

# Epiphytic Macroinvertebrates Along a Gradient of Eurasian Watermilfoil Cover

KENDRA SPENCE CHERUVELIL<sup>1</sup>, PATRICIA A. SORANNO<sup>1</sup> AND JOHN D. MADSEN<sup>2,3</sup>

## ABSTRACT

The exotic macrophyte Eurasian watermilfoil (*Myriophyllum spicatum* L.) has spread throughout North America and has come to dominate macrophyte communities in many North temperate lakes. Because of the central role that macrophytes play in littoral zone foodweb interactions, the spread of this nuisance species and subsequent macrophyte management actions could potentially alter many foodweb

interactions. In particular, Eurasian watermilfoil's morphology and its dense canopies may influence the macroinvertebrate communities associated with macrophytes. In this study, we examined how Eurasian watermilfoil cover affects epiphytic macroinvertebrate biomass in six southern Michigan lakes that range from 20 to 95% cover of Eurasian watermilfoil. The Eurasian watermilfoil gradient was created by treating three lakes with 5 ppb fluridone (Sonar®) in May 1997. We quantified epiphytic macroinvertebrate biomass and macrophyte cover in July and August 1999. Our results show that macroinvertebrate biomass on the dominant plant species in a lake may decrease as percent Eurasian watermilfoil cover increases in lakes. In addition, we did not detect negative effects of the use of fluridone on macroinvertebrate biomass two years post-treatment.

*Key words:* *Myriophyllum spicatum*, exotic, macrophyte, plant architecture, fluridone.

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<sup>1</sup>Department of Fisheries and Wildlife, Michigan State University, 3 Natural Resources Building, E. Lansing, MI 48824. Corresponding author's E-mail: spencek1@msu.edu.

<sup>2</sup>U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180.

<sup>3</sup>Current Address: Department of Biological Sciences, S242 Trafton Science Center, Minnesota State University, Mankato, MN 56001. Received for publication July 17, 2000 and in revised form October 12, 2001.

## INTRODUCTION

Macrophytes are diverse in shape and form, and their role in the foodweb is dependent on their diversity, abundance, and community composition, which are affected by human management practices (Olson et al. 1998). Macrophyte physical structure, also known as architecture (based on the number, morphometry, and arrangement of stems, branches, and leaves; Lillie and Budd 1992), varies across species and has been shown to influence macroinvertebrate colonization of macrophytes (Jackson 1997). Specifically, macrophytes with finely dissected leaves may support more macroinvertebrates than macrophytes with broader, undissected leaves (Krecker 1939, Gerrish and Bristow 1979, Cheruvilil et al. 2000), although this pattern continues to be debated (Cyr and Downing 1988a, b, Brown et al. 1988, Parsons and Matthews 1995).

More macroinvertebrates may colonize dissected-leaf plants because they have a higher surface area to volume ratio (but see Sher-Kaul et al. 1995) and therefore provide more habitat for macroinvertebrate colonization, more food for grazing macroinvertebrates in the form of periphyton, or additional complexity which offers better refuge from predators (Dvorak and Best 1982, Gilinsky 1984, Pardue and Webb 1985). However, dominance by an individual species may alter this pattern between macrophytes and macroinvertebrates. For example, Eurasian watermilfoil (hereafter milfoil, *Myriophyllum spicatum* L.) is an exotic submersed macrophyte found in much of temperate North America (Couch and Nelson 1985). Milfoil forms dense surface canopies that suppress native macrophyte growth and can lead to homogeneous macrophyte beds (Aiken et al. 1979, Madsen et al. 1988, 1991). In fact, milfoil has been found to support fewer invertebrates than native macrophyte species (Soszka 1975, Keast 1984, Cattaneo et al. 1998) even though it is a dissected-leaf plant, it has a higher surface area than four other macrophyte species with the same unit of biomass (*Nitellopsis obtuse* Desv., *Potamogeton lucens* L., *Potamogeton perfoliatus* L., *Potamogeton pectinatus* L., Sher-Kaul et al. 1995), and it has a low frequency of interstices (Dibble et al. 1996). These low macroinvertebrate densities associated with milfoil may be a consequence of milfoil's dense homogeneous canopies that can alter the underlying chemical and physical environment making it inhospitable to macroinvertebrates (Unmuth et al. 2000).

