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Aquatic Plant Control Research Program

Potential for a Native Weevil to Serve as a Biological Control Agent for Eurasian Watermilfoil

by *Robert P. Creed, Jr., Sallie P. Sheldon*
Middlebury College

Prepared for Headquarters, U.S. Army Corps of Engineers

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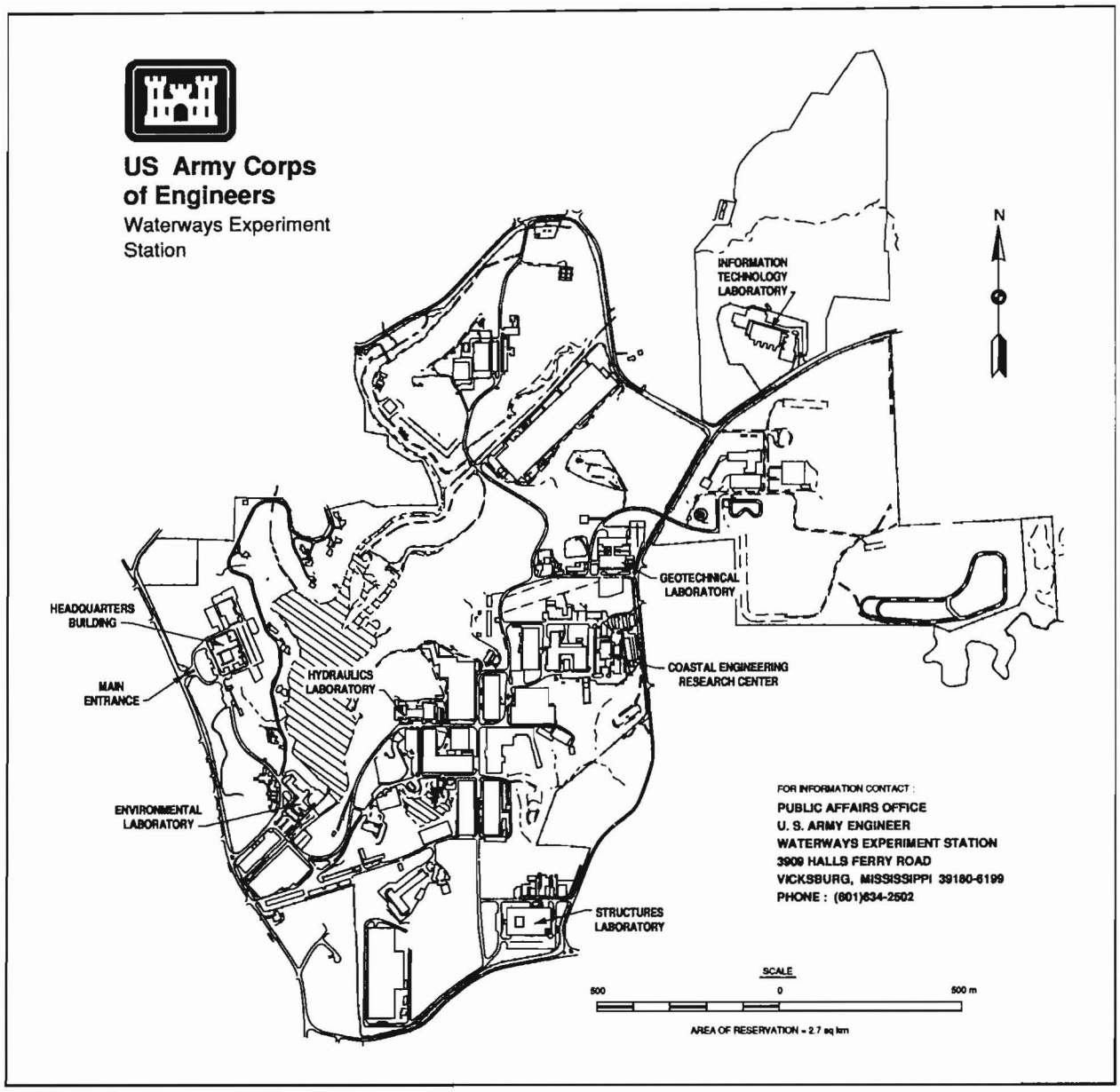

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32739. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Drs. Robert P. Creed, Jr., and Sallie P. Sheldon, Department of Biology, Middlebury College, Middlebury, VT. We are indebted to Mses. Linda O'Bryan and Kristin Henshaw, Middlebury College, for their help with many aspects of this project. Mr. Gabe Gries, University of Vermont, Ms. Diana Cheek, Wellesley College, Ms. Kathy Newbrough, University of Vermont, Mr. Bill Waddell, Middlebury College, and Ms. Wendy Cox, Smith College, helped with the Brownington Pond research. Mses. Kristi Grief and Holly Fryberger, Middlebury College, Mr. David Weedman, Waltham, VT, Ms. Molly Franz and Mr. Creed Clayton, Yale University, Mr. MacDuff Sheehy, Brown University, Ms. Kim Kruise, Ms. Lori Racha, Mr. Chris Alessi, Ms. Sara August, Ms. Ruth Kelty, Ms. Kit van Wagner, and Mr. Jim Rodda, Middlebury College, Mr. Joel Gerwin, Harvard University, Ms. Heidi Van Winkle, Middlebury College, and Mses. Rennie Peddie and Sue Lardner, Middlebury, VT, assisted with the Middlebury research. For the Alberta collections, we wish to thank Mr. John Carr and Mrs. Bertie Carr, Calgary, Alberta, and several people at the Meanook Biological Station for their assistance. We are grateful to Charles O'Brien, Florida A&M University, for verifying the identity of the weevils. Additional funding for this research was provided by the U.S. Environmental Protection Agency Clean Lakes Demonstration Program, the Vermont Department of Environmental Conservation, and Middlebury College.

The study was conducted under the direct supervision of Dr. Alfred F. Cofranceso, Jr., Aquatic Ecology Branch (AEB), Ecological Research Division (ERD), EL, and Dr. Edwin A. Theriot, Chief, AEB, and under the general

supervision of Dr. Conrad J. Kirby, Chief, ERD, and Dr. John W. Keeley, Director, EL, WES.

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1 Introduction

Background

Biological control has been used successfully for controlling a variety of terrestrial and nuisance aquatic plants that have invaded new habitats around the world (Crawley 1989; Debach and Rosen 1991; Harley and Forno 1992; Julien 1992). Most of these successful control programs have been examples of classical biological control (Crawley 1989; Debach and Rosen 1991; Harley and Forno 1992; Julien 1992), which involves the introduction of a control agent from the native range of the nuisance plant into the infested region (Debach and Rosen 1991; Harley and Forno 1992). While introduced insects are frequently used as control agents in these nuisance plant control programs (Julien 1992), native insects, which often colonize introduced plant species (Strong, Lawton, and Southwood 1984), are not commonly utilized (Harley and Forno 1992; Julien 1992). In the majority of cases where native insects have been released as potential control agents for introduced nuisance plants, they have not been successful (Julien 1992). In their handbook on the biological control of nuisance plants, Harley and Forno (1992) do not consider the use of native insects to be a worthwhile approach to the control of exotic plants because their populations do not appear to remain at high enough levels to provide acceptable control of the exotic species. Another potential problem with native insect species is that their life history phenology may be out of phase with that of the exotic plant such that the insect is abundant at a time when it has little effect on the target plant (e.g., Frick and Garcia 1975). The result is a need for augmentation of insect populations at the times when the plant is most susceptible to the insect. Harley and Forno (1992) believe that such augmentation makes the use of native insects extremely expensive and impractical. All native insects may not have these drawbacks, however. Thus, it may be premature to rule out the native entomofauna as a source of potential control agents for introduced plants.

Investigations have been made into the use of a native insect as a control agent for Eurasian watermilfoil (*Myriophyllum spicatum* L.). Hereafter, Eurasian watermilfoil will usually be referred to as watermilfoil. To date, no successful, classical, biological control agent has been found for watermilfoil. Instead, a variety of physical and chemical control efforts (e.g., harvesting, hydroraking, rotovating, drawdowns, bottom barriers, and herbicides) have

been employed in an attempt to maintain the abundance of this nuisance aquatic plant at acceptable levels. While these methods have resulted in short-term reductions in watermilfoil biomass, they are expensive and have not provided long-term control (e.g., Bayley, Rabin, and Southwick 1968; Aiken, Newroth, and Wile 1979; Smith and Barko 1990). The nearctic weevil (*Euhrychiopsis lecontei* (Dietz)) (Coleoptera: Curculionidae) is being evaluated as a potential biological control agent for watermilfoil in North America because it was found associated with a watermilfoil population that had declined.

Purpose and Scope

In this report, the issue is addressed regarding whether the native weevil *E. lecontei* has the potential to be a viable biological control agent for watermilfoil in North America. Chapter 2 presents the results of research conducted at Brownington Pond, Vermont. Surveys and a pond enclosure experiment were conducted to determine if *E. lecontei* had a role in the Brownington Pond watermilfoil decline. Chapters 3 and 4 present the results of studies that evaluated the potential for culturing *E. lecontei* and determined the life history of this aquatic weevil. Chapters 5 and 6 present the results of aquarium and pool experiments that evaluated the effect of *E. lecontei* on watermilfoil and several native aquatic macrophytes. Two surveys of lakes in Vermont and other northeastern states were conducted to assess the distribution of *E. lecontei* and two other watermilfoil herbivores (*Acentria ephemerella* (Denis and Schiffermüller) (=*A. nivea* (Olivier)) and *Parapoynx badiusalis* (Walker)) (Lepidpotera: Pyralidae) in this region. The results of these surveys are presented in Chapter 7. Chapter 8 presents the results of collections of weevils made in Alberta. The determination was made that northern watermilfoil (*Myriophyllum sibiricum* Komarov (=*M. exalbescens* Fernald) is a native host of *Euhrychiopsis*. Chapter 9 presents general conclusions and recommendations.

2 Research at Brownington Pond

Introduction

Watermilfoil declined in abundance in Brownington Pond, a 54-ha, mesotrophic pond in northeastern Vermont, between 1986 and 1989 (Figure 1). A preliminary survey of the remaining watermilfoil found three herbivorous aquatic insects (*E. lecontei*, *A. ephemerella*, and *P. badiusalis*) associated with these damaged plants. This research has focused on *Euhrychiopsis* for two reasons: (a) it was common on watermilfoil in the pond, and (b) it appears to be a watermilfoil specialist. *Parapoynx* was not very abundant on watermilfoil in Brownington Pond and was not studied. *Acentria* is a generalist feeder (Batra 1977; Buckingham and Ross 1981) and thus not a good candidate for a biological control agent.

Materials and Methods

Watermilfoil surveys

Pond surveys. Since 1990, the positions of any watermilfoil beds in Brownington Pond have been qualitatively mapped using snorkeling and boat surveys.

Plant transects. The initial survey of Brownington Pond in 1990 found that there were two watermilfoil beds in water approximately 2.0 to 3.5 m deep (Figure 2). Sampling was begun of all submersed macrophytes along two sets of permanent transects (three per watermilfoil bed) that were perpendicular to shore to document any changes in watermilfoil distribution and abundance. The transects were evenly spaced across the length of the beds (approximately 100 m apart on the West Bed and approximately 60 m apart on the South Bed). Plant samples were taken by SCUBA divers at depths ranging from 0.5 to 3.5 m in 0.5-m increments using a 0.25-m^2 quadrat. All plants within a quadrat were clipped at sediment level and placed in sealable plastic bags.

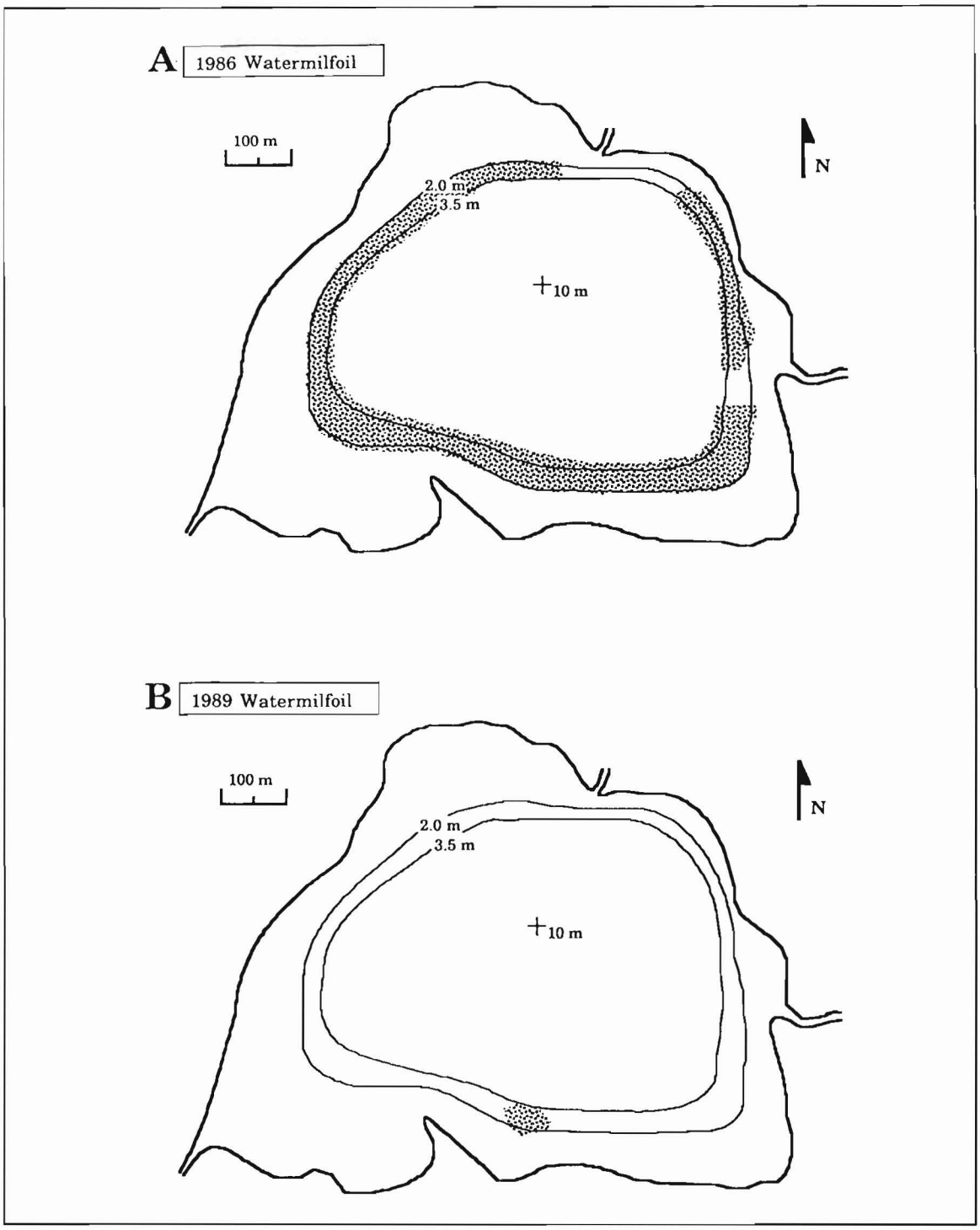

Figure 1. Distribution of Eurasian watermilfoil in Brownington Pond in 1986(A). Data are from a qualitative survey conducted by Vermont Department of Environmental Conservation. Distribution of Eurasian watermilfoil in Brownington Pond in 1989(B). Data are from a qualitative survey conducted by S. Sheldon and D. Smith (Size of watermilfoil beds has been approximated). Maps presented here and in Figure 2 are more accurate than those that appeared in Creed and Sheldon (1991)

Plants were separated by taxa in the laboratory and dried to a constant weight at 80 °C. The transects were sampled on two dates in 1990 and on three dates in 1991 and 1992. As watermilfoil biomass in Brownington Pond is highest in mid to late summer, the biomass data presented here are from the last sample dates (mid-August) in 1990 and 1992, and from the midsummer (late July, early August) samples in 1991. Dry weights for native species were lumped together in the category "Other." Common submersed native macrophytes in Brownington Pond included *Potamogeton amplifolius* Tuckerm., *P. gramineus* L., *Heteranthera dubia* (Jacq.) MacM., *Najas flexilis* (Willd.) Rostk. and Schmidt, *Megalodonta beckii* (Torr.) Greene, *Isoetes* sp., *Chara* sp., and *Nitella flexilis* L.

Permanent grids. In 1990, four permanent grids were placed in the pond (two in each bed) to record fine-scale expansions and contractions of *M. spicatum* beds. The grids were placed on either the ends or the nearshore edges of the beds, as it was believed that watermilfoil would be more likely to spread laterally and into shallow water. The grids covered an area of 8 by 6 m with buoys placed every 2 m in a 4- by 5-m array. Percent cover of watermilfoil was determined by a snorkeler using a 0.5- by 0.5-m quadrat subdivided into 25 subunits. In 1990, percent cover readings were taken along three transects in each grid. In 1991 and 1992, four transects were sampled. For all 3 years, the number of quadrat subunits more than half-filled with watermilfoil plants was recorded for each point along a transect. The percent cover values were grouped into five categories: 0 percent, 1 to 25 percent, 25 to 50 percent, 50 to 75 percent, and >75 percent. The grids were swum only once in 1990 in early September. The grids were swum three times (in June, July, and August) in 1991 and 1992. Because percent cover of watermilfoil increased over the growing season, only present grid data for the last sample of each year are presented.

Weevil surveys

Watermilfoil and weevils were collected in the South and West Beds using a Mobile Invertebrate Sampler (MIS) (Smith and Sheldon, unpublished manuscript) designed for sampling a single watermilfoil stem. A single stem is defined here as an individual shoot emerging from the sediment; lateral stems were included in samples if they branched above the sediment surface. The sampler was a long plastic tube with a removable sieve (500-µm Nitex mesh). A plant was chosen haphazardly and then enclosed in the sampler by a SCUBA diver. Plants were cut at the sediment surface, and the sieve was attached. Samples were placed in sealable, plastic bags, and weevil adults and larvae were removed from the fresh plants. Samples were collected on 6 dates in 1990, 11 dates in 1991, and 12 dates in 1992.

Weevils lay their eggs on watermilfoil meristems. Meristems were sampled in 1991 and 1992 to document changes in the abundance of weevil eggs. Apical pieces of stem approximately 50 cm long with undamaged meristems were collected along three transects in each bed. Eight stems per transect were

collected haphazardly by snorkelers. A total of 24 stems per bed per date were collected. Meristems were dissected under a microscope, and all eggs were counted. Samples were collected weekly from June through August. Additional samples were collected once in September (1991 and 1992) and October (1991).

Water and sediment chemistry

Water chemistry. Water samples were collected on 25 June 1991 and 30 June and 27 August 1992 from the South and West watermilfoil beds and from the east side of the pond in an area with mixed native plant (*Heteranthera dubia* and *Potamogeton amplifolius*) cover. Three or more sites were sampled at each of these three locations using a Kemmerer sampler. Pairs of samples, one shallow (just below the surface) and one deep (just above the bottom), were taken at each site. Samples were placed on ice and transported to the laboratory of the Vermont Department of Environmental Conservation where they were analyzed for concentrations of nitrate, nitrite, and orthophosphate.

Sediment chemistry. Sediment samples were collected by a SCUBA diver on 11 August 1992 in the West Bed, a watermilfoil-free area adjacent to the West Bed (West Shallow), the South Bed, a watermilfoil-free area adjacent to the South Bed (South Shallow), and an area dominated by *H. dubia* and *P. amplifolius* on the east side of the pond. A 3.8-L plastic bag was filled with sediment below the water-sediment interface, sealed, and returned to the surface. Samples were kept cool and sent to the U.S. Army Engineer Waterways Experiment Station (Vicksburg, MS) for analysis within 48 hr of collection. Sediments were analyzed for total phosphorus, total nitrogen, extractable phosphate, exchangeable ammonium-N, exchangeable K, sediment density, and percent organic matter content. Sediment interstitial water was analyzed for concentrations of ammonium-N, soluble reactive phosphorus, iron, and potassium. Sediment data were analyzed using an analysis of variance (ANOVA), and means for each site were compared using Tukey's HSD test (Sokal and Rohlf 1981).

Pond enclosure experiment

In a variety of previous experiments, a determination was made that the adults and larvae of *E. lecontei* can suppress the growth and reduce the buoyancy of small watermilfoil plants in aquaria and pools (see Chapter 5). The following enclosure experiment was conducted to evaluate the effect of *E. lecontei* on larger plants in the pond. The enclosures were 3-m-tall plexiglass cylinders (20 cm OD) with a bottom section (1 m) that was driven into the sediment and a detachable top (2 m). Along the sides of the top were four pairs of ports covered with 202-µm Nitex mesh that allowed for water exchange with the water column. A lid covered with 202-µm Nitex mesh was bolted on the top of each enclosure. There was a vertical-centimeter scale on

the outside of the top. Ten enclosure bottoms were placed in the pond on the nearshore side of the South Bed by a SCUBA diver on 17 June 1992. Two-thirds of each plexiglass cylinder was pushed into the sediment, and the remainder was filled with sediment from the South Bed.

