The Potential for Biological Control of Eurasian Watermilfoil (Myriophyll
Results of the Research Programs Conducted in 1992.

Year 3
Interim Progress Report
April 1, 1993

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Prepared for
Region 1
U.S. Environmental Protection Agency
Boston, Massachusetts

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INTRODUCTION

Eurasian watermilfoil (Myriophyllum spicatum L.) was accidentally introduced into North America sometime between the late 1800's and the 1940's (Bayley et al. 1968, Reed 1977, Aiken et al. 1979, Couch and Nelson 1986). Since its introduction it has spread over much of North America (Aiken et al. 1979, Couch and Nelson 1986, Nichols and Shaw 1986, Painter and McCabe 1988). It was first reported in Vermont in 1962 in Lake Champlain (Holly Crosson, Vermont Agency of Natural Resources (VtANR), pers. comm.). A number of methods, many of them quite costly (Anonymous 1990), have been employed to control watermilfoil in Vermont and elsewhere, including use of drawdowns, herbicides, bottom barriers, and mechanical harvesting. In general, while these control methods may result in short-term reductions in watermilfoil abundance (Bayley et al. 1968, Nichols and Cottam 1972, Aiken et al. 1979) they do not appear to have proven satisfactory for long-term control of this introduced, aquatic weed (Bayley et al. 1968, Spencer and Lekic 1974, Aiken et al. 1979).

Recently, attention has focused on the potential for biological control of Myriophyllum spicatum Aquatic herbivores such as the caterpillar Acentria nivea (=Acentropus niveus)(Lepidoptera; Pyralidae) and the weevil Euhrychiopsis lecontei (Coleoptera; Curculionidae), have been found associated with declining populations of watermilfoil in northeastern North America (Painter and McCabe 1988, Sheldon and Creed, pers. obs.), including Brownington Pond, Vermont. However, the exact role played by these herbivores in bringing about these declines remains undetermined.

We are currently evaluating the potential for insect herbivores to act as biological control agents for Eurasian watermilfoil. There are six main objectives to this research:

1) Determine the probable cause(s) of the Eurasian watermilfoil decline in Brownington Pond (see Figures 1-3, Creed and Sheldon 1991a).

2) Examine the grazing/boring effects of all major herbivores on Eurasian watermilfoil and native aquatic plant species.

3) Determine the feasibility of herbivore introductions into other milfoil-infested lakes in Vermont.

4) Determine if Lake Bomoseen is a suitable site for herbivore introductions/collect pre-introduction baseline data.

5) If determined to be feasible and appropriate based on previous research (a high-likelihood of success and relatively free from causing negative impacts to non-target species), use herbivorous insects to control Eurasian watermilfoil in Lake Bomoseen.

6) Develop a public education program to keep Vermont's citizens abreast of the results of the research.

The research described in this document is from the 1992 field season. This is the third progress report from this five year study.

RESEARCH AT BROWNINGTON POND

Introduction

One of the few declines in a watermilfoil population in North America occurred at Brownington Pond in northeastern Vermont. While the cause of this decline has yet to be determined, initial samples of the watermilfoil found three insect herbivores associated with this pest macrophyte. The goal of the Brownington Pond research is to determine the cause of the watermilfoil decline and ascertain the role the insect herbivores may have played in the decline. In 1990, we initiated research at Brownington Pond. We monitored the abundance of watermilfoil and its associated invertebrates. We also conducted field and laboratory experiments. In 1991, we continued to monitor the watermilfoil population and the abundances of the associated herbivores. We also conducted additional experiments which evaluated the effects of various herbivores on watermilfoil in both lab and field settings. In 1992, we continued to monitor the watermilfoil and herbivore populations in the pond and conducted additional lab and pond experiments.

Study Site

Brownington Pond is a small, mesotrophic lake in northeastern Vermont (Brownington and Derby Townships, 44°53'N, 72°09'W). Total surface area of the pond is 64 hectares, maximum depth is 10.7 m with an average depth of 5.5 m (Figure 1). There are two inlets, one on the north shore and one on the east side, and a single outlet, Day Brook. Less than one quarter of the shoreline has been developed with summer camps, most of which are located along the northeastern shore. There is a public boating access on the west side of the pond.

Materials and Methods

Surveys

Pond Survey

Since the first summer of this project we have been qualitatively mapping the positions of any watermilfoil beds in Brownington Pond (Creed and Sheldon 1991a&b, 1992). The information for these maps has been gathered by snorkeling and boat surveys (Creed and Sheldon 1991a&b). We surveyed the pond in a similar fashion in the summer of 1992.

Water Temperature

Two stations, located in the South and West watermilfoil beds, were established in the pond at which weekly measurements of temperature were made. Temperature was read from pairs of maximum/minimum thermometers suspended from buoys. One thermometer was 0.5 m below the surface and the second was 0.5 m above the bottom. Thermometers were reset after each weekly reading.

Water Chemistry

Two surveys of nutrients (nitrate, nitrite and orthophosphate) in the water column were made on 30 June and 27 August. Samples were collected from the east side of the pond (an area where Potamogeton amplifolius and Heteranthera dubia are the common macrophytes) and from inside the two watermilfoil beds (in the case of the South bed, in the area where the bed used to be). Instead of sampling a fixed point, three or more

locations were chosen to sample a broader array of potential microhabitats within a site. Water samples were collected using a Kemmerer sampler. Pairs of samples, one shallow and one deep were taken at each point. Five pairs of samples were taken at each site in June; three pairs were taken at each site in August. Upon finishing a collection, samples were placed on ice and transported to the lab of the Vermont Department of Environmental Conservation for analysis.

Sediment Chemistry

Sediment samples were taken in Brownington Pond on 11 August 1992. Samples were taken in: 1) the West Bed, 2) a watermilfoil-free area adjacent to the West Bed (West Shallow), 3) in the South Bed, 4) in a watermilfoil-free area adjacent to the South Bed (South Shallow) and 5) on the east side of the pond in an area dominated by H. dubia and P. amplifolius. Pond sediment was collected by a SCUBA diver. A 3.8 l sealable plastic bag was filled with sediment below the water-sediment interface. The bag was sealed and then returned to the surface. All samples were kept cool and sent to the Army Corp of Engineers' Waterways Experiment Station (Vicksburg, Mississippi) for analysis. Samples were sent to Mississippi within 48 hrs of collection.

Plant Transects

In 1990 watermilfoil appeared to be restricted to water between 2.0 - 3.5 m deep (Creed and Sheldon 1991a&b). To see if this distribution pattern persisted in 1991 we established three permanent transects through both of the main beds. An attempt was made to space the transects across the beds. Along each transect, locations were selected at half meter depth intervals ranging from 0.5 m - 3.5 m deep, for a total of twenty one sample points for each bed. At each sample point two PVC pipe T's were pushed into the

sediment at right angles to one another to form a cross. The four ends of the T's were numbered from one to four.

The permanent transects established in 1991 were sampled again on three dates during the 1992 growing season. To ensure that the areas sampled in 1992 were not affected by the 1991 sampling, each transect was shifted 4.5 m; the direction that the transect was shifted was randomly determined. Samples were collected on three pairs of dates in 1992 (West Bed samples taken on the first date of each pair): 10 & 11 June, 8 & 9 July and 12 & 13 August. For each point to be sampled, one of the four numbers from the T's was selected at random from the remaining possible numbers prior to sampling. The samples were collected by SCUBA divers. The divers inserted a 2 m long piece of PVC pipe into the appropriate numbered opening (sampling a quadrat 2 m from the T's minimized the disturbance of the area to be sampled by the diver when reading the numbers on the PVC T's). A 0.25 x 0.25 m quadrat was then placed on the bottom at the end of the pipe and the sample taken. All above sediment plant biomass was clipped and placed into a numbered, plastic bag. Upon returning to the lab, plants from each sample were sorted to species and dried in a drying oven at 80° C. Plants were weighed after drying to a constant weight. For clarity of data presentation, dry weights for native species were lumped together in the category "Other."

Permanent Grids

In addition to determining the location of watermilfoil beds in the littoral zone, we initiated a program to record finer scale expansions and contractions of M. spicatum beds using permanent grids. Four grids were established in the pond in 1990, two in each bed. The grids cover an area of 8 x 6 m with buoys placed every 2 m in a 4 x 5 array. Percent cover of watermilfoil was determined by a diver using a 0.5 x 0.5 m quadrat subdivided into 25 subunits. Placement of the quadrat across the bed was determined using a

transect "line" made of PVC pipe with openings placed every 0.5 m into which the quadrat was inserted. Percent cover was evaluated along four transects for each grid. The position of the transects corresponds to the four lines of buoys that run along the longer dimension of each grid, i.e. A - E. The number of quadrat subunits lying over watermilfoil plants was then recorded. This technique generates percent cover values ranging from 0-100%. For clarity of data presentation, we grouped the percent cover values into five categories - 1) 0% 2) 1-25%, 3) 25-50%, 4) 50-75% and 5) greater than 75% (note: in 1990 and 1991, we grouped the percent cover values into four categories - 1) less than 25%, 2) 25-50%, 3) 50-75% and 4) greater than 75%). The grids set out in 1990 were placed on the ends and nearshore edges of the beds as watermilfoil will be more likely to spread laterally and into shallow water. The grids did not extend into deep water as watermilfoil abundance is probably limited on the deep edge of beds by light availability. The grids were swum on 15 June, 13 July and 24 August during the 1992 growing season.

In 1991, new grid was established in Lake Memphremagog (Newport, Vt) in a bed of watermilfoil just north of the Whipple Bay boat access. Lake Memphremagog is approximately 3 miles northeast of Brownington Pond and is in the same watershed. We qualitatively sampled this grid twice in 1992.

Invertebrate Samples

Super Samples and Minisamples. To describe the watermilfoil invertebrate assemblage quantitative samples of watermilfoil and the associated invertebrates were taken in the South and West Beds. In addition, samples of two abundant native macrophytes (Potamogeton amplifolius and Heteranthera dubia) were taken to compare their invertebrate assemblages with those of watermilfoil. Samples were collected using two sizes of the Mobile Invertebrate Sampler (MIS) developed by Smith and Sheldon

(unpublished manuscript). The larger sampler (the Super Sampler), used for both watermilfoil and the two native macrophytes, samples an area of 0.18 m^2; the smaller version (the Minisampler) was designed for sampling a single stem of watermilfoil. Both samplers were employed by a SCUBA diver. An area or a plant to be sampled was chosen haphazardly. The sampler tube was then slid over the plant(s) as the diver descended. Plants were cut near the sediment surface, the opening of the sampler was then covered with a 500 um mesh sieve and then the sample was returned to the surface. All samples were placed in sealable, plastic bags. Super samples were preserved in 70% ETOH; minisamples were picked soon after sampling while the animals were still alive. Invertebrates were identified to the lowest taxonomic level. Dry weights were recorded for the plants after the invertebrates were removed. Super samples were taken on 8 June, on 29 June, on 20 July and on 10 August. Mini-samples were taken weekly from 9 June to 25 August for a total of 12 sample dates. In 1992, due to there being many small plants, we sampled long (>50 cm, n=3) and short (n=3) plants each date with the minisampler.

Stem Transects. In 1990 we discovered that weevils lay their eggs on the apical meristems of watermilfoil and that the early instar larvae burrow into the meristem upon hatching (Creed and Sheldon 1991a). We initiated "meristem transects" across both watermilfoil beds in 1990 to determine the density of eggs and early instar larvae in the beds. In 1991 we continued taking these stem transects but we sampled larger pieces of stem (approximatley 50 cm long) in order to collect late instar weevil larvae and pupae. In 1992, 16 stems (on average), 8 stems with intact apical meristems and 8 stems without apical meristems, were collected per transect. While it is possible to find all life stages on both stem types (especially as weevils also lay their eggs on lateral meristems), we believed that stems with intact meristems had a greater probability of containing eggs and first instar larvae. We believed that stems without apical meristems were more likely to contain late instar larvae and pupae. These two stem types were collected in pairs

haphazardly by snorkelers swimming across the bed. Three such transects were sampled for each bed on each sample date. Samples were collected weekly for a total of 12 sample dates plus one collection in September. Stems were examined under dissecting microscopes and all lifestages of weevils were recorded for each stem.

Fish Samples

Only five species of fish have been collected from Brownington Pond (Unpubl. State Fisheries Survey 1980). These include yellow perch (Perca flavescens), smallmouth bass (Micropterus dolomieui), chain pickerel (Esox niger), white sucker (Catostomus commersonii) and brown bullhead (Ictalurus nebulosus). The state survey data indicated that the yellow perch is by far the most abundant species numerically in this pond. Because of the abundance of yellow perch and the fact that it is the species most likely to consume macrophyte associated invertebrates, our fish survey focuses on this species.

Gill nets for large perch (>150 mm) were deployed in Brownington Pond on three dates in 1992: 25 June, 2 July and 10 July. These samples were taken concurrently with the fish exclusion experiment (see below) which was located in an area with scattered clumps of watermilfoil 20 m west of the area which had previously supported the south watermilfoil bed. A single net was deployed, perpendicular to shore, approximately 15 m to the west of the western most row of cages in the fish exclusion experiment for all surveys. A net with a 6.4 cm (2.5") stretch mesh was used. The net was deployed for approximately one hour at dawn on all three dates. Captured fish were measured (total length) and weighed. The stomachs were then removed and preserved in 70% ETOH. Stomach contents were examined under a dissecting microscope and identified to the lowest possible taxonomic level.

Experiments

The Effect of Acentria and Euhrychiopsis larvae on Watermilfoil Growth

This experiment was designed to determine the combined effect of these two herbivorous insect larvae on watermilfoil. Several small watermilfoil plants were collected from Brownington Pond. Plants were first checked for herbivore damage. Damaged plants (e.g., with missing meristems, meristem damage, or significant stem damage) were rejected. We selected twenty four of the intact plants which were the most similar in size. All obvious invertebrates and weevil eggs were removed from these plants. These twenty four plants were then weighed (blotted wet weight). We tied a marker around the stem at the base of the plant and the length of the stem from the marker to the tip of the apical meristem was determined. We also counted the number of whorls on each stem above the marker. The initial lengths of the watermilfoil plants ranged from 206-230 mm; initial weights ranged from 0.23-0.88 g. Much of the variation in weight was attributable to differences in root biomass and not above ground biomass (Creed, pers. obs.)

After processing, each watermilfoil plant was planted in a numbered chamber. The chambers consisted of clear plastic tubes (42 mm inside diameter) set in a PVC pipe base. We first placed aquarium gravel in the bases to weight them down. We then filled the remainder of each base with strained pond sediments taken from one of the watermilfoil beds in Brownington Pond. A tight-fitting cap covered with 500 micron, Nitex mesh was then placed on the top of the tube. These are the same type of chambers described in Creed and Sheldon (1991a&b). Plants were planted in the sediment up to the tag on the stem. The chambers were then placed in a large wading pool set out of doors in an unshaded area. The chambers were aerated with a slow trickle of air bubbles

to prevent stagnation. Plants were allowed to acclimate to the chambers for one day before the Acentria and Euhrychiopsis larvae were added.

The experimental design was a randomized complete block design with four treatments per row and six replicates per treatment. The plants were randomly assigned to rows in the wading pool. The determination of treatment within rows was also determined using a random number table. The treatments were as follows: control (no larvae), weevil (1 Euhrychiopsis larva per tube), Acentria (1 Acentria larva per tube) and the combination treatment (1 larva of each species in a tube). Larvae for both species were collected from the west bed and were paired by size for each row. Water temperature in the pool was monitored using a max/min thermometer during the experiment. Water temperatures ranged from 12.8-26.1° C during the experiment (mean minimum temperature was 16.6° C; mean maximum temperature was 22.7° C).

The experiment lasted for 12 days. Plants and larvae were then removed from each chamber. After removing the larvae, the watermilfoil plants were measured (length from tag to tip of rooted stem) and weighed (blotted wet weight). Any plant material not attached to the rooted stem was not included in the final plant weight. We also counted the number of whorls of leaves remaining on each stem. Treatment effects were compared using an ANOVA with planned, orthogonal contrasts (Sokal and Rolhf 1981). The recovered larvae were preserved in 70% ETOH.

The Effect of Larval Weevil Damage on Stem Fragment Viability

Weevil herbivory, particularly larval burrowing, weakens the watermilfoil stem. This can result in stem fragmentation. The production of fragments by other watermilfoil control methods has been a concern as fragmentation can promote the spread of watermilfoil. The following experiments were designed to determine if the viability of stem fragments damaged by weevils was reduced compared to undamaged fragments.

Undamaged fragments are similar to those produced by mechanical control methods (e.g., mechanical harvesting).

Experiment 1. In this experiment, damaged and undamaged stem fragments were collected from the pond on 15 July 1992. Undamaged fragments were checked for weevil eggs; eggs were removed if discovered. Weevil larvae and all other invertebrates were removed as well. The fragments were then cut to a standardized length of four cm. The amount of larval weevil burrowing was not standardized for the damaged fragments but all fragments displayed some degree of larval damage. The two types of stem fragments were then assigned to four groups each containing five fragments. The fragments were then planted in eight 38 l aquaria, four aquaria per treatment. Each aquarium contained well water and strained pond sediment taken from the west watermilfoil bed. The assignment of stems to aquaria and aquaria to treatments was randomized. All aquaria were covered with a tight-fitting translucent lid to prevent herbivore colonization. The lids contained a panel of 500 micron mesh to allow for air exchange and also aid in temperature regulation of the water. All aquaria were aerated. Temperatures were recorded in four of the aquaria twice a day (9:00 am and 6:00 pm). Temperatures during the experiment ranged from 14° to 30° C in the control treatment (mean (± 1 S.E.) morning temperature 17.9° (± 0.32), mean evening temperature 24.2° (± 0.51)) and from 13° to 30° in the damaged stem treatment (mean morning temperature 17.5° (± 0.33), mean evening temperature 24.3° (± 0.47)).

The experiment was terminated 28 days later (12 August). The stems were gently removed from the sediment. Herbivore damage was seen on stems in three of the four control aquaria. These damaged control stems were removed from the analysis; thus n=5 for one control aquarium, n=4 for two of the control aquaria and n=2 for the remaining aquarium. The percentage of stems with roots was determined for each aquarium. Then the roots were removed, blotted dry and weighed. The production of stem tissue was also determined. As stem tissue could be produced either by elongation of the original

stem or by the production of lateral stems final length of both original and lateral stems was determined. Treatment effects were examined using an ANOVA. Due to the varying number of stems in the controls the ANOVA was performed on the means for each variable from each replicate. Root weight data, and original and lateral stem length data were log transformed due to substantial differences between the two treatments for these variables.

Experiment 2. In the previous experiment, the growth of stems was evaluated in small aquaria containing clear water. These stems were subjected to light conditions typical of very shallow water. Watermilfoil is most abundant in water 2.0-3.0 m deep in Brownington Pond and many stem fragments may settle in water with reduced light intensities. This is particularly true of weevil-damaged fragments which have reduced buoyancy (Creed et al. 1992) and probably settle close to the source plants. To see if reduced light intensities had an impact on growth of stem fragments we conducted a second experiment where light intensity was manipulated. Using a portable light meter (Lutron LX-101 Lux meter) we had determined that the light intensity at 2.0 m (at noon on an overcast day) was approximately half that at the surface. To simulate these light levels we made shrouds of window screen for half of the aquaria that reduced the incoming light by half.

The collection and processing of stem fragments and the set up of aquaria in this experiment was similar to the first experiment. There were four treatments: 1)undamaged stems (control), normal light; 2) undamaged stems (control), shaded; 3) weevil-damaged stems, normal light; and 4) weevil-damaged stems, shaded. Each treatment had three replicates. There were five stem fragments per aquarium. Temperatures were recorded using max/min thermometers suspended in four of the aquaria (two shaded and two unshaded) which were read once a week. Temperatures during the experiment ranged from 10.3° to 33.1° C in the unshaded treatment; mean (± 1 S.E.) max temperature 29.5° (± 1.2°), mean min temperature 13.08° (± 1.5°).

Temperatures ranged from 11.1° to 31.1° C in the shaded treatment; mean (± 1 S.E.) max temperature 27.5° (± 1.3°), mean min temperature 13.5° (± 1.7°).

The experiment was started on 19 August and terminated on 17 September. Stem fragments were processed in the same fashion as in the previous experiment. One control stem in a shaded aquarium was damaged so n=4 for that replicate; otherwise n=5 for all other replicates. The data were analyzed using an ANOVA with orthogonal contrasts. The first contrast compared the control stems with the damaged stems. The second two contrasts compared the effect of shading on the control stems and the damaged stems. The ANOVA was performed on the means for each variable from each replicate. Root weights were log transformed.

Fish Exclusion Experiment

Predation by insectivorous fishes may influence either the establishment of a watermilfoil herbivore population in a lake or their distribution and abundance in the system. The effect of fish may be through direct predation. Alternatively, fish may indirectly influence the distribution and abundance of herbivores through their influence on herbivore predators and/or competitors.

In a previous fish exclusion experiment conducted in the summer of 1991, we found no effect of fish on the abundance of E. lecontei or A. nivea. The treatments for this experiment were in place for about two months (mid-June to mid-August). At the time the experiment was sampled, many yellow perch were feeding primarily on open water Cladocera and to a lesser extent on littoral invertebrates. Since yellow perch feed primarily on littoral invertebrates in the early part of the summer we believed that a strong effect of fish on watermilfoil herbivores might be observed at this time. Thus we repeated the fish exclusion experiment, changing only the duration of the study.

The experimental design for this version of the experiment was the same as the previous study and included three treatments; a complete exclusion cage, a cage control and an uncaged control. Complete cages and cage controls were constructed by making cylinders of 0.6 cm mesh that were open on one end. Cylinder ends were held open by wire rings. Four cork floats were attached to the top of each cage to suspend them in the water. Cage controls differed from cages only in that large slits were cut in the sides of the mesh cylinder to permit access to fish. Open controls were simply areas of the watermilfoil bed demarcated by a single buoy. Placement of cages and cage controls involved sliding the cylinder over the watermilfoil. Cages were held in place by both pinning the lower ring into the sediment and placing bricks on top of the ring (four pins and bricks per cage). The position of treatments within a row was randomized.

Six rows containing each of the three treatments were set out on 24 June 1992 in an area with scattered clumps of watermilfoil 20 m west of the area which had previously supported the south watermilfoil bed. The cages were sampled on 6 July. All three treatments were sampled using the large MIS sampler. For cages and cage controls this entailed skin divers removing the top of the cage. Immediately upon removal of the cage top a SCUBA diver descended to the bottom pulling the MIS sampler through the cage. Upon reaching the bottom all plants were clipped at the sediment surface, the sieve was placed over the bottom of the sampler and the sampler was returned to the surface. Samples were removed from the sampler and placed into labeled, sealable plastic bags. Samples were placed in a sieve stack (3 sieves with 8 mm, 2 mm and 0.425 mm openings for the top, middle and bottom sieves, respectively) and sprayed with a jet of water to separate the invertebrates from the larger plant pieces. Each sample fraction was preserved in 70% ETOH. Invertebrates were separated from macrophytes in the laboratory and identified to the lowest feasible taxonomic level. The macrophytes were then dried and weighed. Invertebrate abundance was standardized by watermilfoil biomass for statistical analysis. The data were analyzed using an ANOVA with planned,

orthogonal contrasts which compared 1) the full cage to the cage control and no cage to determine a fish effect and 2) the cage control and no cage treatments to determine if there was a cage effect.

Herbivore Enclosure Experiment

The enclosures used in this experiment were three meter tall plexiglass tubes (20 cm O.D.) which were composed of two parts. The bottom section (1 m tall) was driven into the sediment. The upper portion of the chamber (2 m tall) was then bolted to the bottom section. Along the sides of the upper portion were four pairs of ports covered with 202 um Nitex mesh which allowed for water exchange between the enclosures and the water column. A lid also covered with 202 um Nitex mesh was bolted on the top of each tube. There was a centimeter scale on the outside of the upper portion of each tube.

The bottom sections of ten enclosures were placed in the pond on the nearshore side of the South Bed by a SCUBA diver on 17 June 1992. Due to the depth of the water, the tops of the enclosure bases were not flush with the sediment surface. Extra sediment which was free of other plants was added to each base. The sediment came from the middle of the South Bed. We collected a number of small (approximately 40 cm long shoots) watermilfoil plants from the West Bed on 17 June. The plants were cleaned of obvious macroinvertebrates and any weevil eggs. The plants were then sorted into 13 groups of six and weighed (blotted wet weight) in order to standardize initial biomass. Ten of the groups of plants were randomly assigned to the enclosures; the remaining 3 groups were dried at 80° C for an initial estimate of dry weight. Six plants per enclosure is equivalent to 181 plants/m^2. This value is well within the range of densities determined by surveys of watermilfoil in the two beds during 1990 (Creed and Sheldon 1991a). The initial mean wet weight ($\pm$ 1 S.E.) of plants placed in the tubes was 5.61 $\pm$ 0.16 g.

On 18 June the plants were planted in the tube bottoms by a SCUBA diver. Plants were gently pushed down into the sediments until the roots were buried. The upper portion of the tube was then bolted to the bottom. The lids were then bolted onto each enclosure top. Four days (22 June) after the plants had been placed into the enclosures the maximum height of each plant in each tube was recorded by a SCUBA diver. The height of the plants was measured weekly until the end of the experiment.

The original plan had been to allow the watermilfoil plants inside the enclosures to grow for three weeks before adding the adult weevils. However, during the first three weeks of the experiment larval weevil damage was observed on a single stem in four of the enclosures. These four enclosures were designated as the weevil treatment. As the plants had been randomly assigned to tubes we assumed that the distribution of this treatment across enclosures was also random. On 9 July we added four adult weevils (2 males and 2 females) to these four enclosures. Another three enclosures had been contaminated by single Acentria larvae (Lepidoptera, Pyralidae) so we added an additional, Acentria control treatment. These larvae appear to have entered the enclosures after the watermilfoil had been planted. We assumed that contamination of the three enclosures by Acentria were also random events. The remaining three enclosures were considered uncontaminated controls. At the time the adult weevils were added, the larval weevils and Acentria had not had a significant effect on mean plant height in these enclosures when they were compared with the uncontaminated controls. During the experiment the enclosures were periodically cleaned of external periphyton.

The enclosures were sampled on 20 August. First, the upper portion of the enclosure was removed from the base. The plants were then clipped at sediment level. The shoots either floated or were gently pushed into the upper portion of the enclosure which was then sealed with a screen-covered bottom. The upper portion of the enclosure was then returned to a boat. The tube was lifted out of the water and all of the plant material was collected on the bottom screen. The plants were removed from the tubes and placed in

sealable plastic bags. The roots were gently removed from the sediments, gently shaken to clean off any adhering sediment and then bagged. In the laboratory, shoots were separated into the six original stems (i.e., the plant tissue produced prior to the adult weevil introduction) and the newer lateral stems. Roots were cleaned of any organic debris. Shoots 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

Surveys

Pond Survey

The watermilfoil population in the pond declined substantially over the winter of 1991-1992 (Figure 1A&B). In June of 1992 there were no areas of the pond where dense watermilfoil beds reached the surface. The decline was most dramatic in the South Bed; the bottom of the pond in the area which once supported the South Bed was devoid of any watermilfoil growth. Scattered plants were present in the West Bed. Some of these were taller shoots (aproximately 1.5m high) which were probably survivors from the previous season; most were shorter shoots (<0.5 m) that appeared to have just begun to grow. By the end of the summer, four areas of moderately dense watermilfoil growth were present (Figure 1B). These included the southern portion of the West Bed and three scattered, small patches located along the southern shore of the pond. Watermilfoil only approached the surface in the West Bed; the tops of these plants were still almost a meter

below the surface. Only scattered, small plants were present in the vicinity of the former South Bed by the end of the summer.

Water Temperature

Surface and bottom temperatures remained fairly constant for most of the summer (Figure 2). Mean maximum surface temperature from late June through mid-September was approximately 23° C; mean minimum surface temperature ranged from 17° to 19° C. Bottom temperatures were fairly similar.

Water Chemistry

Concentrations of orthophosphate, nitrite and nitrate varied little in 1992. Concentration of orthophosphate rarely deviated from 0.002 mg/l; concentrations of nitrite and nitrate were always 0.01 mg/l. These values were similar to those obtained in the 1991 water chemistry samples (Creed and Sheldon 1992).

Sediment Chemistry

Ammonium was the only sediment nutrient which varied significantly among sites (Table 1). Interstitial water ammonium concentrations were significantly lower in the South Bed than those for sediments from the native plant sediments or the West Bed sediments. Exchangable ammonium in the South Bed sediments was significantly lower than only the West Bed sediments.

Plant Transects

Watermilfoil biomass was low on both transects in mid-June (Figures 3 & 4). There was a slight increase in watermilfoil biomass in the West Bed over the course of the summer (Figure 3 A-C), i.e., some recovery of the bed occurred. There was little change in watermilfoil biomass in the South Bed (Figure 4 A-C). These data, combined with the permanent grid data (see below) and snorkeling observations demonstrate that the South Bed did not recover immediately from the decline.

Comparing the plant transect data for the last date for each of the three field seasons illustrates the 1991-1992 decline (Figures 5&6). There was a 4-6 fold reduction in watermilfoil biomass in the center of the West Bed between 1991 and 1992 (Figure 5). There was a 15-30 fold reduction in watermilfoil biomass in the center of the South Bed between 1991 and 1992 (Figure 6).

Permanent Grids

The percent cover of watermilfoil on all four grids was very low on 15 June supporting the evidence from the plant transects that a decline had occurred (Figures 7-10). Large sections of all four grids had no watermilfoil present at all (i.e., 0 percent cover). Only two small sections of the South Grid (West Bed) and one on the West Grid (South Bed) had percent cover readings greater than 25%. There was little change in percent cover readings by mid-July for three of the four grids. Only the South Grid (West Bed) showed a substantial increase in watermilfoil cover. By late August there still was little change in watermilfoil cover except on the South Grid (West Bed).

