eagle	513	59	11	0.9	64	Low	Absent
Calhoun	162	31	25	4.6	241	Low	Absent
Cenaiko	12	39	11	3.7	25	Low	Present
Centerville	184	61	5.8	0.9	35	High	Present
Independence	342	50	17.7	0.9	28	Low	Absent
Peltier	188	89	5.5	0.9	60	Low	Absent
Schultz	5	99	4	1.8	55	Low	Absent
Vadnais	159	35	17.7	2.4	20	High	Absent

**Note:** Lake characteristics and fish data are from Minnesota Department of Natural Resources (2004).

\*Fish enclosures (10) and open cages (10); weevils stocked in 5 of each.

†Fish enclosures (4) and enclosures (4); no weevil stocking.

‡Surveys were conducted in 2 years at Otter Lake.

experiment each summer. A subset of sunfish removed from cages after weevils were stocked in June 2001 was retained for diet analysis. The mesh netting on cages was too large to exclude fish <5 cm total length (TL); however, in trials in laboratory aquaria, bluegill <5 cm TL did not consume adult milfoil weevils (Ward 2002). Despite fish invasions, the number of sunfish >5 cm TL noted by snorkelers during biweekly visual cage surveys (2000) or by searching the entire cage for fish at each survey (2001) was lower in enclosures than in open cages (see Results).

We conducted biweekly visual surveys of milfoil weevil abundance in the cages by snorkeling. During surveys, we examined 100–150 milfoil stems evenly distributed within each cage, selecting stems from out of visual range to prevent bias. We counted visible milfoil weevil eggs, adults, pupal chambers, and stems recently mined by weevil larvae as an index of larval abundance. Mined stems show a characteristic darkening, lose rigidity, and exhibit entrance and exit holes for larvae. Tamayo et al. (2004) found that weevil density is positively correlated with counts of damaged stems. Fish in cages were also counted during surveys as noted above. Visual surveys that were conducted during the period of weevil stocking were performed at least 4 days after the most recent stocking.

Macrophytes and associated invertebrates in the cages were sampled via SCUBA prior to weevil stocking and at the conclusion of the experiment each year: 14 June and 31 August 2000 and 30 May and 31 August 2001. Divers clipped all plants in a 0.1 m<sup>2</sup> quadrat at the sediment surface and placed them in sealable plastic bags underwater. When processing samples, we rinsed invertebrates from the plants and later picked them from the washings. Plant species were separated and milfoil weevils, lepidopterans, and other endophytic and epiphytic invertebrates that remained on milfoil were picked under 8× magnification. Plants were spun in a salad spinner to remove excess moisture, weighed, dried to a con-

stant weight at 105 °C (≥48 h) and weighed again. Two samples per cage were obtained on each date and averaged; cages were treated as replicates.

### Otter Lake

Otter Lake is a shallow lake in a suburban park and residential area in Anoka County, Minnesota. Most of Otter Lake is littoral habitat (Table 1) and macrophytes grow over much of the lake area. Sunfish density at Otter Lake was much lower than that at Cedar Lake (Table 1). A near-complete fish kill occurred at Otter Lake in the winter of 2000–2001. In the summers of 2000 and 2001, milfoil weevil and lepidopteran larva densities in Otter Lake increased to some of the highest densities observed in Minnesota, concurrent with a dramatic, lake-wide decline in milfoil (Newman 2004).

In July 2001, we deployed eight cages at sites <2 m deep in a ~900 m<sup>2</sup> area on the west side of Otter Lake. Most milfoil in the study area was heavily damaged by weevil herbivory, but we attempted to enclose plants that were still growing. Cages were similar in construction to the Cedar Lake cages, but enclosed only 4 m<sup>2</sup> of bottom area (PVC frames were 2 m × 2 m). Ambient weevil densities in Otter Lake were very high (0.8 total weevils-stem<sup>-1</sup> in July), so weevils were not introduced into cages.