A number of small (approximatetly 40-cm-long shoots), unbranched watermilfoil plants with intact roots were collected from the West Bed. The plants were cleaned of obvious macroinvertebrates and weevil eggs, sorted into 13 groups of six, and weighed (blotted wet weight) to standardize initial biomass. Ten of the groups of six plants were randomly assigned to the enclosures and three groups were dried at 80 °C for an initial estimate of dry weight. Six plants per enclosure is equivalent to 181 plants/m^2, which was within the range of densities determined by pond surveys in 1990. On 18 June, the plants were gently pushed into the sediments in the enclosure bottoms by a SCUBA diver until the roots were buried. The enclosure top was then bolted to the bottom and the lids were attached. The maximum height of each stem in each enclosure was recorded by a SCUBA diver 4 days after the watermilfoil was planted and then weekly until the end of the experiment.

During the first 3 weeks of the experiment, larval weevil damage was observed on a single stem in four of the enclosures. Weevil eggs are occasionally placed deep inside the meristem and cannot be found without destroying the meristem. These four enclosures were designated as the weevil treatment. Because the plants had been randomly assigned to tubes, the assumption was made that the distribution of this treatment across enclosures was also random. On 9 July, adult weevils (two males and two females) were added to these four enclosures. Another three enclosures each contained a single *Acentria* larva, so these were considered as an *Acentria* treatment. The remaining three enclosures were designated as uncontaminated controls. At the time the adult weevils were added, the mean ($\pm$1 S.E.) height of the stems (n = 6) in the control, *Acentria*, and weevil treatments were 83.00 ($\pm$2.59), 81.06 ($\pm$2.75), and 76.33 ($\pm$3.98), respectively, and the difference between treatments was not significant. The enclosures were periodically cleaned of external periphyton.

The experiment was terminated on 20 August 1992. The plants were clipped at sediment level; the stems were collected inside the enclosure top, which was then sealed with a screen-covered bottom (202-µm Nitex mesh). The enclosure top was lifted out of the water, and the plants and animals were collected on the bottom screen and then placed in sealable plastic bags. The roots were gently removed from the sediments and bagged. In the laboratory, invertebrates were separated from the plant material and enumerated. Watermilfoil stems were separated into the six original stems (i.e., the stems present prior to the adult weevil introduction) and the newer lateral stems. Roots were cleaned of any organic debris. Stems and roots were dried to a constant weight at 80 °C. Weevil larvae were not found in one of the weevil enclosures, so this enclosure was not included in the analysis. Thus, n = 3 for all treatments. Treatment effects were analyzed using an ANOVA, and treatment means were compared using Tukey's HSD test (Sokal and Rohlf 1981).

Results

Watermilfoil surveys

Pond survey. The watermilfoil population in the pond increased from 1989 to 1991 and then declined again during the winter of 1991-1992 (Figure 2). In June of 1992, there were no areas where dense watermilfoil beds reached the surface. The South Bed was devoid of any watermilfoil growth. Scattered watermilfoil plants were present in the West Bed. Some of these were taller shoots (approximately 1.5 m high) that had probably overwintered; most were shorter shoots (<0.5 m) that appeared to have just begun to grow. By the end of August 1992, four areas of moderately dense watermilfoil growth were present (Figure 2C). Watermilfoil only approached the surface in the West Bed; the tops of these plants were approximately 1 m below the surface. Only scattered, small plants were present in the vicinity of the former South Bed by the end of the summer.

Plant transects. Watermilfoil was abundant in 1990 and 1991 (Figures 3 and 4). A decline occurred between 1991 and 1992, and there was a 4- to 6-fold reduction in watermilfoil biomass in the center of the West Bed and a 15- to 30-fold reduction in the center of the South Bed.

Permanent grids. Watermilfoil cover increased on all four grids from 1990 to 1991 (Figures 5 and 6). By the end of 1991, the four grids displayed varying degrees of cover; heavy watermilfoil cover (>50 percent) on the grids ranged from 40 (North Grid, West Bed) to almost 100 percent of the cover on the East Grid, South Bed. At the end of 1992, watermilfoil cover on three of the four grids rarely exceeded 25 percent. In two grids, one-half to three-quarters of the grid area had 0-percent watermilfoil cover. The decline was most striking on the East Grid, South Bed (Figure 6), which had had essentially 100-percent watermilfoil cover at the end of 1991. Little watermilfoil cover was present on this grid in 1992. Only the South Grid from the West Bed had substantial watermilfoil cover by the end of the summer of 1992; approximately 30 percent of the watermilfoil cover on this grid exceeded 50 percent.

Weevil surveys

The number of weevils per stem was relatively low in both watermilfoil beds during 1990 (Figures 7 and 8). In general, weevil abundance on watermilfoil increased through early 1992 and then began to decrease. When watermilfoil abundance is plotted for the same period (Figures 7 and 8), it is apparent that the increase in weevil abundance on watermilfoil coincides with the pronounced decrease in watermilfoil abundance.

There was a steady increase in the mean number of eggs per meristem in the South Bed in 1991, whereas egg number in the West Bed was constant

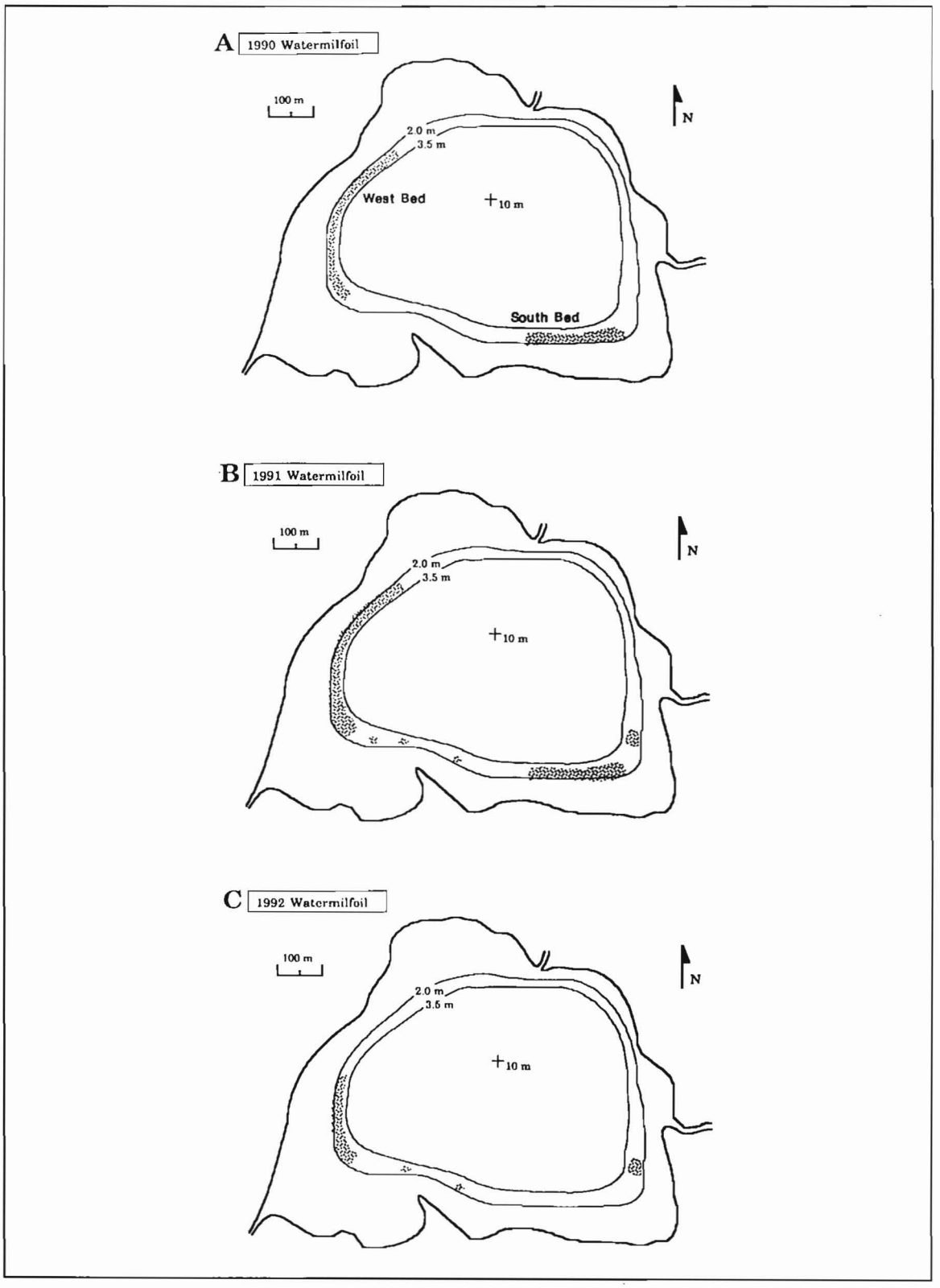

Figure 2. Distribution of Eurasian watermilfoil at end of summer in Brownington Pond in 1990, 1991, and 1992 (Size of watermilfoil beds has been approximated)

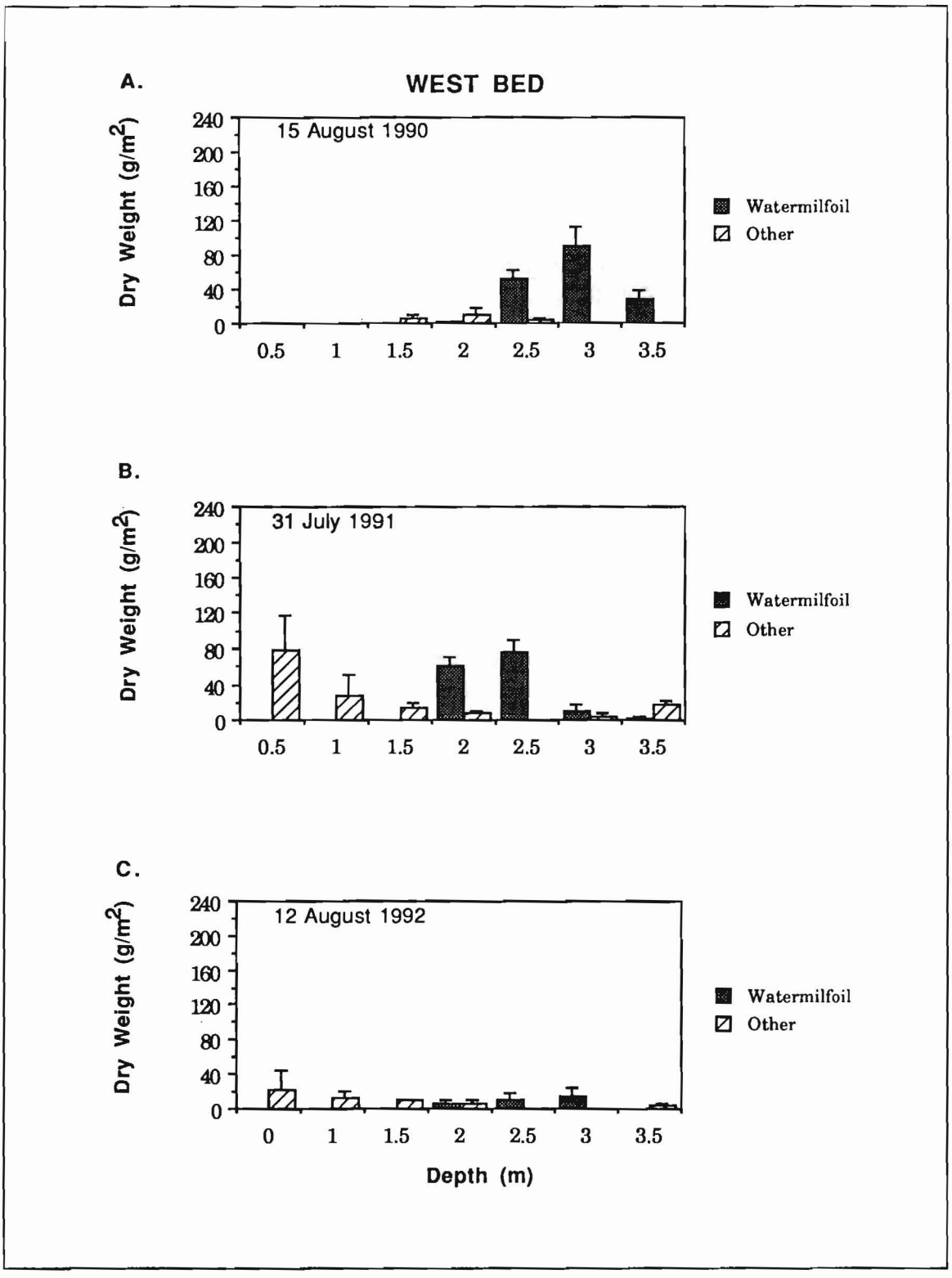

Figure 3. Results of plant transects (n = 3 for each date) for West Bed, 1990-1992 (Bars represent the mean biomass (±1 S.E.) of either Eurasian watermilfoil or combined native macrophyte species (=Other))

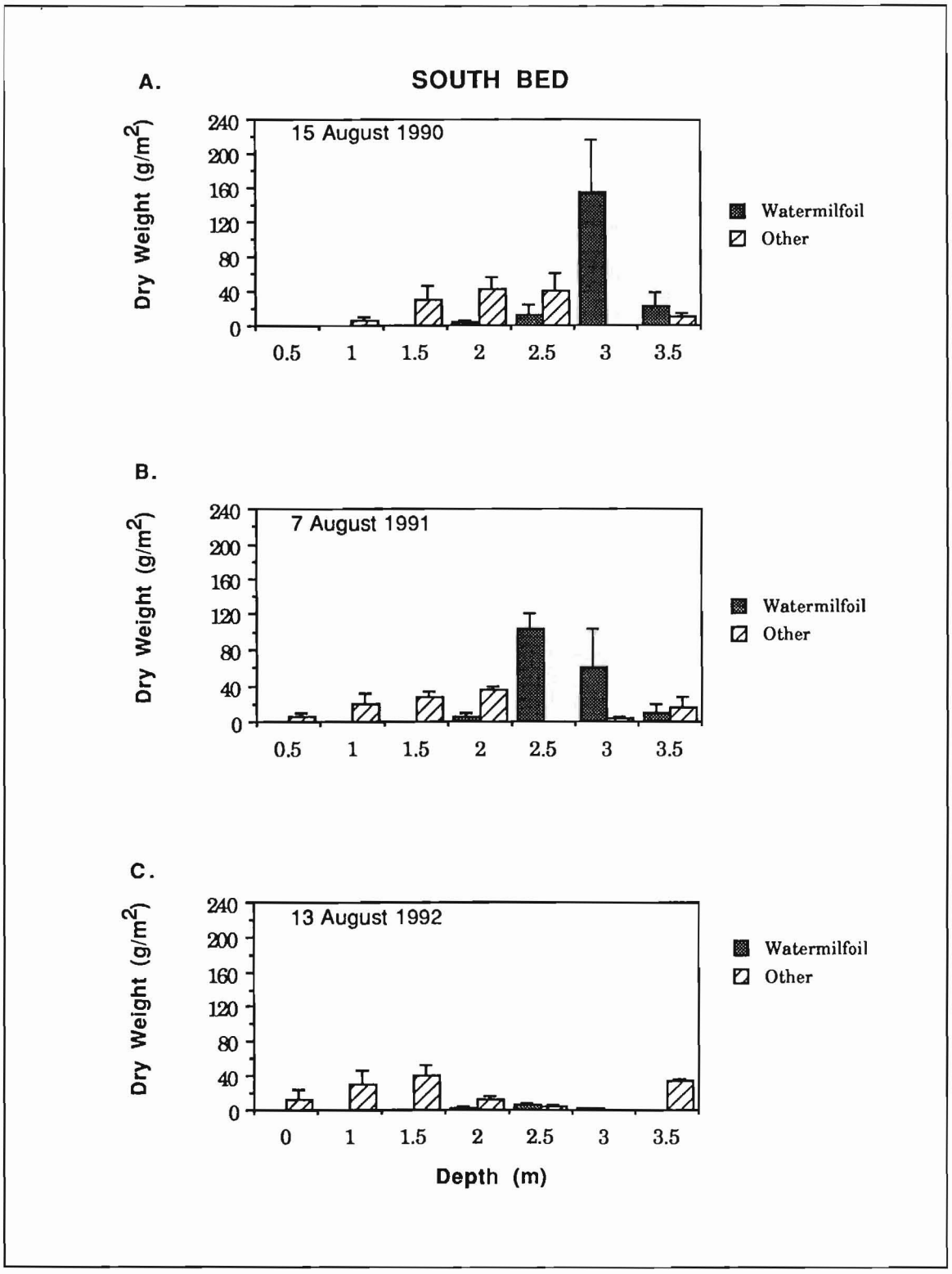

Figure 4. Results of plant transects (n = 3 for each date) for South Bed, 1990-1992 (Bars represent the mean biomass (±1 S.E.) of either Eurasian watermilfoil or combined native macrophyte species (=Other))

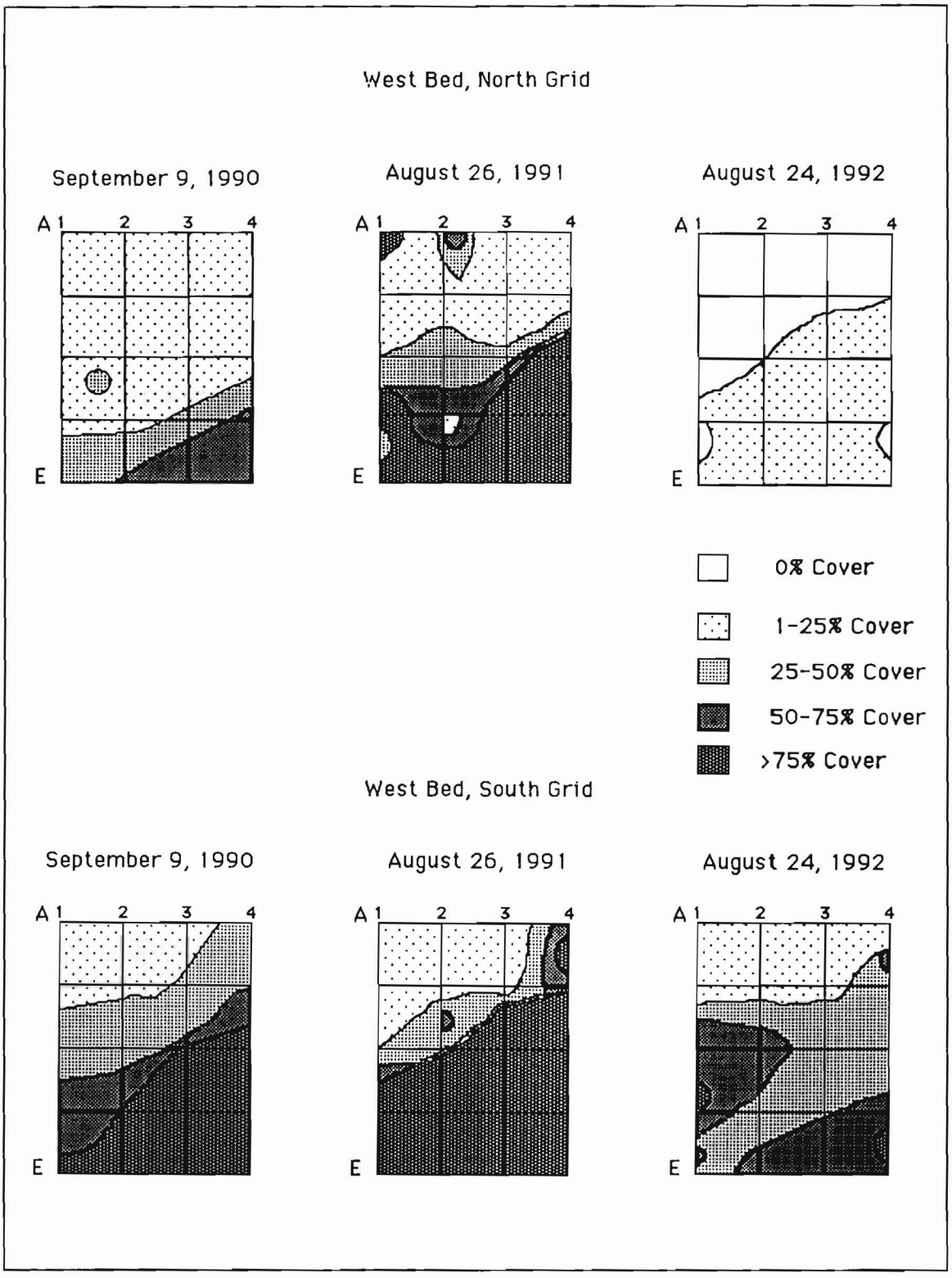

Figure 5. Maps of percent cover of Eurasian watermilfoil in two West Bed grids for last sample date of each summer