When the last reading of the grids for each of the three field seasons are compared the extent of the 1991-1992 decline is more apparent (Figures 11&12). The four grids displayed varying degrees of watermilfoil cover at the end of 1991: heavy watermilfoil

cover (>50%) on the grids ranged from 40% (North Grid, West Bed) to almost 100% of the cover on the East Grid, South Bed. At the end of 1992, three of the four grids had cover values that rarely exceeded 25% (a few small patches of cover >25% were present on the West Grid of the South Bed). In the case of two grids (the east grid on the South Bed and the north grid on the West Bed), anywhere from one half to three quarters of the grid area had 0% watermilfoil cover. The decline was most striking on the east grid of the South Bed (Figure 12). This grid had had essentially 100% watermilfoil cover over the entire grid 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% of the watermilfoil cover on this grid exceeded 50%.

Percent watermilfoil cover on the Memphremagog grid did not exceed 20%. Most of the quadrants in this grid had 0% cover at the end of the summer. One clump of watermilfoil was present in the southwest corner of the grid (near marker E4) and this accounted for the 20% cover reading in this quadrant. While percent cover was not determined for this grid in 1991, the grid had been placed in a sparse watermilfoil bed (i.e., there was watermilfoil in every quadrant of the grid). Thus there appears to have been a decline in watermilfoil abundance at this site.

Invertebrate Samples

Super Samples. Tables 2 and 3 list the dominant taxa in the super samples. The more abundant taxa include the amphipod Hyallela, Hydracarina (water mites), Chironomidae (midges), the mayfly Caenis (early in the season) and the snails Amnicola and Physa. These were also the dominant taxa in 1990 and 1991. Most taxa had similar abundances in both the South and West Beds. Chironomidae, Caenis, and Ceraclea (caddisfly) were significantly more abundant in the South Bed. Oligochaets, Leptocercus

(caddisfly). Planariidae and Physa were significantly more abundant in the West Bed. Significant changes in abundance over the summer were observed in some taxa. Caenis and Enallagma (damselfly) declined in abundance in both beds; Amnicola and Physa increased in both beds. Taxa which increased in abundance in only one bed were the Oligochaets and Oxyethira (SB) and Hyallela (WB); taxa which declined included Ceraclea (SB), and Acentria, Oecetis, Leptocercus and Planorbidae (WB).

Euhrychiopsis was found on both native plant species but the numbers were extremely low compared to the number collected in samples of M. spicatum (Table 4). All three weevils found on P. amplifolius were adults. There was watermilfoil in the 1992 P. amplifolius sample and in one of the 1991 samples which contained weevils. Thus, only one weevil adult appears to have been on P. amplifolius. This weevil may have been resting on the pondweed while searching for more watermilfoil. There was watermilfoil in the one 1992 H. dubia sample which contained weevils. Two weevil larvae were found in an H. dubia sample in 1991. This was the first H. dubia sample taken and may have been the first native plant sample taken on that date. As we always sampled M. spicatum first the presence of these larvae in the H. dubia sample may be the result of contamination. Acentria were most abundant on watermilfoil. However, they were also present, albeit at lower numbers, on the P. amplifolius. No Acentria were collected in these samples of H. dubia. However, some were found feeding on this macrophyte in 1992 (S. Sheldon, pers. obs.). Parapoynx was found on all three macrophytes but was consistently more abundant on P. amplifolius. It was the least common of the three herbivores on watermilfoil.

Minisamples. The same taxa which were abundant in the super samples were the dominant taxa on long watermilfoil stems sampled with the minisampler (Tables 5&6). The only differences were in the abundance of Oligochaets and Hydra. These two taxa appear to be much more abundant in the minisamples. This is probably a result of differences in sample processing. The minisamples are picked by hand while the animals

are alive. The super samples contain much more plant biomass. The animals are separated from the plants using a jet of water which probably fragments the fragile Oligochaets and Hydra. The pattern of Euhrychiopsis abundance over time differed between the super samples and the minisamples. In the minisamples, weevils were more abundant early in the summer. The reverse was observed in the super samples. We are not sure why this is the case. The stem transect data (see below) show a pattern similar to the minisample data.

Both Euhrychiopsis and Acentria were consistently more abundant on longer watermilfoil plants (Tables 7&8). Euhrychiopsis abundance was only greater on short stems on one date in the South Bed. Acentria abundance was greater on short stems on only two dates in the South Bed.

Figures 13&14 are plots of the abundance of weevils (based on minisamples) and watermilfoil biomass for 1990-1992. Weevil abundances were fairly low in both watermilfoil beds during 1990. In general, weevil abundance increased through early 1992 and then began to decrease. When watermilfoil abundance is plotted for the same period it is apparent that the increase in weevil abundance coincides with the pronounced decrease in watermilfoil abundance. The peak in weevil abundance occurs approximately one year after the peak in watermilfoil abundance.

Stem Transects. In general, the stem transect data show an increase in abundance in weevil life stages early in the summer (up to 3 July) followed by a decrease in abundance (Figures 15-20). This pattern was observed in both beds. The number of eggs per stem peaked on 26 June in both beds (Figures 15&18). The increase in eggs in the South Bed almost appears to be exponential up until 26 June. The mean number of eggs per stem in the South Bed on 26 June was 6.0, the highest we have ever observed. The numbers of larvae were similar to those observed in 1991 samples. Larval abundance peaked on 3 July in both beds (Figures 16, 17, 19, 20). Eggs and meristem larvae appeared to be more abundant on watermilfoil stems with intact meristems; stem larvae appeared to be

more abundant on watermilfoil stems with damaged meristems. No E. lecontei pupae were found in the 1992 stem transects (nor were any found in any of the minisamples or supersamples). In 1991, 50 pupae were collected in stem transects.

Fish Samples

Eleven perch were collected on 25 June, thirteen were collected on 2 July and seventeen were collected on 10 July. On 25 June, all of the fish contained prey: the number of fish containing prey on 2 July and 10 July were 10 and 15, respectively. The mean (± 1 S.D) total lengths and weights of fish collected on each date were as follows: 25 June - 231.7 (±21.3) mm and 164.7 (± 47.0) g; 2 July - 232.5 (± 18.5) mm and 161.1 (±36.6) g, 10 July - 237.9 (± 23.8) mm and 175.1 (± 55.4) g.

The dominant prey (determined as having a frequency of occurrence >20% for one or more of the three dates) found in the perch guts were the amphipod Hyallela, Cladocera, chironomids (larvae and pupae), Ceratopogonidae, Chaoborus (larvae and pupae), the mayfly Caenis, the dragonfly Tetragoneuria, the damselfly Enallagma, the snail Physa and perch fry (Table 9). Prey which occurred less frequently in the perch guts included water mites (Hydracarina), Baetid mayfly nymphs, larvae of the beetle Gyrinus, various Trichoptera larvae (Leptocercus, Oecetis and Polycentropus), Neuroptera larvae, small crayfish, and small planorbid snails. No Acentria larvae or Euhrychiopsis (adults or larvae) were found in the perch stomachs.

Experiments

The Effect of Acentria and Euhrychiopsis larvae on Watermilfoil Growth

Both Acentria and Euhrychiopsis larvae had negative effects on all three measures of plant growth (Figure 21 A-C). Watermilfoil plants with just one weevil larva were shorter, had fewer whorls and weighed less than control plants. However, plants with Acentria larvae, either alone or in combination with a weevil larva, exhibited even more damage than plants with just a weevil larva. All measures were negative for plants with Acentria larvae. The damage to plants with both Acentria and weevil larvae was slightly less than that exhibited by plants that had a single Acentria which suggests that the presence of the weevil larva might have had a slight, inhibitory effect on Acentria feeding.

The Effect of Larval Weevil Damage on Stem Fragment Viability

Experiment 1. Slightly fewer damaged stems produced roots compared to the undamaged, control stems but the difference was not significant (Table 10). The biomass of the roots produced by the undamaged, control fragments was 7X greater than that produced by the damaged fragments ($p<0.0008$). Overall, there was no significant difference in the amount of stem tissue produced by the two fragment types. However, undamaged stems produced significantly more stem tissue by elongation of the original stem ($p<0.0005$) while weevil-damaged stems produced more stem tissue by producing lateral stems ($p<0.0012$). There was little elongation of the original stem in the weevil-damaged stems. The lack of difference in total stem production suggests that lateral stem production compensated for reduced stem production that would have occurred at the apical meristem.

Experiment 2. Both weevil damage and shading had a negative impact on root and stem production in stem fragments (Figures 22&23). All of the undamaged stems produced roots regardless of the shade treatment (Figure 22A). A higher percentage of the damaged stems in unshaded aquaria produced roots compared to stems in the shaded aquaria. Undamaged stems (both shaded and unshaded) produced significantly more root biomass than the damaged stems (Figure 22B). Shading reduced root biomass for both damaged and undamaged stems; the difference was significant only for undamaged, control stems Production of total stem tissue was significantly greater for undamaged, control stems (Figure 23A). Most of the stem production in the undamaged stems was due to elongation of the original stem (Figure 23B). There was some production of lateral stems. All of the stem production in the damaged stems was due to the production of lateral stems (Figure 23C). 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, damaged, shaded stems produced 27 mm less lateral stem tissue than unshaded, damaged stems.

Fish Exclusion Experiment

Significant fish effects were found for four of the nineteen taxa evaluated (Table 11). These were the weevil Euhrychiopsis, damselfly larvae of the genus Enallagma, the caddisfly Oxyethira and Oligochaets. Marginally significant ($p<0.10$) fish effects were observed for the water mites (Hydracarina), and two snail genera (Amnicola and Physa).

The Hydracarina and Euhrychiopsis had similar abundances in the cages and cage controls. The highest densities for Physa and Enallagma were in the cages with intermediate densities in the cage controls. The oligochaets and Oxyethira were more abundant in the open watermilfoil bed.

Significant cage effects were observed for Oligochaets, Euhrychiopsis and Amnicola. Marginally significant cage effects were observed for Hyallela, Hydracarina, Acentria, Oxyethira. Hyallela, Hydracarina and Euhrychiopsis were more abundant inside the cages and cage controls. The cage effects for some of these taxa appear to be fish effects which suggests that perch may not have foraged as extensively on the watermilfoil in the cage controls as in exposed watermilfoil. The abundances of Oligochaets, Acentria, Oxyethira and Amnicola all appear to have been depressed by the presence of cages, i.e., they were more abundant in the presence of fish.

Herbivore Enclosure Experiment

Weevils had a significant effect on watermilfoil biomass and plant height in the enclosure experiment. Total biomass was significantly greater in the control and the Acentria treatments compared to the weevil treatment (Figure 24). The differences in total biomass were attributable to differences in root weight and lateral stem weight: there was no significant difference in the weight of the original stems (Figure 24). The weevil-damaged, original stems in the weevil treatment tended to collapse during the experiment. While the mean height of these weevil-damaged original stems in the water column was usually lower than that of the original stems in the control treatments, the difference was not significant until the last three weeks of the experiment (Table 12). The difference in the mean height of original stems between the weevil treatment and the control treatments for this period ranged from 10 - 25 cm (Table 12). The weevil-damaged stems were often supported by the enclosures. In the absence of the enclosures,

the difference in the height of weevil-damaged versus undamaged plants may have been greater.

Discussion

The weevil and watermilfoil survey data support the hypothesis that weevils were involved in the Brownington Pond watermilfoil decline. In 1990, weevil abundance was at its lowest while that of watermilfoil was high. The summer of 1990 was the growing season subsequent to the first observed watermilfoil decline (see Creed and Sheldon 1991b for maps illustrating the first observed Brownington Pond decline). With the marked decline of their major food resource by 1989 (weevils do not appear to feed on any other aquatic macrophyte present in Brownington Pond) it is not surprising that weevil numbers were quite low in 1990. From 1989 through 1991 the areal extent of the watermilfoil beds increased (see Creed and Sheldon 1991b and Figure 1). This expansion was also reflected in the permanent grid data. However, the weevil population also began to increase in abundance in 1991 and watermilfoil biomass did not continue to increase over the 1991 growing season as it had in 1990, i.e., peak watermilfoil biomass was in mid-summer of 1991 and not late summer as was the case in 1990. Overall, the number of weevils per stem increased through 1991 and were high at the onset of the 1992 growing season. Watermilfoil abundance had declined dramatically by this point in time. Subsequent to the watermilfoil decline, weevil abundance began to decline by mid-summer of 1992. Thus, the peak abundances of watermilfoil and E. lecontei appear to be out of phase with one another. These patterns of abundance are similar to that displayed by simple predator-prey or host-parasitoid models (e.g., Begon and Mortimer 1981, Krebs 1985) and suggest that a similar interaction is occurring between Eurasian watermilfoil and E. lecontei. Additional collections over the next 6-9 years are needed to verify this cyclic pattern of weevil and watermilfoil abundance.

The lack of any weevil pupae and observations on watermilfoil size in Brownington Pond in 1992 suggest a reason for the observed decline in the weevil population. Weevil eggs and larvae were very abundant early in 1992. However, very few long (>150 cm) watermilfoil stems were present in the pond early in the summer; all of these long stems were located in the southern portion of the West Bed. Few plants (and practically no long plants) were present in the South Bed. It is possible that there were few stems large enough in diameter in which weevil larvae could construct pupal chambers. Therefore, many larvae may have died in early July. This would explain the lack of pupae in our samples which in turn would explain the lack of other weevil life stages for the remainder of the summer. Furthermore, the high densities of weevils early in the summer would have severely damaged most of the watermilfoil plants in the pond. Many watermifoil plants would have been prevented from growing longer with the result that stems suitable for pupation would be in short supply. This hypothetical scenario suggests that the lack of suitable stems for pupation is a major factor driving the population oscillations of weevils and watermilfoil. High densities of weevils such as those observed early in 1992 could prevent a watermilfoil population which has already declined from producing large plants. What few long plants that might be present might have high densities of weevils (weevils were clearly more abundant on long plants in both beds in the minisamples). Weevil numbers would subsequently crash. With reduced weevil feeding the remaining, small watermilfoil plants could recover and the watermilfoil could spread again. This hypothesis remains to be tested.

The results from both the enclosure experiment and the two lab experiments demonstrate that the weevil E. lecontei can have a significant, negative effect on Eurasian watermilfoil. In both the pond experiment and the wading pool experiment, the primary effect of weevils appears to have been a suppression of watermilfoil growth. In the pond experiment, weevils suppressed production of new stems by damaging lateral shoot meristems. The meristem damage observed in this experiment was due to both

larval and adult feeding. Weevil attacks on the shoots appear to have had a negative impact on root production. Weevil damage may influence root production as the removal of stem vascular tissue by weevil larvae may interupt much of the flow of gases and photosynthate to the root system. Weevil damage to the stem also caused the plants to sink out of the water column. This result with rooted plants confirmed the results of earlier experimental studies (Creed et al. 1992) which demonstrated that weevils could affect the buoyancy of floating watermilfoil fragments. Weevil damage also had a negative impact on the viability of watermilfoil stem fragments. Like the rooted plants in the enclosure experiment, the stem fragments had greatly reduced root production. The reduced viability of these stem fragments suggests that the spread of watermilfoil beds during periods of intense weevil herbivory would not be as great as that observed with other methods of watermilfoil control which produce fragments (e.g., harvesting, rotovating). The results of these experiments suggest that weevils have three effects on Eurasian watermilfoil: 1) weevils damage existing stems, possibly stressing the plants physiologically as a result of disruption of gas balance and loss of vascular tissue, 2) weevils inhibit the production of new stem tissue by destroying meristems and 3) weevils inhibit the spread of watermilfoil beds by reducing stem fragment viability. These data support the hypothesis that weevils played an important role in the Brownington Pond watermilfoil declines.

Changes in water and sediment chemistry do not appear to have been the primary causes of the Brownington Pond decline. Concentrations of the measured nutrients in the water column displayed essentially no change between 1991 and 1992 or within the 1992 growing season. It is possible that a change in some unmeasured waterborn 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

Brownington Pond 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 inside the enclosures readily grew at this site while little watermilfoil growth was observed immediately surrounding the enclosures. The latter observation suggests that some other factor was preventing the reestablishment of watermilfoil in this area.

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 Painter and McCabe (1988) who found that ammonium concentrations were lowest in areas of high watermilfoil abundance. We are not sure why ammonium abundance was lower at the South Bed site. As ammonium is produced by the decomposition of organic matter by heterotrophic bacteria (Wetzel 1983), we would have expected higher sediment concentrations at the South Bed site as there was a layer of decomposing watermilfoil on the sediment surface for much of the summer. Alternatively, 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, we used sediment from the South Bed in the enclosure experiment. As the experimental watermilfoil grew on this sediment we do not believe that change in sediment quality was a primary factor in the Brownington Pond decline. The results from Norton Brook Pond (see section on introductions) also support the hypothesis that herbivory and not changes in sediment quality was primarily responsible for this decline. The sediment was not disturbed in any way in the Norton Brook Pond experiment. The only difference between treatments was the presence of weevils. While there may be an interaction between nutrient availability and the effect of the weevil on Eurasian watermilfoil (e.g., reduced root production in the presence of

weevil herbivory results in reduced sediment nutrient uptake), we do not believe that changes in nutrient availability alone could have produced the Brownington Pond decline. Admittedly, our assertions regarding the effects of sediment nutrients are based on a limited number of samples from a single date. However, our results confirm those of Painter and McCabe (1988) who could find no relationship between sediment quality and the watermilfoil declines observed at the Kawartha Lakes.

The fact that much of the Brownington Pond watermilfoil disappeared during the winter suggests that weevil herbivory stresses the plants in some manner that makes it difficult for watermilfoil to overwinter. For example, weevil damage to stem vascular tissue could prevent movement of nutrients and/or gases from stems to roots (or vice versa) which could physiologically stress the plants (Wetzel 1983). Alternatively, the weevil-damaged plants may be much more susceptible to decomposers than healthy plants. At present, the reason that the watermilfoil in Brownington Pond declined during the winter remains unknown. However, this observation suggests that winter may be the season when the greatest reduction in watermilfoil biomass occurs. Further research is needed to understand this potentially important effect.

Yellow perch do not appear to be a source of mortality for Euhrychiopsis or Acentria. Over the last three summers we have examined the gut contents of 175 large perch plus 25 YOY. We have not found any Euhrychiopsis (adult or larva) or Acentria larvae in any of the guts examined. While we do not have adequate samples of 1+ and 2+ perch it is hard to believe that the contents of their stomachs would be dramatically different from those of the 3+ and 4+ perch we have collected. In the 1991 fish exclusion experiment, E. lecontei showed little response to the treatments. Weevil numbers were actually higher in the watermilfoil bed (controls) and the cage controls (see Creed and Sheldon 1992). At the time that this experiment was sampled (late August), the yellow perch were not feeding as heavily on littoral prey as they had been earlier in the summer (see Creed and Sheldon 1992, Figures 18 and 22). In the 1992 experiment, the opposite

response was observed; weevils were more abundant in the cages and the difference was significant. These results suggest that many weevils were avoiding areas where they might be exposed to fish. This result was observed during a time when yellow perch were feeding extensively on littoral invertebrates (Table 9). Thus, during the early part of the summer when yellow perch feed heavily on littoral invertebrates, weevils may aggregate in potential refugia (e.g., areas with a high density of watermilfoil) even though they are not being consumed by perch. If weevils are introduced into a body of water early in the season then it may be best to introduce them into a region of dense watermilfoil growth. Yellow perch may have had a positive indirect effect on watermilfoil herbivores by consuming potential predators (dragonfly and damselfly larvae). This potential indirect effect needs to be investigated.

Student Research Projects

Two student research projects were carried out in 1992 at Brownington Pond. One project examined the ability of weevils to colonize and damage individual watermilfoil plants at three different distances (10, 30 and 60 m) from a watermilfoil bed. Weevil damage was assessed by determining the amount of stem burrowed by larvae and the number of adult stem bites per stem. Larval damage decreased with increasing plant distance from the bed. There was no significant difference among locations in the number of adult stem bites. A second study was designed to determine what cues female weevils might be using to choose plants on which to lay their eggs. We had noticed that there were more weevils on longer plants. The study evaluated the effect of stem length and depth of the meristems. Weevils laid significantly more eggs on longer stems. There was no significant difference in the number of eggs on shallow and deep stems of the same length although there tended to be more eggs on shallow meristems. These results suggest that female weevils may be actively selecting longer stems.

RESEARCH AT LAKE BOMOSEEN AND MIDDLEBURY

Research at Lake Bomoseen

Introduction

Lake Bomoseen is the largest lake (1128 hectares) contained entirely within the boundaries of the state of Vermont. Eurasian watermilfoil was first reported in Lake Bomoseen in 1982. The lake currently has a serious infestation of this species. Attempts at controlling the watermilfoil have included harvesting and an overwinter drawdown. Hydroraking and bottom barriers have also been used by camp owners on an individual basis. One of the primary objectives of this project is to determine if the herbivores under study could be employed to control the M. spicatum infestation in Lake Bomoseen. The primary goal of the 1992 field season was to collect data on the effects of watermilfoil harvesting on herbivore abundance, primarily the weevil E. lecontei.

Study Sites

Twelve sites were designated in 1992 to be avoided by the harvesters (Figure 25). These sites were marked with permanent buoys. These sites included 1) the eastern shore of the north end, 2) the west side of Eckley Point, 3) the east side of Eckley Point at the southern end (= E. Eckley South), 4) the east side of Eckley Point at the northern end (=E. Eckley North), 5) the east side of Neshobe Island, 6) Green Bay, 7) the top of the Channel (across from Indian Bay), 8) Avalon Point Beach, 9) W. Castleton Bay (south of State Park beach, 10) the NW corner of W. Castleton Bay, 11) north of the slate quarry and 12) eastern Rabbit Island.

Three sites, E. Eckley South, the area south of State Park beach, and the east side of Neshobe I., were previously designated as "no harvest" sites in 1991. All other sites were newly established in May 1992.

The distance each of the no harvest sites extended from shore varied depending on the water depth off shore. At all sites, the buoys were placed close to shore in water no deeper than 3 m. For all of the sites, the area designated to be left unharvested was between the line of buoys and the shore.

The shoreline distance of each of the no harvest sites also varied depending on the available lake shore property and site characteristics. All sites were approximately 50 m along the shore.

Materials and Methods

Stem Transects

Watermilfoil stems were collected weekly from three of the sites (E. Eckley South, E. Eckley North and Neshobe I.). Transects were set-up parallel to the edge of the harvested area (perpendicular to shore). On each side of the line of harvest the first transects were within 1-3 m of the line, and two more transects were placed progressively farther from the harvest line, resulting in six parallel transects, with three transects located in both the harvested and unharvested areas. Along a transect line snorklers removed the 0.3 m uppermost portion of a plant and placed it in a ziplock bag. For each transect, five plants with intact apical meristems and five with damaged apical meristems were collected, resulting in the collection of 60 stem tops per site per day. To determine the distribution of weevils within sites, we separated the plants we collected in each transect into two bags, a shallow bag and a deep bag. In each transect, the four plants collected in the two stops closest to shore were called shallow plants. The other six

plants, collected at the other three stops, were called deep water plants. Separation of stems into deep and shallow bags began on 22 June and continued through the summer. Samples were collected from 26 May through 28 September 1992.

On returning to the lab, plants were examined under a dissecting microscope. From the ten plants within a transect, every weevil was removed, preserved and the number and life stage recorded. Differences between dates, sites and harvest versus unharvested areas were compared using an ANOVA (the data were square root transformed for the analyses). To analyze for depth effects, all sites were combined and we compared the number of weevils per stem in all the shallow regions versus the number of weevils per stem in all the deep regions for the entire summer.

Some of the "harvested" areas near the designated no harvest sites were often not harvested hampering comparisons of harvested and unharvested areas. The "harvested" area near E. Eckley South was not harvested throughout 1992. The "harvested" area near E. Eckley North was harvested once, in early July 1992. The "harvested" area near Neshobe I. was harvested on a regular basis starting mid-July 1992.

Super Samples

Three sites (E. Eckley North and South, and Neshobe I.) were designated as no harvest sites in 1992. Unfortunately, regular harvesting of adjacent watermilfoil was only performed at Neshobe I.; watermilfoil was harvested only once adjacent to the E. Eckley North no harvest area and no harvesting occurred adjacent to E. Eckley South. Therefore, only the data from Neshobe I. will be discussed. Once a month (June through August) six super samples were collected at Neshobe I. (For a description of this sampling method see the Brownington Pond section, page 9). Three samples were taken from the harvested bed and three from the adjacent unharvested area. The sampler was placed haphazardly, although care was taken to distribute sample locations over an area

within 10 m of the line of harvest. Samples were preserved in 70% ethanol. In the lab, all of the invertebrates were removed from the plants, identified and enumerated. The plants were sorted to species, dried at 80° C and weighed. Differences between dates and harvested and unharvested areas for watermilfoil biomass and the abundance of major invertebrate taxa were compared using an ANOVA.

Other Sampling

Three sites (the eastern shore site in the north end (site 1), the western side of Eckley Point (site 2), and the Avalon Point beach site(site 8)) were quantitatively sampled once during the summer to determine the presence of weevils. All of these sites had been harvested the previous summer. Weevil transects were conducted at these sites using the same protocol described above.

Weevil Augmentation

On 3 August approximately 100 weevils (both adults and larvae) were carefully placed on tall M. spicatum plants close to shore at E. Eckley South. The introduced weevils were in all life stages. The weevils were collected in Glen Lake.

Results

Stem Transects

Of the three sites, E. Eckley South had the highest mean (± 1 S.E.) number of weevils per meristem with 0.048 (± 0.010) for the entire summer (Table 13). The other two sites had lower but not significantly different (p=0.804) weevil densities throughout the

summer. Neshobe I. had a mean of 0.042 (± 0.012) weevils per meristem and E. Eckley North had 0.039 (± 0.007) weevils per meristem.

When the data from all of the sites were combined, the mean (± 1 S.E.) number of weevils per meristem in harvested areas (0.026 ± 0.005) was significantly lower (p=0.006) than in the unharvested areas (0.058 + 0.010) (Table 14). The sites did not differ significantly. There was little difference in the number of weevils in harvested and unharvested areas early in the summer (Figure 26); many more weevils were present in samples from unharvested areas later in the summer (Figure 26). Examination of the data for the individual sites indicated that both Neshobe I. and E. Eckley North had significantly more weevils in the unharvested areas (Table 14). More weevils were collected in the unharvested area at E. Eckley North early in the summer; the reverse was true at Neshobe I. (Figure 27). E. Eckley South was never harvested in 1992. Weevil densities in the sites designated as "harvest" and "no harvest" did not differ significantly (Table 14, Figure 27).

All the weevil collection data from all of the sites were combined to test for differences in weevil distribution with respect to water depth. The number of weevils per meristem on watermilfoil in shallow water was significantly higher than the number of weevils on deep water watermilfoil (p<0.001, Table 15). There was an overall mean of 0.071 (± 0.011) weevils per meristem in the shallows vs only 0.029 (± 0.005) weevils per meristem in deep water. There was no significant difference among sites. The difference in weevil abundance with respect to depth was significant at both Eckley Bay sites but not at Neshobe I. (Table 15, Figure 28).

A comparison of the two years (1991 and 1992) of weevil collection data for Neshobe I. and Eckley Bay suggested that weevil abundance declined significantly at Neshobe I. and increased slightly at Eckley Bay (Table 16). When the data from the two sites were combined significantly fewer weevils were collected in 1992 versus 1991. Most of the decrease in abundance was attributable to the decrease in weevil abundance at Neshobe I.

A plot of the abundance of the different weevil life stages over the summer (data from all sites combined) illustrates the decline in numbers of the different life stages between 1991 and 1992 (Figure 29). Three peaks in egg abundance (15 June, 13 July and 24 August) were apparent in 1992; the position of the two later peaks coincides with the 2 peaks in egg abundance observed in 1991 . Larval abundance in 1992 was high in mid-June following the first peak in egg abundance but did not display a clear pattern of abundance afterwards. Pupae were not abundant until late in the summer; adults were never abundant in these samples. Data from both years indicate that weevil egg abundance decreases dramatically at the end of August. No eggs were found in either year after the first week in September and larval numbers dropped to zero by the last week of September.

Super Samples

There was a significant effect of harvesting on watermilfoil biomass; no significant date effect for watermilfoil biomass was observed in 1992 (Table 17). Significant differences between dates were observed for ten of the fourteen most abundant macroinvertebrate taxa. Five of the ten taxa having a significant date effect (Oligochaets, Isopoda, Chironomidae, Caenis, and Zygoptera) were most abundant in June. Three of the ten taxa (Oxyethira, Orthotrichia and Amnicola) were most abundant in July. Only one taxon, Euhrychiopsis, was significantly more abundant in August.

Only three macroinvertebrate taxa (Isopoda, Euhrychiopsis and Caenis) showed a significant response to harvesting in 1992 (Table 17). Both the Isopoda and Caenis were more abundant in the harvested areas; Euhrychiopsis was more abundant in the unharvested areas.

Other Sampling

On 3 August, one weevil larva and six empty pupal chambers were found in a collection of thirty meristems at the eastern shore site in the north end (site 1). No weevils were collected at the other two sites. However, during sampling snorkelers reported signs of weevil damage on watermilfoil at the west side of Eckley Point (Site 2). A local property owner also reported that there appeared to be a large amount of damaged watermilfoil at the west side of Eckley Point and he attributed this damage to weevils.

Discussion

Two of the sites (Neshobe and E. Eckley South) were sampled intensively in both 1991 and 1992 for the months of July, August and September. Although these two sites differed with respect to weevil densities in 1991, our data indicated no difference between the two sites in 1992 because the number of weevils (all life stages) at Neshobe had declined. The mean ($\pm$ 1 S.E.) number of weevils per stem at Neshobe in 1991 was 0.204 ($\pm$ 0.026); in 1992 the mean was 0.072 ($\pm$ 0.016) per stem. This difference was highly significant ($p<0.001$). The mean density of weevils per stem at E. Eckley South did not change significantly: mean number per stem in 1991 = 0.035 ($\pm$ 0.010), mean number per stem in 1992 = 0.056 ($\pm$ 0.013). It is possible that the high number of weevils at Neshobe in 1991 was due to an influx of weevils from Glen Lake into W. Castleton Bay. These weevils may have been emigrating to Lake Bomoseen as a result of the draw down in Glen Lake. Many of the weevils that had previously been at Neshobe may have dispersed to other parts of the lake.