On 30 July 2001, we stocked four randomly selected cages with sunfish seined from a neighboring lake. We initially introduced five fish per enclosure cage (1.25 fish·m<sup>-2</sup>), approximating the upper quartile of densities seen by snorkelers in open cages at Cedar Lake in July 2001 (1.1 fish·m<sup>-2</sup>). The stocked fish were 9–15 cm TL, matching the most prevalent size class in Cedar Lake (MN DNR 2004). Fish density in cages was noted weekly, and if necessary, we replaced fish to maintain a density of five per cage. Some loss of fish occurred, but all fish enclosure cages contained at least three fish throughout the experiment. Many small

(<5 cm TL) sunfish appeared in Otter Lake in July and August 2001, probably the offspring of a few surviving fish. Juvenile fish were found at similar density in enclosure and enclosure cages and could freely pass through the netting. As noted above, sunfish <5 cm TL do not consume adult milfoil weevils. No sunfish >5 cm TL were observed in the experimental area during the experiment except those stocked in fish enclosures.

Visual surveys of milfoil weevil density in cages were conducted on 3, 7, and 23 August and 5 September. Visual surveys were conducted as at Cedar Lake except that all milfoil stems in each cage were examined for weevils because of low milfoil density (10–70 stems·cage<sup>-1</sup>). We could not count weevil pupal chambers at Otter Lake because of the short stature of the milfoil, so pupal counts were not included in total weevil densities for Otter Lake. On 15 September 2001, divers collected two plant samples from each cage. Plant sample collection and processing for plant biomass and invertebrates were the same as that for Cedar Lake.

### Data analysis

We used analysis of variance (ANOVA) to analyze macrophyte community characteristics and herbivore density in plant samples and multivariate repeated measures ANOVA to analyze weevil density from visual surveys. The ANOVA models for Cedar Lake were full factorials for weevil stocking and fish enclosure, blocked by location along the shoreline; the Otter Lake model was by fish enclosure–enclosure only. Here, we present analyses of total (sum of all life stages) weevil density from the visual surveys. Results of analyses of weevil density in plant samples were generally similar when density per unit area or density per unit milfoil mass was used as the response; only analyses of weevil density per unit milfoil dry mass are presented here. Lepidopterans are generalist herbivores, not restricted to milfoil, so density per unit plant mass and areal density were both analyzed. Count data were square-root-transformed to normalize errors. Statistical analyses were conducted using JMP 5.0 (SAS Institute Inc. 2002).

### Survey lakes

We surveyed milfoil herbivore density in eight milfoil-infested Minnesota lakes from Anoka, Carver, and Hennepin counties (Table 1) in midsummer 2002 to characterize the relationship between fish abundance and herbivore density. We selected lakes to span a range in sunfish density based on earlier fish surveys. To increase sample size, we included data from three additional lakes where fisheries surveys were performed and from which we had herbivore survey data from long-term study sites for the same year. For Lake Auburn in 2000, Cenaiko Lake in 2002, and Otter Lake in 2001 and 2002, we averaged our biweekly herbivore surveys to provide an average summer density.

The fish communities in the survey lakes were sampled in 2000, 2001, or 2002 (MN DNR 2004). The standardized Minnesota Department of Natural Resources fish surveys consisted of 24 h littoral trapnet sets using at least five nets per lake. We used the mean catch rate per net of all sunfish species combined in the analyses (bluegill, pumpkinseed, bluegill × pumpkinseed hybrids, and green sunfish (*Lepomis cyanellus*)). Owing to large mesh size, trap nets only captured

fish >6 cm TL, corresponding to the size known to eat adult milfoil weevils. We also compiled lake physical characteristics from the fisheries surveys to determine whether the relationship between fish abundance and herbivore density was confounded with habitat differences (Table 1).

We sampled herbivores in the survey lakes in late July or early August 2002 by snorkeling five transects perpendicular to the shoreline through the plant bed of each lake and collecting eight milfoil meristems (top ~30 cm) at five points on each transect. Sampling points were equally spaced through the width of the plant bed, and transects were equally spaced around the perimeter of the lake, constrained to locations with milfoil beds present. For long-term study site lakes, we sampled herbivores by the same snorkeling method, but along permanently marked transects in a milfoil bed. Milfoil herbivores were picked from stems under 8× magnification.