In this paper, we examine how milfoil cover affects the interactions between macrophytes and epiphytic macroinvertebrates. Specifically, we determined whether macroinverte-

brate density and biomass on the dominant plant species in a lake varies predictably with percent of littoral zone covered with milfoil. We quantified macrophyte cover and macroinvertebrate density and biomass in six lakes with varying percent milfoil cover. This milfoil cover gradient was created by treating three lakes with 5 ppb fluridone (Sonar®, SePRO Corporation, Indianapolis, IN), which has been suggested as a milfoil management tool. Although our study design prevents us from attributing our observed patterns conclusively to fluridone treatment, we can make qualitative comparisons about macroinvertebrate biomass between lakes treated with fluridone and those that were not.

Because milfoil forms dense homogeneous canopies, which may support low macroinvertebrate densities and biomass, we hypothesized that macroinvertebrate density and biomass per plant mass of the dominant plant species in a lake will decrease as percent milfoil cover increases across lakes, even though milfoil is a dissected plant. Thus, we would expect that lakes treated with fluridone for milfoil management would have higher macroinvertebrate density and biomass than lakes not managed using fluridone.

## MATERIALS AND METHODS

*Study Area:* Macrophytes and epiphytic macroinvertebrates were sampled from six lakes in southern Michigan. The lakes are mesotrophic and fall along a gradient of percent milfoil cover (Table 1). The three lakes low on the milfoil gradient (Camp, Big Crooked, and Lobdell) had little milfoil because they were treated in May 1997 with 5 ppb fluridone (Sonar®). The other three lakes (Heron, Big Seven, and Clear) were chosen because they had high percent milfoil cover. All lakes underwent some plant management including harvesting and spot herbicide treatments.

*Sampling:* Macrophytes were sampled in August of 1998 and 1999 in the six lakes using the point intercept method (Madsen 1999). Each lake was mapped using a geographic information system and then overlaid with a grid of points to be surveyed (150 to 250 points per lake). Points were located with a global positioning system. At each survey point, water depth was measured, a two-sided rake was thrown, and macrophyte species presence/absence was recorded (Getsinger et al. 2001).

Using 1998 macrophyte data, the five most common submersed macrophyte species for each lake were selected for epiphytic macroinvertebrate sampling in summer 1999. Less

TABLE 1. LAKE CHARACTERISTICS. TP IS TOTAL PHOSPHOROUS, TN IS TOTAL NITROGEN, AND CHL A IS CHLOROPHYLL A. ALL WATER QUALITY DATA ARE AVERAGES FROM INTEGRATED EPIILIMNETIC SAMPLES TAKEN MONTHLY (JUNE, JULY, AND AUGUST). PERCENT MILFOIL COVER IS THE PERCENT OF THE VEGETATED LITTORAL ZONE OF EACH LAKE WITH MILFOIL PRESENT. THE LITTORAL ZONE IS DEFINED AS THE AREA FROM SHORE TO THE DEEPEST POINT AT WHICH PLANTS CONSISTENTLY OCCUR.

Lake	Latitude, longitude	% Milfoil cover	Lake area (ha)	Mean depth, m (max)	% Littoral area	Secchi depth (m)	Epilimnion depth (m)	Pelagic Chl a (µg/L)	TN (µg/L)	TP (µg/L)
Camp	43.11 N, 85.40 W	20	53.5	7.3 (15)	39	3.0	4.9	11.1	478.6	32.3
Big Crooked	43.03 N, 85.23 W	25	63.9	4.5 (18.3)	55	3.4	4.3	9.2	496.9	25.6
Lobdell	42.47 N, 83.50 W	55	196.9	2.7 (21.3)	83	3.5	4.5	3.6	431.4	16.7
Heron	42.81 N, 83.52 W	54	53.5	3.4 (12.2)	80	4.1	5.0	5.3	380.7	16.8
Clear	42.30 N, 85.16 W	88	72.5	2.2 (4.6)	89	3.6	4.5	11.6	543.9	23.0
Big Seven	42.49 N, 83.40 W	95	64.2	3.2 (15)	82	4.3	4.3	3.9	421.8	18.2