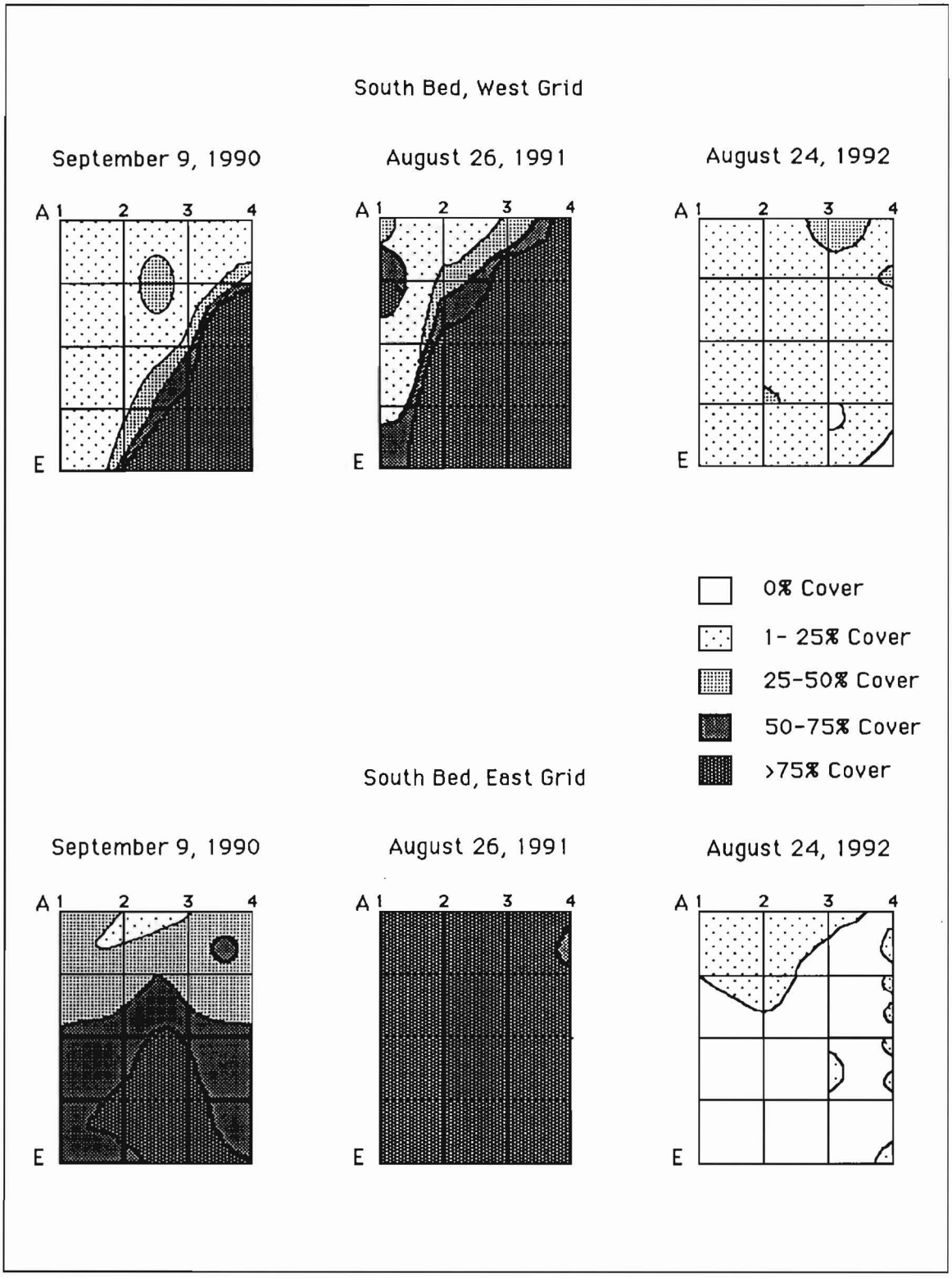

Figure 6. Maps of percent cover of Eurasian watermilfoil in two South Bed grids for last sample date of each summer

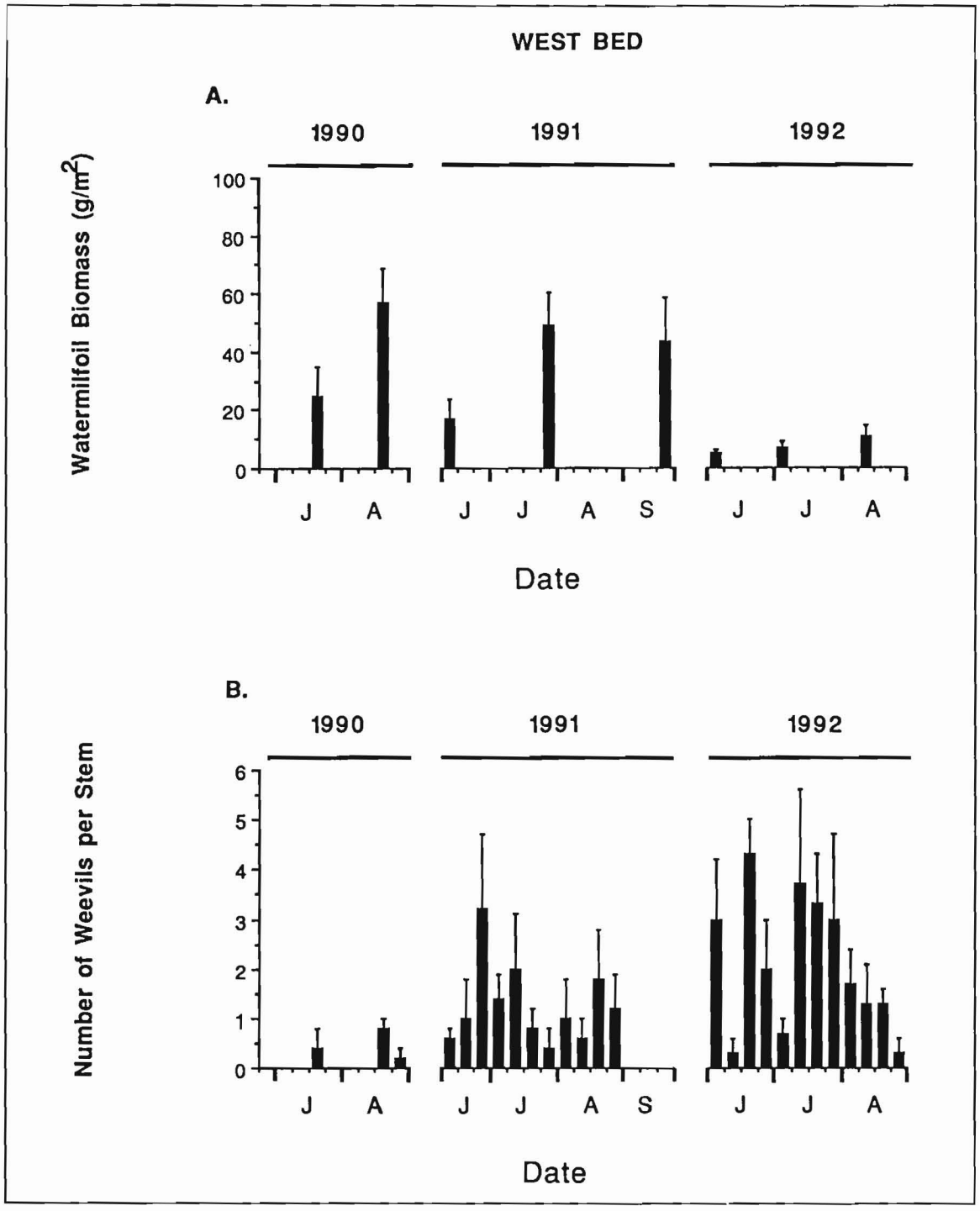

Figure 7. Eurasian watermilfoil and weevil abundance in West Bed from 1990-1992. (A) Watermilfoil biomass (mean ± 1 S.E.). Data are from plant transects. All samples from 2.0- to 3.0-m-depth intervals were used (n = 9 for each date). (B) Weevil abundance as mean (±1 S.E.) number of adults and larvae per stem. Samples were collected using small MIS sampler (N = 5 for all dates in 1990 and 1991; N = 3 for all samples in 1992)

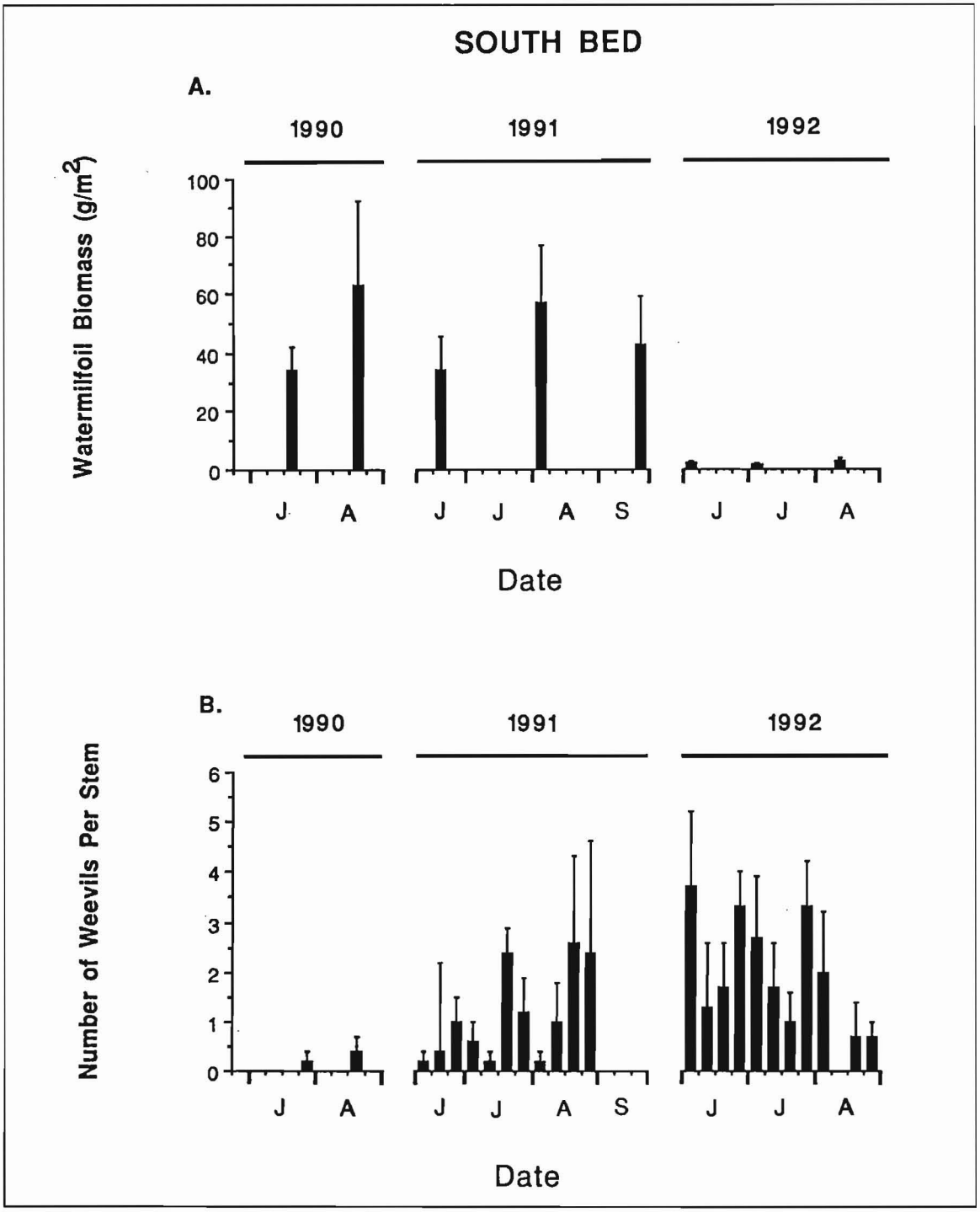

Figure 8. Eurasian watermilfoil and weevil abundance in South Bed from 1990-1992. (A) Watermilfoil biomass (mean ± 1 S.E.). Data are from plant transects. All samples from 2.0- to 3.0-m-depth intervals were used (n = 9 for each date). (B) Weevil abundance as mean (±1 S.E.) number of adults and larvae per stem. Samples were collected using the small MIS sampler (N = 5 for all dates in 1990 and 1991; N = 3 for all samples in 1992)

(Figure 9). The number of eggs per meristem rapidly increased in June of 1992 in both beds and then decreased over the remainder of the summer. Few weevil eggs were collected in September of 1991 in either bed, and no eggs were collected in October 1991 or September 1992.

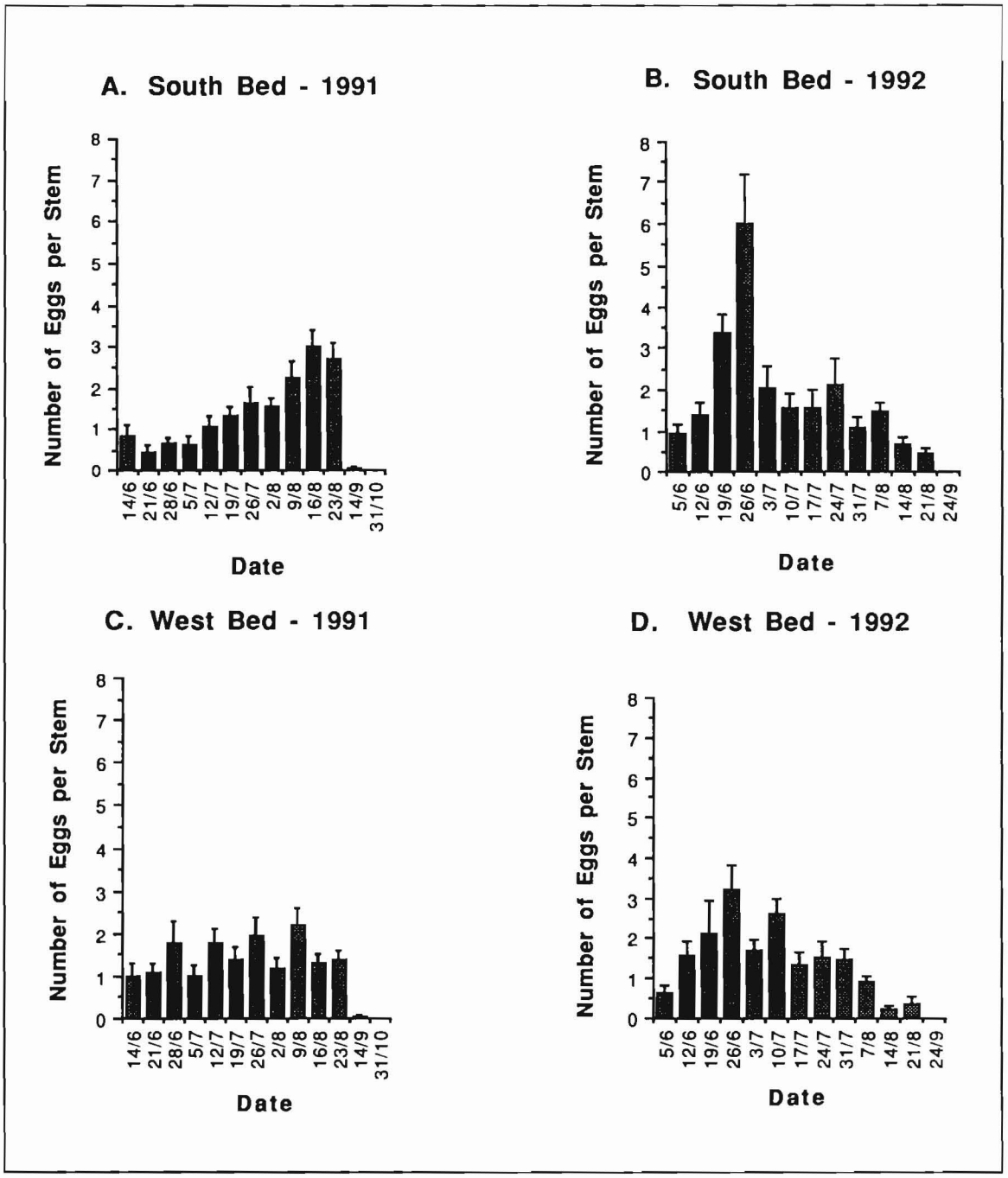

Figure 9. Results of meristem transects in South and West Beds in 1991 and 1992. Data in figure are mean (±1 S.E.) number of eggs found on intact meristems

Water and sediment chemistry

Concentrations of orthophosphate, nitrite, and nitrate varied little in 1991 and 1992. The concentration of orthophosphate (0.002 mg/L) was constant in water collected from both of the watermilfoil beds. Mean orthophosphate concentrations ranged from 0.002 to 0.003 mg/L in water samples collected on the east side of the pond. Concentrations of nitrite and nitrate never deviated from 0.01 mg/L on any date.

Ammonium was the only sediment nutrient that varied significantly among sites (Table 1). Interstitial water ammonium concentrations were significantly lower in the South Bed sediments compared with those in the native plant sediments or the West Bed sediments. Exchangeable ammonium in the South Bed sediments was significantly lower than the West Bed sediments.

Pond enclosure experiment

Total watermilfoil biomass was significantly greater in the control and the *Acentria* treatments compared with the weevil treatment (Figure 10). The differences in total biomass were attributable to differences in root and lateral stem weight; there was no significant difference in the weight of the original stems (Figure 10). Weevil-damaged stems tended to collapse during the experiment. While the mean height of these stems in the water column was usually lower than that of the controls, the difference was not significant until the last 3 weeks of the experiment when the difference ranged from 10 to 25 cm (Table 2). The mean ($\pm$1 S.E.) numbers of weevil adults and larvae recovered from the weevil enclosures were 2.7 ($\pm$0.33) and 5.7 ($\pm$1.2), respectively. The mean ($\pm$1 S.E.) number of weevils per stem for the six stems (originals plus laterals) in the weevil enclosures was 1.4 ($\pm$0.2). Lateral stems were combined with the original stems in the calculation of weevil abundance, as most (88 percent) lateral stems were short (<30 cm) and arose from the original stem just above the sediment surface, which makes these calculations consistent with those in the weevil surveys. Only one *Acentria* larva was recovered from the *Acentria* enclosures.

Discussion

The enclosure experiment demonstrated that *E. lecontei* can significantly suppress watermilfoil growth. Weevils suppressed production of new stems by damaging lateral shoot meristems. Weevil feeding also suppressed root production. Weevil feeding may influence root production, as the destruction of lacunae and stem vascular tissue by weevil larvae may interrupt the movement of gases and photosynthate to the root system. While weevils did not significantly reduce the biomass of existing shoots (i.e., the original stems), they did affect the buoyancy of these stems causing them to settle out of the water column. Most watermilfoil shoot biomass is typically near the surface (Titus and Adams 1979a). The rate of photosynthesis could be substantially reduced

Table 1
Results of Analysis for Sediments Collected from Five Sites in Brownington Pond

Variable	Site				
	Natives	South Bed	South Shallow	West Bed	West Shallow
Sediment Extractions					
Exchangeable NH_4	ab 0.099 (0.014)	b 0.034 (0.016)	ab 0.056 (0.017)	a 0.134 (0.035)	ab 0.069 (0.011)
Exchangeable K	a 0.074 (0.027)	a 0.050 (0.017)	a 0.088 (0.032)	a 0.109 (0.008)	a 0.075 (0.017)
Available PO_4	a 0.147 (0.013)	a 0.131 (0.003)	a 0.145 (0.032)	a 0.173 (0.016)	a 0.162 (0.020)
Total P	a 0.638 (0.056)	a 0.670 (0.050)	a 0.535 (0.109)	a 0.779 (0.029)	a 0.562 (0.071)
Total N	a 15.3 (0.6)	a 13.5 (0.9)	a 12.6 (2.7)	a 13.5 (0.4)	a 14.0 (0.4)
Interstitial Water					
NH4-N	a 2.88 (0.38)	b 0.68 (0.20)	ab 1.17 (0.22)	a 3.16 (0.90)	ab 1.33 (0.20)
SRP	a 0.010 (0.003)	a 0.006 (0.003)	a 0.006 (0.004)	a 0.031 (0.010)	a 0.007 (0.001)
Fe	a 0.24 (0.13)	a 0.35 (0.17)	a 0.63 (0.13)	a 0.45 (0.04)	a 0.17 (0.02)
K	a 2.09 (0.64)	a 1.21 (0.20)	a 1.70 (0.10)	a 1.69 (0.16)	a 2.04 (0.63)
Sediment density	a 0.057 (0.009)	a 0.059 (0.003)	a 0.070 (0.015)	a 0.073 (0.004)	a 0.069 (0.004)
% Organic matter	a 48.28 (0.41)	a 41.26 (1.47)	a 39.46 (6.07)	a 35.46 (1.05)	a 41.63 (0.87)

Note: Values in the table are means (±1 S.E.). The units for the sediment extraction samples are milligrams/gram; the units for the interstitial water samples are milligrams/liter; the units for sediment density are grams/milliliter. The data were analyzed using an ANOVA, and means for each site were compared using Tukey's HSD test. Treatment means that are significantly different ($p < 0.05$) from one another have different letters next to them.