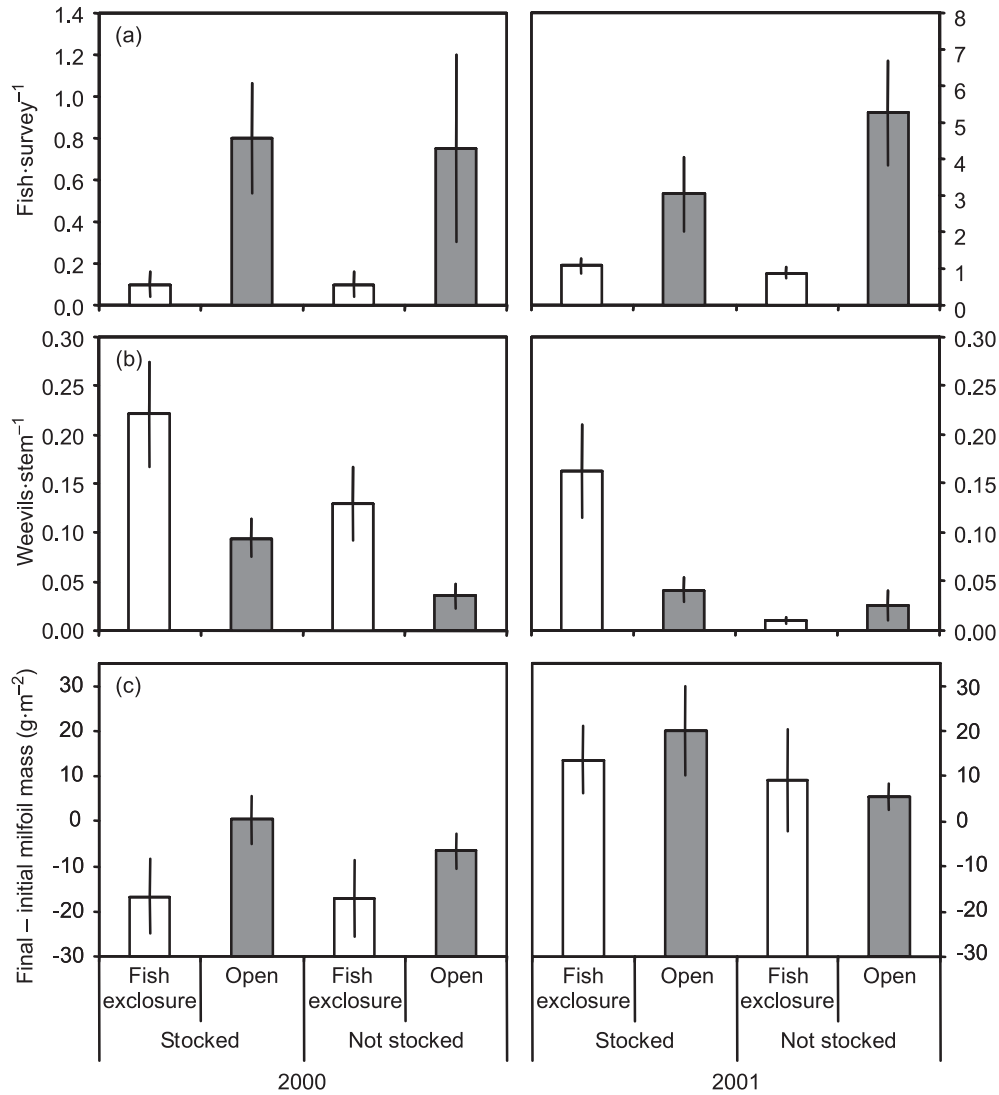
## Results

### Cedar Lake

Snorkelers observed fewer sunfish >5 cm TL in fish enclosures than in open cages during biweekly visual cage surveys in 2000 and when searching the entire cage for fish at each survey in 2001 (Fig. 1; blocked ANOVA for open–closed cages in 2000:  $F_{[1,14]} = 6.44$ ,  $P = 0.02$ ; in 2001:  $F_{[1,14]} = 12.65$ ,  $P = 0.003$ ). Counts of sunfish <5 cm TL were not significantly different between cage treatments in 2000 (mean fish per survey ± standard error (SE): closed cages  $1.33 \pm 0.77$ , open  $0.42 \pm 0.27$ ;  $F_{[1,14]} = 1.20$ ,  $P = 0.30$ ), but were lower in fish enclosures than in open cages in 2001 (closed  $3.89 \pm 0.45$ , open  $5.88 \pm 0.61$ ;  $F_{[1,14]} = 8.21$ ,  $P = 0.01$ ). Sunfish were the most frequently encountered fish in visual cage surveys, but snorkelers also occasionally observed juvenile largemouth bass (*Micropterus salmoides*) and small cyprinids in cages (<10% of total cage surveys). Sunfish in cages preyed on adult milfoil weevils. Twenty-five percent of sunfish >6 cm TL sampled from Cedar Lake in June 2001 had adult weevils in their stomachs ( $N = 20$  fish total).

Weevil density was higher in fish enclosures stocked with weevils than in open cages in both years of the experiment at Cedar Lake (Fig. 1). In 2000, there were significant, positive effects of both fish enclosure (between subjects  $F_{[1,12]} = 12.56$ ,  $P = 0.004$ ) and weevil stocking ( $F_{[1,12]} = 9.05$ ,  $P = 0.01$ ) on season mean weevil abundance, but not a stocking × enclosure interaction ( $F_{[1,12]} = 0.01$ ,  $P = 0.99$ ). In 2001, there was a strong stocking × enclosure interaction (between subjects  $F_{[1,12]} = 8.10$ ,  $P = 0.01$ ), as weevils reached much higher densities in fish enclosures where they were stocked than in other cage types (Fig. 1). Main effects of weevil stocking ( $F_{[1,12]} = 16.48$ ,  $P = 0.002$ ) and fish enclosure ( $F_{[1,12]} = 4.22$ ,  $P = 0.06$ ) were also significant in 2001. There were marginal time effects on total weevil density in 2000 ( $F_{[3,10]} = 2.73$ ,  $P = 0.10$ ) and significant time effects in 2001 ( $F_{[6,7]} = 13.45$ ,  $P = 0.002$ ). Final weevil densities were much higher than initial density in 2000, particularly in fish enclosures, but in 2001 weevil density declined through the season in all cage types following an initial increase in fish enclosure cages stocked with weevils. There were no significant time × treatment interactions for total weevil density in either year (all within interactions

**Fig. 1.** (a) Mean number of sunfish (*Lepomis* spp.) and (b) milfoil weevil (*Euhrychiopsis lecontei*) density (sum of all life stages) observed in experimental cages at Cedar Lake in visual surveys through the season. (c) Change in Eurasian watermilfoil (*Myriophyllum spicatum*) dry mass in cages over the season for experiments in 2000 and 2001.  $N = 5$  cages·treatment<sup>-1</sup>. Error bars are  $\pm 1$  standard error. Larger scale for fish counts in 2001 based on intensive search of cages for fish.



2000: Pillai's trace approximate  $F_{[21,36]} = 0.83, P = 0.61$ ; 2001: Pillai's trace approximate  $F_{[42,72]} = 1.24, P = 0.21$ ).

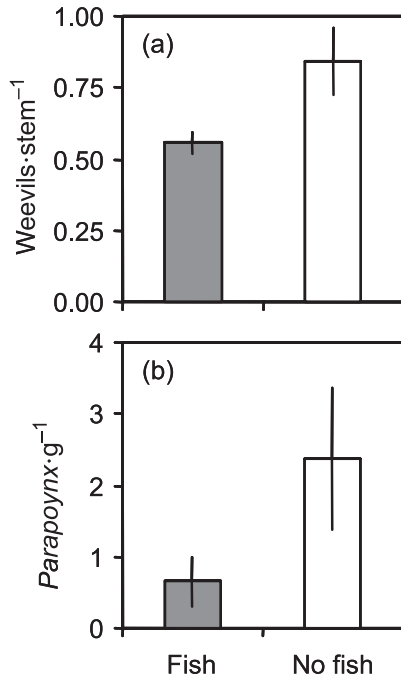
For both years, weevil densities on plants from samples at the end of the experiment followed a similar pattern to visual surveys, but variability was higher (2000 means for weevils per gram dry milfoil  $\pm$  SE: stocked enclosure  $0.17 \pm 0.08$ , unstocked enclosure  $0.05 \pm 0.03$ , stocked open  $0.16 \pm 0.03$ , unstocked open  $0.02 \pm 0.02$ ; 2001 means: stocked enclosure  $0.11 \pm 0.09$ , unstocked enclosure  $0$ , stocked open  $0.02 \pm 0.01$ , unstocked open  $0.004 \pm 0.004$ ). Thus, differences among treatments for these samples were only marginally significant in 2000 (full model test:  $F_{[7,12]} = 2.51, P = 0.08$ ) and not significant in 2001 (full model test:  $F_{[7,12]} = 1.06, P = 0.44$ ).