Our 1992 data (all sites combined) showed that there were significantly more weevils in the unharvested sites in the lake when compared with nearby sites that had been

harvested within the last year. When only the Neshobe data are examined (the only site where regular harvesting occurred in both years) we once again found a dramatic difference between harvested and unharvested areas. These results support our findings from 1991 regarding the effect of harvesters on weevil abundance, i.e., that constant harvesting will prevent the establishment of large weevil populations.

By separating each of the weevil transects into shallow and deep regions in 1992, we were able to test the 1991 hypothesis that weevil densities were higher on watermilfoil in shallow water. Overall, we found less than half as many weevils per meristem on the watermilfoil in deep water as on the shallow water watermilfoil. The difference in weevil density between shallow and deep habitats was especially pronounced at the Eckley Bay sites. At those sites, we found one third to one fifth as many weevils per meristem in deep water as compared to the shallow areas. One main difference in the distribution of watermilfoil between the Eckley Bay sites and the Neshobe I. site was the density of watermilfoil in deep water. At Neshobe I., watermilfoil density was similar throughout the areas sampled. At both the Eckley Bay sites, on the other hand, the watermilfoil was fairly dense near shore but tended to be in small, dense clumps in deeper water. These data suggest that the weevils are not responding to water depth per se but watermilfoil abundance.

The 1992 super sample results from Neshobe I. are very similar to the results we obtained in 1991. Most taxa displayed significant date effects in 1992 which was also the case in 1991 (Creed and Sheldon 1992, see Table 14). Only four taxa (Amphipoda, Isopoda, Planorbidae and Physa) displayed different responses to date in 1992 compared to 1991. Amphipoda and Physa were significantly affected by date in 1991 but not in 1992. The reverse was true for Isopoda and Planorbidae. Of the three taxa which exhibited a significant harvesting effect in 1992, two of the three (Isopoda and Euhrychiopsis) displayed similar responses in 1991. The 1992 results confirm our previous results that showed that harvesting had a negative impact on Euhrychiopsis

abundance in Lake Bomoseen. The Isopoda were again more abundant in the harvested areas and we still can not explain this result. The increased abundance of Caenis in harvested areas in 1992 may have been a response to an increase in the abundance of periphyton on watermilfoil stems which could have occurred due to the removal of the dense watermilfoil canopy. It is not clear why this mayfly taxon displayed different responses to harvesting in the two years. Two snail taxa (Planorbidae and Physa), which were significantly affected by harvesting in 1991, did not show a significant response to harvesting in 1992.

Research at Middlebury

Culture and Life History of E. lecontei

Materials and Methods

Culture of E. lecontei

E. lecontei cultures were first established in June 1991. Batch cultures of weevil eggs, larvae, pupae and adults were maintained in the Middlebury "light room". The room was illuminated with both standard fluorescent and GroLux lamps on a 16h-on, 8h-off photoperiod. Water temperatures ranged from 21.5 - 24°C. Aquaria (approx 100 liters) were filled with aerated tap water. All aquaria were continuously aerated.

M. spicatum plants were collected from Glen Lake or Lake Bomoseen. In some cases, plants were held upright by sliding their roots into weighted down, plastic mesh. Otherwise, plant roots were planted into 100cc cups filled with autoclaved lake sediment. After being planted, all watermilfoil plants regained an upright position. Weevil adults, larvae, and M. spicatum with weevil eggs were added to the aquaria. When M. spicatum plants became heavily damaged (usually larval damage) weevils were moved into new aquaria with undamaged M. spicatum.

Life History of E. lecontei on M. spicatum and M. sibiricum

The lengths of weevil life stages were quantified in the culture room under the conditions described for weevil cultures. M. spicatum was collected from Lake Bomoseen and Glen Lake, and M. sibiricum was collected from Beebe Pond.

Myriophyllum spp. stems were planted into cups of autoclaved lake sediment, were enclosed in clear cylindrical polycarbonate tubes (30cm long, 6cm inside diameter), covered with a lid of 200um Nitex, and set in aquaria containing aerated tap water. Each tube was also individually aerated. Weevil larvae and adults, collected from Glen Lake or reared in the lab were placed in the tubes, one weevil per tube. Plants and weevils were examined daily, often under a microscope at 7-15X magnification, and mortality or metamorphosis noted. In many cases, especially for pupae, this repeated handling weakened the plants, and in some cases the pupae were damaged.

For quantification of the lifetime egg production by a female, unmated females were collected, then mated, and the number of eggs produced recorded. To get an unmated weevil, individual pupae (which reside inside plant stems) were isolated, and after adult emergence, the sex of the weevil was determined. A single, adult virgin female and two males were placed in a tube as described above. Three to six M. spicatum stems with intact meristems were planted into the autoclaved lake sediment. The meristems were removed and examined at 7-15X magnification every 3 or 4 days, and the number of eggs and larvae counted. New meristems were planted in the tube, and the female and two males were returned to that tube. If a male died, he was replaced by another male weevil so that there were always two males in each tube with a single female. Weevils were also grown on M. sibiricum, however these cultures were difficult to establish. Plants were collected from Beebe Pond and weevils housed and treated as described above.

Results and Discussion

Culture of E. leconteı

E. leconteı seemed to do well under these conditions. For the 19 months, eggs, larvae and adults have been continuously produced. The weevils generated were not quantified, so the rate of weevil production under these conditions is not known.

Life History of E. leconteı on M. spicatum and M. sibiricum

Under these lab conditions the duration of the egg phase averaged 3.90 days (±0.20 SE, n=48). Larval duration ranged from 4 to 22 days, averaging 12.98 (± 1.75 SE, n= 9) days. Pupal duration ranged from 7 to 17 days and averaged 13.0 (±1.52 SE, n=5) days. The sum of these averages suggests that the time between egg deposition and emergence as an adult is 29.9 days.

On average, females laid 1.90 (± 0.44 SE, n=7) eggs per day. 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 and if these also had eggs, eggs were deposited on leaves near the plant apex. In general, hatching rate of eggs was 87.3%. While eggs were usually widely distributed, when weevils were enclosed with few plants we found as many as 29 eggs on a single plant in the lab. Eggs were elliptical, 0.52 mm long and 0.39mm wide, and appeared "yolky", i.e.,they were very yellow and viscous.

Two females were both very long lived and fecund. These females as adults lived 160 and 162 days and laid 562 and 469 eggs respectively.

First instar larvae burrow into the meristem, usually destroying the meristem. Later instar larvae spiral down the outside of the stem, then burrowed in. Larvae spend most

of their time inside the stem, burrowing through stem tissue, hollowing out the stem. Sometimes, particularly when they reached the end of an internode, they will burrow out, spiral up or down the stem to a new location, and burrow into the stem again. Larvae were usually found in the top third of the plant. Puparia were formed inside the stem and tended to be found further down in the thicker portions of the stem.

Of the rates quantified in the lab, we are least confident of pupal duration. Repeated handling of M. spicatum plants resulted in plant breakage and pupal mortality. Also, it appears that successful metamorphosis is a function of stem diameter and health of plant. Pupae appear to build their chambers in thick (>2 mm diameter) stems of actively growing M. spicatum. It is difficult to find M. spicatum plants that have thick stems, have roots, and are less than 30 cm long.

We have found that we can put large larvae on thick stems, and within 2 days, larvae will construct a pupal chamber. In the future, we will put large larvae on sufficiently thick M. spicatum stems in the field, enclose the stem in a longer tube, and quantify pupal duration.

The duration of life history stage data collected in the lab are consistent with observations of E. lecontei phenology in the field. There appear to be 3 generations of weevils on M. spicatum each summer in the 2 lakes we have studied in Vermont. 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 at >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. C. O'Brien (pers. comm.) predicted that Euhrychiopsis lecontei may overwinter as adults in leaf litter near lake margins. This prediction is based on observations of other aquatic weevils. This is consistent with the single weevil we found in leaf and soil samples collected on shore in late October, 5 m from the margin of Lake Bomoseen.

Weevils were also grown on M. sibiricum. These cultures were difficult to establish. Seventy percent of the eggs on M. sibiricum hatched. Mean (± 1 SE) hatching time for eggs was 4.7 (± 0.48) days (n=7). For each of the three females placed in tubes on M. sibiricum, no eggs were found on the plants. This is in contrast to the mean (± 1 SE) of 12.75 (±2.26) eggs for weevils (range 2-23 eggs) under these same conditions on M. spicatum for the same period of time. A single puparium was formed. We feel that the stems of the M. sibiricum plants chosen for these cultures were too narrow and this may explain the lack of pupation.

Student Research Projects

A series of student projects were carried out during the summer of 1992 at Middlebury. Project topics included: 1) the effects of Phytobius leucogaster (0, 2, or 4 adults) on M. spicatum and M. sibiricum, 2) the effects of both Phytobius and E. lecontei on M. spicatum (treatments consisted of either 4 Phytobius alone, 4 E. lecontei alone or 2 Phytobius and 2 E. lecontei together, 3) determining the number of weevils associated with floating watermilfoil rafts in Lake Bomoseen, 4) evaluating weevil behavior in the presence of native macrophytes, 5) examining the tendency for weevil larvae to move between plants (both between M. spicatum plants and between M. spicatum and natives), 6) examining the growth rate of different types of watermilfoil fragments (autofragments, weevil-generated fragments and harvester-generated fragments), 7) comparing the buoyancy of rooted watermilfoil fragments (both undamaged and weevil-damaged fragments), 8) identifying weevil damage in a laboratory setting and 9) determining the life history of Phytobius on M. spicatum in Vt.

In the first experiment, Phytobius did not have a significant effect on the growth of either M. spicatum and M. sibiricum. In the second experiment, there was no significant difference among treatments (Phytobius alone, Euhrychiopsis alone, and Phytobius and

Euhrychiopsis together) for either change in length or weight of M. spicatum. However, the behavior of Euhrychiopsis appeared to be affected by the presence of Phytobius. Euhrychiopsis spent less time on flowers in the presence of Phytobius. In the third study, the number of weevils associated with floating watermilfoil mats was correlated with the biomass of the mats. In the fourth study, adult weevils spent most of their time swimming in the presence of most native macrophyte species. Substantial amounts of time were spent on two macrophyte taxa (Ceratophyllum and Chara) which have morphologies similar to that of M. spicatum. In the fifth study, weevil larvae were observed to move between M. spicatum stems. Movement from M. spicatum to Elodea was also observed in one instance and there did appear to be larval damage on the Elodea stem. In the study (Study 6) examining the growth rate of different types of watermilfoil fragments (autofragments, weevil-generated fragments and harvester-generated fragments) growth rate of the autofragments and harvester-generated fragments was greater than weevil-generated fragments but the differences were not significant. In the study (7) which compared the buoyancy of rooted watermilfoil fragments (both undamaged and weevil-damaged fragments), found that the height of weevil-damaged fragments in the water column was significantly lower than that of undamaged fragments. The results of this study confirm the results of the buoyancy experiment conducted at Brownington Pond in 1991 (Creed et al. 1992) and the enclosure experiment conducted in Brownington Pond in 1992 (see Brownington Pond section of this report). In the study (8) which examined weevil damage on watermilfoil in the lab, similar damage (adult stem bites, leaflets removed by adults, larval burrowing etc) to that observed in the field and previous lab experiments was seen. In the last study (9), the life history of Phytobius appeared similar to that reported elsewhere for this weevil. Eggs were laid on and inside of flowers, larvae were observed feeding on flowers and pupae were found on the stem just below the floral spikes. The duration of each of the life history stages was not determined.

The occurrence of Mycoleptodiscus terrestris on watermilfoil in Vermont lakes

Samples of Eurasian watermilfoil from three Vermont lakes (Brownington Pond, Lake Bomoseen and Metcalf Lake) were collected in August 1992 and sent to Judy Shearer at the U.S. Army Corps of Engineers Waterways Experiment Station to determine the presence of the fungus Mycoleptodiscus terrestris on these plants. Mycoleptodiscus terrestris was found on the watermilfoil from Brownington Pond and Lake Bomoseen but not on the watermilfoil from Metcalf Lake.

The Effect of E. lecontei Adults on Native Plants

Introduction

If E lecontei is going to be used as a biological control agent it is necessary to determine if it will have any impact on native macrophytes. To quantify the potential effect of weevils on native plant species a series of feeding experiments were carried out. Plants used for these experiments were some of the more common (frequency, biomass and distribution) macrophyte species in Vermont and included Ceratophyllum demersum, Chara sp., Elodea canadensis, Heteranthera dubia, Megalodonta beckii, Myriophyllum sibiricum, Najas flexilis, Potamogeton amplifolius, Utricularia vulgaris, and Vallisneria americana. The series of experiments was run from 3 July to 26 August.

Materials and Methods

Plants <30 cm total length were collected with roots intact from Beebe Pond and Glen Lake, except Utricularia vulgaris (a species which is not rooted in the sediment) which was collected from Lake Bomoseen. In the lab, all plants were examined under a dissecting microscope (7-15X) and all invertebrates and eggs were removed. Many plants were discarded due to condition, difficulty in invertebrate removal, plant breakage while being handled or other such damage. Of the remaining plants, those that were the most similar in length, weight, number of leaf whorls and number of meristems were selected for use in the experiments. Only plants with intact apical meristems were used. Plants used in the experiment were examined for the initial condition of the meristem(s), leaves and stem. Each leaf was examined, and damaged or missing leaves were recorded by whorl or leaf as appropriate for the species. Each plant stem was marked with a tag at

the point dividing the shoot from the roots. The length of the plant above and below the tag was recorded. Blotted wet weights were also recorded.

Single plants were placed in chambers similar to those used in previous wading pool experiments. Plant roots were inbedded in a container of autoclaved lake sediments to the mark dividing the shoot from the roots. Plants were enclosed in clear polycarbonate cylinders (30cm tall, 6cm inside diameter), except P. amplifolius which was in larger (27cm high, and 12.7cm inside diameter) enclosures. Each chamber was sealed by pushing the polycarbonate cylinder into the sediment and covering the top with a lid of 200 um Nitex. The chambers were placed in large wading pools (375 l) filled with aerated tap water in a greenhouse under ambient light conditions. Each chamber was individually aerated.

The design of each experiment was a randomized block design with three treatments (0, 2 or 4 weevils per chamber) per row and six replicates for each treatment. The chambers containing the eighteen plants were arranged in six rows in a wading pool; the orientation of the rows was perpendicular to a north-south axis. The P. amplifolius and Utricularia vulgaris plants which remained after the initial processing was completed were obviously not homogeneous with respect to length. Relatively short plants (3 for P. amplifolius, 6 for Utricularia) were separated from the other plants and placed in the southern most row(s). The plants in these subsets were randomly assigned to chambers within and between rows. After the chambers were in place, the assigned number of adult weevils (from Glen Lake) were placed in the chambers and the chambers were capped. The treatments were assigned at random to chambers within a row. For each native plant species feeding trial, three chambers of M. spicatum with 4 adult weevils in each were also placed in the pool for the duration of a trial to determine if weevil mortality was due to host plant or environmental conditions in the wading pools.

All trials except three ran for 10 or 11 days. 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 under all conditions including controls (no weevils) some of the plants were starting to fall apart. The Utricularia feeding experiment ran for only 7 days because it was clear that the weevils were not affecting plants by feeding but by knocking off the bladders. The importance of this effect could not be tested under the experimental design used for the feeding experiments.

At the end of each experiment, each tube was opened and the number of surviving weevil adults was recorded. Plants were removed from the sediment and returned to the lab. In some cases, it was difficult to remove the plants from the cylinders without breaking them. Length was difficult to measure accurately for broken plants. Plant length above and below sediment level was recorded and blotted wet weights were determined. Plants were examined under a dissecting microscope and the number of any weevil eggs and larvae were counted. Plants were again examined leaf by leaf and new (relative to initial) leaf and stem damage was recorded. Plants were dried > 4 days at 60°C, and dry weights for shoots and roots recorded.

Due to the breakage of plants, two analyses were performed on the length data for all species: 1) for all plants in a treatment (n=6) and 2) for all intact plants in a treatment. Unless otherwise stated, average length data reported are for intact plants. The data were analysed using ANOVA; differences among treatments were compared using Tukey's HSD test.

Results

The number of intact plants by treatment for each species is presented in Table 18. With the exception of M. sibiricum, there does not appear to be any pattern of broken plants with respect to weevil treatment. The mean numbers of adult weevils surviving on native species and M. spicatum for each experiment are presented in Table 19. The mean number of adult weevils surviving on M. spicatum was always higher than that for native

plant species. The lowest weevil survivorship observed on the M. spicatum controls was 44% (Ceratophyllum trial); survivorship was usually 67% or greater for these controls. On the other hand, weevil survivorship on the native species was usually less than 25%. The exception was weevil survivorship on M. sibiricum which ranged from 46% in the 4 weevil treatment to 58% in the 2 weevil treatment. No weevils survived in the Elodea and Heteranthera trials. No eggs or larvae were found on any of the macrophyte species.

The responses of each macrophyte species to weevils except Najas are presented below. Najas deteriorated during the experiment so the results of this trial will not be discussed. In the results discussed below the numbers of intact plants used to determine length changes was variable (see Table 18); for change in wet weight n=6 in each case.

Ceratophyllum: There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 1.7 to 3.2 cm for the three treatments. Average change in plant wet weight ranged from 0.37 to 0.55 g. There were no significant differences in the average number of new branches (3.7-4.5 per plant) produced by the plants.

Chara. There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 0.05 to 0.32 cm for the three treatments. Average change in plant wet weight ranged from 0.00 to 0.07 g. The average number of whorls added per plant ranged from 0.17 to 0.50 whorls for the three treatments.

Elodea There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 0.08 to 0.50 cm for the three treatments. Average change in plant wet weight ranged from -0.008 to 0.148 g. All but two plants produced new branches. The average number of new branches per plant was significantly higher ($p<0.023$) in the 4-weevil treatment than in the control; the difference between the 2-weevil treatment and the control was marginally significant ($p<0.053$). There was no grazing damage seen on Elodea,

although leaves were missing from three plants in the 2-weevil treatment and one plant in the 4-weevil treatment.

Heteranthera: There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 0.817 to 1.380 cm for the three treatments. Average change in plant wet weight ranged from 0.348 to 0.430 g. There was some length loss recorded in all treatments due to loss of the longest leaf. However, these losses were similar among treatments. All plants added leaves. The mean number of new leaves per treatment ranged from 11 to 14.3 leaves.

Megalodonta. There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 1.56 to 1.83 cm for the three treatments. Average change in plant wet weight ranged from 0.083 to 0.957 g. Some plants lost weight because some root whorls broke off in the sediments. All plants added leaf whorls during the experiment (mean number of whorls added - control: 4.5; 2-weevil treatment: 4.7; 4-weevil treatment: 4.5), and the number of new whorls did not differ among treatments.

M. sibiricum: M. sibiricum did not grow well under these conditions and many plants were broken (Table 18). When broken plants were included in the analysis, mean (± 1 S.E.) change in plant length was +0.667 (± 1.376) cm for the control, -3.750 (± 2.670) cm for the 2-weevil treatment and -6.333 (± 1.470) cm for the 4-weevil treatment. There were no significant differences among treatments, although the difference between the control and the 4-weevil treatment was marginally significant ($p<0.053$). When broken plants were excluded from the analysis the mean changes in length for the three treatments were as follows: control +2.00 (± 1.67) cm, 2-weevil treatment +1.17 (± 0.38), and 4-weevil treatment -3.00 (± 3.00) cm (Figure 30). The differences among treatments for change in length of intact plants were not significant either. Average change in plant weight ranged from 0.095 g in the 2-weevil treatment (n=4) to 0.248 g in

the 0-weevil treatment; the differences among treatments were not significant. Plants without weevils added more leaves than plants with weevils. There was leaf loss at the top of some plants, and damage to apical meristems in both weevil treatments. All treatments plus the M. spicatum controls were covered by variably thick epiphytic algae in this experiment. Plants with weevils were more likely to be covered with algae and broken

P amplifolius: There were no significant differences among treatments for either change in length or weight (Figures 30&31). Average change in plant length ranged from 0.50 to 1.15 cm for the three treatments. Average change in wet weight ranged from 0.68 to 1.17 g. There were no significant differences between the row of short plants and the other five rows for any of the variables measured. On average, plants added 3 to 4 leaves. Nine of the plants added runners. There were no significant differences for either the number of leaves or runners added among the three treatments.

Utricularia: Utricularia had the highest growth rates under these conditions. There were no significant differences in increase in length among the treatments (Figure 30). Average change in plant length ranged from 3.30 to 3.98 cm. There appeared to be an effect of weevils on plant weight. However, there was considerable variability within all treatments so these differences were not statistically significant. Average changes in wet weight for the three treatments were as follows: 0-weevil treatment +0.33 g, 2-weevil treatment -0.59 g, 4-weevil treatment -0.39 g. Most of the weight loss in the weevil treatments appeared to be due to the loss of bladders. The bladders were not counted at the beginning of the experiment, so we could not quantify bladder loss. There was also some loss of bladders in the 0-weevil treatment; half of the plants in the 0-weevil treatment lost weight despite their increase in length. There were no significant differences between the two rows of short plants and the rest of the plants for any of the variables quantified.

Vallisneria: There were no significant differences among treatments in change in plant length or weight (Figures 30&31). Average change in plant length ranged from 0.017 to 0.517 cm. Average change in wet weight ranged from 1.04 to 1.50 g. Most plants in all treatments had new leaves, averaging 0.8 to 1.5 new leaves per plant. In one 0-weevil replicate there was a damaged leaf, likely due to handling. Two plants, one from each of the weevil treatments, lost length. One of these plants had a scar and the other one had no visible scar. A number of the plants in all treatments showed signs of chlorosis which may have been due to handling during the initial processing. The Vallisneria plants were covered with Amnicola eggs. To remove the eggs, plants were examined under lights, and eggs removed with forceps. This process resulted in some desiccated sections of the leaves, and some tears and scars. Later, the desiccated areas became chlorotic. If chlorotic plants are removed from the analysis, mean change in plant length ranged from 0.35 to 1.15 cm; mean change in wet weight ranged from 1.40 to 1.78 g. Again, there were no significant differences among treatments.

Discussion

Adult weevils did not have a significant effect on the growth of any of the macrophyte species tested. The only noticeable effects of weevils were on M. sibiricum length and Utricularia weight. Mean M. sibiricum length decreased with increasing weevil density. There was, however, no significant impact of weevils on M. sibiricum weight. These results are similar to those obtained in a study conducted at Brownington Pond in which E. lecontei adults did not have a significant, negative effect on M. sibiricum length or weight (Creed and Sheldon 1992). Weevils did remove a significant number of leaves in that experiment, though. The effect of weevils on Utricularia weight appeared to be due to the loss of bladders on plants with weevils, however the differences were not significant. Also, there was no significant effect of weevils on Utricularia length. The

most commonly found plant damage was due to effects of pre-experiment processing. For example, there was desiccation of some leaf margins on P. amplifolius. None of this damage was more common on the plants enclosed with weevils than controls.

At the end of an experiment weevils were classified as either alive, dead, or missing. Many weevils (148 out of a total of 324 weevils) were not found at the end of the experiments. In this discussion, only the weevils found alive in the tubes were used to calculate survivorship. Thus the values represent the lowest possible survivorship, assuming that all of the missing weevils were dead. The highest survivorship was on the M. spicatum controls. The highest survivorship on a native plant was on M. sibiricum. There was extensive weevil mortality in the experiments. No weevils survived at either density on either Elodea or Heteranthera. Many more dead weevils (128 of 324) were found associated with the native plants. If weevils on M. sibiricum are not considered, this becomes 128 of 288. In contrast, of the 108 weevils placed in the M. spicatum controls (not including the P. amplifolius trial) only 1 was dead and 24 were missing. Overall, adult weevil survivorship on non-target plants was 15%. If M. sibiricum is excluded from this group, the overall survivorship on native plants was 11%, compared to an average survivorship of 75% on M. spicatum. Forty-five percent of the weevils could not be found. There are a number of possible explanations for their absence: 1) they were not put in the chambers in the first place, 2) they found some way to escape, or 3) they died, fell into the sediment and decomposed. In four sets of the M spicatum controls all of the weevils were found alive. In the other four trials, on average 4.25 weevils of the original 12 were missing. As we have rarely found adult mortality at this rate on M. spicatum, it seems likely that at least some of these weevils may have escaped or been overlooked.

In conclusion, the macrophyte in Vermont which appears to be most vulnerable to E. leconteiis the native watermilfoil, M. sibiricum. We have found E. leconteiand weevil damage on this plant in the field, but the weevils do not appear to have a significant

negative effect on the plant. At one location, Inman Pond in Fair Haven, Vt, it appeared that M. sibiricum responds to weevil damage by increasing the number of lateral branches.

WEEVIL INTRODUCTIONS

Norton Brook Pond

Site Description

Norton Brook Pond is a small (< 8 hectare) impoundment in Bristol township, Vt. M. spicatum was first identified in the lake in 1985, and currently is the dominant (percent cover, biomass, pers. obs., and H. Crosson pers. comm.) macrophyte in the lake. No other submerged plant species were seen. M. spicatum ringed the impoundment.

Materials and Methods

Before weevils were introduced, invertebrates on transects were collected to determine whether E. lecontei was already present in the lake.

Cylindrical enclosures were used for weevil addition. The 30.5 cm diameter, 2.5m tall enclosures were constructed from impermeable 4um polyethylene sheeting held open by external rings. The tops and bottoms of the enclosures were held open by approximately 8 cm tall PVC rings. The tops of the enclosures were covered with 200um Nitex mesh, and were held at the water surface by floats. Six enclosures were placed in a line running north to south on the 2.1m depth contour. The enclosures were placed over dense M. spicatum, and the bottom ring was pushed into the lake sediment enclosing 730 cm2 of sediment. Fifty adult E. lecontei were placed in every other enclosure. No weevils were added to controls. Enclosures were examined weekly, and dissolved oxygen was measured at mid-day at the bottom of the enclosures three times over the course of the experiment with a dissolved oxygen meter.

After 36 days in situ, a diver went to the bottom of each enclosure, cut all plants at sediment level, clamped a 200um mesh sieve to the bottom of the enclosure, and all of the material was brought to the surface. All plants and invertebrates were washed into sealable bags and preserved in 70% ethanol. To quantify the effects of enclosures, three similar samples were also taken under ambient conditions at haphazard locations between enclosure sites on the 2.1 depth contour on the day the enclosures were removed.

At the lab, plants were examined. Meristems were removed and examined under a dissecting microscope (7-15X magnification) for weevil eggs and early instar larvae. All macroinvertebrates were removed, identified and enumerated. Plants were placed in a drying oven for >4 days at 80°C, and dry weights recorded. The data were analyzed using ANOVA and the treatment means were compared using Tukey's HSD test.

Results and Discussion

There were no E. lecontei found in preliminary samples in Norton Brook Pond.

Weevil enclosures had significantly lower M. spicatum biomass compared to control enclosures ($p=0.007$) and to open water ($p=0.043$) (Figure 32). This appears to be a weevil effect as there were no significant differences in M. spicatum dry biomass between controls and open lake. There were also visual differences in the position of the plants in the water column between weevil enclosures and controls when the enclosure tops were removed. In all control enclosures M. spicatum formed a canopy on the surface, as it did in the open water. In the weevil enclosures there were no plants at the surface in any of the weevil enclosures; the plants were approximately one meter below the surface. No plant species other than M. spicatum were found in any of the samples.

There were few differences in invertebrate species and abundance in enclosures compared to open water. There were no significant differences in larger, more benthic macroinvertebrates such as mayflies, caddisflies, mites and chironomids. There were

significantly more macrozooplankton (Cladocerans and Copopods) in enclosures compared to open water ($p < 0.02$). This is not surprizing as there were no planktivores in the enclosures. There were no differences in overall taxa richness, or organism abundance, excluding zooplankton, among the enclosures, and open water (Figure 33).

Dissolved oxygen did not differ between enclosures with and without weevils. Mean ($\pm$ 1 S.E.) dissolved oxygen concentrations at the bottom of the enclosures averaged 8.28 $\pm$ 0.37 mg/l, except on day 6 when one control had a dissolved oxygen concentration of 1.18 mg/l and on day 36 when one weevil enclosure had a dissolved oxygen concentration of 1.46 mg/l. On day 36, dissolved oxygen at the bottom was somewhat lower than under ambient conditions, 7.1 $\pm$ 1.29 mg/l, in enclosures compared to 8.58 mg/l outside the enclosures. On day 36, water temperatures at sediment level and at the surface were 21.5 and 21.9, respectively.

There were very few weevil eggs, larvae and pupae found in the enclosures. There were 6 eggs found in one enclosure (5 eggs on one meristem and 1 on another) and 1 pupa found in another enclosure. No larvae were found. It is unclear why there was apparently so little weevil reproduction. Given the longevity of the weevils seen in the lab it is possible that the adults found in the enclosures, which averaged 30 ($\pm$2.64) represent survivorship of the original 50 adults placed in each enclosure. It is possible that the apparent lack of successful reproduction was due to low dissolved oxygen. We measured dissolved oxygen during the day, at the bottom of the enclosure. We anticipated that dissolved oxygen would be lowest at sediment level both because low light intensities would minimize oxygen from photosynthesis, and because decomposition of organic matter would decrease the available oxygen. On the three dates that we quantified dissolved oxygen, the concentrations were usually high. Furthermore, there was no significant effect of the enclosures on other macroinvertebrate taxa associated with watermilfoil.

One adult E. lecontei was collected in an open water sample. Given that there was none of the characteristic E. lecontei damage found on M. spicatum plants in any area in the lake, it is likely that this was a result of sample contamination. When we took the open water samples, we reused one of the enclosures we had just removed. Unlike the Super Sampler, which is used in the field for brief periods of time and dried between uses, the enclosures remained in the lake for 36 days. The enclosures were encrusted with periphyton making the inside of the tube less smooth, therefore increasing the likelihood of trapping a weevil in the sampler.

Van Vleck's Pond

Site Description

VanVleck's Pond is a small man-made pond (approximately 0.7 hectares) located in Cornwall, Vt. It has a circumference of approximately 240 m. Mean depth is 2.3 m. It has a small stream inflow and an outflow pipe. The pond was built with a drain hole in the NE corner.

Materials and Methods

We divided VanVleck's Pond into eight shoreline sections (30 m of shoreline each) and one center section using permanent stakes and removable ropes. Three corner sections were selected for introduction sites. The NE corner where the drain is located was not used.