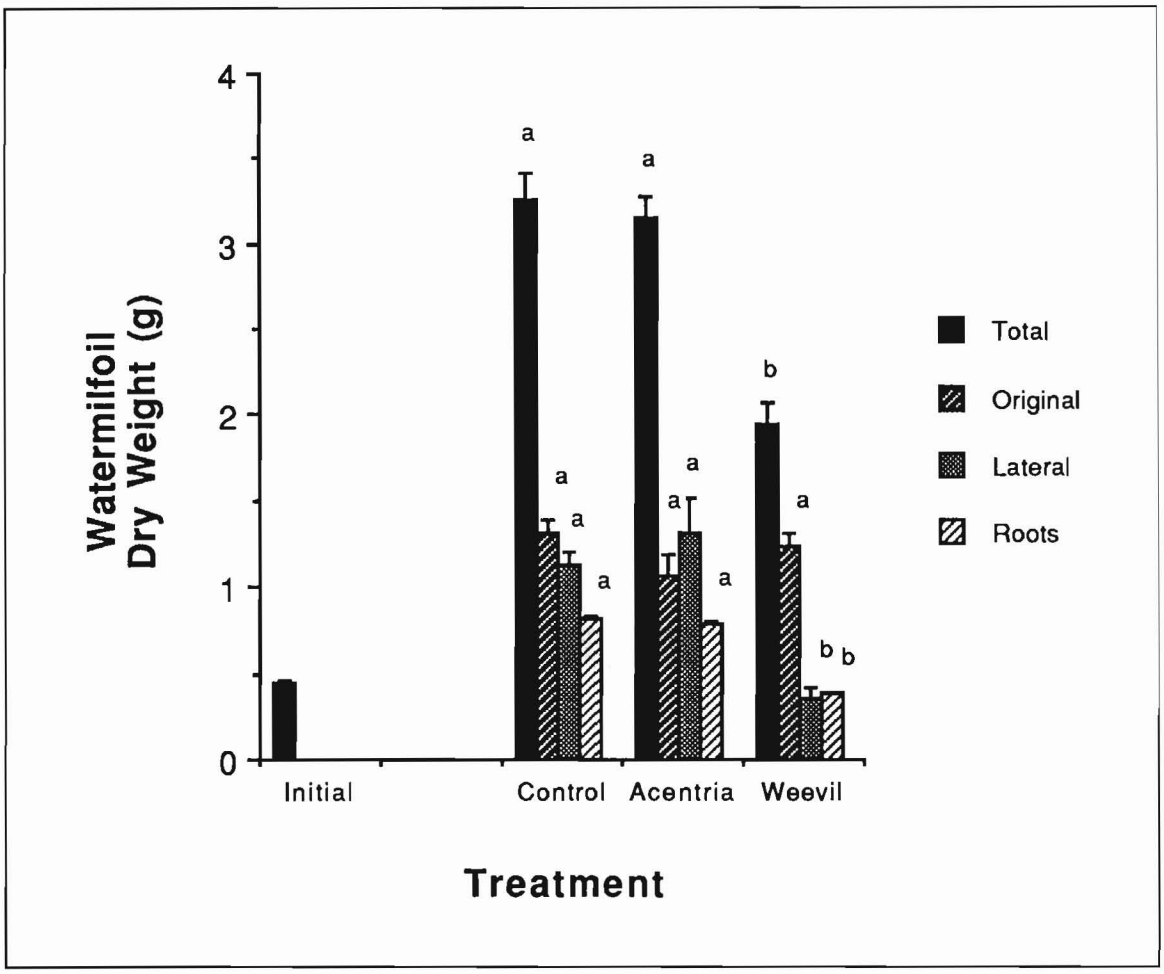

Figure 10. Results of Brownington Pond enclosure experiment. Data shown include total watermilfoil biomass per treatment (solid black bars) plus distribution of that biomass by its components (i.e., original stem biomass, lateral stem biomass, and root biomass). Bars represent mean biomass (±1 S.E.). Treatments with same letter are not significantly different

if the canopy settles into deeper water where light intensities are lower. This effect was probably underestimated in this experiment, as weevil-damaged stems were often supported by the enclosures. The destruction of lacunae and vascular tissue could also reduce stem growth. The lacunal system in watermilfoil functions as a reservoir for respired carbon dioxide (CO_2) (Nichols and Shaw 1986), and the loss of the accumulated CO_2 could slow the rate of photosynthesis. Loss of vascular tissue would halt the translocation of nutrients from the roots to the growing portions of the stems. Therefore, weevil feeding could promote declines by disrupting the physiology of existing plants and suppressing the production of new biomass.

The survey results are consistent with the hypothesis that weevils were involved in the observed watermilfoil declines. Watermilfoil biomass and percent cover were high in 1990 and 1991, but had declined by 1992. Weevil

Table 2
Effect of Weevil and *Acentria* Feeding on Original Stem Height (cm) for Last 3 Weeks of Enclosure Experiment Conducted in Brownington Pond in 1992

Date	Treatment		
	Control	*Acentria*	Weevil
3 August	a 94.61 (3.54)	a 90.78 (1.22)	b 79.29 (3.26)
10 August	a 96.11 (2.47)	a 90.89 (1.68)	b 75.42 (3.12)
17 August	a 94.39 (3.51)	a 91.17 (0.44)	b 70.79 (3.59)

Note: Values in the table are treatment means (±1 S.E.). Treatment means that are significantly different ($p < 0.05$) from one another have different letters next to them.

abundance (expressed as number of weevils per stem), on the other hand, was low in 1990 and high in 1991 and early 1992. Subsequent to the 1992 decline, the number of weevils per stem began to decrease by midsummer of 1992. The density of weevils on watermilfoil in the enclosures was comparable with weevil densities on watermilfoil in the pond in 1991 and 1992, which suggests that the weevil densities observed in the pond were sufficient to suppress watermilfoil growth. These temporal patterns of weevil and watermilfoil abundance are similar to those displayed by simple predator-prey or host-parasitoid models (e.g., Begon and Mortimer 1981; Kuno 1987). When these patterns of abundance are considered in conjunction with the results of the enclosure experiment, they suggest that a similar interaction is occurring between watermilfoil and *E. lecontei.*

While weevils appear to have been important in reducing the abundance of watermilfoil in both of the observed declines, they did not eradicate the watermilfoil. One mechanism that could prevent weevil populations from completely eliminating watermilfoil populations is a lack of adequate pupation sites. In 1992, many of the surviving plants in the pond were relatively short (<50 cm high). *Euhrychiopsis lecontei* constructs a puparium entirely inside the stem, and the stem diameter of these short plants may not have been large enough for puparia. This hypothesis is supported by the fact that while *E. lecontei* eggs and larvae were common in 1992, especially early in the summer, no pupae were collected in any samples in 1992; pupae were collected in 1990 and 1991. The decline in the number of eggs per meristem during 1992 also suggests that the population of adults had decreased in the pond despite the increase in watermilfoil biomass and cover in some parts of the pond, particularly the south end of the West Bed. Such a reduction in pupation sites

could be important in generating the temporal patterns of watermilfoil and weevil abundance described above.

The absence of considerable watermilfoil biomass from both beds in early 1992 suggests that weevil herbivory affects the ability of watermilfoil to overwinter and produce new stem tissue in the spring. While entire watermilfoil plants can overwinter, shoots typically die back to the root crowns that may or may not have attached short stems (Aiken, Newroth, and Wile 1979; Titus and Adams 1979b; Nichols and Shaw 1986). Watermilfoil stores nonstructural carbohydrates in the lower stem and root crown, and much of this stored carbohydrate is utilized during the spring growth flush (Titus and Adams 1979b; Madsen 1993). Destruction of stem vascular tissue by weevils during the summer could disrupt the movement of carbohydrates to storage sites, which normally occurs in the summer and fall (Madsen 1993). Production of new stem tissue in the spring might be greatly curtailed if reserves of nonstructural carbohydrates are low. In addition, stems that might have successfully overwintered might be more susceptible to decomposers if they are damaged by weevils.

Change in water chemistry does not appear to have been the primary cause of the Brownington Pond watermilfoil decline. Concentrations of the measured nutrients in the water column were essentially constant between 1991 and 1992 and within the 1992 growing season. It is possible that a change in some unmeasured waterborne micronutrient could have caused the decline. However, observations from Brownington Pond suggest that this was not the case. First, watermilfoil did not disappear throughout the pond, which is fairly small and appears to have a well-mixed epilimnion (e.g., temperatures are nearly uniform around the epilimnion of the pond). Second, the enclosure experiment was conducted adjacent to the site of the former South Bed where the reduction in watermilfoil abundance was greatest between 1991 and 1992. The watermilfoil grew inside the enclosures, while little watermilfoil growth was observed in the area immediately surrounding the enclosures.

Similarly, changes in sediment chemistry also do not appear to have been important in producing the decline. Only one sediment variable, the concentration of ammonium, was found to vary significantly among sites. Ammonium concentrations in both the sediment and the interstitial pore water were lowest in the sediments of the former South Bed. These results were the opposite of those of Carignan (1984, 1985) and Painter and McCabe (1988), who found that ammonium concentrations were lowest in areas of high watermilfoil abundance. As ammonium is produced by the decomposition of organic matter by heterotrophic bacteria (Wetzel 1983), higher sediment concentrations were expected at the South Bed site, as there was a layer of decomposing watermilfoil on the sediment surface for much of the summer. It is possible that the watermilfoil bed that had previously been present at this site may have severely depleted sediment ammonium concentrations with the result that watermilfoil was unable to grow here. However, Carignan (1985) observed ammonium regeneration in sediments beneath watermilfoil beds during the autumn. Watermilfoil had been present at both the South and West Bed sites

in the autumn of 1991, where it subsequently declined. Also, sediment from the South Bed was used in the enclosure experiment. Because the watermilfoil grew on this sediment, change in sediment quality is not believed to be the cause of the declines. The results of a similar experiment conducted at Norton Brook Pond (Creed and Sheldon 1993b), a pond that had not been colonized by *E. lecontei*, also support the hypothesis that herbivory, and not changes in sediment quality, was primarily responsible for the Brownington Pond decline. In the Norton Brook Pond experiment, existing clumps of watermilfoil were enclosed. There was significantly less watermilfoil biomass in the enclosures containing weevils. While there may be an interaction between sediment nutrient availability and the effect of the weevil on watermilfoil (e.g., reduced root production in the presence of weevil herbivory could result in reduced sediment nutrient uptake during the summer and reduced nutrient regeneration because of root decomposition in the fall), changes in nutrient availability alone is not believed to have produced the Brownington Pond declines. Neither Carignan (1984) nor Painter and McCabe (1988) could find a relationship between sediment quality and the watermilfoil declines in the lakes they studied.

3 Weevil Culture

Introduction

If *Euhrychiopsis lecontei* is to be used in a biological control program, rearing large numbers of weevils for sustained releases would be preferable rather than having to collect them from the field. Weevil cultures were initiated at Middlebury in the summer of 1991. These cultures were maintained into 1993. Successfully establishing weevil cultures in the laboratory and maintaining these cultures through the winter were goals.

Materials and Methods

Weevils and watermilfoil were cultured in a room illuminated with both standard fluorescent and GroLux bulbs (light:dark schedule 16:8). Weevils and watermilfoil were cultured in aquaria filled with tap water that was continuously aerated. Water temperatures ranged from 13 to 27 °C.

Watermilfoil rhizomes were planted in autoclaved sediment. *Euhrychiopsis lecontei* were collected from *M. spicatum* from several Vermont lakes and added to aquaria containing watermilfoil. Extra *M. spicatum* cultures without weevils provided a source of watermilfoil for the weevil cultures. Plants were added as necessary. Algal growth in aquaria was controlled by snails (*Physella*), hand removal, and in-tank filters. Cultures were examined periodically to determine the presence of weevil life stages.

Results and Discussion

Eggs, larvae, pupae, and adults were continuously produced in these cultures for 19 months. Notation was made that adult weevils will mate readily in a laboratory environment. The biggest problem encountered with weevil cultures was maintaining an adequate supply of undamaged watermilfoil, especially during the winter months. Also, weevils escaped from aquaria during the first fall of culturing. These weevils had been collected in late summer

and may have already received an environmental cue that caused them to leave the water. Thereafter, adult emigration was not a problem.

The authors of this report believe that both laboratory cultures and breeding ponds should be used for culturing weevils. Weevils can be reared in laboratory cultures during the summer when watermilfoil is easy to obtain. Larvae could be quickly produced. Adults could also be produced, but it would take twice as long to obtain them (see Chapter 4). Breeding ponds might provide a reliable long-term supply of weevils.

4 Weevil Life History Studies

Introduction

Understanding the life history of *Euhrychiopsis lecontei* is important if this weevil is to be used in a biological control program. Weevil life history information could be combined with information regarding potential control points in the annual cycle of watermilfoil (e.g., Madsen 1993) in an attempt to determine the best times to release weevils. The goals in these studies were to determine (a) the duration of the different phases of *E. lecontei*'s life history (on both *M. spicatum* and *M. sibiricum*) and (b) the fecundity of female weevils.

Materials and Methods

Watermilfoil was collected from local lakes and planted in chambers. Chambers were clear, polycarbonate cylinders (30-cm-long, 6-cm-ID) set into cups of autoclaved lake sediment. Each chamber was capped with a lid of 202-µm Nitex mesh. Chambers were set in aquaria filled with aerated tap water, and each chamber was also individually aerated. Chambers were housed in the light room and illuminated with both standard flourescent and GroLux lights under a 16 hr:8 hr light:dark regime. Water temperatures ranged from 21.5 to 24.0 °C.

Adult *E. lecontei* were also collected from local lakes and placed in the chambers. Immediately after an egg was laid on a watermilfoil plant, the plant and egg were transferred to a new chamber and the eggs examined daily until hatching. Each newly hatched larva was transferred to an undamaged watermilfoil plant in a new chamber and new plants added as needed, usually every second or third day. Late instar larvae formed puparia inside stems, and the duration of the pupal phase was followed. Newly emerged adults were removed from the chambers, and their sex was determined. For quantification of lifetime egg production, each newly emerged female was placed in a chamber with two males and three to six watermilfoil stems with intact meristems. Plants and dead males were replaced as needed. The number of eggs each female laid was recorded until she died.

To see whether weevils collected from Eurasian watermilfoil would complete their life cycle on a native watermilfoil, the attempt was made to rear *E. lecontei* on northern watermilfoil (*M. sibiricum*) in chambers similar to those described above. Two batch cultures were also set up in which weevils were cultured on northern watermilfoil and on Eurasian watermilfoil from Minnesota, which is presumably a different genetic stock of *M. spicatum*. Weevils were collected from local Eurasian watermilfoil and placed in aquaria containing either northern watermilfoil collected in Vermont or Minnesota watermilfoil. These cultures were periodically inspected; adult survival, deposition of eggs, and evidence of larval burrowing in the stems were noted.

Results

Eggs were laid on apical meristems, and first instar larvae fed on mersistematic tissue for 3 to 5 days. Late instar larvae spent most of their time inside the stem feeding on stem tissue. Sometimes, particularly when larvae reached the end of an internode, they burrowed out, spiraled up or down the outside of the stem to a new internode, and burrowed back into the stem. Larvae were usually found in the top third of the plant. Puparia tended to be found further down in the thicker portions of the stem. Adults were usually found on the top third of the plants, where they fed on both leaves and stem tissue.

In transferring weevils to new plants, many plants broke; thus sample sizes were low. Under these laboratory conditions, the mean ($\pm$1 S.E.) duration of the egg phase was 3.90 ($\pm$0.20) days (n = 48). Larval duration averaged 12.98 ($\pm$1.75) days (n = 9). Pupal duration averaged 13.00 ($\pm$1.52) days (n = 5). The sum of these averages suggests that the average time between egg deposition and emergence as an adult is approximately 30 days.

Females lived from 11 to 162 days, and they produced from 3 to 562 eggs, respectively. On average, females laid 1.90 ($\pm$0.44) eggs per day (n = 7). Eggs appeared to be preferentially laid on the apical meristem. If eggs were already present on the apical meristem, eggs were often laid on the uppermost lateral meristems; if these also had eggs, eggs were deposited on leaves near the plant apex. Hatching rate of eggs was 87.3 percent. Normally, a few eggs were laid on each meristem in a chamber. However, when weevils were enclosed with few plants, as many as 29 eggs were found on a single plant.

Rearing *E. lecontei* on northern watermilfoil was more difficult. No eggs were laid on the northern watermilfoil planted in the chambers in contrast to 1.9 eggs laid per day on Eurasian watermilfoil under the same conditions. Ten eggs were found on northern watermilfoil in the batch cultures. Seven of the ten eggs hatched. The duration of the egg phase on northern watermilfoil was a half day longer than that observed on Eurasian watermilfoil. For the batch cultures of Vermont weevils on Minnesota Eurasian watermilfoil, weevil larvae and adults fed on the Minnesota plants, and eggs were laid and hatched.

Discussion

The life history data collected in the laboratory are consistent with observations of *E. lecontei* phenology in the field. There appear to be three generations of weevils on *M. spicatum* each summer in two lakes studied in Vermont. If the generation time for *E. lecontei* in the field is approximately 30 days, then three generations per summer is feasible. In the field, eggs are found primarily on meristems near the surface; larvae are found in the top meter of the plant; and pupae are typically found 0.5 m or more down the stem. The first weevils found in the spring are adults; thereafter, eggs and then larvae are found. In September, weevil densities decline. Charles O'Brien predicted that *Euhrychiopsis lecontei* may overwinter as adults in soil and leaf litter near lake margins.[1] This prediction was based on observations of other aquatic weevils. Terrestrial collections in November 1993 around a lake containing watermilfoil and *E. lecontei* in Minnesota have verified this prediction. Densities of adult weevils in soil samples were as high as 360/square meter.[2]

[1] Personal Communication, 1990, Charles O'Brien, Florida A&M University, Tallahassee, FL.
[2] Personal Communication, 1993, David Ragsdale, University of Minnesota, St. Paul, MN.

5 Effect of *E. Lecontei* on Watermilfoil in Pool and Aquarium Experiments

Introduction

Several pool and aquarium experiments were conducted that were designed to determine how *E. lecontei* adults and larvae damaged watermilfoil and suppressed its growth. Experiments examining the effect of weevil herbivory on the bouyancy and viability of watermilfoil stem fragments were also conducted. Because the caterpillar *Acentria* was frequently found associated with damaged watermilfoil plants, it was included in one of the experiments.

Materials and Methods

Effect of *Euhrychiopsis* adults and first instar larvae on watermilfoil growth

This experiment was conducted in a 750-L outdoor pool. Each plant was placed in a separate chamber that consisted of a clear, plastic cylinder (height 30 cm, ID 42 mm) set in a polyvinyl chloride pipe base filled with sieved pond sediment taken from one of the watermilfoil beds in Brownington Pond. A tight-fitting cap with 500-µm Nitex mesh was placed on the top of the tube.

Eighteen *M. spicatum* autofragments (portions of stem that have developed roots prior to natural fragmentation) were collected from Brownington Pond. All visible invertebrates were removed from the autofragments under a dissecting microscope. The autofragments were then measured from a marked point at the base of the stem and weighed (blotted wet weight). Initial lengths of autofragments ranged from 175 to 267 mm; initial weights ranged from 0.87 to 1.89 g. A single plant was then planted into the sediment in the base of each chamber. Chambers were continuously aerated with a slow trickle of air bubbles. Plants were allowed to acclimate to the conditions for 1 day before the weevils were added.

The chambers were placed in six rows with three chambers per row in the pool. There were three treatments: 0, 2, and 4 weevils per chamber. The experimental design was a randomized complete block design with three treatments per block and six replicates per treatment. The plants were subjected to the ambient light regime. Water temperatures in the pool were recorded every morning and evening.

After 13 days, plants and weevils were removed from each chamber. Plants were measured and weighed. Also, the number of whole leaves removed and their location along the shoot were recorded. Weevil larvae were found in both of the weevil treatments (in five of the 2-weevil treatment replicates and four of the 4-weevil treatment replicates). Two larvae were also found on two of the control plants. Because both the number of adults and larvae were negatively correlated with change in length and weight, the effect of adults were analyzed using analysis of covariance (ANCOVA) with orthogonal contrasts, with number of larvae as the covariate (Sokal and Rohlf 1981). Prior to ANCOVA analysis, tests were made for homogeneity of slopes; no significant adult X larva interaction was found. The identification of the weevils as *Euhrychiopsis lecontei* was verified by Charles O'Brien, Florida A&M University.

Effect of late instar *Euhrychiopsis* larvae on watermilfoil growth

The design of this experiment and the processing of plants were the same as that described above. Instead of using autofragments, small plants with intact roots were used. The initial length of the plants ranged from 158 to 223 mm; initial weights ranged from 0.33 to 0.74 g. Late instar larvae (approximately 3- to 4-mm-long) were collected from watermilfoil plants in Brownington Pond. Treatments consisted of a control, 1 and 2 late instar, weevil larvae per plant. Densities of one to two weevils per stem were frequently observed in Brownington Pond. Water temperature in the pool was monitored with a maximum/minimum thermometer.

The experiment lasted 9 days. Change in plant length and weight was quantified, and the amount of stem that had been burrowed by the larvae was measured. Because weevil larvae do not appear to feed extensively on leaves, changes in the number of leaves or leaf whorls were not quantified. Treatment effects were compared using an ANOVA with planned, orthogonal contrasts (Sokal and Rohlf 1981). Weevil larvae died in both the 1- and 2-larvae treatments in one row ($n = 5$ for these two treatments; $n = 6$ for the control).