Caterpillars were rare in Cedar Lake during the experiment. We found four *A. ephemera* larvae in plant samples in 2000, two *A. ephemera* in 2001, and no *Parapoynx*

spp. in either year. All *A. ephemera* larvae we found at Cedar Lake were in plant samples from fish enclosure cages.

Milfoil was present in all 160 plant samples from Cedar Lake in 2000 and 2001 and constituted 81% of the total plant biomass sampled. Ten other macrophyte species were collected in Cedar Lake, but *Ceratophyllum demersum* was the only common macrophyte besides milfoil (12% of total plant biomass). Final total plant dry mass and milfoil dry mass were highly variable, and there were no significant treatment effects on final mass in either year (all  $P > 0.25$ ). However, in 2000 milfoil dry mass declined marginally more in fish enclosures than in open cages through the season (Fig. 1;  $F_{[1,12]} = 3.92, P = 0.07$ ). There was no effect of weevil stocking ( $F_{[1,12]} = 0.28, P = 0.61$ ) or a stocking  $\times$  enclosure interaction ( $F_{[1,12]} = 0.23, P = 0.64$ ) in 2000, and there were no significant treatment effects on change in milfoil mass in 2001 (Fig. 1; full model test:  $F_{[7,12]} = 0.45, P = 0.86$ ).

**Fig. 2.** (a) Mean number of milfoil weevils (*Euhrychiopsis lecontei*) per stem from visual surveys of experimental cages (sum of all life stages) and (b) density of *Parapoynx* spp. caterpillars from final plant samples in cages for the experiment at Otter Lake in 2001.  $N = 4$  cages-treatment<sup>-1</sup>. Error bars are  $\pm 1$  standard error.