To determine if weevils were present prior to the introductions, the three introduction sections were systematically sampled immediately prior to the weevil introduction. In each section, two (2 m apart) transects parallel to shore were swum by snorkelers. On each transect, ten milfoil meristems (top 50 cm) were collected. Of these, five meristems had signs of invertebrate damage and five were undamaged Three (3 m apart) transects running north to south were also sampled in the middle of the pond. Overall, nine transects were sampled, with a total of 90 meristems collected.

Weevils were introduced on the same day as the pre-introduction sampling, 9 July 1992. Weevils were added to three sites using three different strategies. At site A (the SE corner), 50 adult weevils were added on the first day. Fifty more adults were added every other week until 19 August 1992. At site B (the SW corner), 50 adult weevils

were added on this date only. At site C (the NW corner), 50 adult weevils were added with approximately 50 eggs and larvae on this first day only.

All introduction sites were examined on 19 August 1992, Day 41 after the introduction. Observations of weevil presence were recorded but no samples were collected.

We conducted extensive sampling of the entire pond on 25 August 1992, Day 47 after the introduction. Collection transects were made in all portions of the pond. Two (site B) or three (sites A and C) transects were taken at each of the three introduction sites. One transect was taken in each of the other five pond sections. Three transects were taken in the pond center as described above. On each transect, fifteen meristems (top 50 cm) were collected. Of these, five meristems had signs of invertebrate damage, five were undamaged, and five were flowering meristems. (Flowering meristems were not sampled in the pre-introductory sampling because they were not present. We added them to the sampling regime to be certain we were not overlooking any possible weevil habitat.) We also collected five meristems (top 50 cm) from some of the floating watermilfoil from each of the shoreline sections. In total, sixteen transects of rooted watermilfoil were sampled and 240 attached meristems collected. Forty meristems from floating watermilfoil in were collected.

Results

In pre-introduction sampling on Day 0 (9 July 1992), we collected one unidentifiable weevil larva from one of the center transects. Because we collected 90 meristems and one weevil, this suggests a pre-introduction weevil density of 0.011 weevils per meristem.

Observational data from Day 41 after introduction indicated one weevil pupal chamber and some larval damage at site A, possible larval damage at site B, and 3 adult E. lecontei and a fair amount of larval damage at site C. No eggs were seen at any site.

In extensive sampling on Day 47 (25 August 1992), we collected 27 weevils (seven adults, one pupa, eighteen larvae, and one egg). Based on 240 collected meristems, we found a final weevil density of 0.113 weevils per meristem for the entire pond. The highest numbers of weevils were collected near sites C and A. By examining these two sites independent of the rest of the pond, weevil densities in the weevil transects at these two sites were 0.29 (site C) and 0.20 (site A) weevils per meristem. No weevils were collected from site B.

A total of five weevils (one adult and four larvae) were collected from six non-introduction sites. Five weevils collected on 120 meristems suggests a weevil density on Day 47 of 0.042 weevils per meristem. This density is higher than that initially measured in the pond. No weevils were collected from floating milfoil pieces.

Discussion

We collected twelve larvae at introduction site C. Weevil eggs and larvae under laboratory conditions need approximately 30 days at 21.1°C to go from egg to adult. Mean surface temperature on Day 47 after the introductions was 27.4°C. At 30 cm from surface, water temperature was 25.5°C. This suggests that the larvae collected on Day 47 were not the same larvae we placed in the pond.

Based on the large total number of weevils (13) collected from site C (the site with a single introduction of 100 eggs, larvae, and adults), this type of mixed life stage introduction appears to be most productive. While we did collect nine weevils from site A, this site had biweekly introductions of 50 adult weevils, or 200 weevils in the summer.

Overall, the density of weevils throughout the pond appears to be increasing. Excluding the three introduction sites, our final sampling suggested a four fold increase in the number of weevils per meristem in the pond.

Betourney's Pond

Site Description

Betourney's Pond is an oblong, man-made pond located in West Salisbury, Vt. It is small, measuring only 12 m across at its widest point, and only 24 m long. It has a mean depth of 1.5 m and a maximum depth of 2.1 m.

Materials and Methods

We divided Betourney's Pond into eight shoreline sections (9 m of shoreline each) and one center section. The SE corner section was selected as an introduction site. This corner was fairly shallow (mean depth of 0.75 m) and had M. spicatum growing to the surface.

The entire pond was systematically sampled immediately prior to the weevil introduction to determine pre-introduction weevil abundance. Snorkelers swam two parallel transects along the long axes and two transects along the short axes of the pond. Along the long axis, for each transect, ten milfoil meristems (top 50 cm) were collected. Along the short axis, eight milfoil meristems were collected per transect. For each transect, half of the total meristems collected had signs of invertebrate damage, the other half of the collection was undamaged meristems. In total, four transects were sampled, with a total of 36 meristems collected.

Weevils were introduced on the same day as the pre-introduction sampling, 30 July 1992. One hundred weevil larvae (with a small number of eggs and pupae) were added to the shallow, south end of the pond. Fifty weevils (both adults and larvae) were added on 13 August 1992, fourteen days after the initial introduction.

The pond was examined on 19 August 1992, twenty days after the introduction. No weevils or weevil damage were observed.

We conducted extensive sampling of the entire pond on 26 August 1992, 27 days after the introduction. The same four transects were sampled. Three transects were also taken parallel to the shore at the introduction site. As with the pre-introductory sampling, ten meristems were collected in each of the long axis transects, eight meristems were collected in the short axis transects. Ten meristems were also collected in each of the near-shore transects taken at the introduction site.

Results

In pre-introduction sampling, no weevils were collected.

In extensive observations on Day 20 after the introduction, we found no weevils and no evidence of weevils.

In extensive sampling on Day 27 (26 August 1992), we collected no weevils. Within our collection, we found seven plants (out of 66 collected plants) that had signs of adult weevil damage to the leaves.

Water temperature on the day of introduction was 22.5°C.

Discussion

The fate of the weevils in Betourney's Pond remains a mystery. On the final collection day, we noted that most of the watermilfoil in the center of the pond was cut up and no longer rooted to the bottom. While we have been assured by the property owner that the pond remained untouched, it appeared that much of the watermilfoil had been "harvested" or cut at the bottom by some means. Other invertebrates appear to have survived in the pond.

COMMUNICATION OF RESEARCH RESULTS WITH PUBLIC GROUPS, STATE AND FEDERAL AGENCIES, AND AT SCIENTIFIC MEETINGS

Public Groups

S. Sheldon made a presentation on our research to an audience comprised of state and federal aquatic plant managers and the general public in Minneapolis, Minn. (sponsored by the University of Minnesota). R. Creed also presented a seminar on the research to a wetlands ecology class in the Fisheries and Wildlife Dept. at the University of Vermont. R. Creed made an informal presentation of research results to the board of directors of the Lake Bomoseen Association and Castleton town officials. The purpose of this meeting was to discuss the impending drawdown on Lake Bomoseen and its potential impacts on the weevil research. The slide show on the prospect of biological control of Eurasian watermilfoil was not updated this year. A workshop for the general public was held at Middlebury College during the summer. Information about the research has also been made available to the public in the Vt. Department of Environmental Conservations' biannual newsletter. We have also responded to numerous queries from the public regarding the research (primarily in the form of phone calls) and have sent out materials describing the project when they are requested.

State and Federal Agencies

In November, S. Sheldon and R. Creed attended the annual Aquatic Plant Control Research Program (APCRP) meetings sponsored by the Army Corps of Engineers in Bellevue, Washington. The results of research conducted at Brownington Pond and at Middlebury College were presented by S. Sheldon. S. Sheldon gave an informal

presentation to the Vermont Eurasian Watermilfoil Study Committee which is charged with formulating an aquatic herbicide use policy in Vermont.

R. Creed presented a paper on recent research results at the annual New England Association of Environmental Biologists meetings in Meridan, Conn. in March 1993.

Scientific Meetings

S. Sheldon attended a workshop at the annual meetings of the Aquatic Plant Management Society in Daytona, Florida. She also wrote a paper on the distribution of exotic aquatic plants in New England for this workshop. S. Sheldon also attended a symposium on biological control in Minnesota and presented a paper on biological control of aquatic macrophytes (sponsored by the University of Minnesota). S. Sheldon gave a talk at the annual meeting of the New England Botanical Society in Boston.

SUMMARY DISCUSSION

The research undertaken during 1992 addressed five of the six primary objectives proposed for this project (Table 20). Considerable progress was made at the Brownington Pond (BP) field site where we are examining the watermilfoil decline (Objective 1). We continued the plant, invertebrate and fish surveys begun in 1990. We also continued monitoring water chemistry and temperature and obtained usable sediment samples. Laboratory experiments documented strong negative effects of herbivores (E. leconteі and A. nivea) on watermilfoil. The herbivore exclusion experiment demonstrated that weevils can have strong effects on established watermilfoil plants. The second fish exclusion experiment also demonstrated that yellow perch have little direct effect on the abundance of weevils. Once again, we did not work with Parapoynx as this species was still rare on watermilfoil in BP. While we have not yet demonstrated the cause of the decline at Brownington Pond the results of the 1992 field season lend further support to the hypothesis that Euhrychiopsis, and possibly other herbivores such as the Acentria, played an important role in both watermilfoil declines.

Research conducted at both Brownington Pond and at Middlebury (M) examined the effect of herbivores on watermilfoil and native macrophytes (Objective 2). Strong effects of Acentria and Euhrychiopsis on M. spicatum were observed in the BP experiment. Weevil larvae did not have as strong an effect on M. spicatum as Acentria, however, burrowing by larval weevils may contribute the most to the reduction in Eurasian watermilfoil buoyancy. Weevils were not very common on native macrophytes in BP in 1991 and 1992. Weevils had no significant effect on native macrophytes in wading pool experiments conducted at Middlebury.

Weevils were successfully introduced into Norton Brook Pond and Van Vleck's Pond (Objective 3). We have had continued success at maintaining a small culture of E.

lecontei at Middlebury and documenting their life history. Successful culturing is important if we are to undertake controlled introductions in the future.

Our data from L. Bomoseen indicate that this lake already supports a population of the weevil E. lecontei which suggests that a natural weevil introduction has already begun (Objective 4). In 1991, the weevils had begun to reduce watermilfoil abundance in certain parts of the lake (Objective 5). Weevil abundances were much lower in Lake Bomoseen in 1992. As mentioned previously (Creed and Sheldon 1992), extensive harvesting could prevent this weevil population from expanding and affecting watermilfoil throughout the lake. We have had considerable difficulty in influencing watermilfoil management in Lake Bomoseen. The severity of the watermilfoil problem in the lake makes lakeshore property owners reluctant to suspend watermilfoil control activities long enough for a weevil population to become established.

We have presented the results of our work to public groups and at scientific meetings. We have also prepared a slide show on watermilfoil control that is available to the public through the Vermont DEC (Objective 6).

Finally, a list of equipment purchased on the grant from June 1992 to April 1993 is presented in Table 21.

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TABLES

Table 1. Results of the analysis for sediments collected from five sites in Brownington Pond. Values in the table are means (± 1 S.E.). The units for the sediment extraction samples are mg/g; the units for the interstitial water samples mg/l; the units for sediment density are g/ml.

Variable	Site				
	Natives	South Bed	South Shallow	West Bed	West Shallow
Sediment Extractions					
Exch. 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)
Exch. 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)
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)

Table 1. Continued.

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)

Table 2. Dominant macroinvertebrate taxa associated with M. spicatum in the South Bed in Brownington Pond in 1992. Samples were collected using the Super Sampler (MIS). Data in the table are mean number (± 1 S.E.) of individuals per gram (dry weight) of M. spicatum. Five samples were taken on each date. Statistical comparisons were made using an ANOVA with Tukey's test on log transformed data. Means with the same letter are not significantly different.

Taxon	Date			
	8/6	29/6	20/7	10/8
Annelida				
Oligochaeta	0.4 a (0.3)	3.5 ab (1.5)	5.4 ab (2.7)	3.9 b (1.1)
Arthropoda				
Amphipoda				
Hyallela	12.1 a (4.5)	13.5 a (4.5)	18.0 a (7.0)	20.8 a (3.4)
Cladocera	5.1 a (1.9)	1.4 a (1.4)	0.2 a (0.2)	12.2 a (10.3)
Hydracarina	9.0 a (1.8)	7.8 a (2.7)	2.2 a (0.7)	11.6 a (4.5)
Insecta				
Coleoptera				
Euhrychiopsis	1.8 a (1.4)	1.2 a (0.7)	2.3 a (0.7)	2.1 a (0.7)
Diptera				
Chironomidae	42.5 a (12.5)	74.4 a (34.7)	39.1 a (6.7)	14.6 a (2.9)
Ephemeroptera				
Caenis	63.3 a (13.1)	48.8 ab (12.4)	13.4 b (2.0)	2.7 c (1.2)
Lepidoptera				
Acentria	2.4 a (1.0)	0.4 a (0.4)	0.6 a (0.6)	0.4 a (0.4)

Table 2 Continued.

Odonata								
Anisoptera	0.4 (0.2)	a	0.6 (0.5)	a	0.3 (0.1)	a	0.2 (0.2)	a
Zygoptera								
Enallagma	6.6 (2.1)	a	3.0 (2.0)	ab	0.2 (0.2)	b	0.8 (0.5)	b
Trichoptera								
Oxyethira	0.0 (0.0)	a	0.0 (0.0)	a	0.9 (0.6)	ab	2.8 (1.0)	b
Oecetis	3.2 (2.2)	a	1.9 (1.4)	a	<0.1 (<0.1)	a	0.0 (0.0)	a
Triaenodes	1.1 (0.6)	a	0.4 (0.4)	a	0.2 (0.1)	a	0.6 (0.6)	a
Leptocercus	0.2 (0.2)	a	0.0 (0.0)	a	0.1 (0.1)	a	0.0 (0.0)	a
Ceraclea	6.5 (2.2)	a	3.0 (1.7)	ab	<0.1 (<0.1)	b	0.0 (0.0)	b
Platyhelminthes								
Planariidae	0.6 (0.5)	a	0.1 (0.1)	a	0.3 (0.3)	a	1.5 (0.9)	a
Mollusca								
Gastropoda								
Amnicola	37.1 (7.2)	ab	9.7 (6.1)	c	24.4 (10.1)	bc	155.3 (60.7)	a
Physa	0.1 (0.1)	a	0.8 (0.4)	ab	5.6 (2.6)	bc	11.5 (4.0)	c
Planorbidae	4.3 (0.5)	a	1.4 (0.9)	a	5.4 (5.1)	a	0.6 (0.4)	a

Table 3. Dominant macroinvertebrate taxa associated with M. spicatum in the West Bed in Brownington Pond in 1992. Samples were collected using the Super Sampler (MIS). Data in the table are mean number (± 1 S.E.) of individuals per gram (dry weight) of M. spicatum. Five samples were taken on each date. Statistical comparisons were made using an ANOVA with Tukey's test on log transformed data. Means with the same letter are not significantly different.

Taxon	Date			
	8/6	29/6	20/7	10/8
Annelida				
Oligochaeta	6.0 ab (1.8)	13.3 a (1.6)	0.9 b (0.2)	8.3 a (4.1)
Arthropoda				
Amphipoda				
Hyallela	13.5 ab (4.2)	8.8 b (2.3)	21.6 ab (5.1)	36.2 a (4.3)
Cladocera	2.6 a (1.2)	1.8 a (1.7)	0.0 a (0.0)	0.5 a (0.3)
Hydracarina	14.6 a (3.7)	10.3 a (4.7)	3.2 a (1.4)	11.5 a (4.0)
Insecta				
Coleoptera				
Euhrychiopsis	1.1 a (0.2)	1.8 a (0.6)	3.6 a (0.8)	3.0 a (0.8)
Diptera				
Chironomidae	10.6 a (3.5)	5.2 a (1.7)	8.7 a (3.1)	1.6 a (0.4)
Ephemeroptera				
Caenis	8.0 a (2.5)	3.4 a (0.8)	0.2 b (0.1)	0.2 b (0.2)
Lepidoptera				
Acentria	5.2 a (1.7)	2.5 ab (1.6)	0.4 bc (0.2)	0.0 c (0.0)

Table 3 Continued.

Odonata				
Anisoptera	1.3 a (0.6)	0.0 a (0.0)	0.3 a (0.1)	0.9 a (0.3)
Zygoptera				
Enallagma	2.1 a (0.5)	1.4 a (0.4)	0.3 b (0.1)	0.1 b (0.1)
Trichoptera				
Oxyethira	0.2 a (0.2)	0.5 a (0.5)	0.2 a (0.2)	2.2 a (1.5)
Oecetis	2.1 a (0.5)	2.3 ab (1.0)	0.1 b (0.1)	0.3 ab (0.2)
Triaenodes	0.9 a (0.3)	0.8 a (0.5)	0.4 a (0.3)	0.0 a (0.0)
Leptocercus	2.0 a (0.6)	1.2 ab (0.7)	0.0 b (0.0)	0.0 b (0.0)
Ceraclea	0.7 a (0.3)	0.9 a (0.7)	0.1 a (0.1)	0.0 a (0.0)
Platyhelminthes				
Planariidae	1.9 a (1.0)	11.0 b (3.0)	0.6 a (0.3)	2.7 ab (0.9)
Mollusca				
Gastropoda				
Amnicola	35.4 a (3.7)	42.3 a (6.0)	24.2 a (5.1)	100.8 b (19.2)
Physa	0.6 a (0.2)	15.9 b (5.5)	7.9 ab (3.1)	24.5 b (7.3)
Planorbidae	3.2 a (1.1)	2.7 a (0.9)	1.2 ab (0.4)	0.7 b (0.3)

Table 4. The total number of E. lecontei, A. nivea and P. badiusalis collected in the Super Samples (both M. spicatum and native plants) taken in 1991 and 1992. For M. spicatum n=38 in 1991 and n=40 in 1992; for P. amplifolius n=11 in 1991 and n=16 in 1992; for H. dubia n=11 in 1991 and n=12 in 1992.

Euhrychiopsis lecontei

	P. amplifolius	H. dubia	M. spicatum
1991	2	2	296
1992	1	2	188

Acentria nivea

	P. amplifolius	H. dubia	M. spicatum
1991	20	0	352
1992	12	0	72

Parapoynx badiusalis

	P. amplifolius	H. dubia	M. spicatum
1991	72	11	23
1992	85	16	3

Table 5A. Dominant macroinvertebrate taxa associated with long M. spicatum stems from the South Bed in Brownington Pond (9 June - 14 July, 1992). Samples were collected using the smaller MIS sampler (minisampler). Data in the table are mean number of individuals per stem (± 1 S.E.). Three samples were taken on each date.

Taxon	Date					
	9/6	16/6	23/6	30/6	7/7	14/7
Annelida						
Oligochaeta	1.0 (0.6)	12.3 (12.3)	4.3 (0.3)	40.3 (16.3)	41.3 (11.9)	49.3 (5.2)
Arthropoda						
Amphipoda						
Hyallela	4.0 (0.6)	-	1.0 (0.6)	3.3 (0.9)	4.0 (2.0)	1.3 (0.3)
Cladocera	26.7 (0.9)	2.3 (2.3)	1.0 (0.6)	-	-	-
Hydracarina	1.7 (0.3)	1.0 (0.6)	0.7 (0.3)	1.3 (0.9)	0.3 (0.3)	0.3 (0.3)
Insecta						
Coleoptera						
Euhrychiopsis	3.7 (1.5)	1.3 (1.3)	1.7 (0.9)	3.3 (0.7)	2.7 (1.2)	1.7 (0.9)
Gyrinus	0.3 (0.3)	0.7 (0.3)	-	0.3 (0.3)	0.3 (0.3)	-
Diptera						
Chironomidae	2.3 (1.5)	0.7 (0.3)	3.3 (1.7)	10.3 (7.9)	8.0 (2.5)	3.3 (1.5)
Lepidoptera						
Acentria	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.7 (0.7)	0.3 (0.3)
Odonata						
Anisoptera	-	-	-	-	-	-
Zygoptera						
Enallagma	1.0 (1.0)	0.3 (0.3)	0.7 (0.3)	-	-	-

Table 5A. Continued.

Trichoptera						
Oxyethira	-	-	-	-	0.3 (0.3)	1.0 (1.0)
Oecetis	1.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.7 (0.7)	0.3 (0.3)	0.3 (0.3)
Coelenterata						
Hydra	31.0 (16.7)	78.3 (19.8)	4.3 (1.9)	-	-	-
Platyhelminthes						
Planariidae	0.3 (0.3)	-	0.7 (0.3)	2.0 (0.6)	2.3 (1.2)	3.7 (1.5)
Mollusca						
Gastropoda						
Amnicola	6.7 (2.2)	2.0 (1.0)	1.3 (0.9)	1.7 (0.9)	5.0 (0.6)	6.0 (1.0)
Physa	-	-	-	-	0.3 (0.3)	0.3 (0.3)
Planorbidae	0.7 (0.3)	-	0.3 (0.3)	1.0 (0.6)	0.7 (0.3)	0.7 (0.7)

Table 5B. Dominant macroinvertebrate taxa associated with long M. spicatum stems from the South Bed in Brownington Pond (21 July - 25 August, 1992). Samples were collected using the smaller MIS sampler. Data in the table are mean number of individuals per stem (± 1 S.E.). Three samples were taken on each date.

Taxon	Date					
	21/7	28/7	4/8	11/8	18/8	25/8
Annelida						
Oligochaeta	73.3 (17.2)	87.0 (4.0)	63.0 (10.4)	9.7 (4.8)	12.0 (5.5)	5.0 (0.6)
Arthropoda						
Amphipoda						
Hyallela	4.0 (1.0)	6.7 (3.7)	0.3 (0.3)	-	-	1.3 (1.3)
Cladocera	-	0.7 (0.7)	0.3 (0.3)	-	17.3 (5.4)	0.3 (0.3)
Hydracarina	0.7 (0.3)	2.3 (1.5)	0.7 (0.3)	-	-	0.7 (0.3)
Insecta						
Coleoptera						
Euhrychiopsis	1.0 (0.6)	3.3 (0.9)	2.0 (1.2)	-	0.7 (0.7)	0.7 (0.3)
Gyrinus	-	0.7 (0.3)	-	-	-	-
Diptera						
Chironomidae	2.3 (0.7)	1.7 (1.2)	1.3 (0.3)	1.0 (0.0)	2.0 (0.6)	1.7 (1.2)
Lepidoptera						
Acentria	0.7 (0.3)	0.7 (0.7)	-	-	0.7 (0.7)	0.3 (0.3)
Odonata						
Anisoptera	-	-	-	-	-	-
Zygoptera						
Enallagma	-	-	-	0.3 (0.3)	0.3 (0.3)	-

Table 5B. Continued.

Trichoptera						
Oxyethira	4.0 (1.2)	2.7 (1.5)	0.7 (0.7)	2.7 (1.2)	0.7 (0.3)	-
Cecetis	0.3 (0.3)	0.3 (0.3)	-	-	0.3 (0.3)	0.7 (0.7)
Coelenterata						
Hydra	-	-	-	-	-	-
Platyhelminthes						
Planariidae	5.7 (2.3)	15.0 (8.1)	7.3 (3.0)	1.0 (0.6)	2.0 (1.0)	6.0 (1.5)
Mollusca						
Gastropoda						
Amnicola	5.7 (0.9)	10.3 (3.0)	9.3 (3.8)	5.7 (0.3)	10.3 (5.3)	25.3 (2.9)
Physa	0.7 (0.3)	4.3 (3.3)	2.0 (1.0)	1.0 (0.6)	1.3 (0.7)	2.3 (1.2)
Planorbidae	1.0 (0.6)	1.7 (0.9)	1.0 (0.6)	0.3 (0.3)	-	0.7 (0.3)

Table 6A. Dominant macroinvertebrate taxa associated with long M. spicatum stems from the West Bed in Brownington Pond (9 June - 14 July, 1992). Samples were collected using the smaller MIS sampler. Data in the table are mean number of individuals per stem (± 1 S.E.). Three samples were taken on each date.

Taxon	Date					
	9/6	16/6	23/6	30/6	7/7	14/7
Annelida						
Oligochaeta	27.3 (6.0)	25.7 (12.0)	63.3 (7.9)	36.7 (21.2)	54.3 (22.9)	25.7 (19.9)
Arthropoda						
Amphipoda						
Hyallela	1.0 (1.0)	0.7 (0.7)	0.3 (0.3)	0.3 (0.3)	0.7 (0.3)	0.7 (0.7)
Cladocera	4.0 (2.3)	1.7 (0.9)	9.0 (6.7)	0.7 (0.7)	0.3 (0.3)	0.7 (0.7)
Hydracarina	-	-	1.7 (0.9)	0.7 (0.7)	1.0 (1.0)	3.3 (0.9)
Insecta						
Coleoptera						
Eubrychiopsis	3.0 (1.2)	0.3 (0.3)	4.3 (0.7)	2.0 (1.0)	0.7 (0.3)	3.7 (1.9)
Gyrinus	2.0 (2.0)	-	-	0.7 (0.3)	-	0.3 (0.3)
Diptera						
Chironomidae	1.3 (0.7)	2.0 (0.6)	1.7 (0.9)	2.0 (1.2)	0.3 (0.3)	3.3 (0.7)
Lepidoptera						
Acentria	1.3 (0.9)	2.0 (1.5)	-	1.0 (1.0)	1.0 (0.0)	1.0 (0.0)
Odonata						
Anisoptera	-	-	-	-	-	-
Zygoptera						
Enallagma	-	-	0.3 (0.3)	-	-	-

Table 6A. Continued.

Trichoptera						
Oxyethira	-	-	-	0.3 (0.3)	-	1.3 (0.3)
Oecetis	2.0 (0.6)	0.3 (0.3)	0.3 (0.3)	0.7 (0.3)	-	-
Coelenterata						
Hydra	130.7 (76.5)	24.3 (6.3)	0.3 (0.3)	-	-	-
Platyhelminthes						
Planariidae	8.0 (3.5)	4.3 (1.7)	15.7 (3.2)	5.3 (2.0)	6.0 (1.5)	39.0 (8.9)
Mollusca						
Gastropoda						
Amnicola	3.7 (0.9)	4.3 (3.4)	16.3 (3.5)	11.7 (7.3)	8.0 (2.5)	21.0 (4.5)
Physa	-	-	-	0.3 (0.3)	5.0 (3.1)	2.0 (2.0)
Planorbidae	2.3 (1.9)	0.3 (0.3)	-	0.7 (0.7)	-	1.0 (0.6)

Table 6B. Dominant macroinvertebrate taxa associated with long M. spicatum stems from the West Bed in Brownington Pond (21 July - 25 August, 1992). Samples were collected using the smaller MIS sampler. Data in the table are mean number of individuals per stem (± 1 S.E.). Three samples were taken on each date.

Taxon	Date					
	21/7	28/7	4/8	11/8	18/8	25/8
Annelida						
Oligochaeta	62.0 (8.1)	62.7 (39.7)	99.0 (39.1)	15.0 (4.0)	23.0 (9.3)	24.0 (11.5)
Arthropoda						
Amphipoda						
Hyallela	3.7 (0.7)	0.7 (0.7)	3.3 (1.5)	3.3 (1.9)	1.0 (1.0)	0.3 (0.3)
Cladocera	0.3 (0.3)	0.3 (0.3)	-	1.7 (0.9)	0.7 (0.7)	1.7 (0.9)
Hydracarina	0.3 (0.3)	0.3 (0.3)	1.0 (0.6)	0.7 (0.7)	1.3 (0.7)	-
Insecta						
Coleoptera						
Euhrychiopsis	3.3 (0.9)	3.0 (1.7)	1.7 (0.7)	1.3 (0.9)	1.3 (0.3)	0.3 (0.3)
Gyrinus	-	1.0 (1.0)	-	-	-	-
Diptera						
Chironomidae	2.0 (0.6)	1.0 (1.0)	0.7 (0.7)	-	-	1.0 (0.6)
Lepidoptera						
Acentria	0.3 (0.3)	1.3 (0.3)	0.3 (0.3)	0.3 (0.3)	-	0.3 (0.3)
Odonata						
Anisoptera	-	-	0.3 (0.3)	-	-	-
Zygoptera						
Enallagma	0.3 (0.3)	-	-	-	-	0.3 (0.3)

Table 6B. Continued.

Trichoptera						
Oxyethira	1.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	2.0 (1.2)	0.7 (0.3)
Oecetis	-	0.3 (0.3)	0.3 (0.3)	-	-	0.7 (0.7)
Coelenterata						
Hydra	-	-	-	-	-	-
Platyhelminthes						
Planariidae	19.7 (5.8)	39.0 (14.8)	57.7 (35.0)	11.0 (7.0)	9.7 (9.0)	20.3 (10.8)
Mollusca						
Gastropoda						
Amnicola	14.7 (4.6)	11.0 (2.0)	30.7 (8.9)	19.7 (0.3)	20.3 (5.2)	37.0 (11.7)
Physa	2.7 (1.2)	1.0 (0.6)	1.3 (0.9)	4.7 (2.7)	6.3 (5.8)	5.0 (1.5)
Planorbidae	-	0.7 (0.3)	-	1.3 (0.9)	1.0 (0.6)	0.3 (0.3)

Table 7. The abundance of E. lecontei and A. nivea on long (>50 cm) and short watermilfoil stems in the West Bed. The samples were taken with the small MIS sampler (Minisampler). Values in the table are means (± 1 S.E.). N=3 for all samples.

Date	E. lecontei		A. nivea	
	Long	Short	Long	Short
9 June	3.00 (1.15)	0.67 (0.67)	1.33 (0.88)	0.33 (0.33)
16 June	0.33 (0.33)	0.00 (0.00)	2.00 (1.53)	0.00 (0.00)
23 June	4.33 (0.67)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
30 June	2.00 (1.00)	1.67 (1.67)	1.00 (1.00)	0.33 (0.33)
7 July	0.67 (0.33)	0.33 (0.33)	1.00 (0.00)	0.00 (0.00)
14 July	3.67 (1.86)	0.00 (0.00)	1.00 (0.00)	0.00 (0.00)
21 July	3.33 (0.88)	1.00 (0.00)	0.33 (0.33)	0.00 (0.00)
28 July	3.00 (1.73)	0.67 (0.67)	1.33 (0.33)	0.00 (0.00)
4 August	1.67 (0.67)	1.33 (1.33)	0.33 (0.33)	0.00 (0.00)
11 August	1.33 (0.88)	0.00 (0.00)	0.33 (0.33)	0.33 (0.33)
18 August	1.33 (0.33)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
25 August	0.33 (0.33)	0.00 (0.00)	0.33 (0.33)	0.00 (0.00)

Table 8. The abundance of E. lecontei and A. nivea on long (>50 cm) and short watermilfoil stems in the South Bed. The samples were taken with the small MIS sampler (Minisampler). Values in the table are means (± 1 S.E.). N=3 for all samples.