common species were sampled in a few cases in order to collect at least two species within each plant architecture type (dissected and undissected) and to include milfoil in each lake. We adapted the final list on-site for seasonal and inter-annual changes that occurred from 1998 to 1999. Epiphytic macroinvertebrates were sampled in each lake twice during summer 1999 (June 28 to July 7 and August 16 to August 24). A snorkeller sampled individual plant stems with a 500  $\mu$ m mesh bag sampler measuring 65 cm long by 24 cm in diameter (Cheruvilil et al. 2000). In each lake, epiphytic macroinvertebrates were sampled at 3 to 5 sites separated by greater than 100 m. Each site was approximately 2 m deep and consisted of heterogeneous macrophyte beds. Based on power and sample size analyses from data collected in one of the lakes in August 1998 (Cheruvilil et al. 2000), 2 to 4 stems from each of the five macrophyte species were randomly sampled from approximately a 10 m radius around an anchored boat at each site. This sampling scheme resulted in 13 individuals of each macrophyte species, or 65 samples per lake per date (except Camp Lake in July when only four macrophyte species were sampled), totaling ~800 samples. Individual samples (macrophyte stem, associated macroinvertebrates, and water) were stored in a sealed plastic bag and kept cool and dark until further processing.

In the lab, individual macrophyte stems were rinsed with water to detach macroinvertebrates; the macrophytes were dried at 105C for 48 hours and weighed to estimate plant mass. Macroinvertebrates were preserved in 95% ethanol. For each lake and plant species, the 13 replicate samples were pooled and subsampled using methods developed by Waters (1969). Subsamples were counted until at least 140 individuals had been counted, which resulted in density estimates within 20% of the mean. Macroinvertebrates were identified to the lowest possible taxonomic level (genus, tribe, or family). Each individual was measured to the nearest  $\mu$ m with a drawing tube and digitizing tablet. Macroinvertebrate biomass was estimated from body lengths using length-dry weight regressions from the literature (Rogers et al. 1977, Smock 1980, Meyer 1989, Burgherr and Meyer 1997, G. G. Mittelbach, unpublished data).

*Data Analysis:* We analyzed macroinvertebrates along a gradient of percent milfoil cover across six lakes. Using macrophyte data collected in 1999, we calculated a milfoil gradient that included all submersed and floating-leaved macrophytes, (we excluded emergent and free-floating macrophytes from analysis). For each lake, the littoral zone was defined as the zone from shore to the deepest point at which macrophytes consistently occurred. Within the littoral zone, we calculated the percent of sites that were vegetated. To calculate the milfoil gradient we calculated the percent of the vegetated littoral zone that had milfoil present (sum of milfoil points divided by the number of vegetated sites in the littoral zone multiplied by 100).

For all analyses, macroinvertebrate densities and biomass were standardized by plant dry mass (g), which allows for the comparison of macroinvertebrates among different macrophyte species and architecture types. We report results for July and August separately rather than as an average because macroinvertebrate life cycles are short and periodic; thus, density, biomass, and species composition change throughout

the summer (Gaufin et al. 1956, Mracheck 1966, Merritt and Cummins 1996). Macroinvertebrates (expressed as biomass (mg) per gram plant mass) were natural log transformed and regression analyses were performed to determine if macroinvertebrate biomass was related to the percent cover of milfoil.