Combined effect of *Acentria* and *Euhrychiopsis* larvae on watermilfoil growth

The collection and processing of the plants and the experimental chambers were the same for this experiment as for the previous one. The initial lengths of the 24 watermilfoil plants ranged from 206 to 230 mm; initial wet weights ranged from 0.23 to 0.88 g. Much of the variation in weight was attributable to differences in root biomass and not aboveground biomass. The experimental design was a randomized complete block design with four treatments per row and six replicates per treatment. The treatments were as follows: control (no larvae), weevil (one *Euhrychiopsis* larva per chamber), *Acentria* (one *Acentria* larva per chamber), and the combination treatment (one larva of each species in a chamber). Late instar *Euhrychiopsis* larvae and *Acentria* larvae (approximately 5- to 6-mm-long) were collected in Brownington Pond and were paired by size for each row.

The experiment lasted for 13 days. Plants and larvae were then removed from each chamber. After removing the larvae, the watermilfoil plants were measured and weighed. Any plant material not attached to the rooted stem was not included in the final plant weight. The number of whorls of leaves remaining on each stem was counted. Weevil larvae in the single weevil treatment died in rows 1 and 6, and an *Acentria* larva died in the single *Acentria* treatment in row 6. These two rows were removed from the analysis (n = 4 for all treatments). Treatment effects were compared using an ANOVA with planned, orthogonal contrasts (Sokal and Rolhf 1981).

Effect of herbivores on watermilfoil buoyancy

Adult weevils and undamaged, apical portions of watermilfoil stems were collected from Brownington Pond. The length of the stems was standardized, and they were sorted into 10 groups of six stems each. The blotted wet weight was determined for each of the groups. The mean initial wet weight ($\pm$1 S.E.) of the groups of stems was 5.08 $\pm$ 0.15 g. Watermilfoil stems were placed into ten 38-L aquaria filled with aerated well water. The aquaria were placed in a line (north to south) on the ground in an area where they received direct sunlight from midmorning to midafternoon. The weevils were sexed and sorted into five groups of four weevils (each with three females and one male). Weevils were added to 5 of the 10 aquaria; the remaining 5 aquaria served as controls. Assignments of treatments to aquaria and watermilfoil and weevils to aquaria were randomized. All aquaria were covered with a tight-fitting, translucent lid to prevent the escape of the weevils. The lids also contained a panel of 500-µm mesh Nitex to allow for air exchange and also aid in temperature regulation of the water.

After 21 days, all watermilfoil that was not resting on the bottom was considered as floating. All watermilfoil settled to the bottom on cool, overcast days, so the experiment was terminated on a sunny day. Floating watermilfoil was separated from that which had settled to the bottom of the aquaria. All

herbivores were removed from the aquaria and from the plant material. Four of the five weevil aquaria were contaminated with *Acentria* larvae. Therefore, the effect on buoyancy will be referred to as an herbivore effect and not simply a weevil effect. All watermilfoil was then weighed (blotted wet weight). Most of the watermilfoil in one of the control aquaria had settled to the bottom. The watermilfoil in this aquarium was encrusted with what appeared to be iron precipitation. Using Dixon's test (Sokal and Rohlf 1981), this replicate was determined to be a statistical outlier, and it was removed from the analysis. Treatment effects were compared using an ANOVA (Sokal and Rohlf 1981). The data used in the ANOVA were the weight of the floating watermilfoil. Weight data were log transformed prior to performing the analysis.

Effect of larval weevil damage on stem fragment viability

Weevil herbivory, particularly larval burrowing, weakens watermilfoil stems, which can result in stem fragmentation. Fragments are generated by other watermilfoil control methods (e.g., mechanical harvesting) (Nichols and Shaw 1986; Smith and Barko 1990); and, as such fragments are usually viable (Aiken, Newroth, and Wile 1979), their production could promote the spread of watermilfoil. The following experiment was designed to determine if the viability of stem fragments damaged by weevils was reduced compared with undamaged fragments. Undamaged fragments were similar to those produced by mechanical macrophyte harvesters. Because many fragments may settle in deeper water where light intensity is reduced, the effect of light intensity on fragment growth was also evaluated.

Damaged and undamaged pieces of stem (meristem plus stem) were removed from watermilfoil plants in Brownington Pond. These fragments were checked for either weevil eggs (undamaged fragments) or larvae (damaged fragments) that were removed along with all other macroinvertebrates. The fragments were cut to a standardized length of 4 cm. The amount of larval weevil burrowing was not standardized for the damaged fragments, but all fragments displayed some degree of larval damage (meristem damage plus stem burrowing). The fragments were planted in twelve 38-L aquaria containing aerated well water and sieved pond sediment from the West watermilfoil bed in Brownington Pond. The aquaria were in a line (north to south) on the ground and were exposed to ambient light. To prevent herbivore colonization, all aquaria were covered with a tight-fitting translucent lid that contained a panel of 500-µm mesh to allow for air exchange and also aid in temperature regulation of the water. Aquaria selected for the reduced light treatments were covered with a shroud of window screen, which reduced light intensity in these aquaria to half that of the unshaded aquaria. Unshaded aquaria simulated light levels (3,750 to 4,000 lux) for shallow water (<0.5 m) in Brownington Pond; shaded aquaria had light levels (1,500 to 2,000 lux) comparable with those at 2.0 m, where much of the watermilfoil is located in the pond. Light levels were measured in the pond and the aquaria using a Lutron LX-101 lux meter.

There were four treatments: undamaged stems (control), ambient light; undamaged stems (control), shaded; weevil-damaged stems, ambient light; and weevil-damaged stems, shaded. Each treatment had three replicates, and there were five stem fragments per aquarium. The assignment of aquaria to treatments was randomized. Temperatures were recorded weekly using maximum/minimum thermometers suspended in four of the aquaria (two shaded and two unshaded).

After 29 days, the stems were gently removed from the sediment. The percentage of stems with roots was determined for each aquarium. Roots were removed from the stem, blotted dry, and weighed. The final length of both original and lateral stems was measured. Treatment effects were analyzed using an ANOVA with orthogonal contrasts, which was performed on the means for each variable from each replicate aquarium. Root weights were log transformed.

Results

Effect of *Euhrychiopsis* adults and first instar larvae on watermilfoil growth

Feeding by adult weevils resulted in significant reductions in change in plant weight compared with the control (Figure 11A). Both the control and the 2-weevil treatment plants gained weight compared with their initial weights. The control plants gained almost twice as much weight as the 2-weevil treatment plants. The plants from the 4-weevil treatment, on the other hand, lost weight during the experiment. The comparison for change in weight between the control and the weevil treatments was highly significant, as was the difference between the 2- and 4-weevil treatments.

A similar pattern was observed in the change in plant length (Figure 11B). The comparison between the control and both weevil treatments was marginally significant ($p < 0.08$), while the difference between the 2- and 4-weevil treatment was not significant. When the effect of the larvae was not factored out using the ANCOVA, i.e., the data were analyzed using an ANOVA, the difference between the control and the weevil treatments was significant ($p < 0.03$). The difference between the 2- and the 4-weevil treatments was marginally significant ($p < 0.08$). Thus, there was a significant overall weevil effect (adults plus larvae) on change in plant length. The effect of adults alone, however, was not significant.

Leaves were lost from plants in all three treatments (Figure 11C). The comparison between the control and both weevil treatments and between the two weevil treatments were highly significant. The loss of leaves in the control treatment was primarily the result of leaves dying at the point where the stems were pushed into the sediment (Figure 12A). While plants from both of the weevil treatments lost leaves at sediment level, the vast majority of the

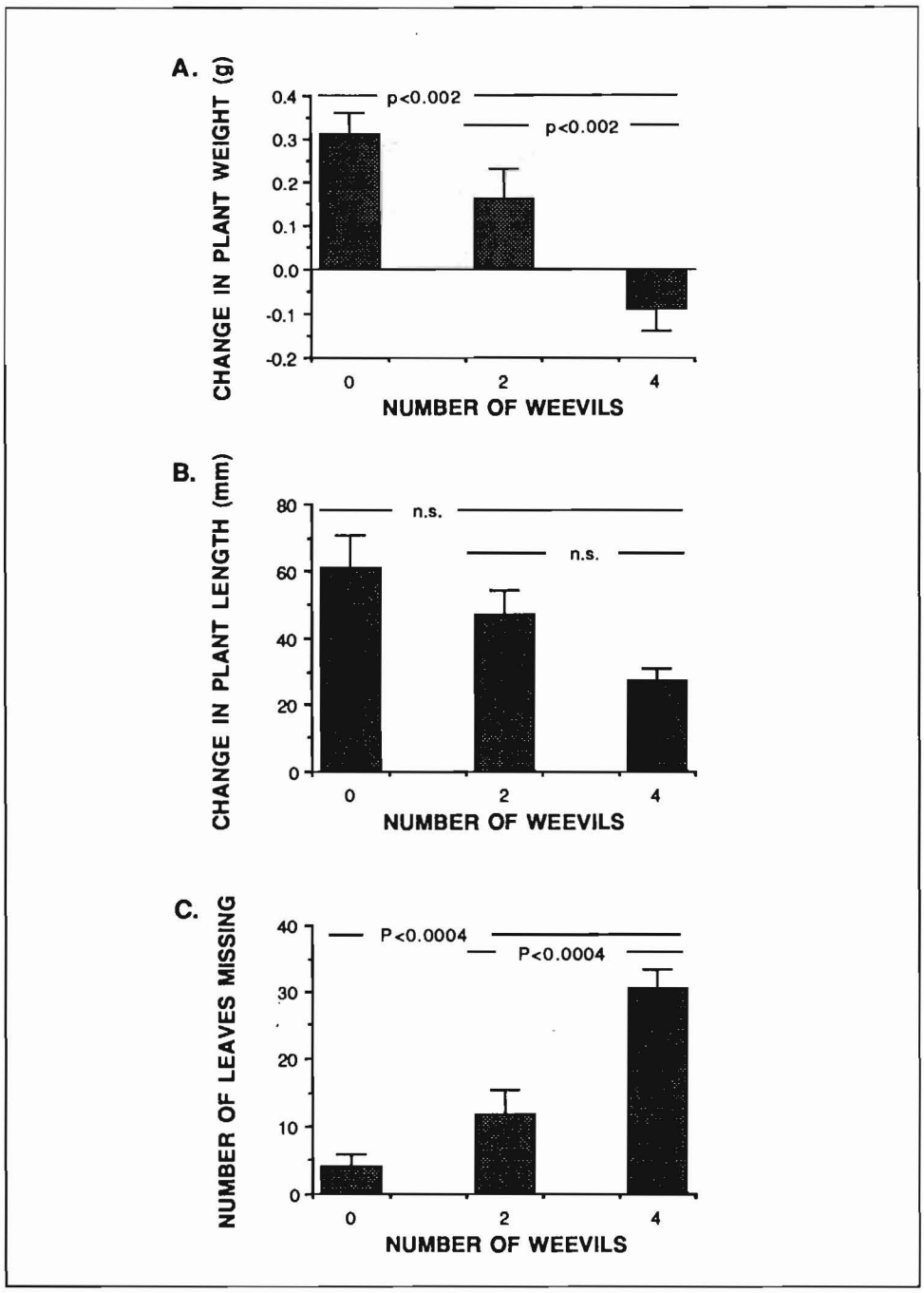

Figure 11. Effect of feeding by adult weevils (*E. lecontei*) on growth of watermilfoil autofragments and leaf loss. Bars in histograms represent mean change in a variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANCOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of control (0 weevils) versus weevil treatments; lower line represents comparison of 2- versus 4-weevil treatment. (A) Change in autofragment weight (g). (B) Change in autofragment length (mm). (C) Number of leaves lost per autofragment

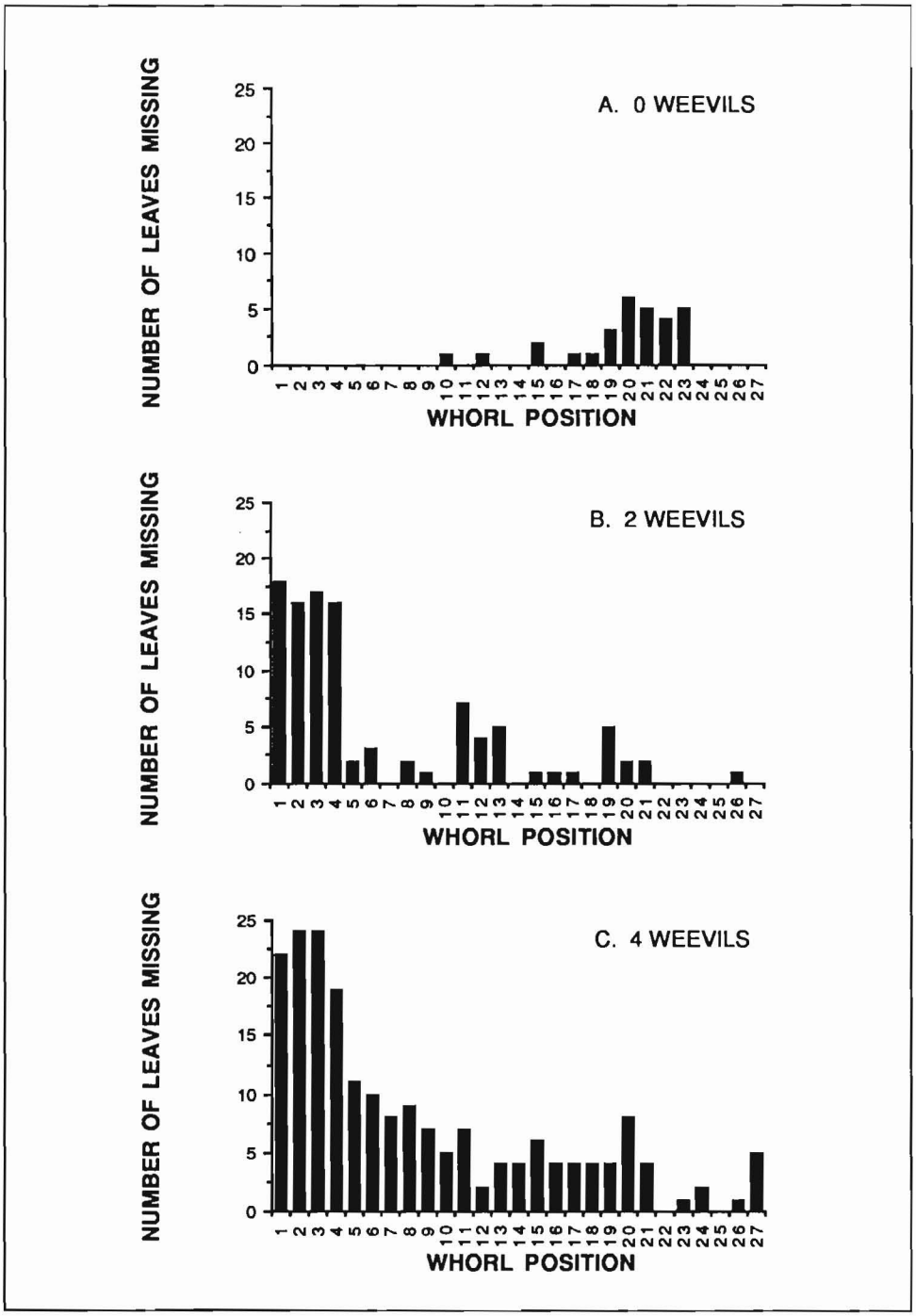

Figure 12. Distribution of leaves removed from watermilfoil autofragments by adult weevils for each treatment. Bars in histograms represent total number of leaves lost from a given whorl for all six replicate plants in each treatment (maximum number of leaves that could be removed is 24). Whorl position (X axis) denotes location of leaf whorls on stem with whorl 1 being whorl adjacent to apical meristem. (A) Control (0 weevils) treatment. (B) 2-weevil treatment. (C) 4-weevil treatment

leaves were removed from the tops of the plants by the weevils (Figure 12B and C). In the 2-weevil treatment, the weevils concentrated their feeding activity on the first four whorls of leaves with scattered removal of leaves further down the stem (Figure 12B). In the 4-weevil treatment, the tops of all the plants were stripped of leaves; there was considerable removal of leaves further down on the stems (Figure 12C). While there was some variation in autofragment length, the range in the number of whorls per plant for each treatment was similar (0 weevils: 17 to 26 whorls; 2 weevils: 19 to 27 whorls; 4 weevils: 20 to 27 whorls). Water temperatures in the pool ranged between 17 and 25 °C during the experiment.

Effect of late instar *Euhrychiopsis* larvae on watermilfoil growth

Euhrychiopsis larvae did not have a consistent effect on watermilfoil growth in this experiment (Figure 13A and B). The presence of one larva reduced watermilfoil change in length compared with the control, but there was no difference in change in length between the 2-larva treatment and the control. Because of this varied response, the contrast between the control and the weevil treatments was not significant. However, the contrast between the weevil treatments was marginally ($p < 0.10$) significant (Figure 13A). Weevil larvae did have a consistent effect on change in weight (Figure 13B). Control plants gained about twice as much weight as either of the weevil treatments; the greater weight of the control plants appears to be due to increased root production, as there was no significant difference in length. The contrast between the control and both weevil treatments was marginally significant. The contrast between the weevil treatments was not significant. The mean ($\pm$1 S.E.) amount of stem hollowed by weevil larvae in the weevil treatments was as follows: 1-larva treatment, 75.4 $\pm$ 6.7 mm (range 59 to 98 mm); 2 larvae, 106.2 $\pm$ 18.6 mm (range 59 to 160 mm). These values translate into burrowing rates of 8.4 mm/day for single larvae and 11.8 mm/day for two larvae. No stem burrowing was observed in internodes 1 (just beneath the meristem) to 5. Watermilfoil stems often buckled in the regions where they were burrowed by weevil larvae. The mean minimum and maximum water temperatures were 15.6 and 22.5 °C, respectively (range 11 to 27 °C).

Combined effect of *Acentria* and *Euhrychiopsis* larvae on watermilfoil growth

Both *Acentria* and *Euhrychiopsis* larvae had significant negative effects on all three measures of plant growth (Figure 14A-C). Watermilfoil plants with just one weevil larva were shorter, had fewer whorls, and weighed less than control plants. The mean ($\pm$1 S.E.) amount of stem hollowed out by weevil larvae was 93.5 $\pm$ 24.9 mm (range 50 to 147 mm), which translates into a mean burrowing rate of 7.8 mm/day. As in the previous experiment, watermilfoil stems often buckled in the regions where they were burrowed by weevil larvae. Only 0.01 $\pm$ 0.006 g of unattached plant material was found in this treatment. Plants with *Acentria* larvae, either alone or in combination with

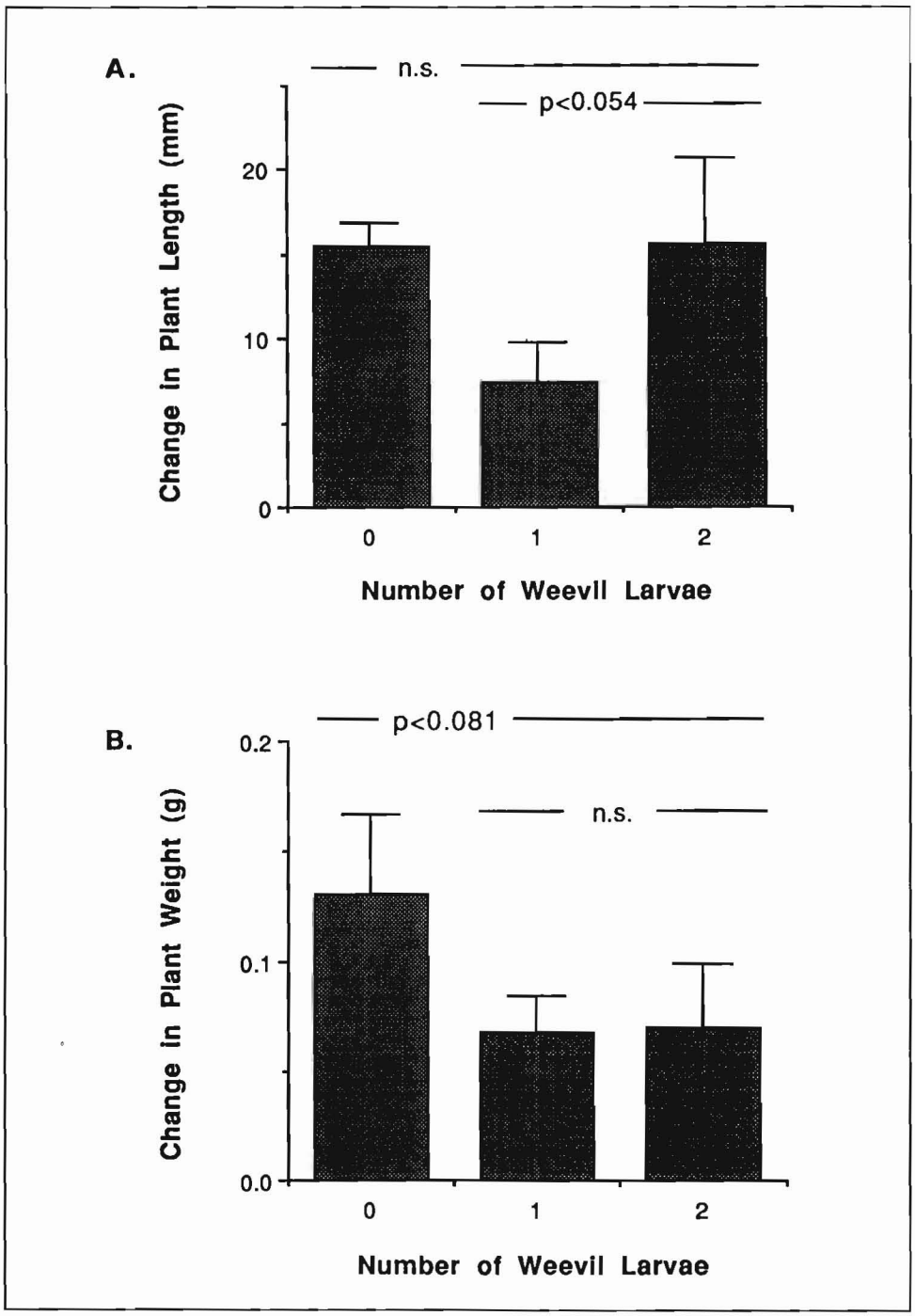

Figure 13. Effect of feeding by *Euhrychiopsis* larvae on watermilfoil plants. Bars in histogram represent mean change in a response variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of control versus weevil treatments; lower line represents comparison of 1- versus 2-weevil treatment. (A) Change in plant length (mm). (B) Change in plant weight (g)