Date	E. lecontei		A. nivea	
	Long	Short	Long	Short
9 June	3.67 (1.45)	0.00 (0.00)	0.33 (0.33)	0.67 (0.67)
16 June	1.33 (1.33)	0.00 (0.00)	0.33 (0.33)	0.00 (0.00)
23 June	1.67 (0.88)	0.00 (0.00)	0.33 (0.33)	0.67 (0.33)
30 June	3.33 (0.67)	0.00 (0.00)	0.33 (0.33)	0.00 (0.00)
7 July	2.67 (1.20)	1.00 (0.58)	0.67 (0.67)	0.00 (0.00)
14 July	1.67 (0.88)	0.67 (0.33)	0.33 (0.33)	0.33 (0.33)
21 July	1.00 (0.58)	0.33 (0.33)	0.67 (0.33)	0.33 (0.33)
28 July	3.33 (0.88)	2.67 (0.88)	0.67 (0.67)	0.00 (0.00)
4 August	2.00 (1.15)	0.33 (0.33)	0.00 (0.00)	0.00 (0.00)
11 August	0.00 (0.00)	0.67 (0.33)	0.00 (0.00)	0.00 (0.00)
18 August	0.67 (0.67)	0.33 (0.33)	0.67 (0.67)	0.00 (0.00)
25 August	0.67 (0.33)	0.00 (0.00)	0.33 (0.33)	0.00 (0.00)

Table 9. Dominant prey found in the guts of yellow perch collected in Brownington Pond in June and July of 1992. Values in the table are frequencies of occurrence.

Prey Taxon	Date		
	25 June	2 July	10 July
Crustacea			
Amphipoda			
Hyallela	46	31	35
Cladocera	36	0	35
Insecta			
Diptera			
Chironomidae L.[1]	73	31	71
Chironomidae P.	55	46	47
Ceratopogonidae[2]	46	31	29
Chaoborus L.	27	15	24
Chaoborus P.	27	0	0
Ephemeroptera			
Caenis	73	31	59
Odonata			
Tetragoneuria	46	23	48
Enallagma	46	54	29
Mollusca			
Physa	27	8	12
Chordata			
Perch fry	27	39	18

L. = Larvae, P. = Pupae
Ceratopogonidae = Heleidae (used in previous reports).

Table 10. The effect of weevil damage on stem fragment viability: results of Experiment 1. Values in the table are means (± 1 S.E).

Variable	Treatment		F Value	P Value
	Control	Damaged		
Percent of Stems with Roots	100.0 (0.0)	85.0 (9.6)	2.80	-
Root Weight (g)	0.257 (0.018)	0.036 (0.011)	37.90	0.0008
Change in Stem Length (mm)				
Total	114.8 (6.5)	90.7 (13.0)	3.22	-
Original	111.7 (5.6)	5.1 (5.6)	45.52	0.0005
Lateral	3.1 (1.8)	85.6 (15.4)	33.26	0.0012

Table 11. The response of the dominant macroinvertebrates found on M. spicatum in Brownington Pond to the exclusion of fish. Values in the table are mean number of a taxon per gram of watermilfoil (± 1 S.E.). The treatments were compared using an ANOVA with planned, orthogonal contrasts. The Fish contrast in the table is the comparison of fish vs no fish, i.e., the cage treatment vs the cage control and the control treatments. The Cage contrast tests for a cage effect and compares the cage control vs the control. The ANOVA was performed on log(X+1) transformed data. Significance levels are as follows: # $p<0.1$ (marginally significant), * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Ct=Control, Cc=Cage Control, Ca=Cage.

Taxon	Treatment			Contrast	
	Ct	Cc	Ca	Fish	Cage
Insecta					
Coleoptera					
Euhrychiopsis[1]	2.82 (0.64)	6.02 (1.03)	6.84 (0.40)	*	**
Diptera					
Chironomidae	8.85 (3.98)	9.34 (2.51)	5.02 (1.08)	-	-
Ceratopogonidae	6.03 (2.17)	2.87 (0.65)	3.49 (1.11)	-	-
Ephemeroptera					
Caenis	8.26 (2.90)	10.94 (3.66)	5.02 (0.65)	-	-
Lepidoptera					
Acentria	0.06 (0.06)	0.23 (0.12)	0.05 (0.03)	-	#
Odonata					
Anisoptera	0.27 (0.09)	0.46 (0.19)	0.29 (0.60)	-	-
Zygoptera					
Enallagma	0.42 (0.23)	0.87 (0.31)	1.58 (0.16)	**	-
Trichoptera					
Oecetis	0.57 (0.20)	0.25 (0.12)	0.33 (0.12)	-	-

Table 11. Continued.

Taxon	Treatment			Contrast	
	Ct	Cc	Ca	Fish	Cage
Oxyethira	0.99 (0.36)	0.28 (0.15)	0.03 (0.02)	*	#
Triaenodes	0.09 (0.09)	0.29 (0.07)	0.34 (0.11)	-	-
Ceraclea	0.38 (0.20)	0.50 (0.15)	0.22 (0.06)	-	-
Crustacea					
Amphipoda					
Hyalella	12.27 (4.95)	19.19 (2.50)	23.04 (2.86)	-	#
Hydracarina	3.09 (1.11)	6.16 (1.99)	6.52 (0.67)	#	#
Gastropoda					
Amnicola	12.68 (3.53)	7.17 (1.57)	12.99 (2.40)	#	*
Physa	9.62 (1.56)	11.95 (2.00)	15.45 (1.48)	#	-
Immature Planorbidae[2]	7.78 (2.00)	10.36 (1.98)	11.96 (1.66)	-	-
Platyhelminthes					
Planaria	0.43 (0.18)	0.32 (0.19)	0.18 (0.08)	-	-
Oligochaeta	13.32 (5.24)	1.92 (0.54)	2.12 (0.86)	*	*
Hirudinea	1.84 (0.76)	1.46 (0.39)	0.87 (0.08)	-	-

1 - E. lecontei adults and larvae combined.
2 - Immature Planorbidae consists of Gyraulus sp. and Helisoma sp.

Table 12. The effect of weevil and *Acentria* feeding on the height of original stems(cm) for the last three weeks of the enclosure experiment conducted in Brownington Pond in 1992. 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.

Date	Treatment		
	Control	*Acentria* Control	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 (3.19)
17 August	a 94.39 (3.51)	a 91.17 (0.44)	b 70.79 (3.59)

Table 13. The mean (± 1 S.E.) number of weevils (larvae, pupae and adults) per meristem collected at three sites in Lake Bomoseen in 1992 during stem transects. Data are from all samples taken at a site, i.e., samples from harvested and unharvested sites are combined.

Site	N	Number of weevils per meristem
Neshobe Island	19	0.042 (0.012)
East Eckley N.	17	0.039 (0.007)
East Eckley S.[1]	19	0.048 (0.010)
All Sites Combined	19	0.042 (0.006)

1- This site was not harvested in 1992.

Table 14. The effect of mechanical harvesting on weevil abundance (larvae, pupae and adults) in 1992. Data are from the stem transects at three sites in Lake Bomoseen. Values in the table are the mean (± 1 S.E.) numbers of weevils per meristem. Differences between harvested and unharvested areas compared using ANOVA; data were square root transformed for the analysis.

Site	N	Harvested	Unharvested	p value
Neshobe I.	19	0.011 (0.004)	0.074 (0.024)	0.012
East Eckley N.	17	0.024 (0.006)	0.055 (0.012)	0.043
East Eckley S.[1]	19	0.046 (0.011)	0.051 (0.013)	0.772
All Sites Combined [2]	19	0.026 (0.005)	0.058 (0.010)	0.006

1- This site was not harvested in 1992.
2- There were no significant differences among sites (p=0.444) when harvest and no harvest data were pooled.

Table 15. The distribution of weevils (larvae, pupae and adults) with respect to water depth in 1992. Data are from the stem transects at three sites in Lake Bomoseen. Values in the table are the mean (± 1 S.E.) numbers of weevils per meristem. Differences between shallow and deep areas compared using ANOVA; data were square root transformed for the analysis.

Site	N	Shallow	Deep	p value
Neshobe I.	15 [1]	0.056 (0.016)	0.050 (0.017)	0.896
East Eckley N.	15	0.078 (0.016)	0.009 (0.004)	0.001
East Eckley S.[2]	15	0.081 (0.016)	0.028 (0.010)	0.013
All Sites Combined [3]	15	0.071 (0.011)	0.029 (0.005)	0.001

1- Shallow-deep comparisons were started later in the summer thus the reduced number of samples compared with Tables 13 and 14.
2- This site was not harvested in 1992.
3- There were no significant differences among sites (p=0.578) when shallow and deep data were pooled.

Table 16. The mean number (± 1 S.E.) of weevils (all life stages) collected per date at each site in Lake Bomoseen for 1991 and 1992. The data are from the stem transects and samples from approximately the same dates for each year were compared. For each comparison n=12.

Year	Site		
	Neshobe	Eckley[1]	Neshobe + Eckley
1991	12.08 (1.48)	2.08 (0.57)	14.17 (1.92)
1992	4.94 (0.88)	3.58 (0.77)	8.53 (1.06)
T Statistic	3.966	1.501	2.395
p value	0.001	0.148	0.026

1- Eckley samples are just for E. Eckley South which was the only area in Eckley Bay which was sampled both years.

Table 17. Results of the ANOVA of the super sample data from the harvested and unharvested areas at Neshobe Island in Lake Bomoseen in 1992. Samples were collected on 30 June, 27 July 31 August. Three samples were taken in both the harvested and unharvested areas on each date. The ANOVA was performed on log transformed data. The table presents the level of significance (i.e., based on p values) of watermilfoil biomass and major taxa for the effects of date and harvesting.

Taxon	Effect	
	Date	Harvesting
Milfoil Dry Weight	-	**
Invertebrates		
Oligochaeta	*	-
Arthropoda		
Crustacea		
Amphipoda	-	-
Isopoda	*	*
Hydracarina	#	#
Insecta		
Euhrychiopsis	*	*
Chironomidae	*	-
Caenis	**	*
Zygoptera	*	-
Agraylea	-	-
Oxyethira	*	-
Orthotrichia	*	-
Mollusca		
Gastropoda		
Planorbidae	***	-
Amnicola	***	-
Physa	#	-

Significance levels: - not significant, # marginally significant ($p<0.10$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 18. The number of intact plants for each macrophyte species in each of the three treatments for the native macrophyte trials.

Macrophyte Species	Treatment		
	0 weevils	2 Weevils	4 Weevils
Ceratophyllum	5	6	4
Chara	4	4	6
Elodea	5	4	4
Heteranthera	6	6	6
Megalodonta	5	6	6
M. sibiricum	4	3	2
P. amplifolius	6	6	6
Utricularia	6	5	6
Vallisneria	6	6	6

Table 19. The average number of weevils surviving per chamber (2 and 4 weevil treatments for natives and the M. spicatum controls (4 weevils/chamber)) in the native plant trials. N=6 for the native plant treatments and n=3 for the M. spicatum controls.

Native Species Trial	Treatment		
	2 Weevils	4 Weevils	M. spicatum Control
Ceratophyllum	0.50	0.00	1.75
Chara	0.33	1.00	4.00
Elodea	0.00	0.00	2.00
Heteranthera	0.00	0.00	2.67
Megalodonta	0.33	0.67	4.00
M. sibiricum	1.17	1.83	2.67
P. amplifolius[1]	-	-	-
Utricularia	0.66 [2]	1.33 [2]	3.00
Vallisneria	0.17	0.33	4.00

1- Weevil survivorship not recorded for P. amplifolius.
2- The Utricularia experiment was terminated early (after only seven days).

Table 20. A list of the six primary objectives of this study and the work conducted during the 1992 field season that addresses these objectives. As the ideas in the objectives overlap, some projects are listed under two or more objectives.

Objective 1. Determine the probable cause(s) of the Eurasian watermilfoil decline in Brownington Pond.

-all Brownington Pond (BP) research

Objective 2. Examine the grazing/boring effects of all major herbivores on Eurasian watermilfoil and native aquatic plant species.

-Wading Pool Experiment (BP)
-Stem Fragment Viability Experiment (BP)
-Herbivore Enclosure Experiment (BP)
-Native Plant Experiments (Middlebury (M))

Objective 3. Determine the feasibility of herbivore introductions into other milfoil-infested lakes in Vermont.

-Weevil culture data (M)
-Weevil transect data (Lake Bomoseen (LB))
-Introductions at Norton Brook, Van Vleck's and Betourney's (M)

Objective 4. Determine if Lake Bomoseen is a suitable site for herbivore introductions/collect pre-introduction base-line data.

-Weevil transect data (LB)

Objective 5. If determined to be feasible and appropriate based on previous research (a high likelihood of success and relatively free from causing negative impacts to non-target species), use herbivorous insects to control Eurasian watermilfoil in Lake Bomoseen.

-augmentation of weevils at Eckley Bay (LB)

Objective 6. Develop a public education program to keep Vermont's citizens abreast of the results of the research.

-presentations given by Sheldon and Creed
-slide show on watermilfoil control prepared (M)

Table 21. Equipment purchased on the EPA grant from June 1991 to April 1992.

Item	Amount
Post Script Option for IBM Printer	329.00
Statistix (Statistical package for Zenith laptop computer)	201.00
Underwater Camera	252.86

FIGURE LEGENDS

Figure 1. The distribution of watermilfoil in Brownington Pond in 1991 (A) and 1992 (B).

Figure 2 A and B. Water temperatures in Brownington Pond for 1992. Temperatures were recorded with maximum/minimum thermometers suspended 0.5 m below the surface and 0.5 m above the bottom. Values in figures are means (+ 1 S.E.) for two pairs of thermometers located around the pond. A. Surface temperatures. B. Bottom temperatures.

Figure 3 A - C. Results of the plant transects for the West Bed in 1992. Bars represent the mean (± 1 S.E.) biomass of watermilfoil or combined native macrophyte species (=Other).

Figure 4 A - C. Results of the plant transects for the South Bed in 1992. Bars represent the mean (± 1 S.E.) biomass of watermilfoil or combined native macrophyte species (=Other).

Figure 5 A - C. Results of the plant transects for the West Bed: 1990-1992. The figures for each year represent samples taken at about the same time of year. Bars represent the mean (± 1 S.E.) biomass of watermilfoil or other macrophyte species.

Figure 6 A - C. Results of the plant transects for the South Bed: 1990-1992. The figures for each year represent samples taken at about the same time of year. Bars represent the mean (± 1 S.E.) biomass of watermilfoil or other macrophyte species.

Figure 7. Maps of the percent cover of Eurasian watermilfoil in the South Grid, West Bed for one date in 1991 and three dates in 1992.

Figure 8. Maps of the percent cover of Eurasian watermilfoil in the North Grid, West Bed for one date in 1991 and three dates in 1992.

Figure 9. Maps of the percent cover of Eurasian watermilfoil in the West Grid, South Bed for one date in 1991 and three dates in 1992.

Figure 10. Maps of the percent cover of Eurasian watermilfoil in the East Grid, South Bed for one date in 1991 and three dates in 1992.

Figure 11. Maps of the percent cover of Eurasian watermilfoil for the West Bed: 1990-1992. The figures for each grid represent the last map for each year.

Figure 12. Maps of the percent cover of Eurasian watermilfoil for the South Bed: 1990-1992. The figures for each grid represent the last map for each year.

Figure 13. Eurasian watermilfoil and weevil abundance in the West Bed from 1990-1992. A. Watermilfoil biomass (mean ± 1 S.E.). Data for 1990 are from a series of quadrat samples. The number of samples for a given date ranges from three to six. Data for 1991 and 1992 are from the plant transects. All samples from the 2.0-3.5 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 14. Eurasian watermilfoil and weevil abundance in the South Bed from 1990-1992. A. Watermilfoil biomass (mean ± 1 S.E.). Data for 1990 are from a series of quadrat samples. The number of samples for a given date ranges from three to six. Data for 1991 and 1992 are from the plant transects. All samples from the 2.0-3.5 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 15 A and B. Results of the stem transects in the West Bed in Brownington Pond in 1992. The data in the figure are the mean (± 1 S.E.) number of eggs found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 16 A and B. Results of the stem transects in the West Bed in Brownington Pond in 1992 The data in the figure are the mean (± 1 S.E.) number of meristem larvae found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 17 A and B. Results of the stem transects in the West Bed in Brownington Pond in 1992. The data in the figure are the mean (± 1 S.E.) number of stem larvae found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 18 A and B. Results of the stem transects in the South Bed in Brownington Pond in 1992. The data in the figure are the mean (± 1 S.E.) number of eggs found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 19 A and B. Results of the stem transects in the South Bed in Brownington Pond in 1992. The data in the figure are the mean (± 1 S.E.) number of meristem larvae found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 20 A and B. Results of the stem transects in the South Bed in Brownington Pond in 1992. The data in the figure are the mean (± 1 S.E.) number of stem larvae found associated with A) watermilfoil stems with intact apical meristems and B) watermilfoil stems without intact apical meristems.

Figure 21 A - C. The effect of feeding by Euhrychiopsis and Acentria larvae on watermilfoil plants. The bars in the histogram represent the mean change in a response variable (± 1 S.E.) for each treatment. The lines with significance values above the histograms show the results of ANOVA comparisons with orthogonal contrasts. In each figure, the upper line represents the comparison of the control vs the herbivore treatments; the middle line represents the comparison of the weevil treatment versus the two treatments containing Acentria larvae; The lowest line represents the comparison of the Acentria alone treatment versus the treatment with both the Acentria and the Euhrychiopsis larvae (combined). A. Change in plant weight (in grams). B. Change in plant length (in millimeters). C. Change in the number of whorls per plant.

Figure 22. A and B. The effect of weevil damage on the viability of watermilfoil stem fragments. The bars in the histogram represent the mean change in a response variable (± 1 S E.) for each treatment. The lines with significance values above the histograms show the results of ANOVA comparisons with orthogonal contrasts. In each figure, the upper line represents the comparison of the undamaged control fragments (C) vs the damaged fragments (D); the lower line on the left represents the comparison of the unshaded control treatment (CU) vs the shaded control treatment (CS); the lower line on the right represents the comparison of the unshaded damaged stem treatment (DU) vs the shaded damaged stem treatment (DS) A. Percent of stems with roots. B Root weight (in grams).

Figure 23. A - C. The effect of weevil damage on the viability of watermilfoil stem fragments. The bars in the histogram represent the mean change in a response variable (± 1 S.E.) for each treatment. The lines with significance values above the histograms show the results of ANOVA comparisons with orthogonal contrasts. In each figure, the upper line represents the comparison of the undamaged control fragments (C) vs the damaged fragments (D); the lower line on the left represents the comparison of the unshaded control treatment (CU) vs the shaded control treatment (CS); the lower line on the right represents the comparison of the unshaded damaged stem treatment (DU) vs the shaded damaged stem treatment (DS) A. Total stem tissue produced. B. Stem tissue produced by the original stem. C. Stem tissue produced by the lateral stems.

Figure 24. Results of the Brownington Pond enclosure experiment. The data shown include the total watermilfoil biomass per treatment (solid black bars) plus the distribution of that biomass by its components (i.e., original stem biomass, lateral stem biomass and root biomass). The bars represent mean biomass (± 1 S.E.). Treatments connected by the same letter are not significantly different.

Figure 25. The location of study sites in Lake Bomoseen, Vermont.

Figure 26. The total number of all E. leconteі life stages (larvae, pupae and adults) sampled in harvested and unharvested areas for all three sites (Neshobe I., East Eckley North and East Eckley South) combined. Data are from the stem transects.

Figure 27. A comparison of the total number of all E. lecontei life stages (larvae, pupae and adults) sampled in harvested and unharvested areas at the three sites. Data are from the stem transects. A. Neshobe I. B. East Eckley North. C. East Eckley South.

Figure 28. The mean number of weevils per meristem on watermilfoil in shallow and deep water at the three sites (Neshobe I., East Eckley North and East Eckley South) in Lake Bomoseen in 1992. Data are from the stem transects. The bars represent the mean (± 1 S.E.) abundance of weevils in each of these habitats (n=15 for each site).

Figure 29. The number of each life stage of E. lecontei, summed for all sites, for each week in A) 1991 and B) 1992.

Figure 30. The effect of adult weevils on change in length (cm) for nine species of native aquatic macrophytes. Bars in the histogram represent the mean (±1 S E.) length of intact plants for each of the species for each of the three weevil treatments.

Figure 31. The effect of adult weevils on change in wet weight (g) for eight species of native aquatic macrophytes. Bars in the histogram represent the mean (±1 S.E.) wet weight of each of the species for each of the three weevil treatments. Utricularia is not included in this figure in order to expand the scale for the remaining seven species. Utricularia weight data are discussed in the text. N=6 for all species except for the 2-weevil treatment for M. sibiricum where n=4.

Figure 32. Myriophyllum spicatum average dry weight (± 1 S.E., n=3) in enclosures with and without weevils, and in open water in Norton Brook Pond.

Figure 33. Macroinvertebrates in enclosures with and without weevils, and in open water in Norton Brook Pond. A. Average number (± 1 S.E.) of macroinvertebrates excluding zooplankton. B. Average taxa richness (± 1 S.E., n=3).

FIGURES

Figure 1.

BROWNINGTON POND VT

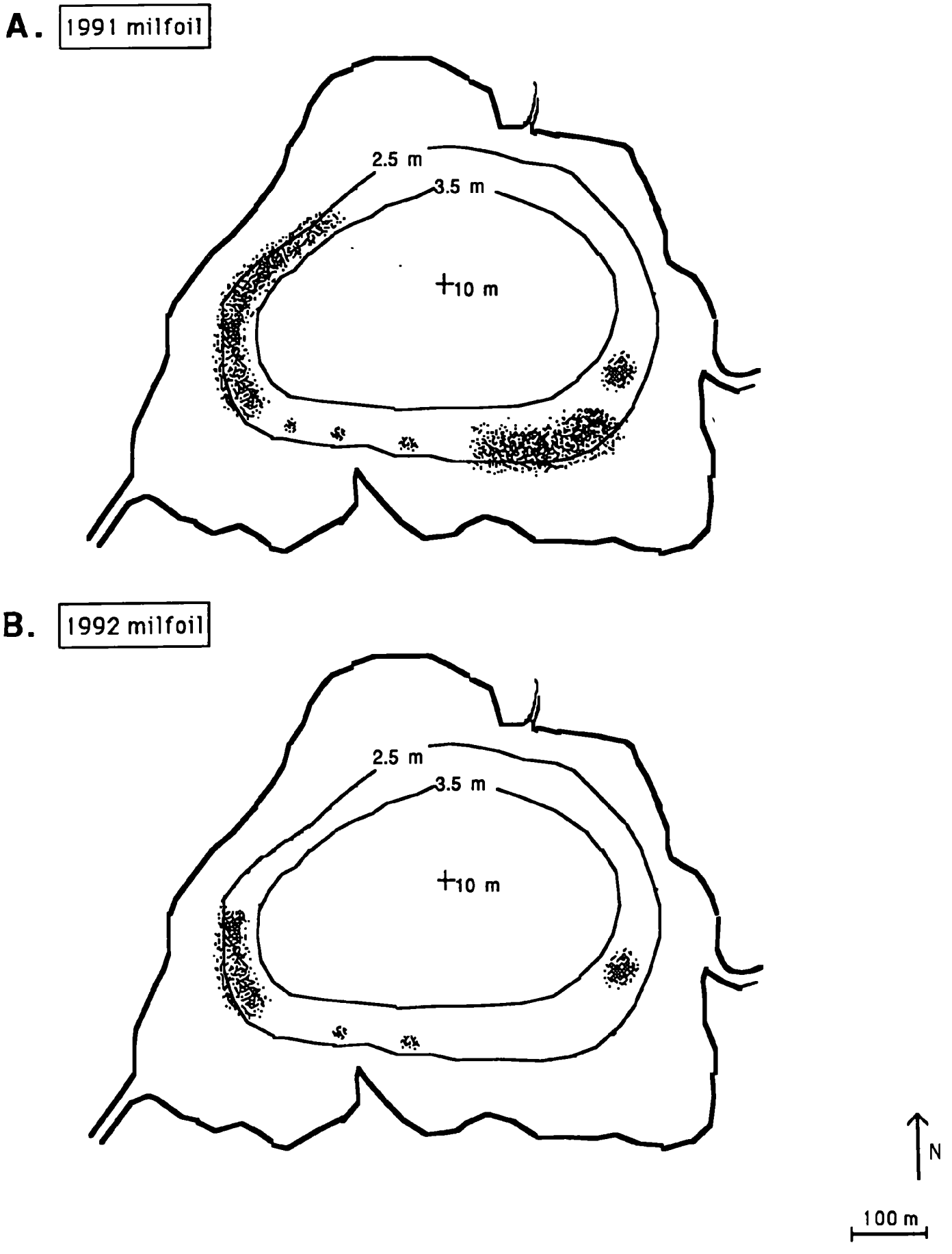
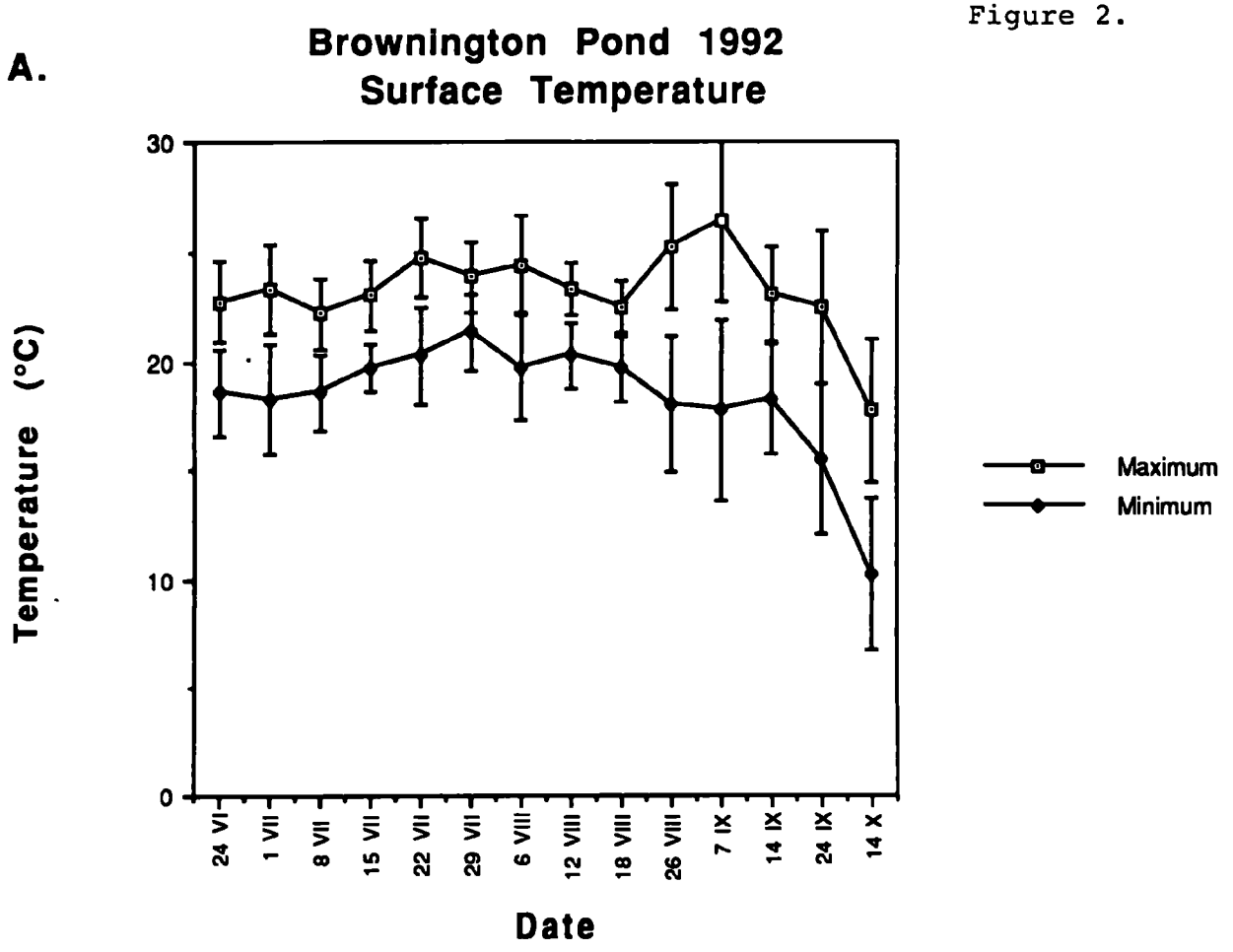


Figure 2.

A.


Brownington Pond 1992
Surface Temperature
Temperature (°C)
30
20
10
0
24 VI
1 VII
8 VII
15 VII
22 VII
29 VII
6 VIII
12 VIII
18 VIII
26 VIII
7 IX
14 IX
24 IX
14 X
Date
Maximum
Minimum

B.