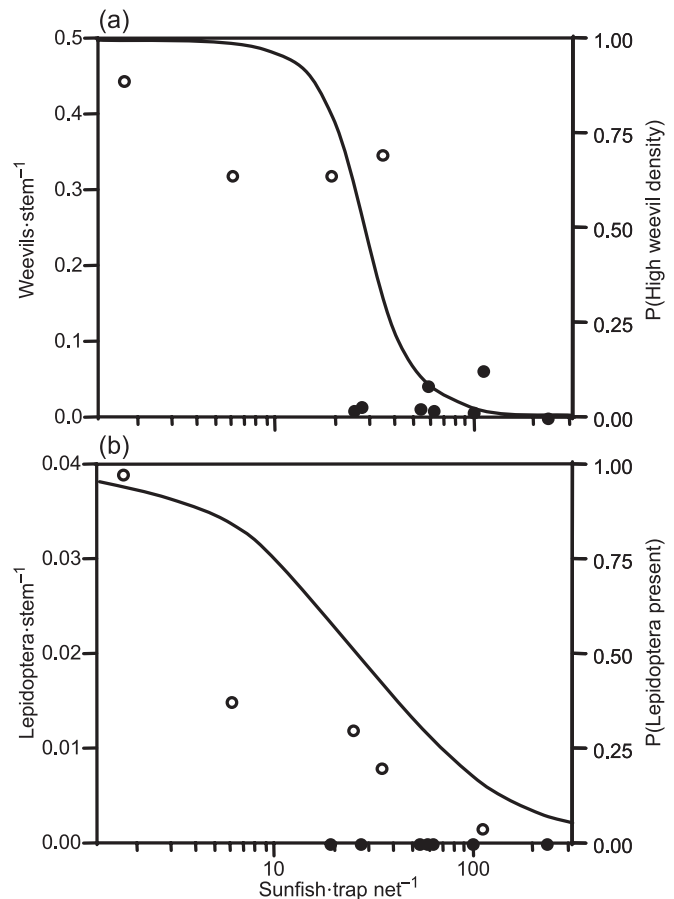


### Otter Lake

Total mean weevil density through the season from visual surveys was lower in fish enclosures than in fishless cages at Otter Lake (Fig. 2;  $F_{[1,6]} = 5.17$ ,  $P = 0.06$ ), primarily because of reduced density of eggs in fish enclosures (mean eggs per stem  $\pm$  SE: fish enclosures  $0.21 \pm 0.03$ , fishless cages  $0.08 \pm 0.01$ ). As at Cedar Lake, weevil density in plant biomass samples followed a similar trend but was more variable (density per gram dry milfoil  $\pm$  SE: fish enclosures  $0.63 \pm 0.28$ , fishless cages  $1.1 \pm 0.15$ ;  $F_{[1,6]} = 1.84$ ,  $P = 0.22$ ). *Parapoynx* spp. density was also marginally lower in fish enclosures (Fig. 2; density per square metre:  $F_{[1,6]} = 5.74$ ,  $P = 0.05$ ; density per gram dry milfoil:  $F_{[1,6]} = 3.81$ ,  $P = 0.09$ ). *Acentria ephemera* were relatively rare and density was similar across treatments (mean *Acentria* per gram dry milfoil  $\pm$  SE: fish enclosures  $0.073 \pm 0.037$ , fishless cages  $0.17 \pm 0.09$ ;  $F_{[1,6]} = 1.07$ ,  $P = 0.34$ ).

We collected 14 macrophyte species from cages in Otter Lake; milfoil was only 24% of total plant biomass at Otter Lake, and the plant community was dominated by *Chara* spp. (47% of plant mass). Although mean final milfoil dry mass in fish enclosures was double that in fishless cages (fish cages:  $2.5 \pm 1.1$  g·m<sup>-2</sup>; fishless cages:  $1.2 \pm 0.21$  g·m<sup>-2</sup>), the difference between treatments was not significant ( $F_{[1,6]} = 1.29$ ,  $P = 0.30$ ). Dry mass of *Zosterella dubia*, a relatively minor component of the macrophyte community, was higher in cages with fish (enclosures:  $0.17 \pm 0.06$  g·m<sup>-2</sup>; enclosures:  $0.02 \pm 0.01$  g·m<sup>-2</sup>;  $F_{[1,6]} = 5.36$ ,  $P = 0.06$ ), associated with

**Fig. 3.** (a) Scatterplot of total milfoil weevil (*Euhrychiopsis lecontei*) density and sunfish (*Lepomis* spp.) catch rate across lakes. The curve is the probability of high weevil density from the logistic fit of categorical weevil density on sunfish catch rate, with categorical grouping indicated by solid (low weevil density) and open (high weevil density) circles. (b) Scatterplot of lepidopteran density and sunfish catch rate across lakes. The curve is the probability that lepidopterans were present in samples from the logistic fit of lepidopteran presence (open points) or absence (solid points) on sunfish catch rate.



suppression of the generalist herbivore *Parapoynx* spp. There was a strong negative relationship of *Z. dubia* biomass with *Parapoynx* spp. density (linear regression equation:  $\ln(Z. dubia \text{ mass}) = -1.95 - 0.021 \times (Parapoynx \text{ spp.} \cdot \text{m}^{-2})$ ;  $F_{[1,6]} = 13.1$ ,  $P = 0.02$ ,  $r^2 = 0.72$ ), excluding one cage with a sandy substrate in which all plants were at very low density.