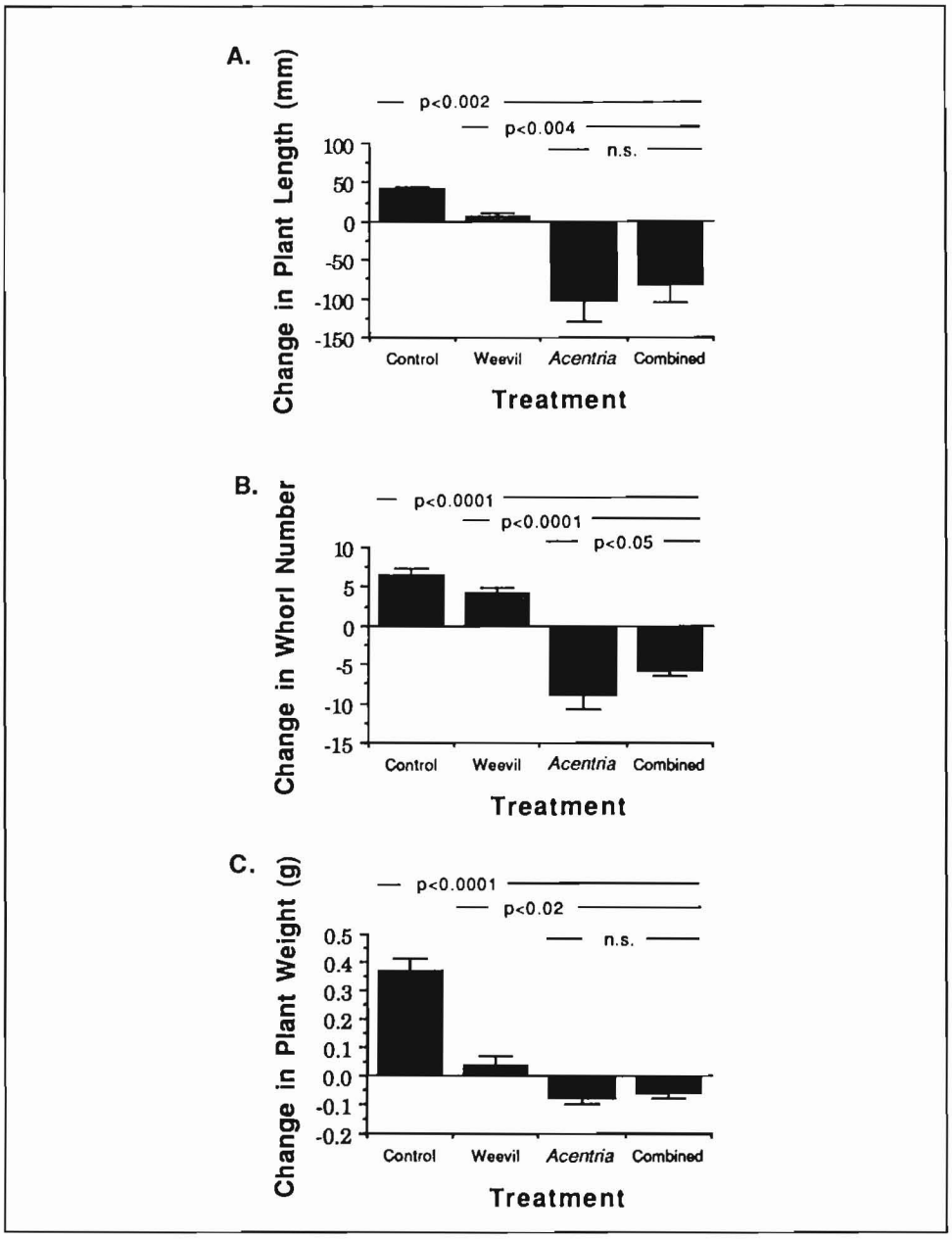

Figure 14. Effect of feeding by *Euhrychiopsis* and *Acentria* larvae on watermilfoil plants. Bars in histogram represent mean change in a response variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of control versus three herbivore treatments; middle line represents comparison of weevil treatment versus two treatments containing *Acentria* larvae; lowest line represents comparison of *Acentria* alone treatment versus treatment with both *Acentria* and *Euhrychiopsis* larvae (combined). (A) Change in plant length (mrn). (B) Change in number of whorls per plant. (C) Change in plant weight (g)

a weevil larva, exhibited even more damage than plants with a single weevil larva. All measures were negative for plants with *Acentria* larvae. The mean amount of unattached plant material found in the chambers containing only *Acentria* was 0.06 $\pm$ 0.03 g wet weight. The damage to plants with both *Acentria* and *Euhrychiopsis* larvae was slightly less than that exhibited by plants that had a single *Acentria*, although more unattached plant material was found in this treatment (0.09 $\pm$ 0.03 g). The mean minimum and maximum water temperatures were 16.6 and 22.7 °C, respectively (range 12.8 to 26.1 °C).

Effect of herbivores on watermilfoil buoyancy

Significantly more watermilfoil was floating in the control aquaria than in the aquaria with herbivores ($F = 19.97$, $p < 0.003$). Almost all of the watermilfoil (98.6 $\pm$ 1.3 percent) was floating in the controls, but only 18.5 $\pm$ 7.5 percent was floating in the herbivore treatments (Figure 15). The mean morning and evening water temperatures were 18.7 and 23.8 °C, respectively (range 16 to 29 °C).

Not all of the weevil adults were recovered from the aquaria at the end of the experiment. All four adults were recovered from two of the aquaria, but only two were found in each of the remaining three aquaria with weevils. An average of 26.4 weevil larvae were removed from the five aquaria with weevils (range 11 to 43). The numbers of *Acentria* larvae found in the weevil treatment aquaria were 0, 1, 2, 3, and 18. Three dead weevil pupae and one *Acentria* larva were found in the control aquaria.

Effect of larval weevil damage on stem fragment viability

Both weevil damage and shading had a negative impact on stem and root production by stem fragments (Figures 16 and 17). All of the undamaged stems produced roots regardless of the shade treatment (Figure 16A). A significantly higher percentage of the damaged stems in unshaded aquaria (DU) produced roots compared with stems in the shaded aquaria (DS). Undamaged stems produced significantly more root biomass than the damaged stems (Figure 16B). Shading reduced root biomass for only undamaged stems. Production of total stem tissue was significantly greater for undamaged, control stems, and most was due to apical elongation of the original stem (Figure 17A and B). All of the stem production in the damaged stems was due to the production of lateral stems (Figure 17C). The difference between the undamaged control stems and the damaged stems was highly significant for all three measures of stem production. Shading appeared to have a positive effect on stem elongation in the undamaged stems; on average, the shaded control stems had original stems that were 27 mm longer than the unshaded ones. However, the shaded control stems produced less lateral stem tissue with the result that the two treatments were almost identical in total stem tissue produced. Shading inhibited the production of lateral stem tissue by damaged stems. On average,

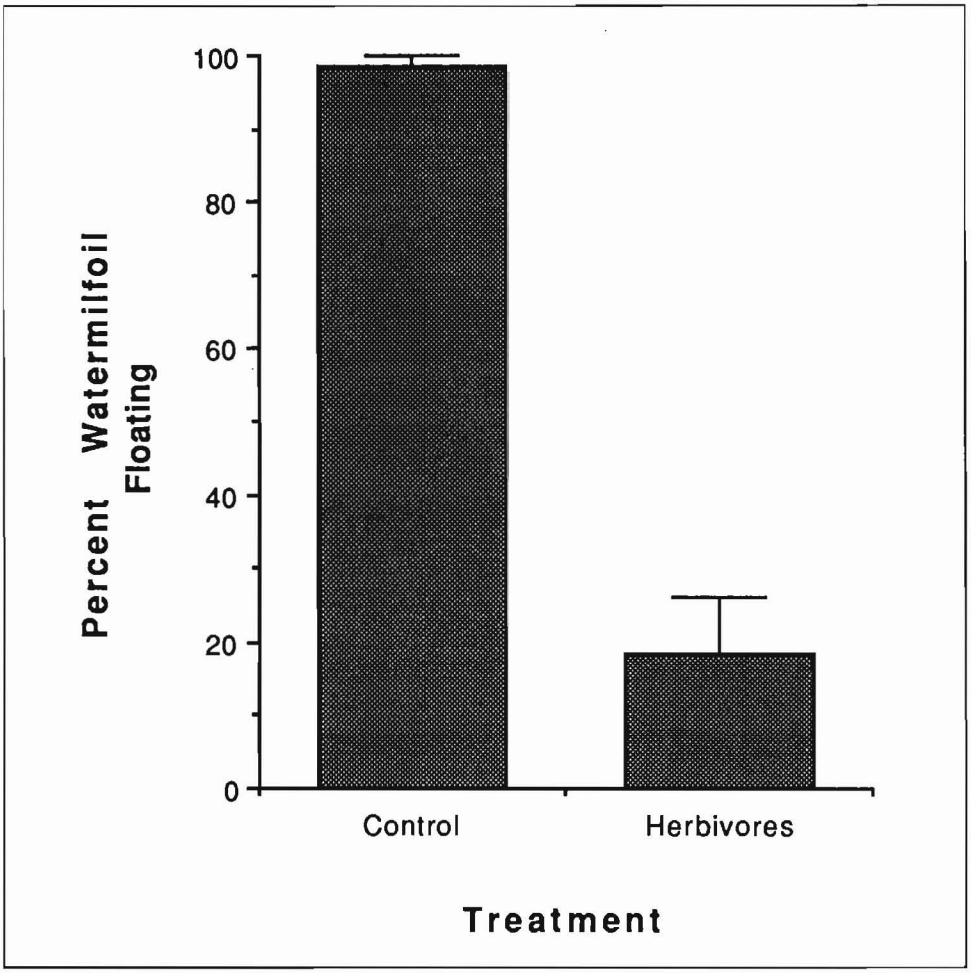

Figure 15. Effect of herbivores on watermilfoil buoyancy in aquarium experiment. Bars in histogram represent mean (±1 S.E.) percent of total watermilfoil weight found floating in each of two treatments

damaged, shaded stems produced 27 mm less lateral stem tissue than unshaded, damaged stems. The mean (±1 S.E.) minimum and maximum water temperatures were 13.1 (±1.5) °C and 29.5 (±1.2) °C in the unshaded treatment, and 13.5 (±1.7) °C and 27.5 (±1.3) °C in the shaded treatment during the experiment.

Discussion

Euhrychiopsis lecontei adults and first instar larvae can have a significant negative effect on watermilfoil growth. Adult weevils fed on the meristem, leaves, and stem of Eurasian watermilfoil. Destruction of the meristem has consequences for watermilfoil growth, including slowing the increase in plant length by preventing further apical growth. If the plant cannot increase in length, then weight change will also be affected. The extensive feeding by

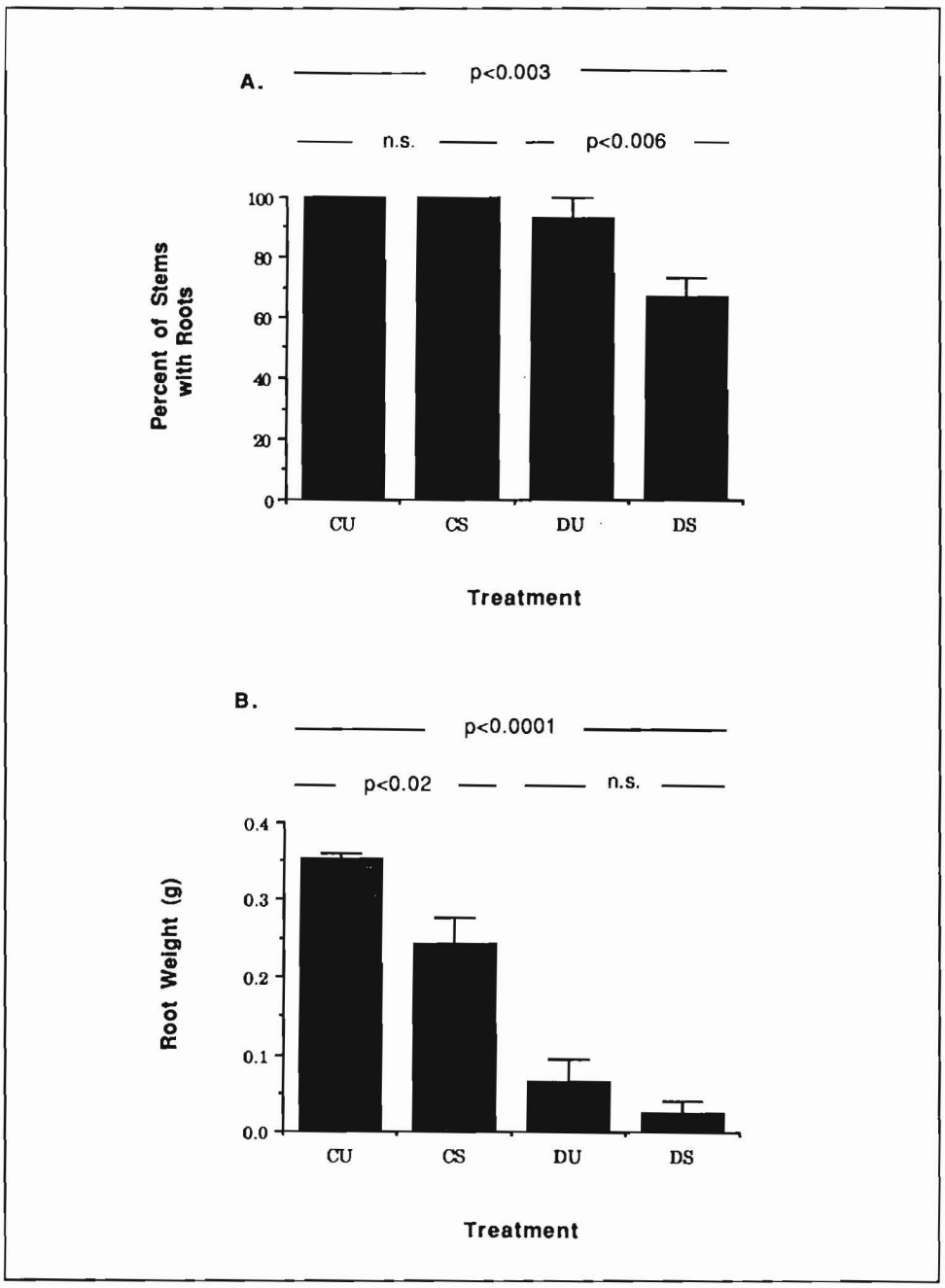

Figure 16. Effect of weevil damage on viability of watermilfoil stem fragments: Production of root tissue. Bars in histogram represent mean change in a response variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of undamaged control fragments (C) versus damaged fragments (D); lower line on left represents comparison of unshaded control treatment (CU) versus shaded control treatment (CS); lower line on right represents comparison of unshaded damaged stem treatment (DU) versus shaded damaged stem treatment (DS). (A) Percent of stems with roots. (B) Root weight (g)

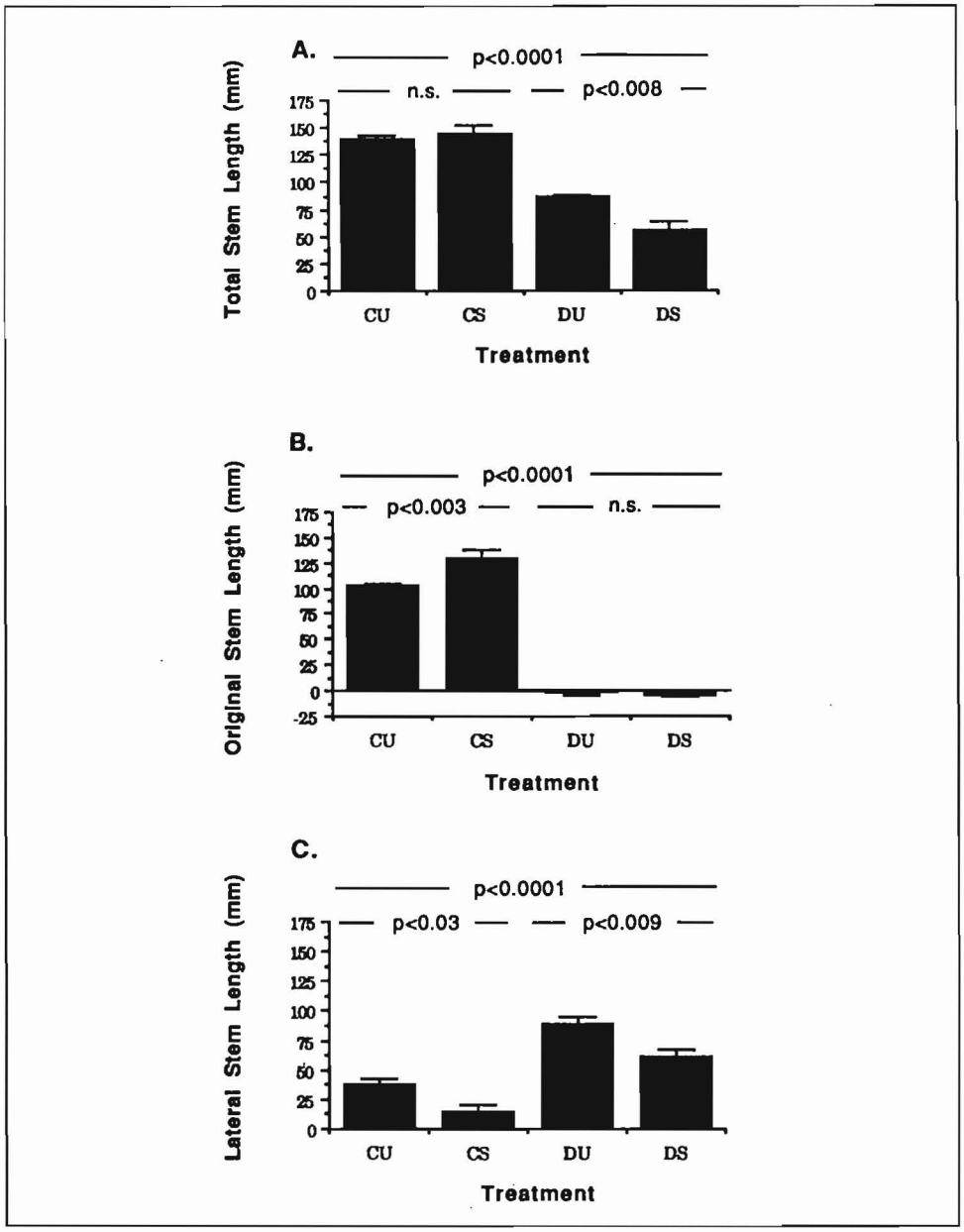

Figure 17. Effect of weevil damage on viability of watermilfoil stem fragments: Production of stem tissue. Bars in histogram represent mean change in a response variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of undamaged control fragments (C) versus damaged fragments (D); lower line on left represents comparison of unshaded control treatment (CU) versus shaded control treatment (CS); lower line on right represents comparison of unshaded damaged stem treatment (DU) versus shaded damaged stem treatment (DS). (A) Total stem tissue produced (mm). (B) Stem tissue produced by original stem (mm). (C) Stem tissue produced by lateral stems (mm)

adults on meristems in the pool study may be an experimental artifact. Field observations suggest that adults feed primarily on the stem and leaves. If the adults are confined to a single stem for an extended period of time, however, they will feed on the meristem. Most meristem destruction in natural settings is thought to be the result of larval weevil feeding. The contribution of first instar larvae to slowing apical growth was apparent in the experiment. Adults may actually avoid feeding on the meristem to preserve this important resource for the first instar larvae (Creed and Sheldon 1993a). Thus, first instar larvae may actually have a greater effect on watermilfoil growth than adults as a result of meristem destruction. This is not to imply that the effect of adults on watermilfoil is negligible. Adults removed significant numbers of whole leaves and leaflets in these experiments, especially from the top of the plant. In a canopy-forming macrophyte such as *M. spicatum* (Smith and Barko 1990), loss of the upper leaves could have severe consequences for the energy balance of the plant if it must rely on deeper leaves for photosynthesis. Also, the lesions created by adult weevil feeding may make the plants more susceptible to attacks by bacteria and fungi. Finally, stem feeding by both adults and larvae can cause watermilfoil to lose its buoyancy and sink.