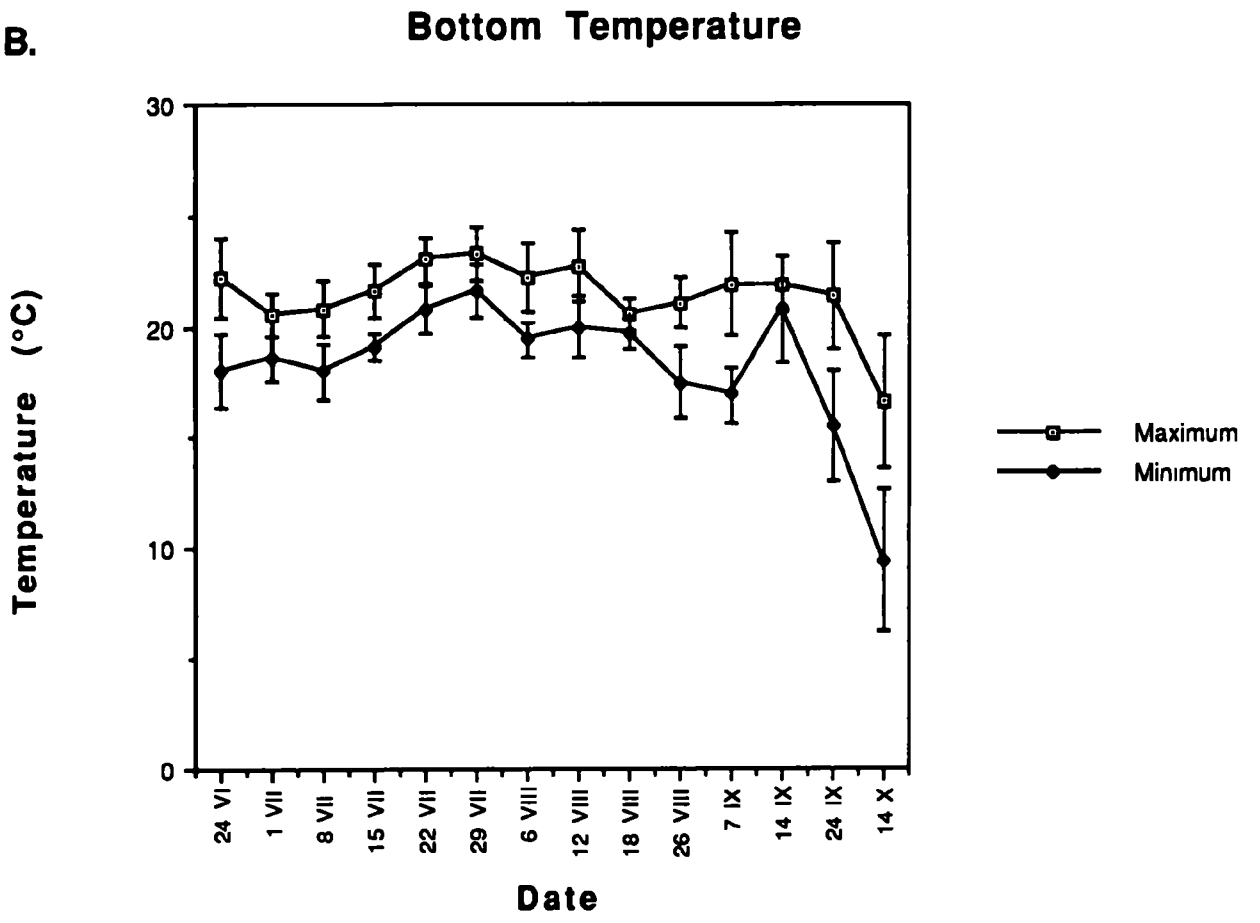

Bottom Temperature
Temperature (°C)
30
20
10
0
24 VI
1 VII
8 VII
15 VII
22 VII
29 VII
6 VIII
12 VIII
18 VIII
26 VIII
7 IX
14 IX
24 IX
14 X
Date
Maximum
Minimum

Figure 3.

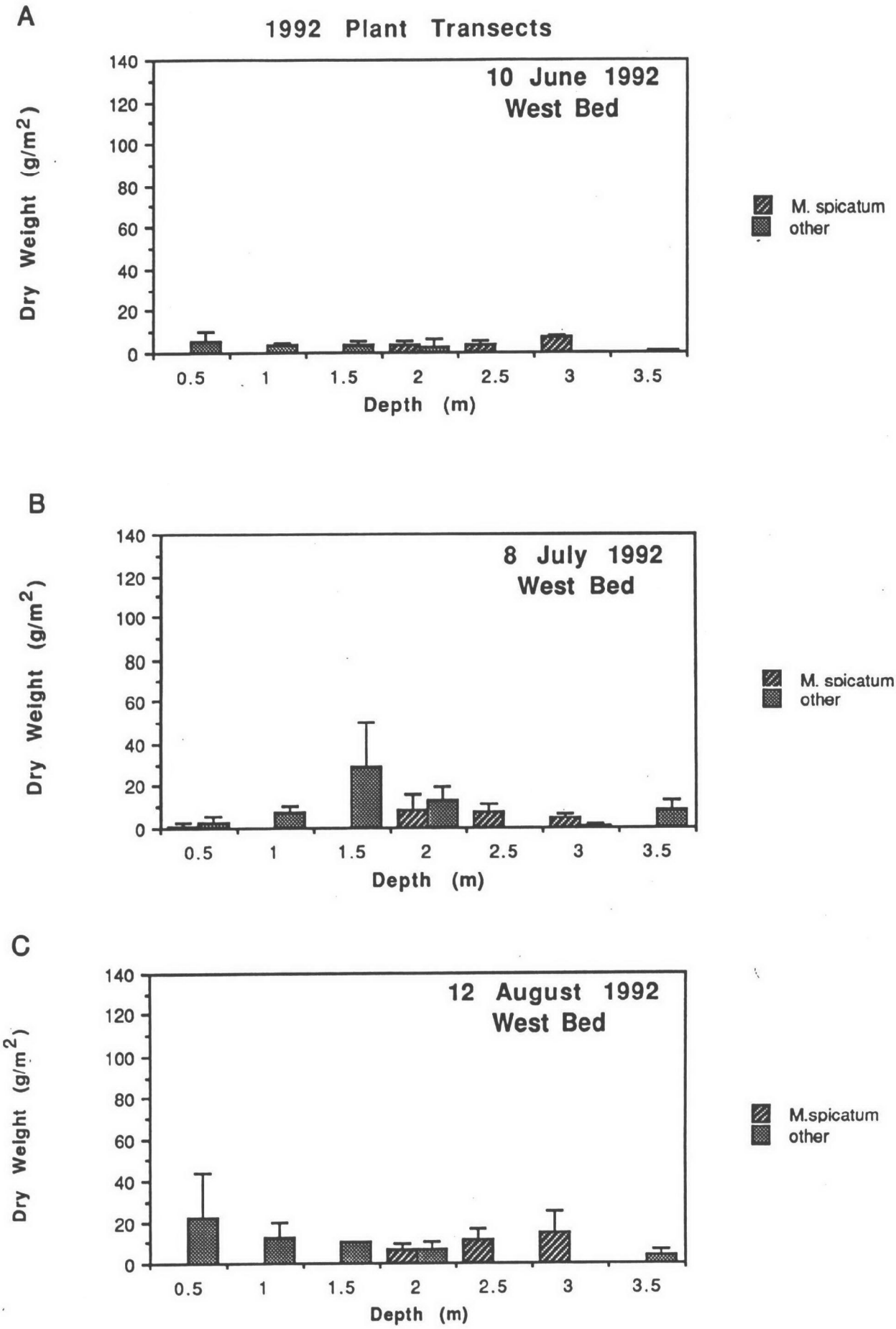

A
1992 Plant Transects
10 June 1992
West Bed
Dry Weight (g/m2)
140
120
100
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M. spicatum
other
B
8 July 1992
West Bed
Dry Weight (g/m2)
Depth (m)
M. spicatum
other
C
12 August 1992
West Bed
Dry Weight (g/m2)
Depth (m)
M.spicatum
other

Figure 4.

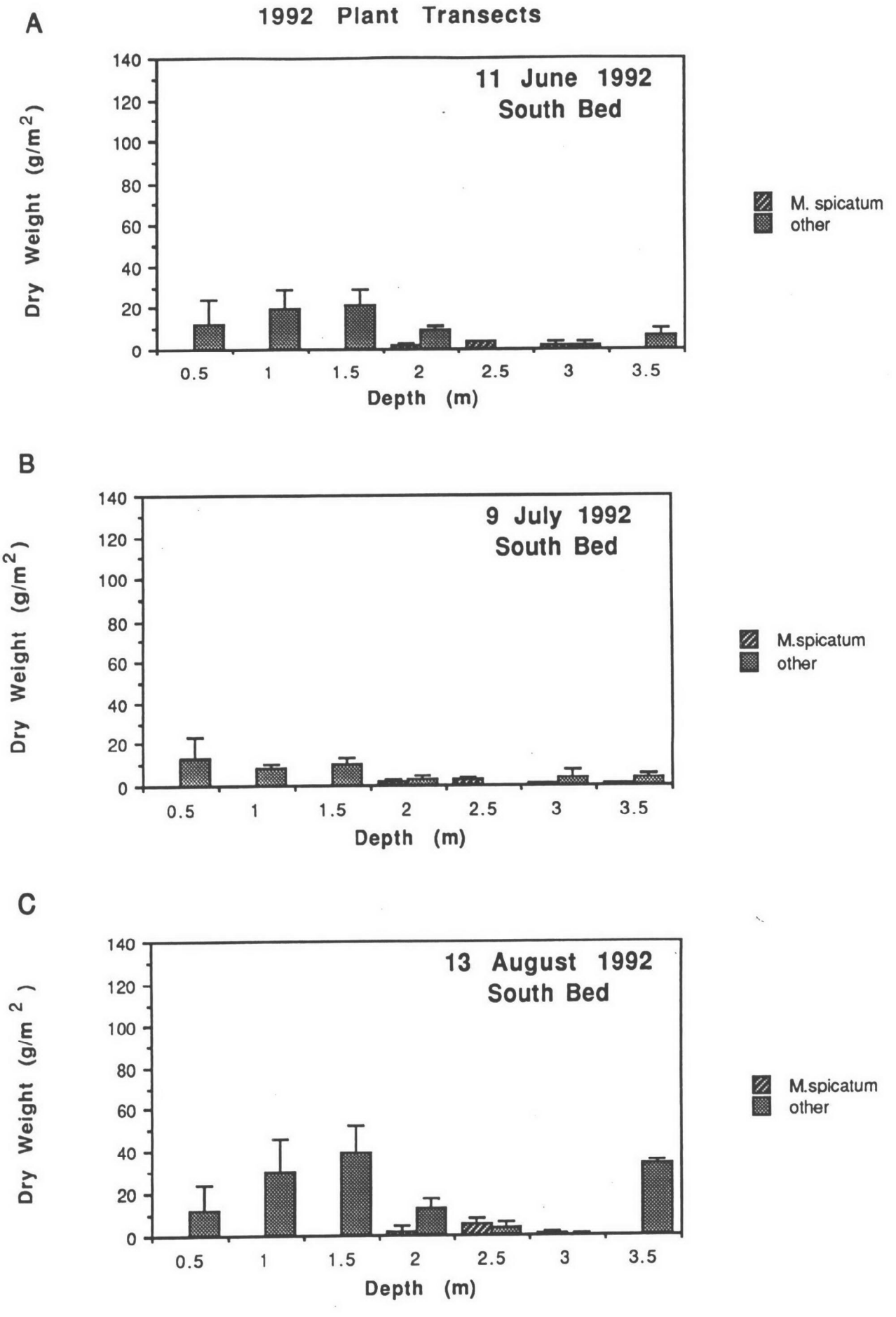

A
1992 Plant Transects
11 June 1992
South Bed
Dry Weight (g/m2)
140
120
100
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M. spicatum
other
B
9 July 1992
South Bed
Dry Weight (g/m2)
M.spicatum
other
C
13 August 1992
South Bed
Dry Weight (g/m 2)
M.spicatum
other

Figure 5.

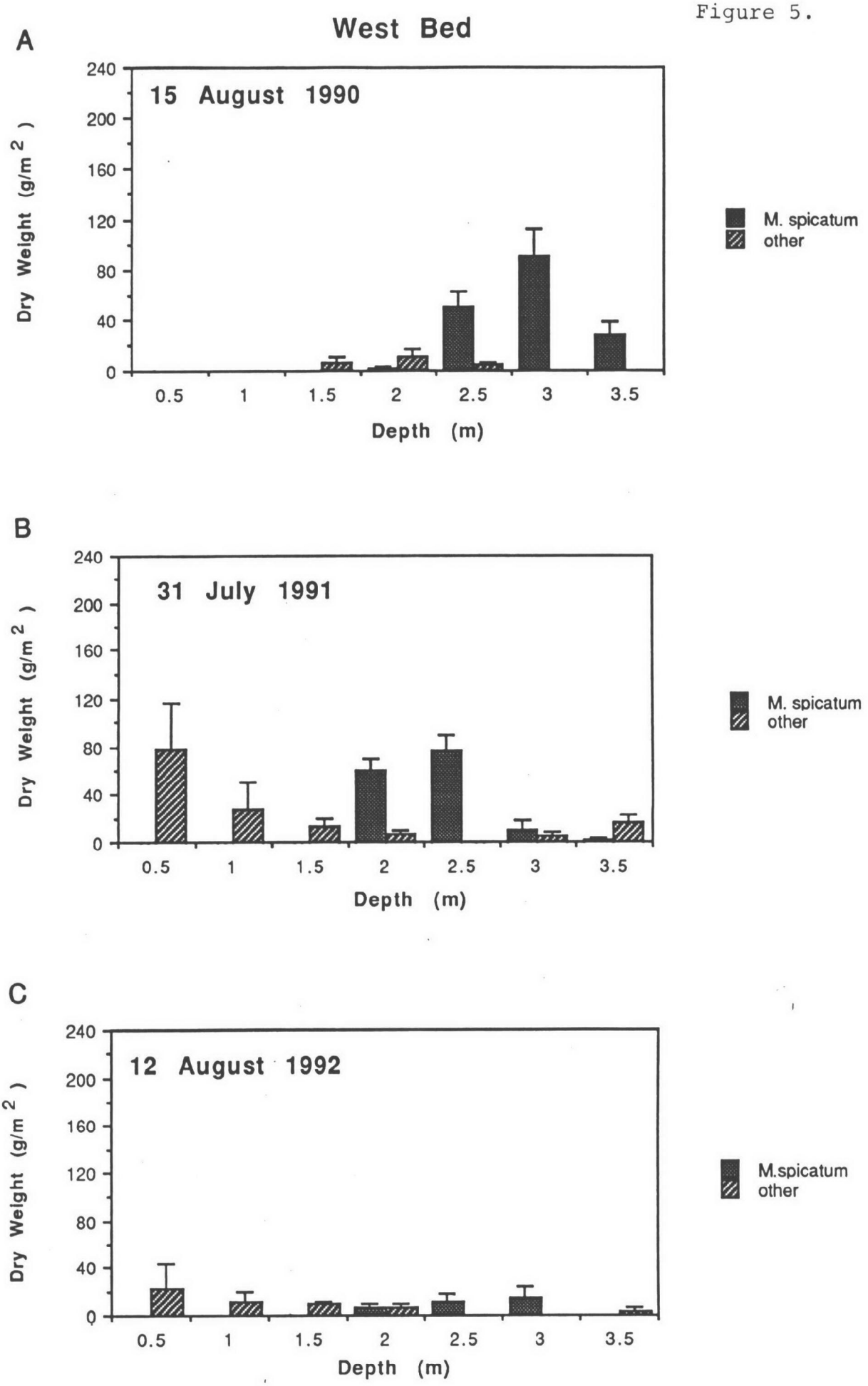

A
West Bed
15 August 1990
Dry Weight (g/m 2)
240
200
160
120
80
40
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M. spicatum
other
B
31 July 1991
Dry Weight (g/m 2)
Depth (m)
M. spicatum
other
C
12 August 1992
Dry Weight (g/m 2)
Depth (m)
M.spicatum
other

Figure 6.

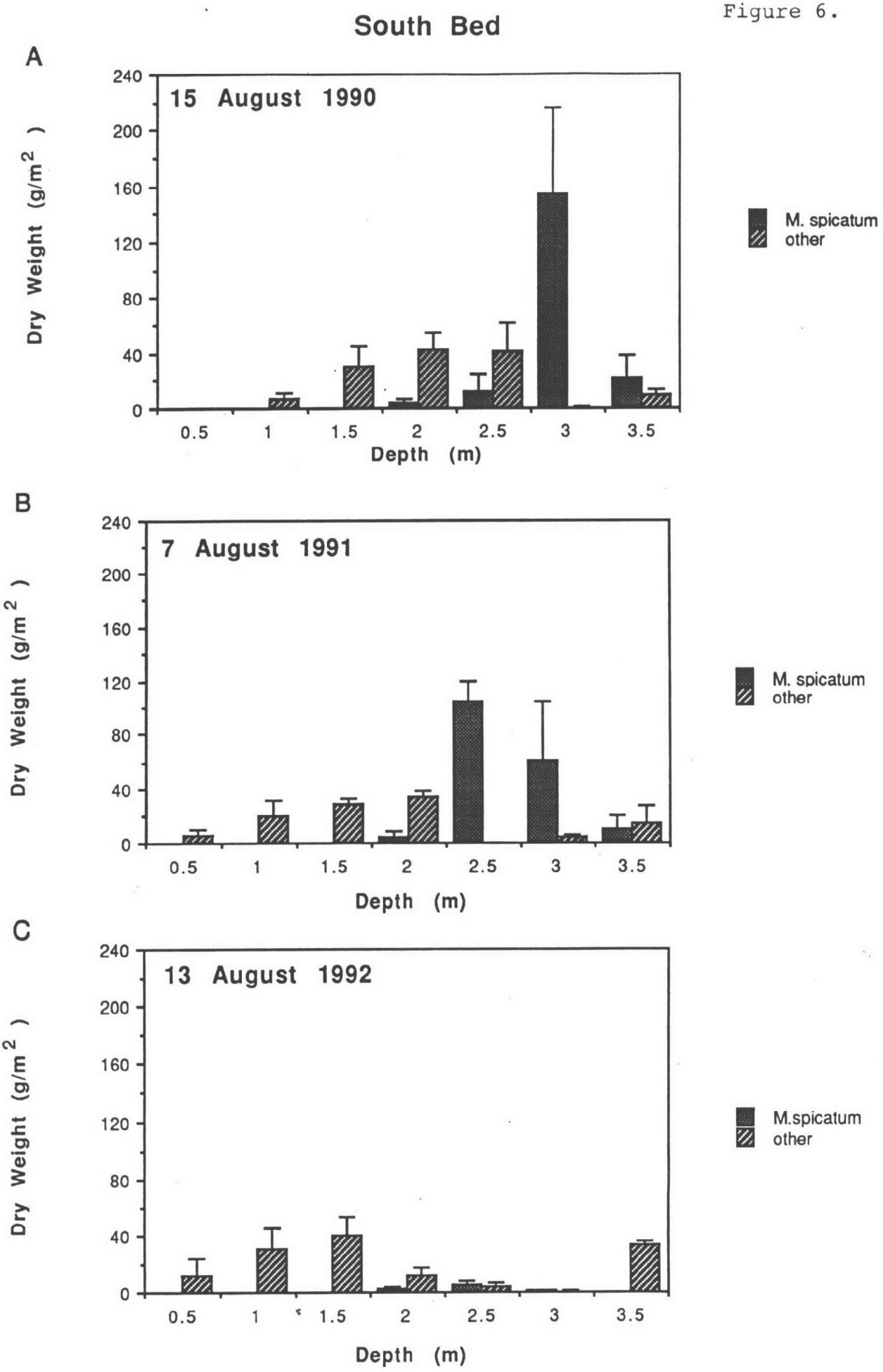

South Bed
A
15 August 1990
Dry Weight (g/m2)
240
200
160
120
80
40
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M. spicatum
other
B
7 August 1991
Dry Weight (g/m2)
240
200
160
120
80
40
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M. spicatum
other
C
13 August 1992
Dry Weight (g/m2)
240
200
160
120
80
40
0
0.5
1
1.5
2
2.5
3
3.5
Depth (m)
M.spicatum
other

Figure 7.

West Bed, South Grid

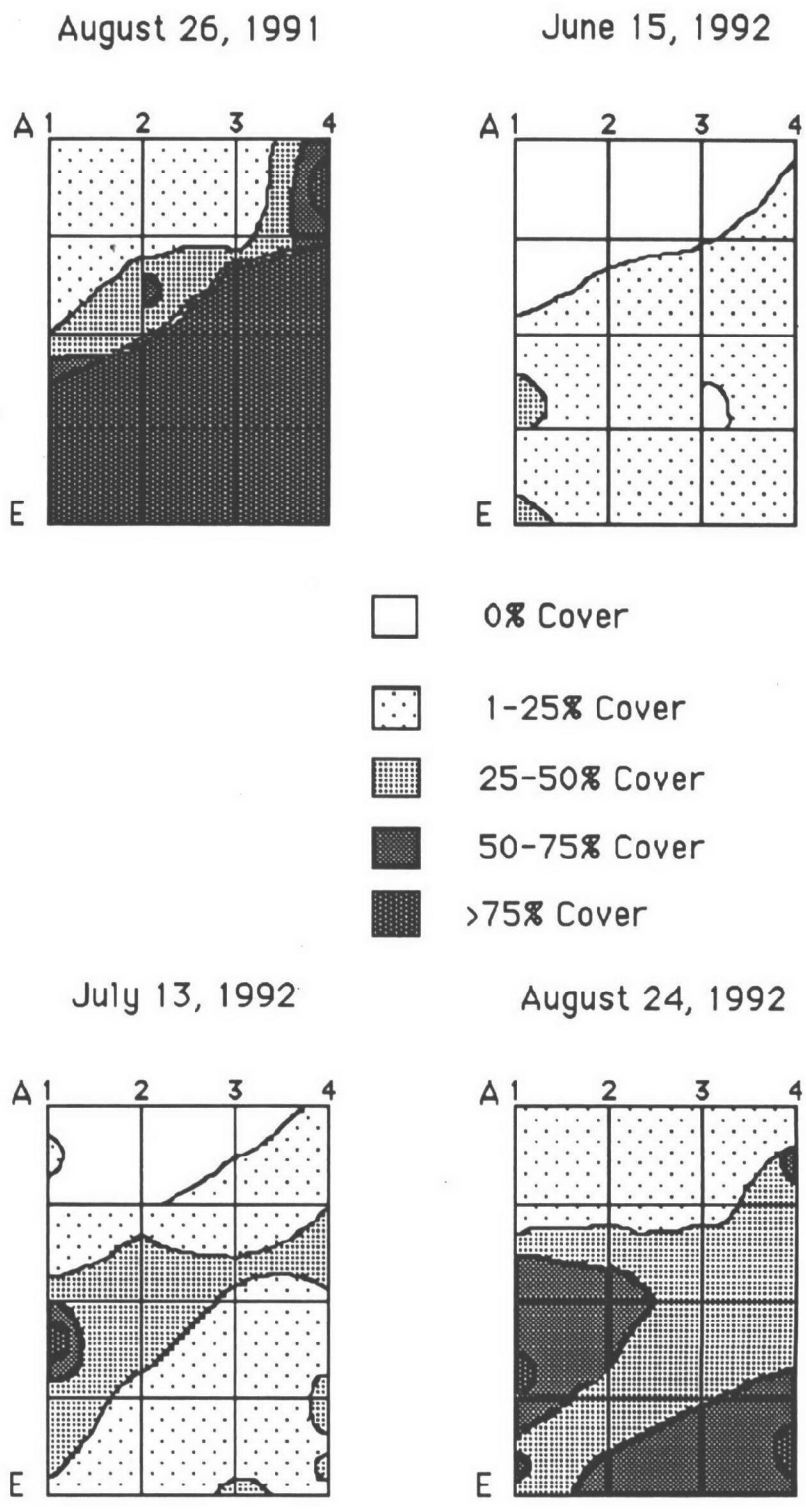


Figure 8.

West Bed, North Grid

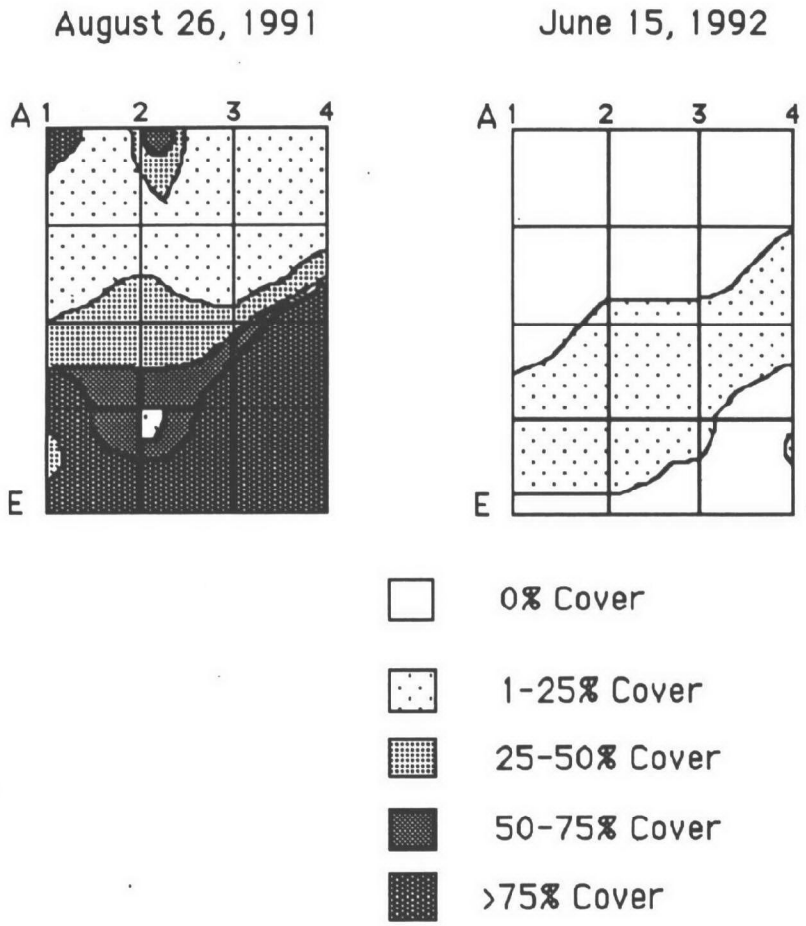


Figure 9.

South Bed, West Grid

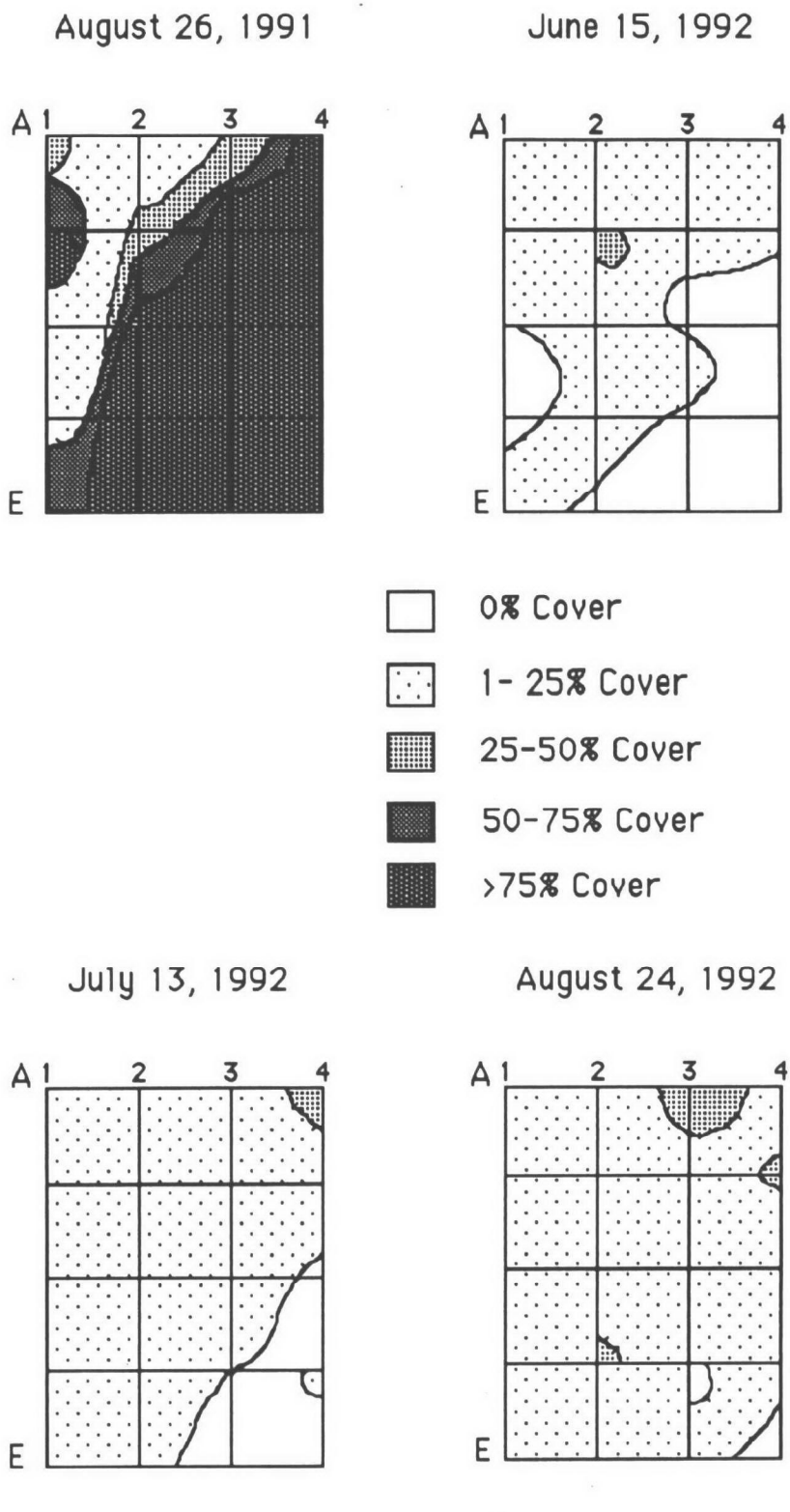


Figure 10.