## RESULTS AND DISCUSSION

*Milfoil and macroinvertebrates:* We found that as percent milfoil cover increased in lakes, the proportion of dissected plants significantly increased ( $r^2 = 0.885$ ,  $p = 0.005$ ). If we consider the relationship between macroinvertebrates and plant architecture alone, then as the proportion of dissected plants increases with increasing percent milfoil cover, we might expect macroinvertebrate biomass to increase as well. But, because milfoil forms dense homogeneous canopies that may support low macroinvertebrate biomass, we hypothesized that macroinvertebrate biomass would decrease lake-wide as percent milfoil cover increases. Our results in July showed that macroinvertebrate biomass significantly decreased along the percent milfoil gradient and that lakes treated with fluridone had higher macroinvertebrate biomass than lakes not treated with fluridone (Figure 1a, b). Because we sampled 2 to 3 species of dissected plants in all six lakes along the percent milfoil gradient, we cannot attribute this trend to the sampling of more dissected plants in high milfoil lakes. In August, no significant trend in macroinvertebrate biomass along the milfoil gradient was observed. Patterns for macroinvertebrate density showed similar results (Cheruvilil et al. in press), so we only discuss the biomass results here.

Epiphytic macroinvertebrates exhibit high natural variability (Gaufin et al. 1956, Mracheck 1966, Merritt and Cummins 1996), which may have contributed to the lack of trend in August. For example, the lake lowest on the milfoil gradient (Camp Lake) experienced a decrease in macroinvertebrate densities and biomass from July to August. Upon further inspection, this decrease was likely due to an emergence of two of the dominant taxa, odonates and chironomids, between the two months. Therefore, seasonal macroinvertebrate fluctuations may have contributed to our inability to detect a pattern between macroinvertebrates and percent milfoil cover across the six lakes in August.

To better understand the factors driving the patterns of decreasing macroinvertebrate biomass with increasing percent milfoil cover, we examined macroinvertebrate biomass on individual plant species that were present in at least three of the lakes (*C. demersum* L., *P. zosteriformis* Fernald., *P. illinoensis* Morong, and milfoil). After pooling the six lakes, milfoil had similar macroinvertebrate biomass as each of the other three plant species. However, we then regressed macroinvertebrate biomass per g plant mass on milfoil alone along the six-lake gradient. We found that macroinvertebrate biomass on milfoil decreased as percent milfoil cover increased, although only marginally significant ( $r^2 = 0.536$ ,  $p = 0.098$ ). This pattern of decreasing macroinvertebrate biomass as percent milfoil cover increased did not occur for the other three plant species, suggesting that as milfoil becomes more dense along the gradient and perhaps inhospitable physical and chemical conditions under the canopy increases, fewer macroinvertebrates may be able to use the milfoil habitat.