The effect of late instar *Euhrychiopsis* larvae on watermilfoil length and weight varied between the pool experiments. This variation in response appears to be due in part to differences in watermilfoil growth rates in the two experiments. In the experiment involving only *Euhrychiopsis* larvae, mean change in stem length for control plants was only 15 mm, and mean change in weight was only 0.13 g. In the experiment with both *Acentria* and *Euhrychiopsis* larvae, mean change in stem length and stem weight for control plants were three times that of the first experiment (mean change in length was 43 mm and mean change in weight was 0.38 g). Changes in plant length and weight in the 1-weevil treatment in both experiments were fairly similar. Thus, while weevil larvae can suppress the growth of slow-growing plants, the inhibition of watermilfoil growth by weevil larvae is more pronounced when watermilfoil plants have the potential to grow at faster rates. The cause of the difference in growth rates in the two experiments is not known. Water temperatures were similar for both experiments, and the same water source was used to fill the wading pools. Possible explanations include either differences in sediment nutrient concentrations or plant condition.

One way that late instar weevil larvae may influence watermilfoil growth is by destroying stem vascular tissue. Removal of vascular tissue could result in reduced or halted translocation of nutrients from roots to actively growing portions of shoots. However, if adequate quantities of nutrients can be removed directly from the water by the plant, then this effect should be negligible. While the sediments are an important source of nutrients for rooted, aquatic macrophytes (e.g., Barko and Smart 1978, 1981; Carignan and Kalff 1980), *M. spicatum* can absorb nutrients from the water column through stem and leaf tissue (Nichols and Keeney 1976; Best and Mantai 1978; Carignan and Kalff 1980). The ability of *M. spicatum* to take up water column nutrients appears to be a function of nutrient concentration (Nichols and Keeney 1976; Best and Mantai 1978; Carignan and Kalff 1980), with nutrients being

absorbed from the water when at higher concentrations. In their review of watermilfoil biology, Smith and Barko (1990) concluded that most of the N and P taken up by this macrophyte species comes from the sediments. When the results of this study are considered in conjunction with the nutrient uptake data, they suggest that larval weevil burrowing might have a more pronounced effect on *M. spicatum* growth in nutrient-poor water bodies if the growing portion of the stems cannot obtain sediment nutrients.

Acentria significantly reduced watermilfoil growth, and much of their effect appeared to have been attributable to cutting the stem while feeding and for retreat construction. *Acentria* feed largely on leaves and stems (Batra 1977; Buckingham and Ross 1981). Early instar larvae usually construct retreats by folding over a single leaf and attaching it to the stem with silk. While late instar larvae may also dwell in such retreats (Batra 1977), they have frequently been seen to cut the stem, slide the upper portion of the stem down, and construct a silk retreat between the two stem pieces. In this experiment, *Acentria* larvae frequently cut the stems additional times above their retreats, which accounted for the reduced lengths of the plants and the detached stem material in the chambers. Although *Acentria* larvae consumed watermilfoil in the chambers, it is not possible to determine the amount consumed, as the difference between *Acentria* treatments and controls could have been because of consumption of tissue, inhibition of growth, and decay of some of the unattached material. The results of this study with rooted stems confirm those of Painter and McCabe (1988), who examined the effects of *Acentria* on floating watermilfoil fragments. They noted that watermilfoil continued to grow when *Acentria* densities were less than one per stem. When *Acentria* densities exceeded one per stem, a dramatic reduction in watermilfoil weight was observed (Painter and McCabe 1988).

While *Acentria* consistently reduced watermilfoil growth compared with *Euhrychiopsis* in these experiments, field observations suggest that *E. lecontei* may do more damage in lakes and ponds. When one of the watermilfoil beds (the West Bed) in Brownington Pond collapsed in mid-July 1991, mean weevil abundance in this bed ranged from 2.0 to 3.2 weevils per stem. Mean weevil abundance in the South Bed, which did not collapse, ranged from 0.2 to 1.0 weevils per stem. There was no difference in the average abundance of *Acentria* between the two beds: South Bed, 0.0 to 0.6 larvae per stem; West Bed, 0.2 to 0.4 larvae per stem (Creed and Sheldon 1992). *Acentria* can damage the stem, especially during construction of the late instar retreat/ puparium (see above); however, retreat construction does not appear to have any long-term effect on stem buoyancy. Watermilfoil plants have frequently been seen with one or two late instar *Acentria* cases that did not appear to have suffered any loss of buoyancy. While *Acentria* larvae in meristems have occasionally been encountered, *Acentria* appear to concentrate their feeding on leaves below the meristem (but see Painter and McCabe 1988). Thus, apical elongation may continue despite the *Acentria* feeding. First instar *Euhrychiopsis* larvae, on the other hand, destroy meristems, which can result in a suppression of stem elongation. The pond enclosure experiment (Chapter 2) also demonstrated that weevil feeding suppressed the production of lateral

stems and root tissue. The pool experiments suggest that *Acentria* can have a strong, negative effect on watermilfoil as a result of cutting the stem and biomass consumption. Field observations, on the other hand, suggest that *Acentria* may not be as important in producing declines as *Euhrychiopsis*. *Euhrychiopsis* larvae and adults may suppress watermilfoil growth to a greater extent than *Acentria* by affecting the physiology of the entire plant.

Feeding by *Acentria* and *Euhrychiopsis* larvae on watermilfoil may result in stem fragmentation. This effect was clearly seen in the treatments containing *Acentria*, but not in the *Euhrychiopsis* treatments. Larval weevil burrowing does weaken the watermilfoil stem, with the result that burrowed stems fragment easily. Such broken stems have been commonly encountered in lakes and ponds. This effect was not observed in treatments with weevil larvae, as the stems were protected from physical disturbance by the chambers. It is possible that this herbivore-induced fragmentation could promote the spread of watermilfoil. However, the meristem experiment demonstrated that some stem fragments damaged by weevil larvae were still viable, but produced significantly less stem and root tissue than undamaged control fragments. Growth rates of damaged fragments in the shaded treatments were reduced even further. Watermilfoil is most abundant in water 2.0 to 3.0 m deep in Brownington Pond, and many stem fragments may settle in deep water where light levels are lower. This may be particularly true of weevil-damaged fragments, which have reduced buoyancy and probably settle close to the source plants. Therefore, while weevil herbivory can generate stem fragments, the potential for these fragments to produce extensive, new watermilfoil beds appears to be reduced. The viability of stem fragments produced by *Acentria* larvae has not been determined.

6 Effect of *E. Lecontei* on Native Macrophytes

Introduction

A series of feeding experiments were conducted to quantify the effect of adult weevils on a native species of watermilfoil (*Myriophyllum sibiricum*) and other native macrophyte species (*Ceratophyllum demersum* L., *Chara* sp., *Elodea canadensis* Michx., *Heteranthera dubia* (Jacq.) MacM., *Megalodonta beckii* (Torr.), *Najas flexilis* (Willd.) Rostk. and Schmidt, *Potamogeton amplifolius* Tuckerm., *Utricularia vulgaris* L., and *Vallisneria americana* Michx.). These native species were some of the more common (frequency, biomass, and distribution) macrophyte species in Vermont.

Materials and Methods

Effect of *E. lecontei* adults on a native watermilfoil

The effects of adult weevils on the native northern watermilfoil (*Myriophyllum sibiricum* Komorov) were quantified in an experiment similar to the growth experiments described in Chapter 5. Plants with intact roots were collected from a local Vermont lake. All invertebrates and eggs were removed from the plants. The condition of each leaf, internode, and meristem were described at the beginning of the experiment. The length of the plants above a marker on the stem and blotted wet weights were recorded. The initial length of the plants ranged from 168 to 217 mm; initial weights ranged from 0.57 to 1.97 g. The experimental design was a randomized block design with three treatments (0, 2, or 4 weevils per chamber) and six replicates per treatment. The experiment was terminated after 13 days. Treatment effects were compared using an ANOVA with planned, orthogonal contrasts (Sokal and Rolhf 1981).

Effect of *E. lecontei* adults on other native macrophytes

The same experimental design was used to quantify the effect of weevils on other native, aquatic macrophytes. Plants <30 cm total length with intact roots except *Utricularia vulgaris* (a species that is not rooted in the sediment) were collected from three Vermont lakes. Plants were examined under a dissecting microscope, and all invertebrates and eggs were removed. The condition of each leaf, internode, and meristem by whorl or leaf as appropriate for each species was described. Only plants with intact apical meristems were used. Blotted wet weights and stem length were recorded. The 18 plants that were the most similar in length, weight, number of leaf whorls, and number of meristems were chosen for these experiments.

All species except *P. amplifolius* were placed in chambers similar to the ones used before. The chambers for *P. amplifolius* were 27 cm high, and the inside diameter was 12.7 cm. The chambers were placed in 375-L pools filled with aerated tap water in a greenhouse under ambient light. As before, there were three treatments, 0, 2, or 4 adult *E. lecontei* per chamber, and six replicates of each treatment. Three additional chambers, each with an *M. spicatum* plant and four adult weevils, served as a control for environmental conditions during the experiment.

All trials ran for 10 or 11 days except three. The *Elodea* experiment was ended after 8 days because all of the weevils enclosed with *Elodea* were dead. The *Chara* experiment was terminated after 8 days because plants in all treatments were deteriorating. The *Utricularia* experiment ran for only 7 days because it was clear that the weevils were affecting the plants by knocking off the bladders (structures used for catching animals by this carnivorous plant), and not by feeding.

At the end of each experiment, any new (relative to initial) leaf and stem damage was recorded. Plant lengths and weights were determined as described above. In some cases, plants fragmented when removed from the chambers. Consequently, the length data was analyzed two ways—once with all plants (n = 6 per treatment) and once using only intact plants (n = 3 to 6). All 18 plants were used for the determination of change in plant weight. The data were analyzed with an ANOVA with planned orthogonal contrasts (Sokal and Rohlf 1981). The discussion of results for plant change in length is based on the analysis of intact plants only.

Results

Effect of *E. lecontei* adults on a native watermilfoil

Weevils did not have a significant negative effect on either change in length or weight of *M. sibiricum* (Figure 18). There was significantly

($p < 0.002$) more leaf loss at the tops of plants and damage to apical meristems in both weevil treatments compared with plants grown without weevils. Weevils did not have a significant effect on the mean number of leaf whorls added. Significantly more ($p < 0.002$) lateral branches were produced in the 0-weevil treatment than in the other two treatments. One egg and six larvae were found on *M. sibiricum* plants. The mean minimum and maximum water temperatures were 17.0 and 22.9 °C, respectively (range 15.6 to 26.7 °C).

Effect of *E. lecontei* adults on other native macrophytes

All of the species grew in the chambers, although the growth rates of some were slow. Some plants broke while being removed from the chambers, often at the root-shoot interface. Since initial lengths were measured relative to a marker, change in plant length was difficult to measure accurately for broken plants. There was no increased probability of plant breakage with or without weevils. There were no significant differences in change in plant length among treatments for any plant species using either all of the plants in a trial or only intact plants (Figure 19A). Neither were there significant differences among treatments in change in plant weight, although there was a trend of decreasing weight in the *Utricularia* trial (Figure 19B). Most of the weight loss in *Utricularia* appeared to be due to the loss of bladders.

On average, all macrophyte species added either new leaves, leaf whorls, or side branches. There were no significant differences in the production of new plant tissue except for *Elodea* (Table 3). There were significantly ($p \leq 0.05$) more side branches on *Elodea* plants in the 4-weevil treatment compared with the 0- and 2-weevil treatments.

There was extensive weevil mortality on the native macrophytes. No weevils survived on either *Elodea* or *Heteranthera*. The highest weevil survivorship (33 percent) was on *Utricularia* in the 4-weevil treatment; the *Utricularia* trial was terminated after only 7 days. No weevil eggs, larvae, or weevil grazing damage were found on any of the native macrophytes. In contrast, weevil survivorship was high on the *M. spicatum* controls. Only one dead weevil was found in the *M. spicatum* controls over all eight trials.

Discussion

Adult weevils did not have a significant effect on the growth of any of the native macrophyte species tested, although weevils did remove a significant number of leaves from *M. sibiricum* in that experiment. In the field, weevil damage has only been seen on *M. spicatum* and *M. sibiricum*. These observations and the low survivorship of weevils on the other macrophytes suggest that *E. lecontei* is a watermilfoil specialist. This supports its use as a biological control agent for Eurasian watermilfoil. The only negative effect

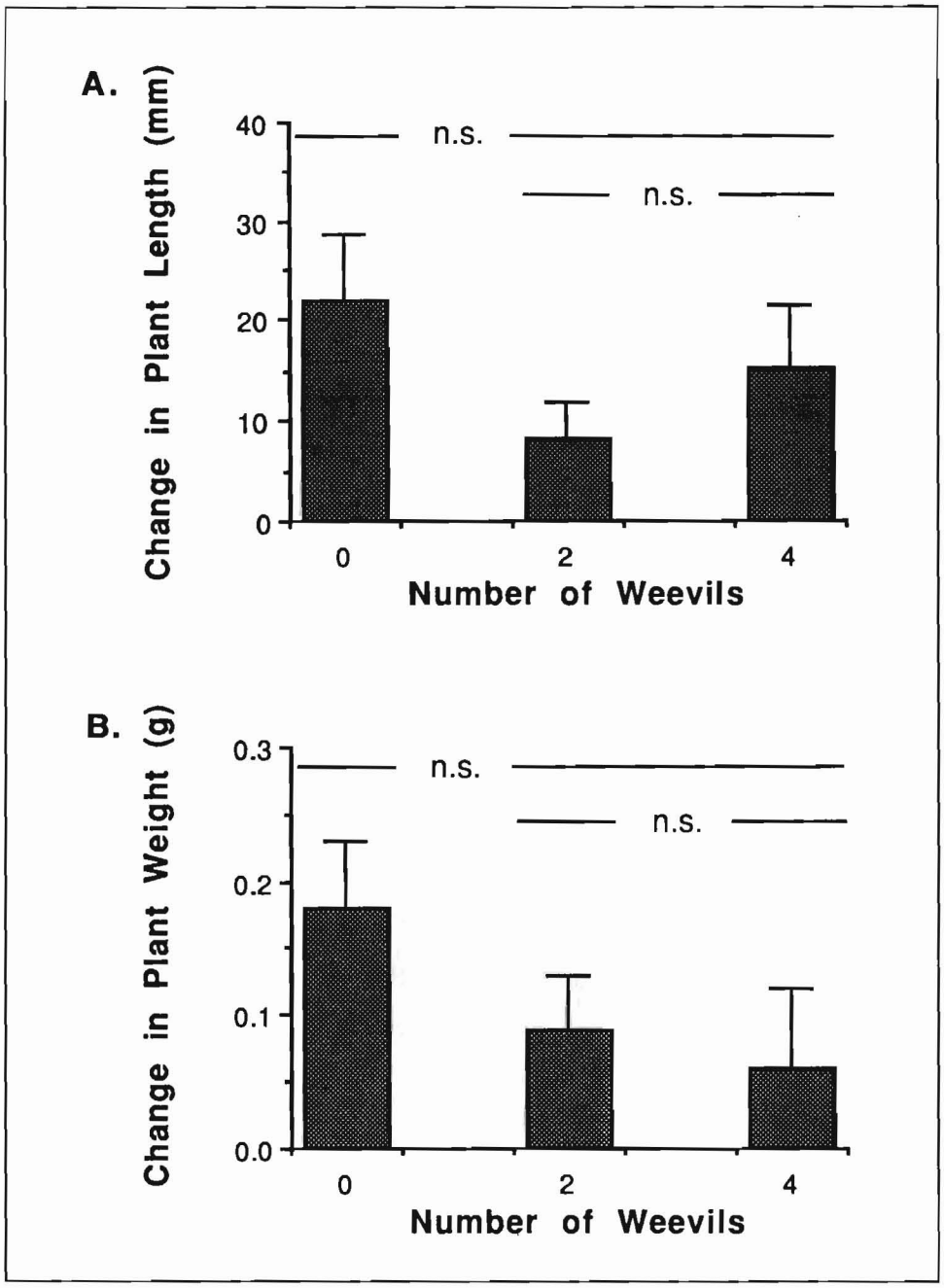

Figure 18. Effect of feeding by *Euhrychiopsis lecontei* adults on native water-milfoil *Myriophyllum sibiricum*. Bars in histogram represent mean change in a response variable (±1 S.E.) for each treatment. Lines with significance values above histograms show results of ANOVA comparisons with orthogonal contrasts. In each figure, upper line represents comparison of control versus weevil treatments; lower line represents comparison of 2- versus 4-weevil treatment. (A) Change in plant length (mm). (B_s) Change in plant weight (g)

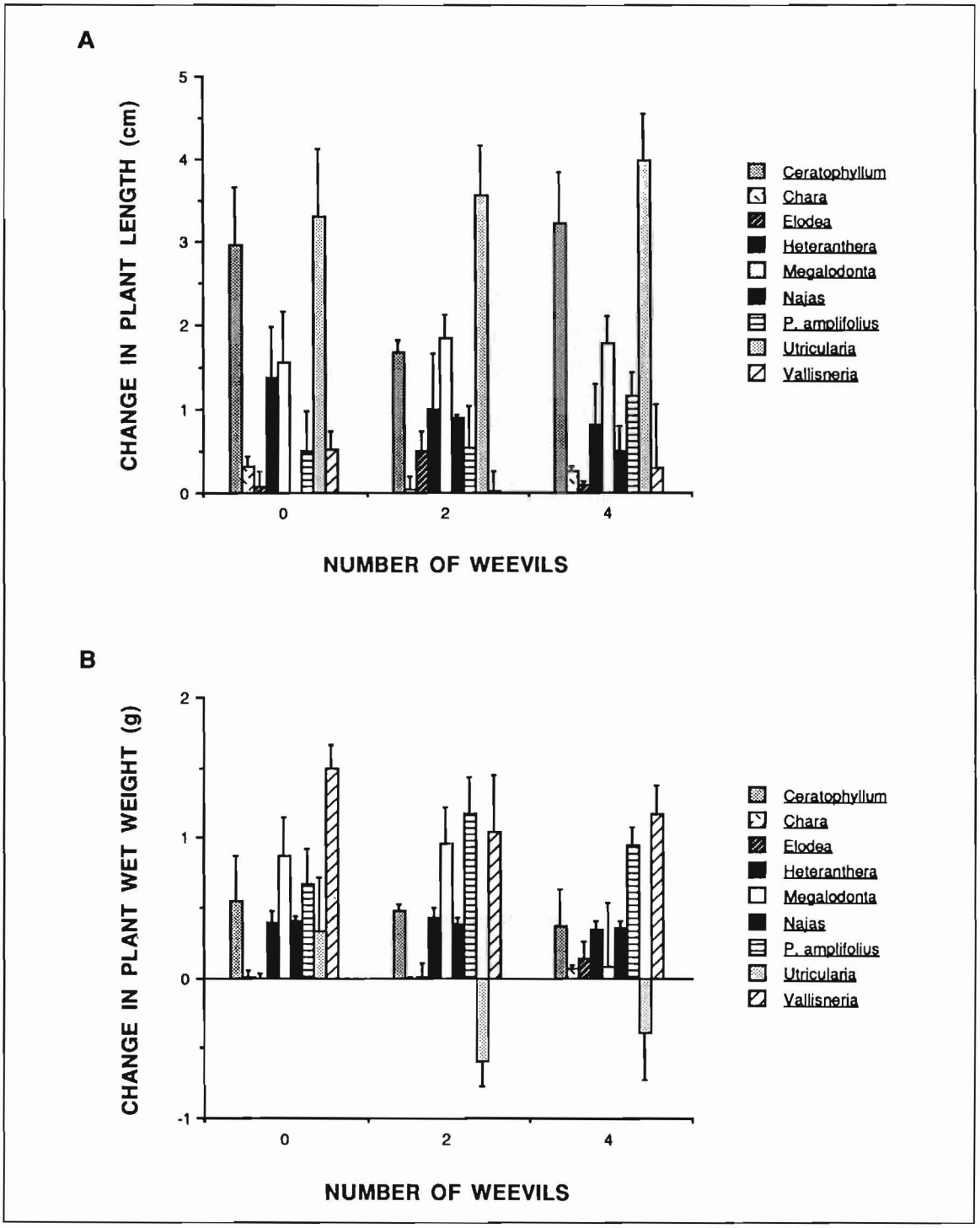

Figure 19. Effect of adult *E. Lecontei* on nine native, aquatic marcophyte species. (A) Change in plant length (cm). Values are means (±1 S.E.) for each treatment for intact plants. (B) Change in plant wet weight (g). Values are mean (±1 S.E.) for each treatment for all plants

anticipated is increased weevil damage on *M. sibiricum* and possibly other species of *Myriophyllum*.