### Survey lakes

There was a negative relationship between sunfish abundance and herbivore density across lakes (Fig. 3). Total density of milfoil weevils (sum of all life stages) across lakes was strongly bimodal, so we classified lakes as high ( $>0.3$  weevils·stem<sup>-1</sup>) or low ( $<0.1$  weevils·stem<sup>-1</sup>) weevil density and analyzed qualitative weevil density using logistic regression. Sunfish catch rate was a highly significant predictor of qualitative weevil density ( $G^2 = 8.77$ , 1 df,  $P = 0.003$ ) and explained 57% of the uncertainty in qualitative total weevil density. The logistic model suggests a threshold catch rate of 25–30 sunfish-trap net<sup>-1</sup>, above which weevil populations

will be at low density ( $<0.1$  weevil-stem<sup>-1</sup>; Fig. 3). Adult weevil density was negatively related to  $\log_{10}$  sunfish catch rate (linear regression equation: adult weevils per stem =  $0.146 - 0.071 \times \log_{10}(\text{sunfish catch rate})$ ;  $F_{[1,10]} = 24.41$ ,  $P < 0.001$ ,  $r^2 = 0.71$ ). None of the lake habitat characteristics were associated with weevil density (all  $G^2 < 0.97$ ,  $P > 0.33$ ).

We only found aquatic caterpillars (*A. ephemere* or *Parapoynx* spp.) in 5 of the 12 lake surveys, and they were always at low density when present ( $<0.04$  total larvae-stem<sup>-1</sup>). Lepidopterans were more likely to be absent from lakes with high fish density (Fig. 3; logistic regression of presence or absence on fish density:  $G^2 = 3.48$ , 1 df,  $P = 0.06$ ), and total lepidopteran density was negatively related to  $\log_{10}$ -transformed sunfish catch rate (linear regression equation: lepidopteran larvae per stem =  $0.032 - 0.017 \times \log_{10}(\text{sunfish catch rate})$ ;  $F_{[1,10]} = 25.50$ ,  $P < 0.001$ ,  $r^2 = 0.72$ ). Lepidoptera were marginally more likely to be absent from deeper lakes ( $G^2 = 2.79$ , 1 df,  $P = 0.09$ ), but no other lake habitat characteristics were associated with absence of lepidopterans (all  $G^2 < 1.57$ ,  $P > 0.21$ ).

## Discussion

Predatory sunfish reduced the density of milfoil herbivores. Milfoil weevils were suppressed by fish in our manipulative experiments in both Cedar Lake (high fish density) and Otter Lake (low fish density). *Parapoynx* spp. was suppressed by fish in the experiment at Otter Lake, and lepidopterans were rare and found only in fish enclosures at Cedar Lake. Across lakes, both milfoil weevil and lepidopteran densities were negatively related to sunfish abundance. As in some other studies (Newman et al. 2001; Tamayo et al. 2004), we found that general lake habitat characteristics did not explain variation in herbivore density across lakes. Other recent studies in New York and Washington also suggest that sunfish can limit herbivorous insect densities (Lord 2004; R.L. Johnson, Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA, personal communication; J. Parsons, Washington State Department of Ecology, Yakima, WA 98902, USA, personal communication).

This and other studies have demonstrated that sunfish do consume milfoil weevils (Newbrough 1993; Sutter and Newman 1997). However, predator avoidance or behavioral changes may also explain some of the impacts of predators on herbivore density. Reduced weevil density in the Otter Lake fish enclosures was due primarily to reduced density of weevil eggs, suggesting a potential effect of predators on weevil oviposition rate. Weevils also moved in and out of experimental cages (D. Ward, personal observation), so movement in response to local predation risk may have been a factor in fish effects on weevil densities in our small-scale experiments (Englund 1997). However, the strong relationship of fish abundance and herbivore density in our across-lakes survey indicates that the interaction has important large-scale consequences. The potential for behaviorally mediated interactions between fish and macrophyte herbivores merits further investigation.

Although milfoil herbivores were suppressed by fish, we only saw marginally significant evidence of a cascading effect on milfoil in one experiment. In the 2000 experiment

at Cedar Lake, milfoil mass declined marginally more over the season in cages that excluded fish. Final weevil densities in fish enclosures in our experiment at Cedar Lake in 2000 ( $0.40 \pm 0.18$  total weevils-stem<sup>-1</sup>) were slightly lower than densities associated with weevil suppression of milfoil in other lakes ( $>0.5$  weevils-stem<sup>-1</sup>; reviewed in Newman 2004). Even with local-scale protection from predators, weevil populations did not increase sufficiently to dramatically suppress milfoil within the time frame of our experiments. At Otter Lake, fish suppressed herbivores, but not sufficiently to allow milfoil to recover; final weevil density in fish enclosures at Otter Lake was still high ( $0.54 \pm 0.04$  weevils-stem<sup>-1</sup>). The temporal scale of our cage experiments may have been too short to detect the strongest effects of milfoil weevils on milfoil, as they do not directly consume much plant biomass and may influence milfoil density primarily by reducing overwinter survival (Creed and Sheldon 1995; Newman et al. 1996; Newman and Biesboer 2000). We also observed a positive response of another macrophyte, *Z. dubia*, to fish suppression of the generalist lepidopteran herbivore *Parapoynx* spp., suggesting that our proposed effect of fish on macrophytes is not limited to milfoil and specialist herbivores.