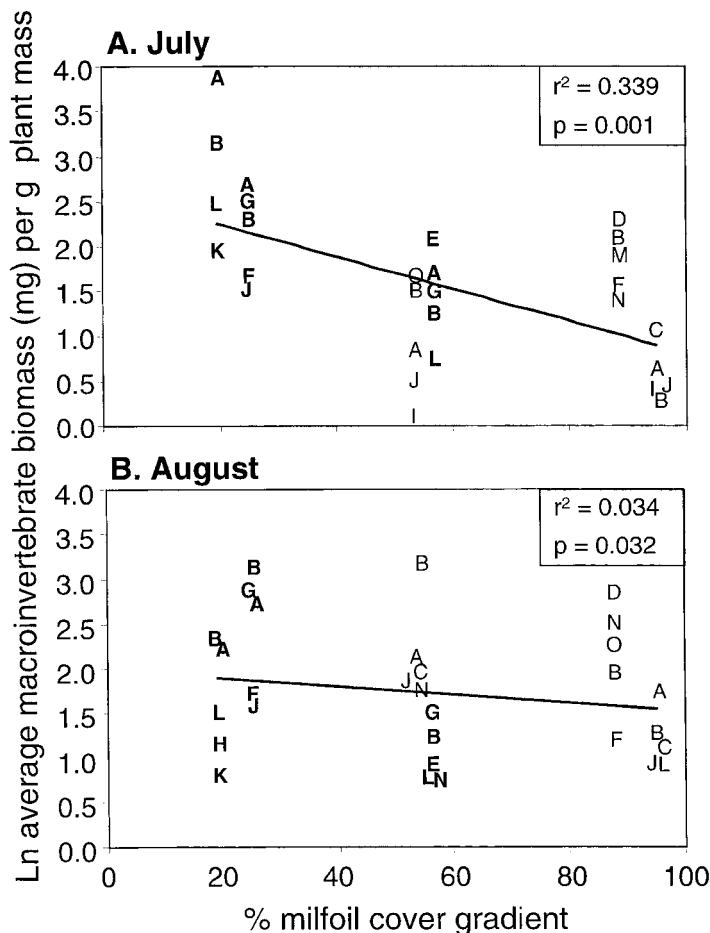


Figure 1. Average macroinvertebrate biomass (mg) per g plant mass along the percent milfoil cover gradient in July (A) and August (B). Each letter represents the average macroinvertebrate biomass for each plant species sampled within a given lake. Bold letters indicate lakes treated with 5 ppb fluridone in May 1997, two years prior to macroinvertebrate sampling. Plant species letter codes are as follows. Dissected species: A = *Ceratophyllum demersum* L., B = *Myriophyllum spicatum* L., C = *Potamogeton pectinatus* L., D = *Cabomba caroliniana* Gray, E = *Utricularia* spp. Undissected species: F = *Potamogeton amplifolius* Tuckerm., G = *Najas* spp., H = *Heteranthera dubia* Jacq., I = *Eloidea canadensis* Michx., J = *Potamogeton zosteriformis* Fernald., K = *Potamogeton praelongus* Wulf., L = *Valisneria americana* Michx., M = *Potamogeton foliosus* G., N = *Potamogeton illinoensis* Morong., O = *Potamogeton pusillus* L.

We also considered juvenile bluegill (*Lepomis macrochirus* Rafinesque) densities as a potential driver of macroinvertebrate density and biomass because juvenile bluegill feed on epiphytic macroinvertebrates within the vegetated littoral zone (Werner and Hall 1988, Olson et al. 1995). If fish densities controlled macroinvertebrate densities and biomass rather than percent milfoil cover, we would expect to see an increase in juvenile fish density with increasing percent milfoil cover (and decreasing macroinvertebrate biomass). However, juvenile bluegill density and percent milfoil cover were not related ( $R^2 = 0.210$ ,  $p = 0.437$ ; Valley 2000), thus bluegill densities alone do not appear to influence macroinvertebrate biomass.

Because the relationship between macroinvertebrate biomass and percent milfoil cover in our study lakes was equivocal, we considered additional factors that may have confounded

our results. For example, one potential reason we failed to see strong relationships between macroinvertebrate biomass and percent milfoil cover may be found in our sampling technique. We sampled epiphytic macroinvertebrates from plants in relatively heterogeneous macrophyte beds. However, in some lakes, milfoil forms dense homogeneous beds within which macroinvertebrate density, biomass, and taxa richness is higher in the upper and edge areas than lower and center areas (Sloey et al. 1987). In addition, Brown et al. (1988) found that homogeneous macrophyte beds support lower abundance of macroinvertebrates than heterogeneous macrophyte beds. Therefore, we may have underestimated the true effects of milfoil by sampling plants from heterogeneous rather than homogeneous macrophyte beds. Thus, if anything, our results may be conservative.

**Management implications:** The possible recreational and ecological ramifications of the continued spread of milfoil have prompted much research into its ecology, biology, and management (e.g., Chilton 1990, Smith and Barko 1990, Trebitz et al. 1993). Due to its multiple propagation mechanisms (Madsen and Smith 1997), traditional management tools such as harvesting without plant removal, derooting, dredging, and drawdown can actually promote expansion of milfoil (Cooke et al. 1990, Smith and Barko 1990). Alternatively, selective aquatic herbicides are potential management options for controlling milfoil. Fluridone is a candidate for such an approach because, relative to most native aquatic plant species, milfoil is highly susceptible to low concentrations of fluridone, increasing the potential for selective plant control (Netherlands et al. 1997). The direct and indirect effects of fluridone on lake foodwebs have not been fully explored, however, and therefore its use is debated.