Table 3
Effect of Adult Weevils on Number of New Side Branches, Leaves, or Ramets Produced by Native Macrophyte Species in Feeding Trials

	Treatment		
Macrophyte Species	**0 Weevils**	**2 Weevils**	**4 Weevils**
Ceratophyllum (side branches)	3.67 ± 0.92	4.00 ± 0.45	4.50 ± 0.43
Chara (leaf whorls)	0.27 ± 0.49	0.50 ± 0.22	0.12 ± 0.31
Elodea (side branches)	0.83 ± 0.31[a]	1.00 ± 0.26[a]	2.00 ± 0.26[b]
Heteranthera (leaves)	11.0 ± 3.5	14.3 ± 2.5	12.6 ± 2.3
Megalodonta (leaf whorls)	4.50 ± 0.67	4.67 ± 0.80	4.50 ± 1.12
P. amplifolius (leaves)	3.33 ± 0.96	3.20 ± 0.37	3.00 ± 0.26
P. amplifolius (ramets)	1.00 ± 0.63	0.40 ± 0.24	0.67 ± 0.21
Utricularia (side branches)	0.17 ± 0.16	0.17 ± 0.16	0.33 ± 0.20
Vallisneria (leaves)	1.50 ± 0.34	1.50 ± 0.56	0.83 ± 0.31

Note: Values in the table are means ±1 S.E. For *Elodea*, treatment means with the same letter are not significantly different from one another.

7 Multistate Lake Survey

Introduction

In 1989, *Euhrychiopsis lecontei* was found in 4 Vermont lakes, and caterpillars (*Acentria ephemerella* and *Parapoynx badiusalis*) were found in 10 lakes. To determine the distribution of *M. spicatum* herbivores throughout Vermont and neighboring states (Connecticut, Maine, Massachusetts, New Hampshire, and New York), a number of lakes were visited in 1990 and 1991. *Myriophyllum spicatum* was not reported from Maine or New Hampshire in 1990 and 1991. A North American species of watermilfoil (*Myriophyllum heterophyllum*), which is not native to New England,[1] was reported to be extremely abundant in several lakes in these two states and was sampled for *M. spicatum* herbivores.

Materials and Methods

In both 1990 and 1991, the section of the littoral zone of each lake where watermilfoil had previously been observed was surveyed by a pair of snorkelers. Upon locating the *Myriophyllum*, they examined the plants for the presence of herbivores and evidence of herbivore damage (e.g., *Acentria* day refugia and puparia, weevil stem damage). Presence of herbivores and their relative abundance were noted as were observations of herbivore damage. Specimens of all potential invertebrate herbivores associated with the watermilfoil were collected and preserved. In 1991, in addition to the surveys, watermilfoil plants were collected and transferred to the laboratory. If possible, the macroinvertebrates were removed from the fresh plants and preserved. Otherwise, the entire sample was preserved for later enumeration of the invertebrates.

A second species of weevil, *Phytobius leucogaster* (Marsham), was occasionally found associated with watermilfoil. Distinction can be made between the adults and pupae (in puparia) of *E. lecontei* and *P. leucogaster*. Larvae can usually be assigned to species with a high degree of certainty based upon

[1] Personal Communication, 1990, Barre Hellquist, N. Adams State College, N. Adams, MA.

where they are located on a plant (e.g., *Phytobius* larvae usually feed on emergent floral spikes, while *Euhrychiopsis* larvae are normally found burrowing through submersed portions of the stem). However, a morphological feature that allows us to readily differentiate between the larvae has not yet been found if they are not on the plants. If weevil larvae were collected but their location on a plant was not noted, they were not assigned to either species.

Results

Twenty-nine lakes with *M. spicatum* were surveyed in Vermont in 1990 and 1991. One or more of the herbivore species were found in 20 of these lakes (Table 4). *Eurhychiopsis* was found in 18 lakes located throughout the state. *Acentria* and *Parapoynx* were present in 13 and 5 lakes, respectively. *Euhrychiopsis* was the only herbivore associated with watermilfoil in six lakes. *Acentria* was almost always found together with *Euhrychiopsis* (12 of 13 lakes). In the collections, *Parapoynx* was always found in lakes containing the other two watermilfoil herbivores (n = 5). *Eurhychiopsis* was also collected and/or observed in lakes in Connecticut, Massachusetts, and New York (Table 5). Unidentified weevil larvae were collected from *M. heterophyllum* in New Hampshire and Maine. *Acentria* larvae were found associated with *M. spicatum* in Massachusetts and with *M. heterophyllum* in New Hampshire. *Parapoynx* was only found in one lake in Connecticut.

Discussion

All three herbivores were found throughout Vermont. Most collections (n = 17) of herbivores were from lakes in the Lake Champlain drainage on the western side of the state. Lake Champlain drains into the St. Lawrence River. The exceptions were Lake Memphremagog, Brownington Pond, and Round Pond. Lake Memphremagog and Brownington Pond are on the Canadian border and are also in the St. Lawrence River drainage. Round Pond is on the east side of the state and is in the Connecticut River drainage. *Euhrychiopsis* and *Acentria* were found associated with watermilfoil in lakes in western Massachusetts; *Euhrychiopsis* was found on watermilfoil in lakes in western Connecticut. The lakes in both western Massachusetts and western Connecticut were in the Housatonic River drainage.

Northern watermilfoil (*Myriophyllum sibiricum*), which appears to be one of the native hosts for *Euhrychiopsis* (see Chapter 8), is common in western Vermont, western Massachusetts, and western Connecticut (Crow and Hellquist 1983). The proximity of these two similar aquatic macrophytes may have facilitated the host shift observed in this native weevil. *Acentria* and *Parapoynx* are generalist feeders and are found on a variety of aquatic macrophytes (e.g., McGaha 1952, 1954; Batra 1977; Buckingham and Ross 1981). These two moths may have shifted onto *M. spicatum* from various hosts.

Table 4
Lakes Visited in Vermont in 1990 and/or 1991 with Indications of Presence or Absence of *Euhrychiopsis lecontei, Acentria ephemerella,* and *Parapoynx badiusalis*

Lakes	*Euhrychiopsis*	*Acentria*	*Parapoynx*
Arrowhead Mtn.	R	R	
Berlin	X	X	X
Black			
Bomoseen	X	X	
Brownington	X	X	X
Burr			
Carmi			
Champlain -McCuen Slang -Shelburne Bay	 X X	 X X	 X
Dunmore			
Echo	R		
Glen	X		
Hortonia			
Iroquois	X	R	
Little			
Love's	R		
Lower	X	X	
Memphremagog	X	X	
Metcalf		X	
Mill (Kennedy)			
North Montpelier	X	X	
Norton Brook			
Paran	X		
Parson's Mill			
Richville			
Round	X		

(Continued)

Note: An "X" indicates collection and identification of invertebrates. An "R" indicates a field siting but no collected individuals. "Lar" appears only in the weevil column and indicates the collection of unidentifiable weevil larvae.

Table 4 (Concluded)

Lakes	*Euhrychiopsis*	*Acentria*	*Parapoynx*
St. Catherine	Lar		
Sunrise	R	R	R
Sunset	R		
Winona	X	X	X

In Vermont, many of the lakes that did not have herbivores had watermilfoil control programs. For example, bottom barriers and manual weeding were used to control *M. spicatum* in Black Pond and Lake Dunmore. Both mechanical harvesting and bottom barriers were employed in Little Lake. Watermilfoil was being harvested mechanically from Lake St. Catherine and Lake Hortonia. In Lake Bomoseen, where adjacent sections of watermilfoil beds were designated as either harvest or no-harvest areas, the effect of mechanical harvesting was evaluated on herbivore abundance. Densities of both *Euhrychiopsis* and *Acentria* were significantly higher in unharvested areas (Creed and Sheldon 1992). Herbivore damage was also more extensive in these unharvested areas. Since adults concentrate their feeding at the tops of plants and weevil eggs and first instar larvae are found in and on the meristems, mechanical harvesting may prevent these herbivores from becoming abundant by removing the upper portion of the watermilfoil.

Table 5
Lakes Visited in Other States in Multistate Surveys in 1990 and 1991 with an Indication of Presence or Absence of *Euhrychiopsis lecontei, Acentria ephemerella*, and *Parapoynx badiusalis*

State Lakes	*Euhrychiopsis*	*Acentria*	*Parapoynx*
CT			
Candlewood			
Long	X		
West Twin	X		X
Wononskopomuc	X		
MA			
Buell			
Cheshire	Lar		
Garfield			
Laurel			
Onota	X	X	
Pleasant Valley			
Pontoosuc	X	X	
Richman		R	
Shaker Mill	R	R	
Stockbridge	X	X	
ME			
Sebago[1]			
Thompson[1]	Lar		
NH			
Winnepesaukee			
- Wolfeboro Bay[1]			
- Moultonboro Bay[1]	Lar		
- Opechee Bay[1]		R	
NY			
Augur	R		
Cossayuna			

Note: An "X" indicates collection and identification of invertebrates. An "R" indicates a field siting but no collected individuals. "Lar" appears only in the weevil column and indicates the collection of unidentifiable weevil larvae.
[1] *Myriophyllum heterophyllum* was sampled in this lake.

8 Collections of Aquatic Weevils Associated with Northern Watermilfoil in Alberta

Introduction

The identity of *E. lecontei's* native host (or hosts) and its life history on its native host(s) are unknown. Blatchley and Leng (1916) report *Potamogeton* sp. and *Myriophyllum spicatum* as hosts. However, Blatchley and Leng incorrectly synonymized this weevil species with the palearctic weevil *Eubrychius velatus* (Beck).[1] Since the host-use information reported by Blatchley and Lang (1916) may be derived from European records of *E. velatus*, it is questionable. More recently, Kissinger (1964) reported that one species of *Euhrychiopsis* lived on *M. spicatum*. Kangasniemi (1983) reported collecting *E. lecontei* on *M. spicatum* in British Columbia. The repeated collection of *E. lecontei* on the introduced *M. spicatum* suggests that the native host(s) might be one or more of the native watermilfoils.

Collections of *E. lecontei* have been made from northern watermilfoil (*Myriophyllum sibiricum*) in three lakes in Vermont. *Myriophyllum spicatum* was also present in two of the three lakes, so it was unclear if the weevils had been present on the northern watermilfoil when Eurasian watermilfoil invaded the lakes or if they had entered the lakes with Eurasian watermilfoil and had then begun to feed on the native watermilfoil, which is morphologically similar to Eurasian watermilfoil (Aiken, Newroth, and Wile 1979). To determine if northern watermilfoil is a native host, weevils were collected in Alberta, Canada, where both northern watermilfoil and the weevil are present but Eurasian watermilfoil is absent. Previous collections of *Euhrychiopsis* had been made in Alberta (Brown 1932; Kissinger 1964; O'Brien and Wibmer 1982), but the native host was not determined.

[1] Personal Communications, 1993, Charles O'Brien, Florida A&M University, Tallahassee, FL.

Currently, two species of *Euhrychiopsis*, *E. Lecontei* and *E. albertanus* (Brown), are recognized in North America. However, Dr. Charles O'Brien (Florida A&M University, Tallahassee, FL) has examined the weevils ollected in Alberta and other specimens in his collection and believes that *E. lecontei* and *E. albertanus* may be a single species, based on a lack of differentiation in male genitalia. Because of the present uncertainty in the taxonomic status of these two species, reference will be made to the weevils collected in Alberta as *Euhrychiopsis*. These two species are not being synonymized in this report.

Materials and Methods

Collections of weevils on northern watermilfoil were made in mid-July to late July of 1992. Weevils were usually collected from northern watermilfoil while snorkeling. Only lakes where the visibility exceeded 1 m were surveyed intensively by snorkeling. In very shallow water or in very turbid water bodies, collections were made by inspecting northern watermilfoil while wading. Lakes with extensive algal blooms that made visual collection impossible were not examined. Approximately 1 hr was spent examining northern watermilfoil in lakes where collecting was possible. While the primary goal of these collections was to obtain adult specimens, some eggs, larvae, and pupae were collected. The identity of the adult weevils was verified by Dr. Charles O'Brien of Florida A&M University, and most of the specimens are now in his collection. The identity of eggs, larvae, and pupae was based on field and laboratory observations of these life stages of *E. lecontei* and *P. leucogaster* on *M. spicatum* and *M. sibiricum* in eastern North America.

Results and Discussion

Adult *Euhrychiopsis* were found on *M. sibiricum* in 10 of the 13 lakes that were sampled (Table 6). *Myriophyllum sibiricum* was present in all 13 lakes. *Euhrychiopsis* adults were always collected beneath the surface of the water. They were usually located near apical or lateral meristems, although they were occasionally found further down the stem. *Euhrychiopsis* eggs, larvae, and pupae were always found underwater on *M. sibiricum*. Eggs were found on northern watermilfoil in six of the lakes; larvae and pupae were each collected in two lakes (Table 6). Eggs were found on meristems, and only one was found per meristem (n = 16). This is unlike the observation for *E. lecontei*, which may lay several eggs on a Eurasian watermilfoil meristem (see Chapter 4). First instar *Euhrychiopsis* larvae were not collected in Alberta, but the presence of eggs on the meristems suggests that the first instar larvae of western *Euhrychiopsis* feed on northern watermilfoil meristems. Older larvae (n = 4) were found burrowing in the stem well below the surface of the water. Pupae (n = 2) were found inside the stem below the region burrowed by the larvae. The puparium consisted of a small chamber entirely within the stem

Table 6
Lakes in Alberta from Which *Euhrychiopsis* and *Phytobius* Were Collected

Lake	Collection Method	*Euhrychiopsis*	*Phytobius*
Winchell	Snorkeling	E, A (3,4)	
Pine	Wading	E, L, A (4, 1, 6)	
Hofmann	Snorkeling	E, L, P, A (2, 3, 1, 8)	A (2)
Newall	Snorkeling	*	
MacGregor	Snorkeling		
Narrow	Snorkeling	E, A (4,13)	
Long	Snorkeling	E, P, A (2, 1, 5)	
Island	Wading	A (5)	E, L, P, A (1, 2, 8, 4)
N. Buck	Wading	A (1)	
Chump	Snorkeling	A (1)	
Lac la Biche	Wading	*	A (4)
Beaver	Snorkeling	E, A (1, 9)	A (1)
Hasse	Wading	A# (1)	

Note: "E" refers to the collection of eggs, "L" to the collection of larvae, "P" to pupae, and "A" to adults. The numbers in parentheses beneath the letters refer to the number collected.
(1)* = previously collected at this site by John Carr. (2)# = observed but not collected

with a sealed entrance hole. The location of western *Euhrychiopsis* eggs, larvae, and pupae on *M. sibiricum* was the same as that observed for *E. lecontei* on *M. sibiricum* and *M. spicatum* in eastern North America. It is highly likely that these eggs, larvae, and pupae are those of *Euhrychiopsis*, as all three life stages were collected in lakes in which *Euhrychiopsis* was the only adult weevil found on *M. sibiricum* (Table 6).

The weevil *Phytobius leucogaster* (Marsham) (=*Litodactylus griseomicans* (Schwarz) and *Litodactylus leucogaster* (Marsham)), a species with a holarctic distribution, was found on *M. sibiricum* in four of these lakes (Table 6). *Phytobius* adults (n = 11) were found both above and below the surface of the water. All life stages were collected at Island Lake, which was the only lake

where large numbers of the *M. sibiricum* plants were flowering. Eggs (n = 2) and larvae (n = 2) were collected on *M. sibiricum* floral spikes above the water surface. Pupal chambers (n = 8) were found on the stem a short distance below the floral spike and were either above or just below the water surface. The puparium consisted of a shallow excavation with a dark, translucent cover and was similar to that described by Buckingham and Bennett (1981). The locations of *Phytobius* eggs, larvae, and pupae on northern watermilfoil were similar to the locations reported by Buckingham and Bennett (1981) for *Phytobius* on Eurasian watermilfoil. Hatch (1971) and Buckingham and Bennett (1981) speculated that a native watermilfoil was the native host of *P. leucogaster*. Observations confirm that *M. sibiricum* is one host for *P. leucogaster*. While this weevil may use other native macrophyte species as hosts, they have yet to be reported. The observations during this study and those of Buckingham and Bennett (1981) suggest that *Phytobius*, like *Euhrychiopsis*, may be a watermilfoil specialist.

9 Conclusions and Recommendations

The native weevil *Euhrychiopsis lecontei* appears to meet all of the criteria Harley and Forno (1992) attribute to successful classical biocontrol agents. These criteria include (a) becoming a permanent part of the biota, (b) being self-regulating and self-perpetuating, and (c) providing acceptable control of the target plant. *Euhrychiopsis lecontei* is already a permanent member of the North American biota. The survey data suggest that the weevil population in Brownington Pond is self-perpetuating and self-regulating. Weevils also appear to have reached high enough densities in Brownington Pond to provide acceptable control of watermilfoil. When Brownington Pond was first surveyed in 1986, watermilfoil beds covered approximately 30 to 35 percent (10 to 11 ha) of the littoral zone. By 1989, watermilfoil cover had been reduced to approximately 1.0 percent of the littoral zone (approximately <0.5 ha). In 1991, watermilfoil cover had increased to 7 percent of the littoral zone (approximately 2.5 ha). Approximately 1 ha of nuisance watermilfoil was present in the pond by the end of 1992. In addition, *E. lecontei* adults and larvae were abundant on watermilfoil throughout much of the watermilfoil growing season in Brownington Pond. In 1991 and 1992, weevils were found on watermilfoil on the first sample dates in early June. Weevils were no longer present on watermilfoil by mid-October. Weevils have been found on Eurasian watermilfoil as early as mid-May in Glen Lake, another Vermont lake with a watermilfoil infestation. *Euhrychiopsis* phenology is different from that of the North American moth *Bactra verutana* (Zeller), a native insect that has been used for biological control of the introduced purple nutsedge (*Cyperus rotundus* L.) (Frick and Garcia 1975; Frick and Chandler 1978; Frick 1982). *Bactra* is normally most abundant in late summer and early autumn (Frick and Garcia 1975). *Bactra* larvae have the greatest effect on small nutsedge shoots, which are present in the spring. Thus, early-season releases of large numbers of laboratory-reared *Bactra* larvae are required. The data from Brownington Pond suggest that weevils will naturally reach high enough densities to reduce watermilfoil abundance. While augmentation of weevil numbers during years of low weevil abundance could increase the level of watermilfoil control in the pond, weevils appear to be providing acceptable control without augmentation. Finally, *Euhrychiopsis* is easily cultured, and it appears to have no effect on native macrophytes with the exception of northern watermilfoil, *Myriophyllum*

sibiricum. Based on all results and observations of this study, these authors believe that *E. lecontei* should be considered for use as a biological control agent for Eurasian watermilfoil in North America.

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12a. DISTRIBUTION/AVAILABILITY STATEMENT
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13. ABSTRACT *(Maximum 200 words)*

The potential of the North American weevil *Euhrychiopsis lecontei* to serve as a biological control agent for Eurasian watermilfoil (*Myriophyllum spicatum*) was evaluated. Attention has focused on *E. lecontei* because it was found associated with a watermilfoil population in Brownington Pond, Vermont, United States, which had declined from approximately 10 to 11 ha in 1986 to <1 ha in 1989. Watermilfoil abundance was monitored in Brownington Pond from 1990 through 1992. Watermilfoil abundance increased from 1989 through 1991 and decreased again in 1992. Samples of weevils, water, and sediment chemistry suggested that this second decrease in watermilfoil abundance was caused by weevils and not changes in either water or sediment nutrient concentrations. Weevils significantly suppressed lateral stem and root production of watermilfoil in an enclosure experiment conducted in the pond. Damaged stems also lost their buoyancy and settled out of the water column.

Weevils were readily cultured in the laboratory. All life stages were produced, and under culture conditions, mean weevil generation time was approximately 1 month. Weevil eggs are laid on meristems; the first instar larvae burrow into and destroy the meristems; older larvae burrow through the stem; and pupation also occurs in the stem. Weevil adults feed and mate on the plants.

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In a series of pool and aquarium experiments, weevil adults and larvae significantly suppressed watermilfoil growth and reduced stem buoyancy. Adult weevils damaged the plants by feeding on the stem and leaf tissue. Adults removed a considerable number of leaves from the tops of the stems in one experiment. First instar larvae suppressed stem elongation by destroying meristems. Late instar larvae significantly reduced watermilfoil growth in one experiment but not another. Late instar larvae significantly reduced plant growth when watermilfoil exhibited a faster growth rate. The caterpillar *Acentria ephemerella* also suppressed watermilfoil growth in one of these experiments. In another experiment, stem fragments damaged by weevil larvae produced significantly less stem and root tissue compared with undamaged, control fragments. In similar experiments, weevils did not have a significant effect on the growth of native macrophytes commonly found in Vermont, although they did remove a significant number of leaves from the native, northern watermilfoil (*Myriophyllum sibiricum*). Weevil survivorship was often considerably lower on some of these native macrophyte species.

Collections of three herbivorous insects on watermilfoil were made in the northeastern United States in 1990 and 1991. *Euhrychiopsis lecontei* was found on watermilfoil in 18 lakes in Vermont, 3 lakes in Connecticut, 4 lakes in Massachusetts, and 1 lake in New York. *Acentria* was found in 13 lakes in Vermont, 5 lakes in Massachusetts, and 1 lake in New Hampshire. The caterpillar *Parapoynx badiusalis* was found in five Vermont lakes and in one lake in Connecticut. Collections in Alberta, Canada, suggest that northern watermilfoil is a native host for *Euhrychiopsis*, as eggs, larvae, pupae, and adults were collected from northern watermilfoil in 10 lakes.