South Bed, East Grid

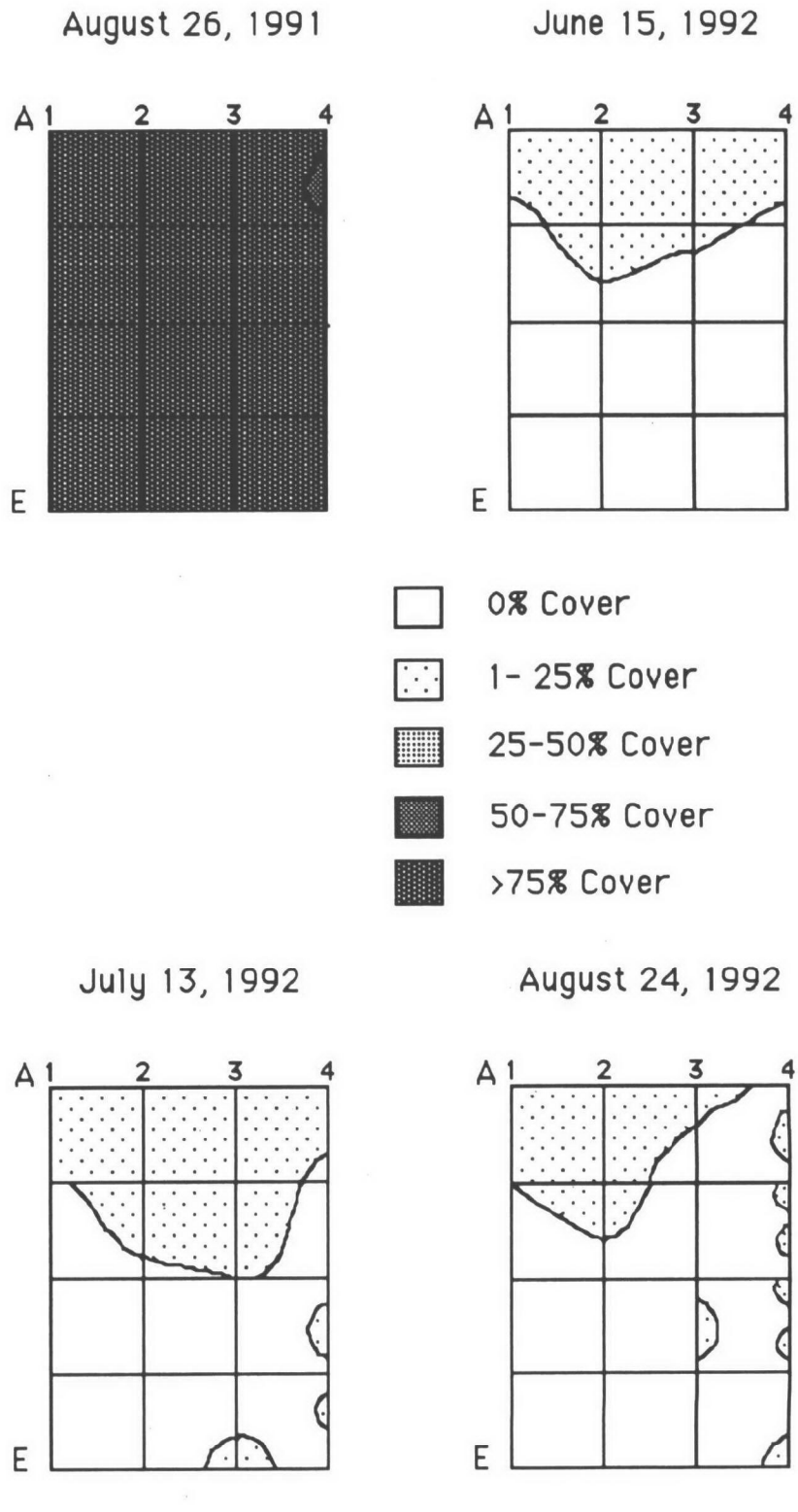
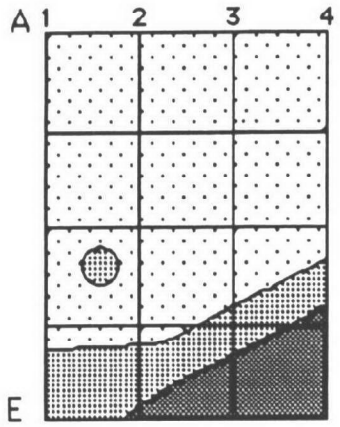


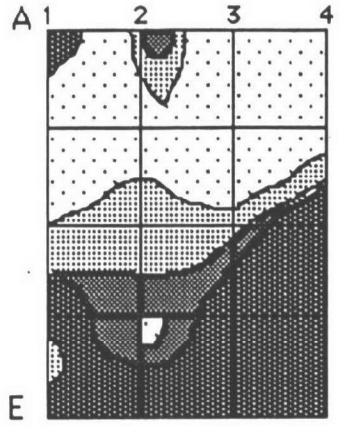
Figure 11.

West Bed, North Grid

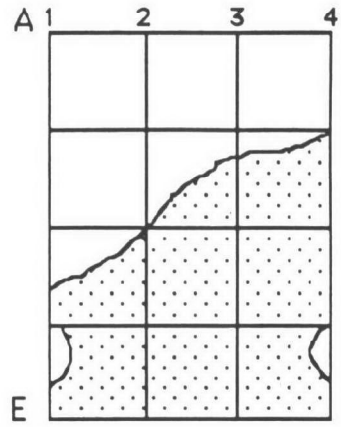
September 9, 1990



August 26, 1991

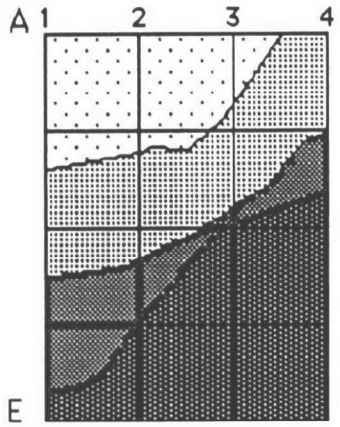


August 24, 1992

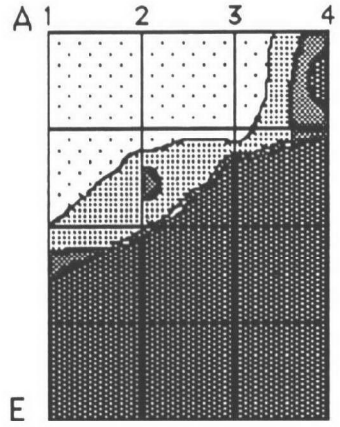


West Bed, South Grid

September 9, 1990



August 26, 1991



August 24, 1992

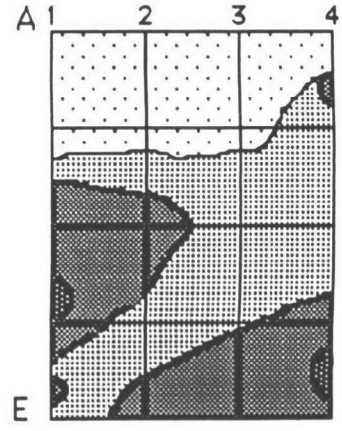
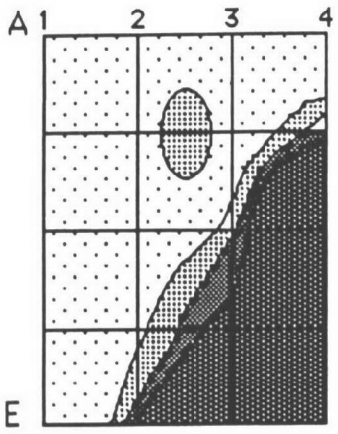


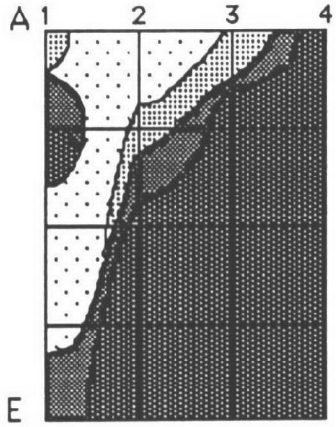
Figure 12.

South Bed, West Grid

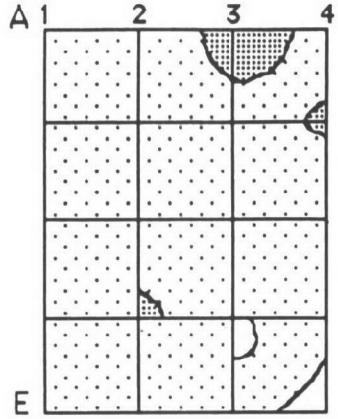
September 9, 1990



August 26, 1991

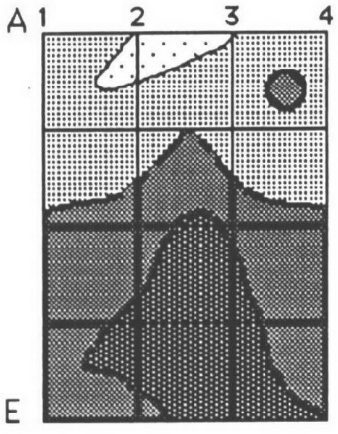


August 24, 1992

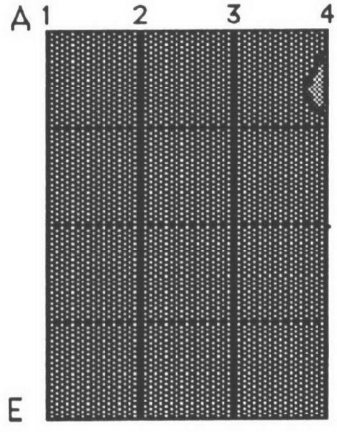


South Bed, East Grid

September 9, 1990



August 26, 1991



August 24, 1992

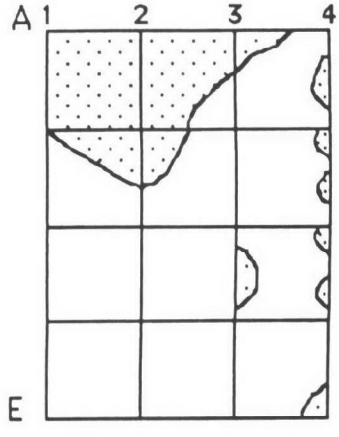


Figure 13.

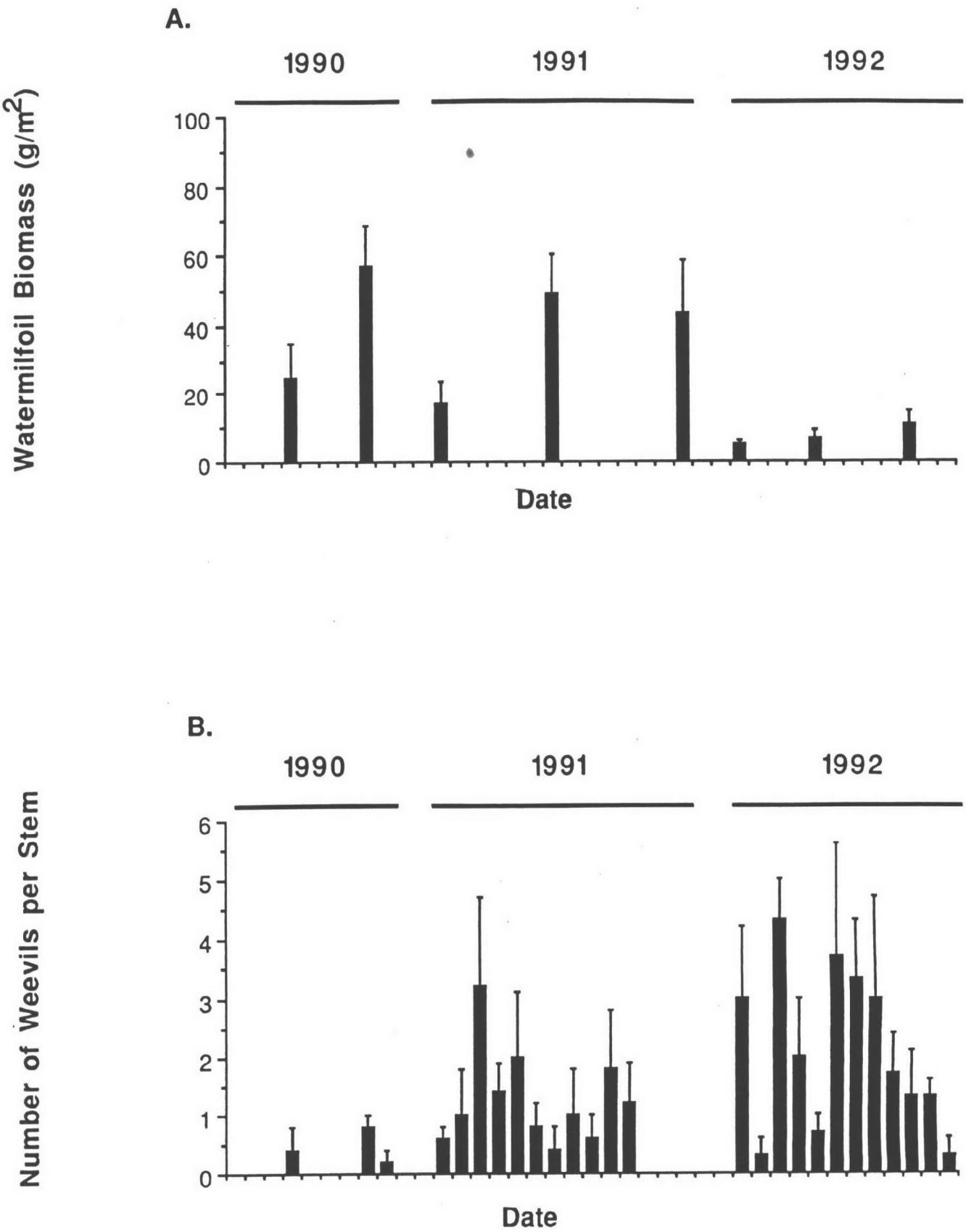

WEST BED
A.
1990
1991
1992
Watermilfoil Biomass (g/m2)
100
80
60
40
20
0
Date
B.
1990
1991
1992
Number of Weevils per Stem
6
5
4
3
2
1
0
Date

Figure 14.

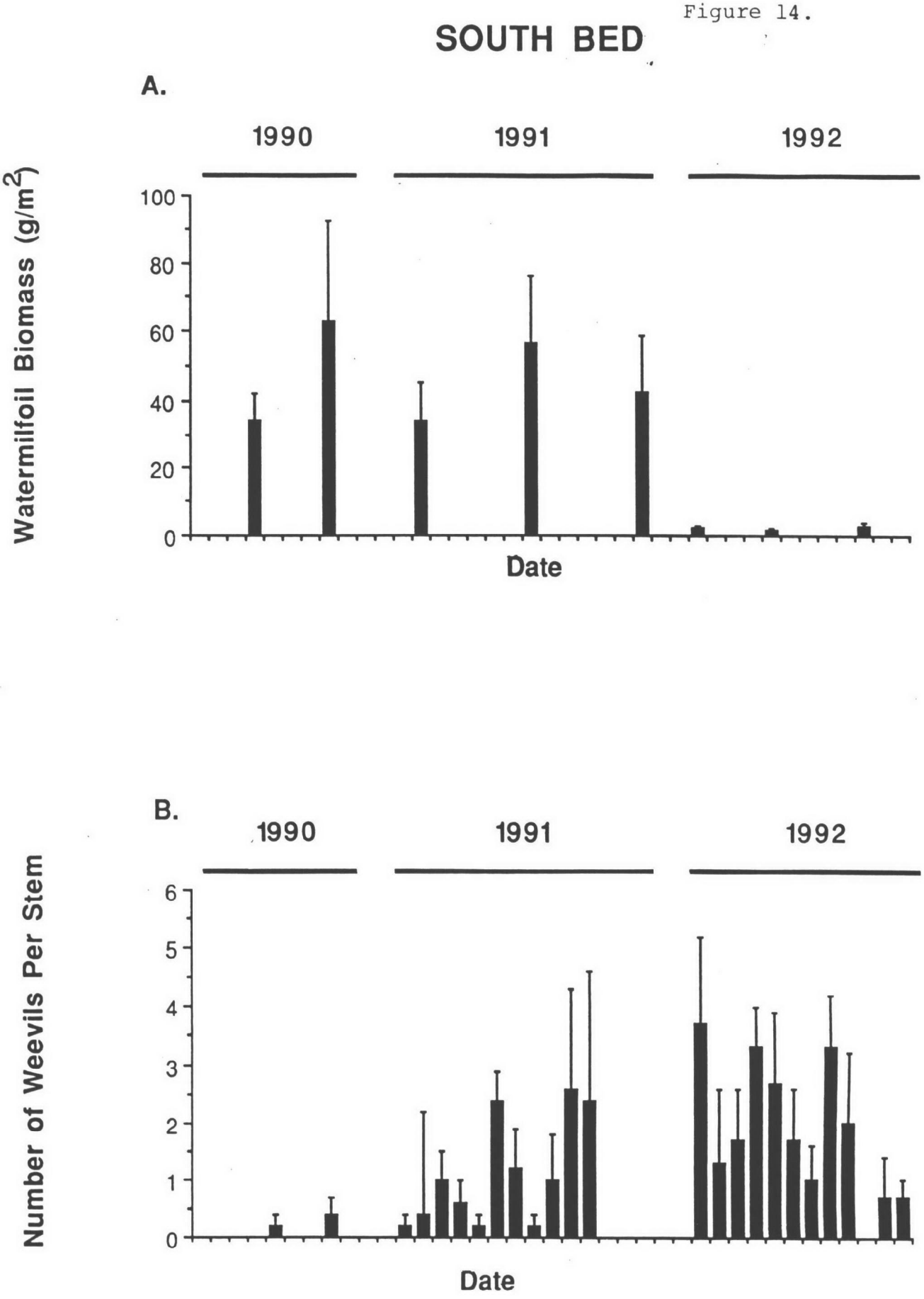

SOUTH BED
A.
1990
1991
1992
Watermilfoil Biomass (g/m2)
100
80
60
40
20
0
Date
B.
1990
1991
1992
Number of Weevils Per Stem
6
5
4
3
2
1
0
Date

Figure 15.

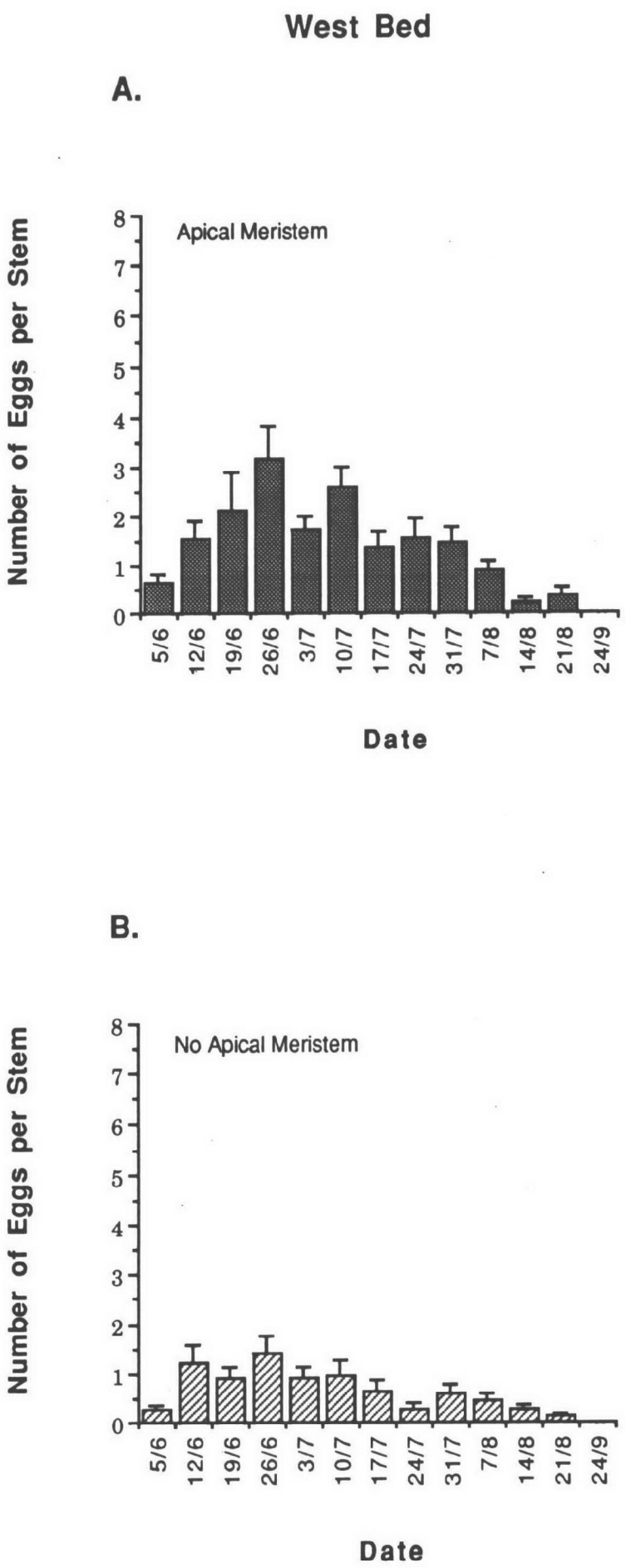
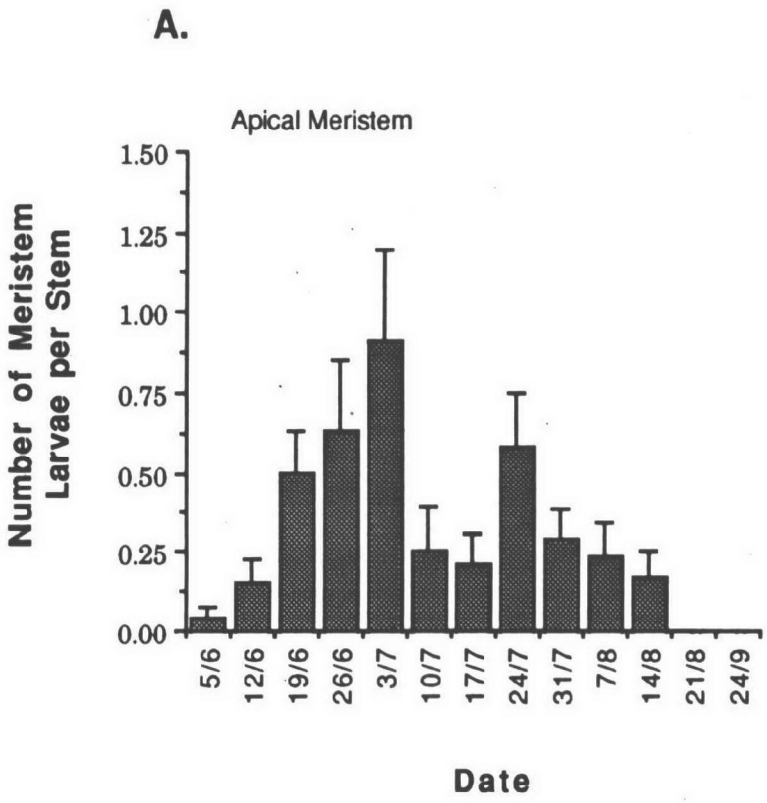

West Bed
A.
Apical Meristem
Number of Eggs per Stem
0
1
2
3
4
5
6
7
8
5/6
12/6
19/6
26/6
3/7
10/7
17/7
24/7
31/7
7/8
14/8
21/8
24/9
Date
B.
No Apical Meristem
Number of Eggs per Stem
0
1
2
3
4
5
6
7
8
5/6
12/6
19/6
26/6
3/7
10/7
17/7
24/7
31/7
7/8
14/8
21/8
24/9
Date

Figure 16.

West Bed



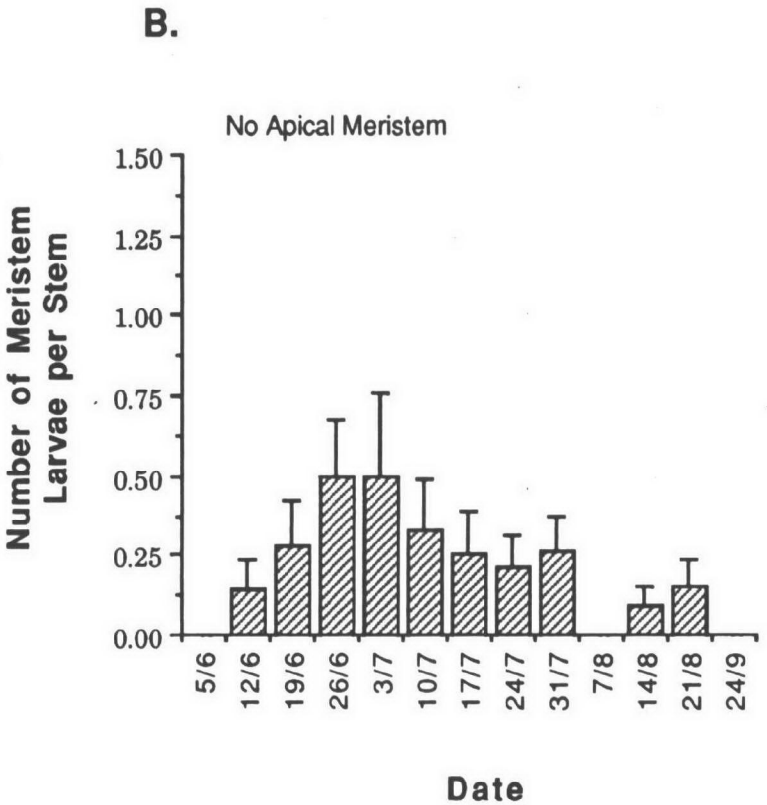
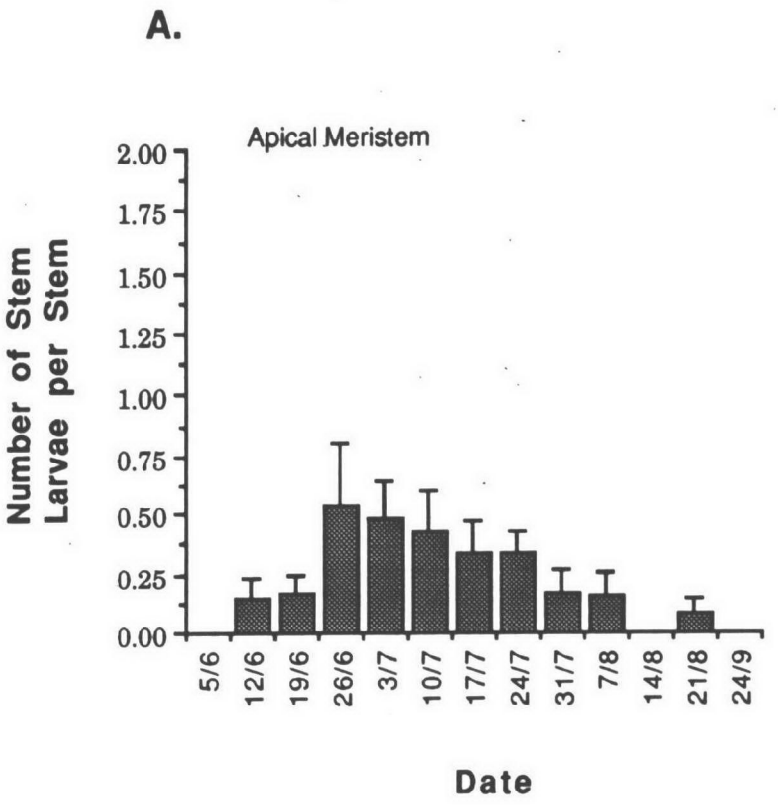


Figure 17.

West Bed



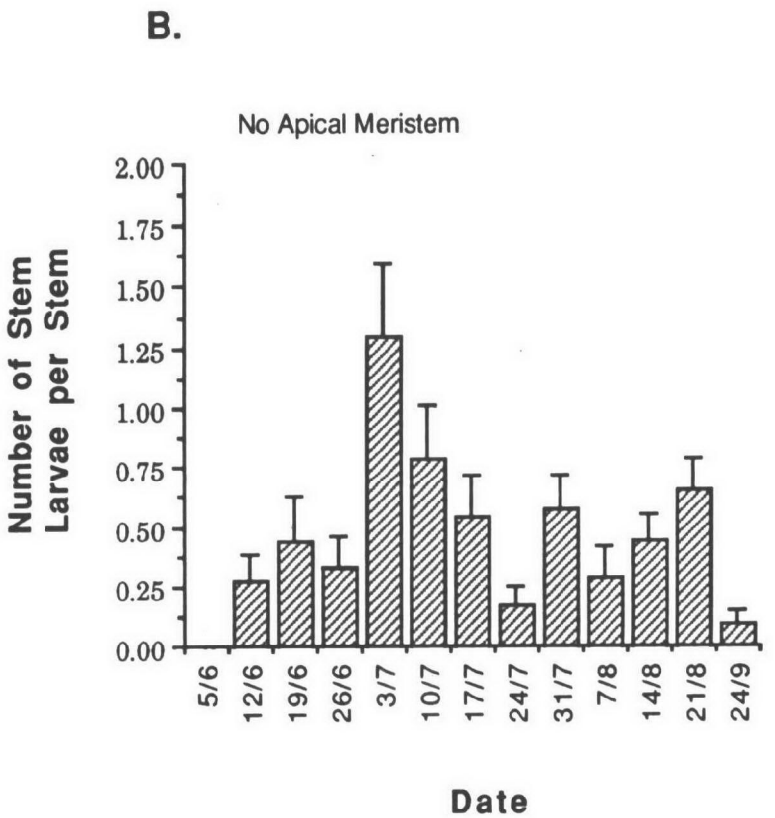
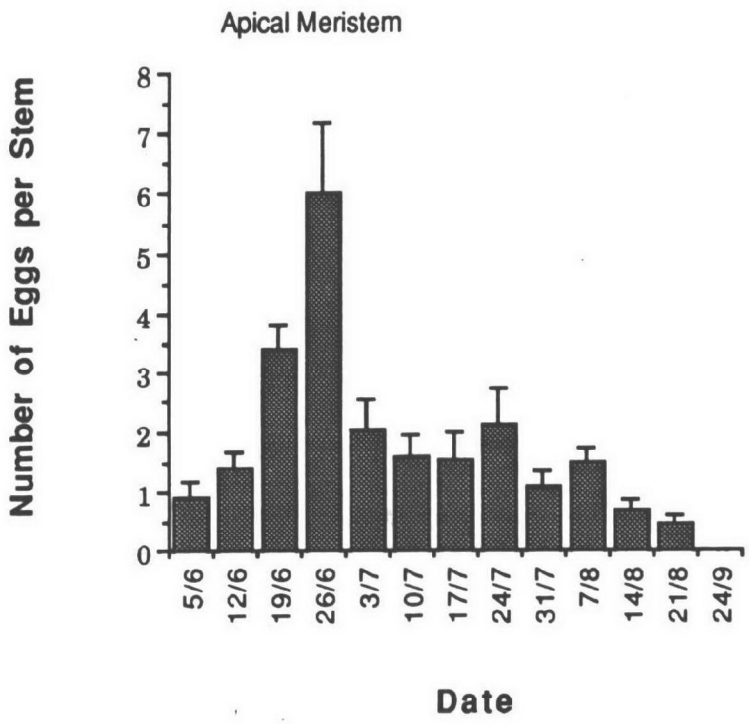


Figure 18.