Studies assessing the direct and indirect effects of fluridone on native macrophyte communities and the subsequent indirect effects on the associated macroinvertebrate, littoral fish, and zooplankton communities are few, especially at the whole-lake scale with multiple lakes. Small-scale fluridone toxicity studies have found negligible direct toxic effects of fluridone on benthic macroinvertebrates. Two studies of *Chironomus tentans* larvae found that interactions of fluridone with suspended solids or sediment had relatively little effect on herbicide accumulation (Muir et al. 1982) and that fluridone assimilation by larvae from ingested sediments was negligible (Muir et al. 1983). Hamelink et al. (1986) also found a favorable safety margin between the concentration that affects *Gammarus pseudolimnaeus* and *Chironomus plumosus* and the fluridone label rate (100 ppb). The only study that found direct toxic effects of fluridone on macroinvertebrates (fly larvae; *Hydrellia*) used the herbicide at concentrations of 4600-9200 ppb (Haag and Buckingham 1991). Therefore, although few taxa have been studied, fluridone appears to have minimal direct toxic effects on macroinvertebrates.

There have been even fewer studies examining indirect effects of fluridone on lake foodwebs. Studies of two Minnesota lakes reported negative impacts of fluridone (application rate of 23 ppb) on native macrophyte species, water quality, macroinvertebrates, and small littoral fish diversity, but positive effects on growth of larger fish (Delong and Mundahl 1996, Pothoven et al. 1999). In contrast, in our study, we found that lakes low on the gradient (those treated with flu-

ridone) had greater macroinvertebrate biomass than lakes higher on the gradient. Although we cannot attribute the difference between treated and non-treated lakes in our study to fluridone applications alone, our results do not suggest negative indirect effects of fluridone treatments on macroinvertebrate biomass two years post-treatment.

There are two important reasons why our results may contradict the only other study examining the indirect effects of fluridone on macroinvertebrates (DeLong and Mundahl 1996). First, the effects of fluridone on epiphytic macroinvertebrates may depend on its effects on overall plant structure or biomass within a lake, which differed between these two studies. In DeLong and Mundahl's treatment lake, not only was milfoil reduced dramatically following the 23 ppb application rate, but overall plant cover in the lake was reduced by 30% (Welling et al. 1997), thus causing an overall reduction in epiphytic macroinvertebrate habitat. In our study lakes, percent plant cover was not reduced two years post-treatment with milfoil, and native plant species thrived before and after treatment (Getsinger et al. 2001). Milfoil is highly susceptible to low fluridone concentrations, which allows native species to persist and increases the potential for selective plant control (Netherland et al. 1997), thus minimizing the potential effects on macroinvertebrates and other organisms utilizing plants. Overall in our study, we may not have found negative effects of fluridone on epiphytic macroinvertebrate biomass because we considered multiple treated and reference lakes with relatively heterogeneous macrophyte communities and the lakes were treated with a lower concentration of fluridone (5 ppb). Our study found adequate milfoil control (Getsinger et al. 2001) and no negative indirect effects on macroinvertebrate biomass.

At the beginning of this study, insufficient data regarding the direct and indirect effects of fluridone had been collected and synthesized, and fluridone use in Michigan had been restricted and fiercely debated for nearly a decade. Currently, the state of Michigan has decided to allow fluridone use at low concentrations ( $\leq 6$  ppb) as a milfoil management tool (Batterson 2000). Based on the conflicting results of the only two studies examining the indirect effects of fluridone on macroinvertebrates (DeLong and Mundahl 1996, our study), it is difficult to conclude whether fluridone use has indirect effects (positive or negative) on macroinvertebrates. Therefore, indirect effects should continue to be monitored, and lake management plans should be adapted as new data are gathered and analyzed.

## ACKNOWLEDGMENTS

This project was supported by funds from the U.S. E.P.A. S.T.A.R. Fellowship Program (N-915342-01-0, U-91595801-0), Michigan State University Agricultural Experimental Station, and Michigan State University Extension. Thanks to Mary Bremigan for her review of early drafts. Kristy Rogers, Steve Hanson, Tamara Brunger Lipsey, Marla Sanborn, Sarah Walsh, Rebekah Serbin, Abby Mahan, Vern Moore, Jason Stockwell, George Klemolin, and Ray Valley provided invaluable sample collection and processing. We wish to thank Marla Sanborn, in particular, for her enormous help processing macroinvertebrate samples.

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