Our results showing suppression of milfoil herbivores by sunfish indicate the potential for an important sunfish-herbivore-macrophyte trophic cascade in lake littoral zones. This is supported by other studies that have shown dramatic declines of milfoil associated with milfoil herbivores, in both controlled experiments (e.g., Sheldon and Creed 1995; Newman et al. 1996; Gross et al. 2001) and natural lake systems (e.g., Creed and Sheldon 1995; Johnson et al. 2000; Newman and Biesboer 2000). The milfoil weevil and herbivorous Lepidoptera can cause dramatic reductions in milfoil biomass (50%–95%) in lakes when adequate densities of herbivores are attained (reviewed in Newman 2004). Creed (2000a) even suggested that the milfoil weevil is a keystone species in North American lakes because of its ability to suppress competitively dominant milfoil. Thus, fish predation on macrophyte herbivores may have important consequences for lake ecosystems.

Most investigations of the indirect effects of fish predators on littoral macrophytes have focused on pelagic trophic cascades affecting water clarity (Scheffer et al. 1993) or on fish predation on snails releasing periphyton (Brönmark and Weisner 1992; Martin et al. 1992; Brönmark and Vermaat 1998), with negative, indirect effects on macrophytes via shading (but see Sheldon 1987 relating to direct snail herbivory on macrophytes). In contrast with these negative, indirect effects of fish on macrophytes mediated by shading, littoral fish can have a positive, indirect effect on macrophytes in the trophic cascade we propose. As dense macrophyte stands are often associated with overpopulation and high littoral density of sunfish (Mittelbach 1988; Valley and Bremigan 2002), high milfoil density could set up a self-reinforcing feedback loop; littoral sunfish density increases when milfoil invades and the fish maintain a low density of herbivorous insects.

Sunfish predation likely accounts for the observed failure of native and naturalized herbivorous insects to control milfoil in many lakes. Adequate densities of herbivores must be sustained over the season to control the plant (Newman

2004). Our results suggest that sunfish densities >25–30 per 24-hour trapnet set will limit herbivore densities to very low levels, which are inadequate to effect control. The two cases of successful lake-wide suppression of milfoil by herbivores we have observed in Minnesota occurred during periods of low sunfish density (Newman and Biesboer 2000; Newman 2004). Similarly, strong effects of weevils on milfoil were observed in Brownington Pond, Vermont (Creed and Sheldon 1995), a lake with no bluegill or pumpkinseed. Furthermore, predation by sunfish may be an important factor limiting the success of insects as biological controls of other submersed macrophytes, such as *Hydrilla*. Although numerous aquatic macrophytes have been controlled with insects, all of the well-documented successes are with floating-leafed or emergent plants, where control agents are not susceptible to predation by fish (Newman 2004).

## Acknowledgements

We thank John Foley, Aaron Berger, Chris Lemmon, and numerous undergraduates for assistance in the field. Michelle Marko, Bruce Vondracek, and Sandy Weisberg reviewed an earlier draft. Comments by Rob Creed, Carol Folt, Julia Butzler, Roxanne Karimi, Kate Schofield, and several anonymous reviewers improved the manuscript. Rick Walsh and others at the Minnesota Department of Natural Resources provided fish community data. This research was funded by grants from the Minnesota Department of Natural Resources, based on funds appropriated by the Minnesota Legislature as recommended by the Legislative Commission on Minnesota Resources from the Minnesota Future Resources Fund, and by the Minnesota Agricultural Experiment Station. DMW was also supported by a University of Minnesota Graduate School fellowship.

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