South Bed

A.



B.

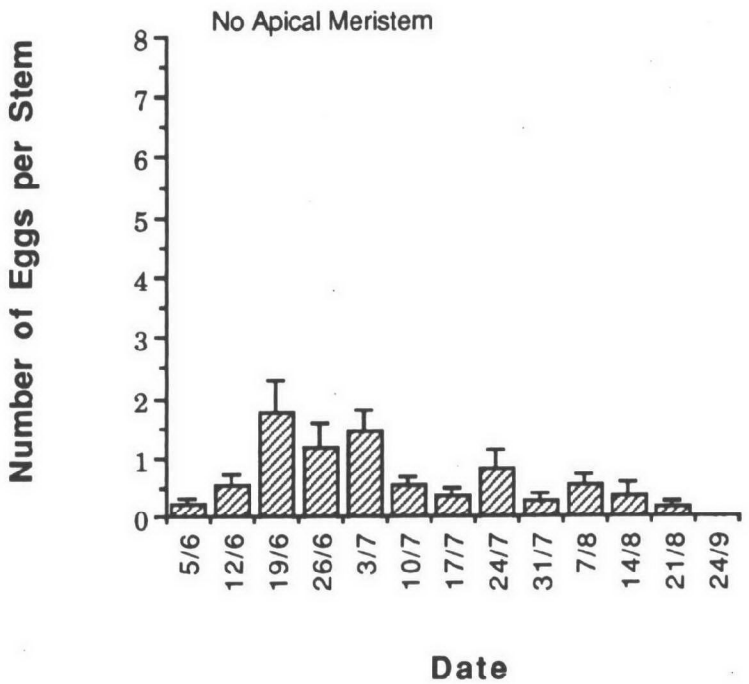
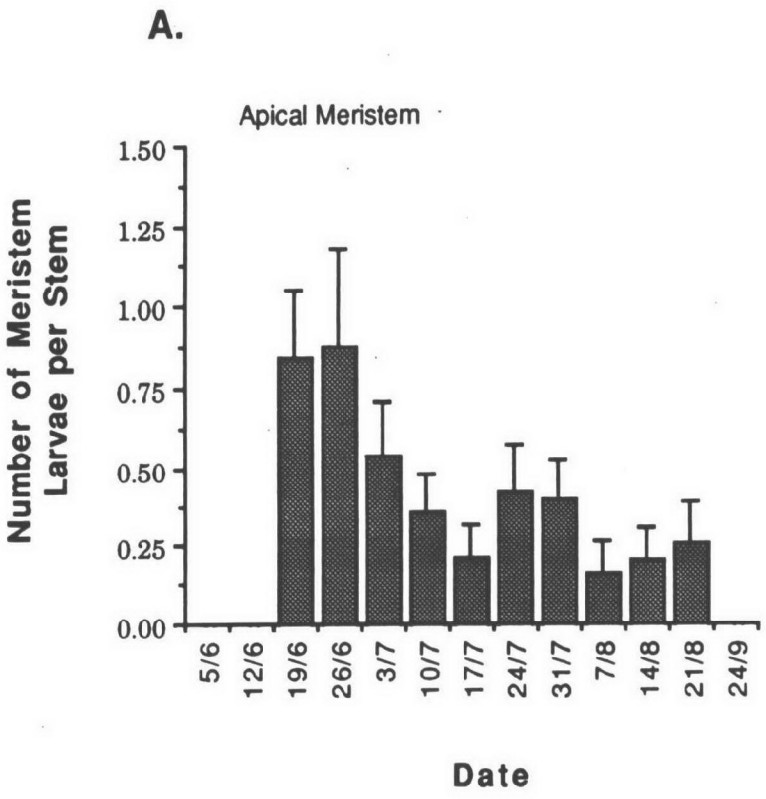


Figure 19.

South Bed



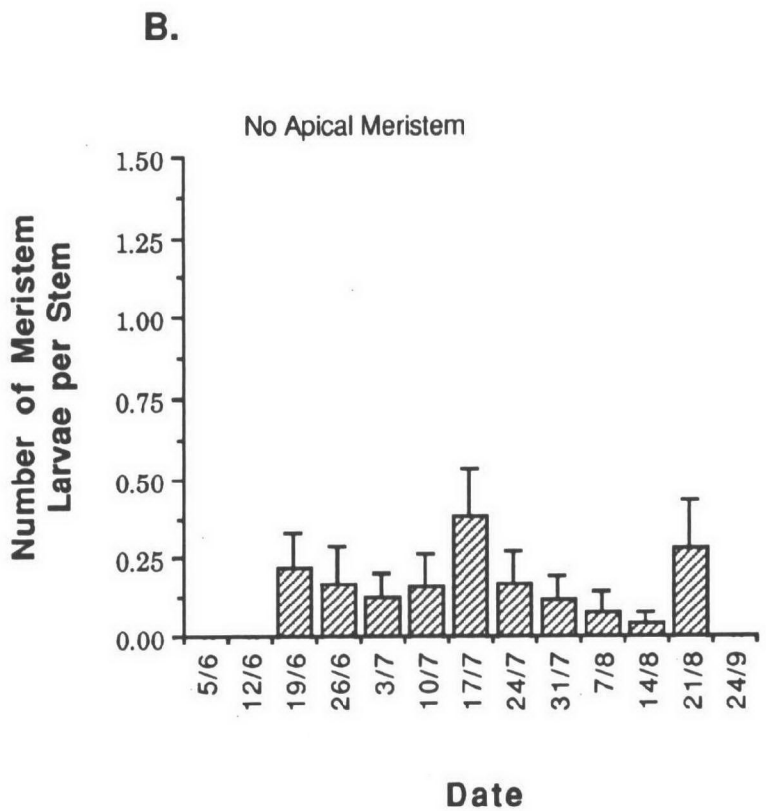
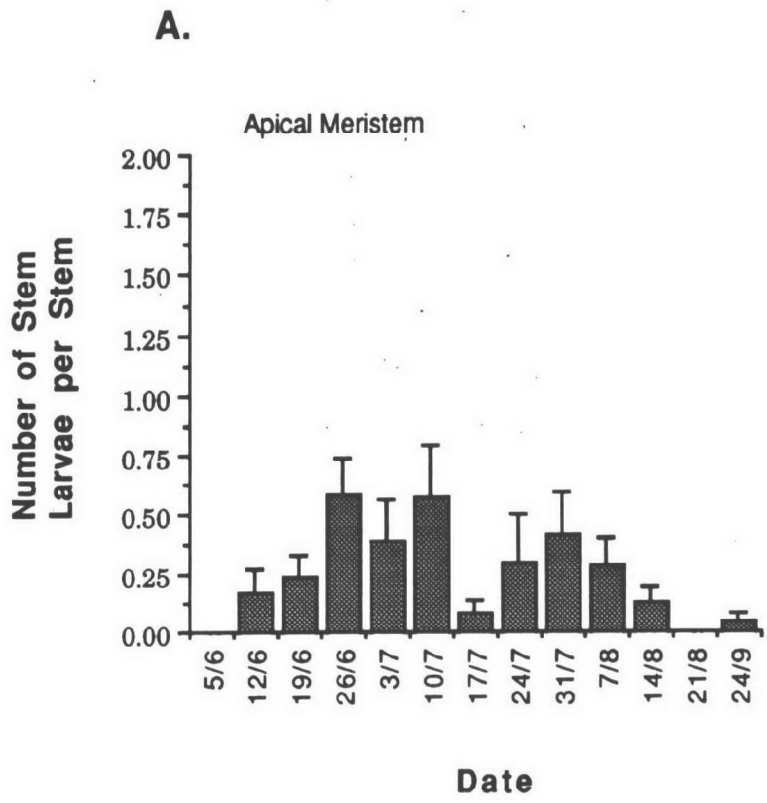


Figure 20.

South Bed



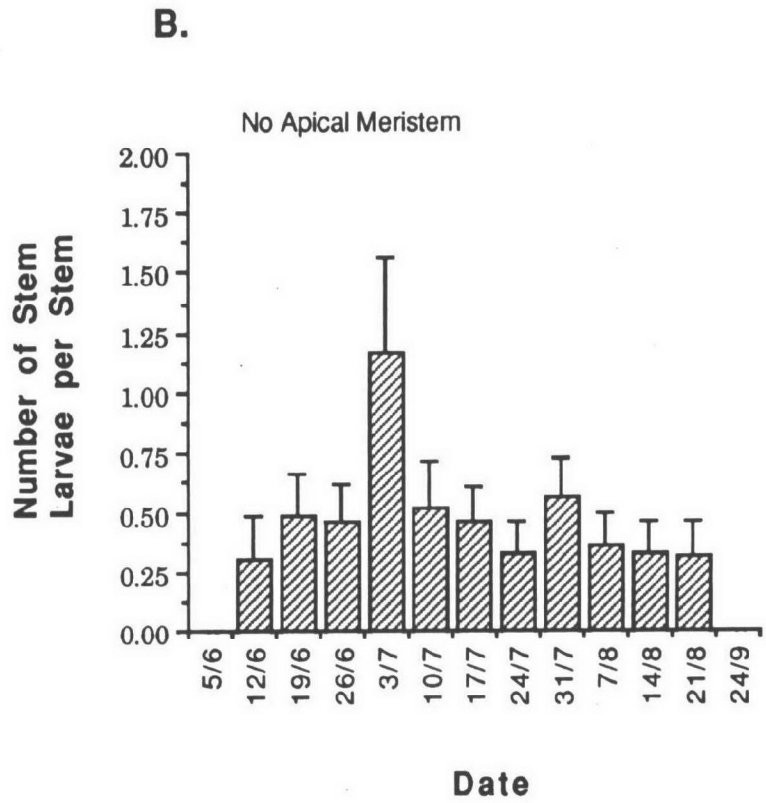


Figure 21A.

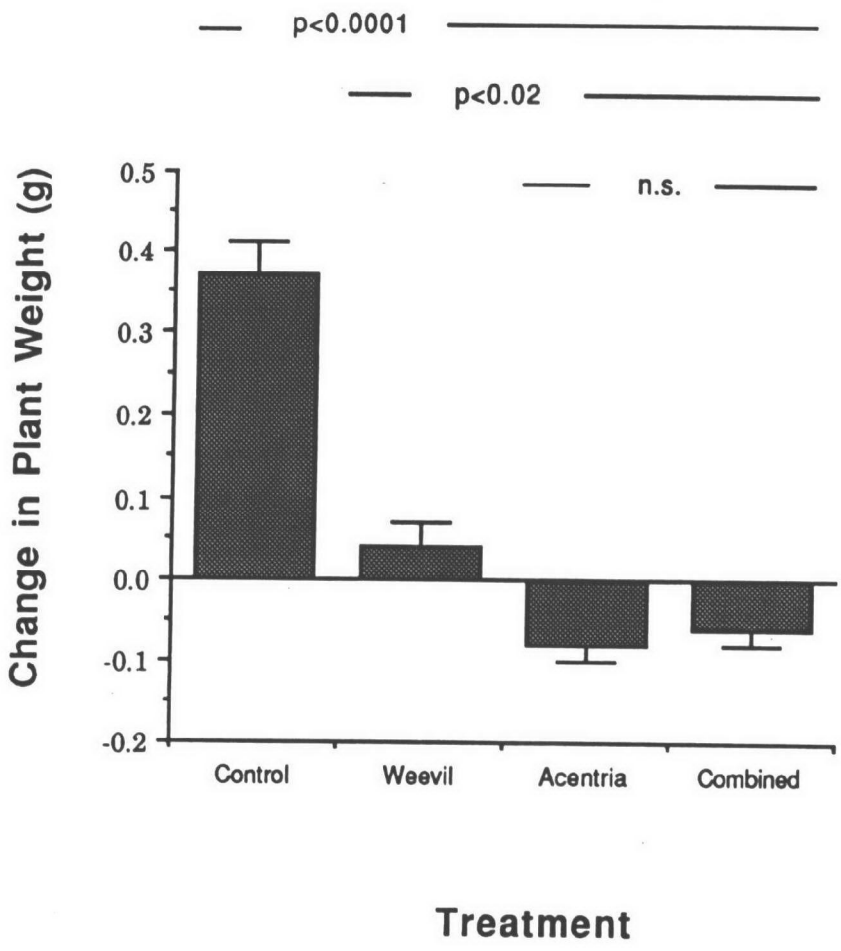

p<0.0001
p<0.02
n.s.
Change in Plant Weight (g)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
Control
Weevil
Acentria
Combined
Treatment

Figure 21B.

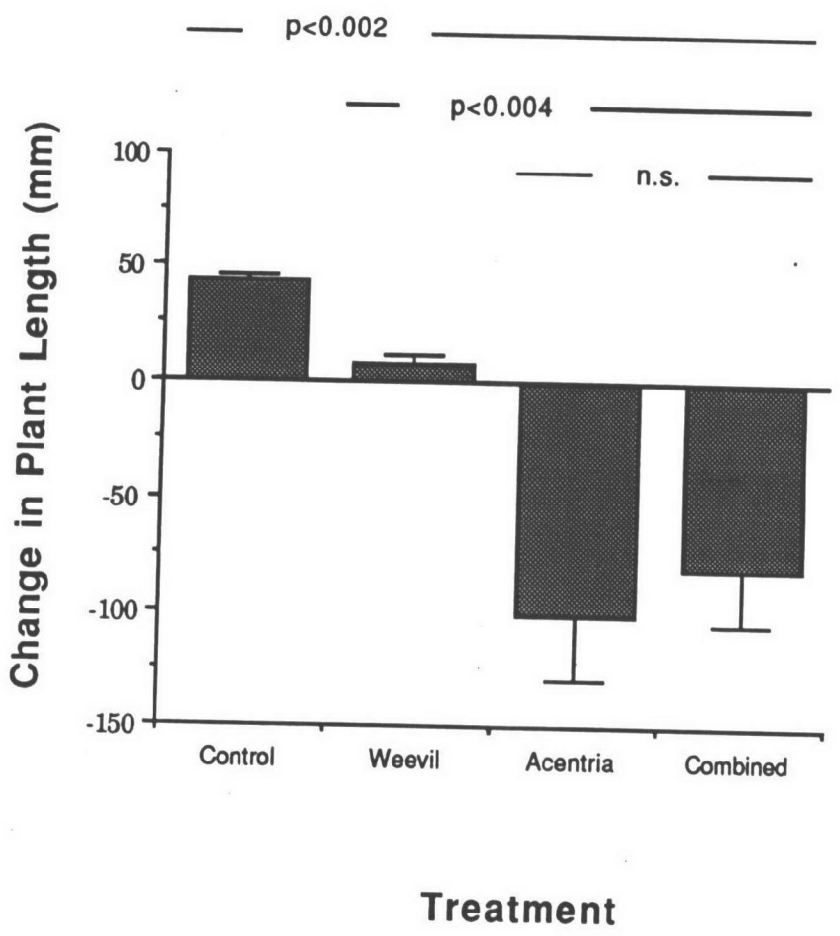

p<0.002
p<0.004
n.s.
Change in Plant Length (mm)
100
50
0
-50
-100
-150
Control
Weevil
Acentria
Combined
Treatment

Figure 21C.

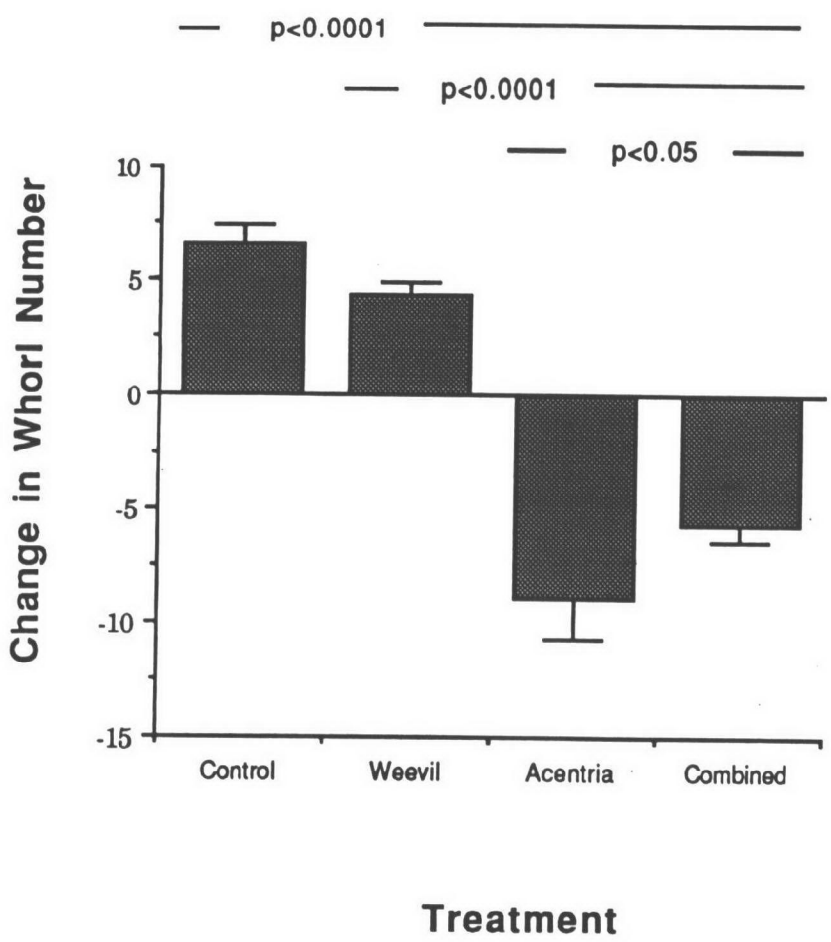
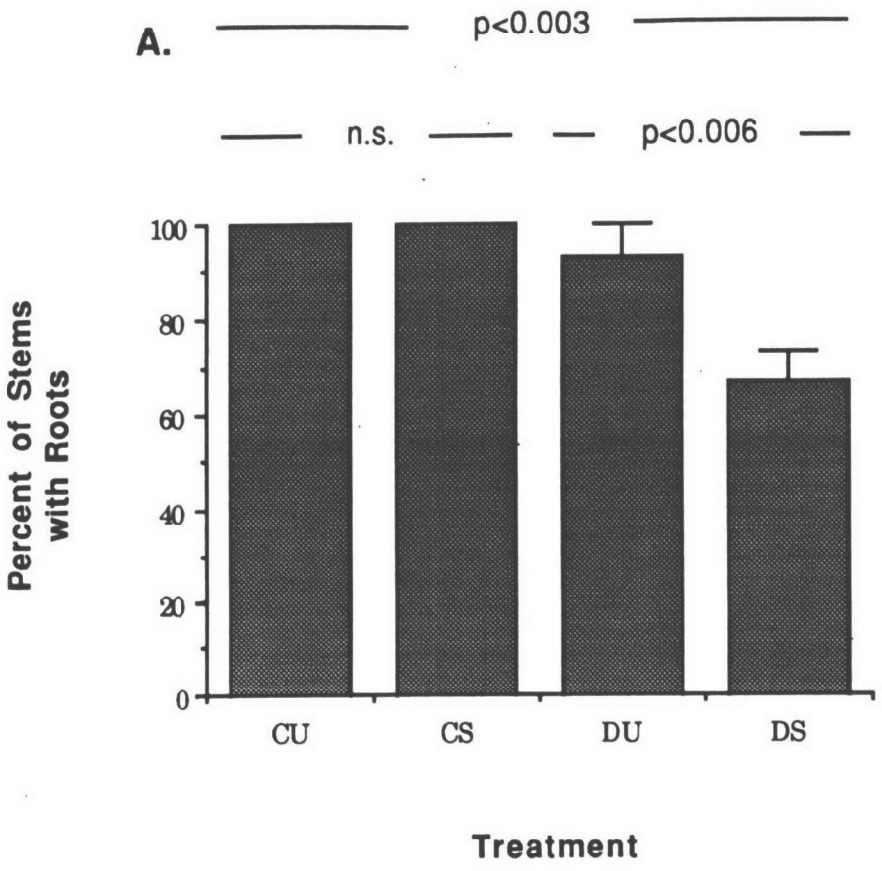

p<0.0001
p<0.0001
p<0.05
Change in Whorl Number
10
5
0
-5
-10
-15
Control
Weevil
Acentria
Combined
Treatment

Figure 22.


A.
p<0.003
n.s.
p<0.006
Percent of Stems with Roots
100
80
60
40
20
0
CU
CS
DU
DS
Treatment

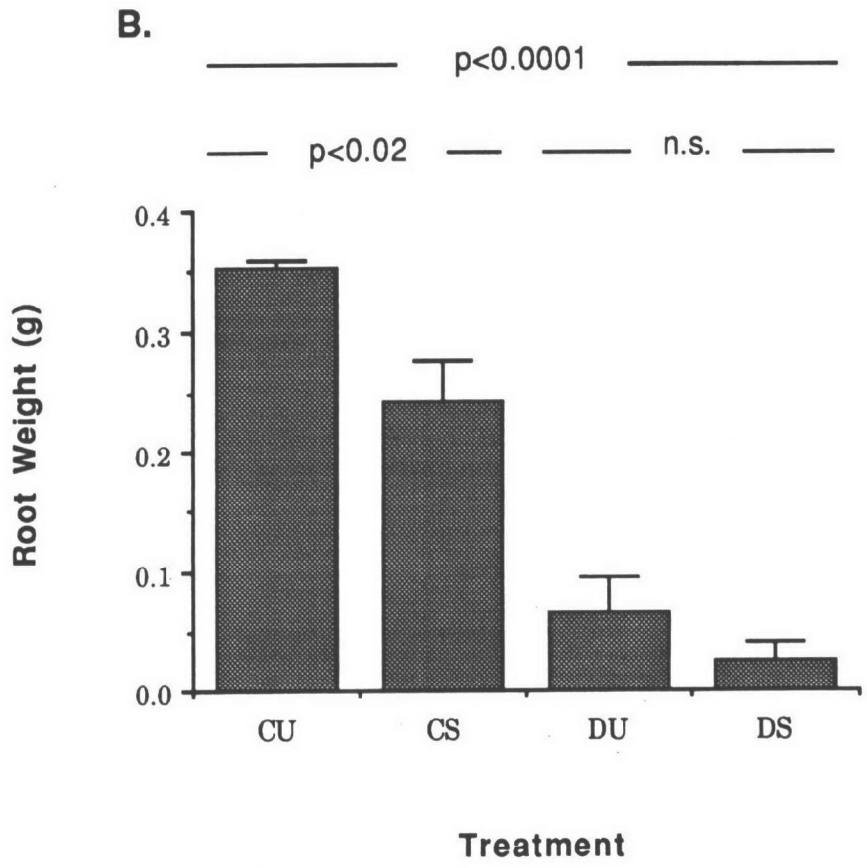

B.
p<0.0001
p<0.02
n.s.
Root Weight (g)
0.4
0.3
0.2
0.1
0.0
CU
CS
DU
DS
Treatment

Figure 23.

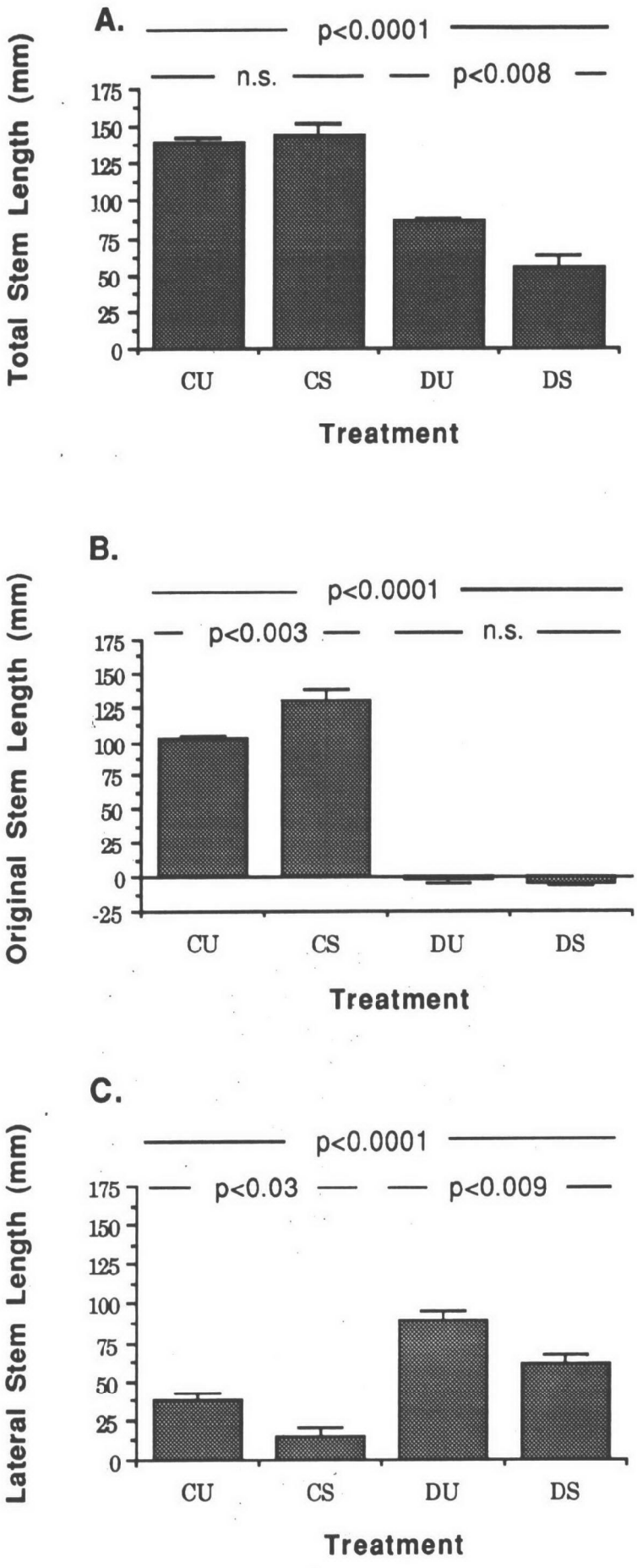

A.
p<0.0001
n.s.
p<0.008
Total Stem Length (mm)
175
150
125
100
75
50
25
0
CU
CS
DU
DS
Treatment
B.
p<0.0001
p<0.003
n.s.
Original Stem Length (mm)
175
150
125
100
75
50
25
0
-25
CU
CS
DU
DS
Treatment
C.
p<0.0001
p<0.03
p<0.009
Lateral Stem Length (mm)
175
150
125
100
75
50
25
0
CU
CS
DU
DS
Treatment

Figure 24.

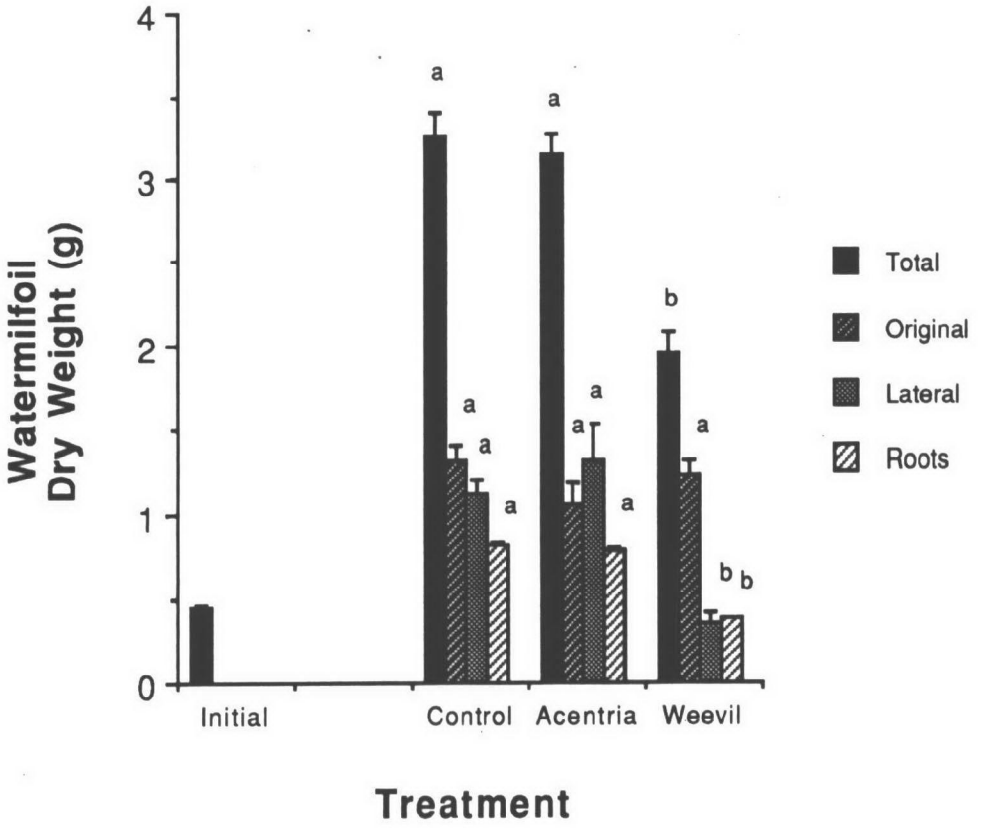

Watermilfoil
Dry Weight (g)
4
3
2
1
0
a
a
a
a
a
a
a
a
b
a
b b
Initial
Control
Acentria
Weevil
Treatment
Total
Original
Lateral
Roots

Figure 25.

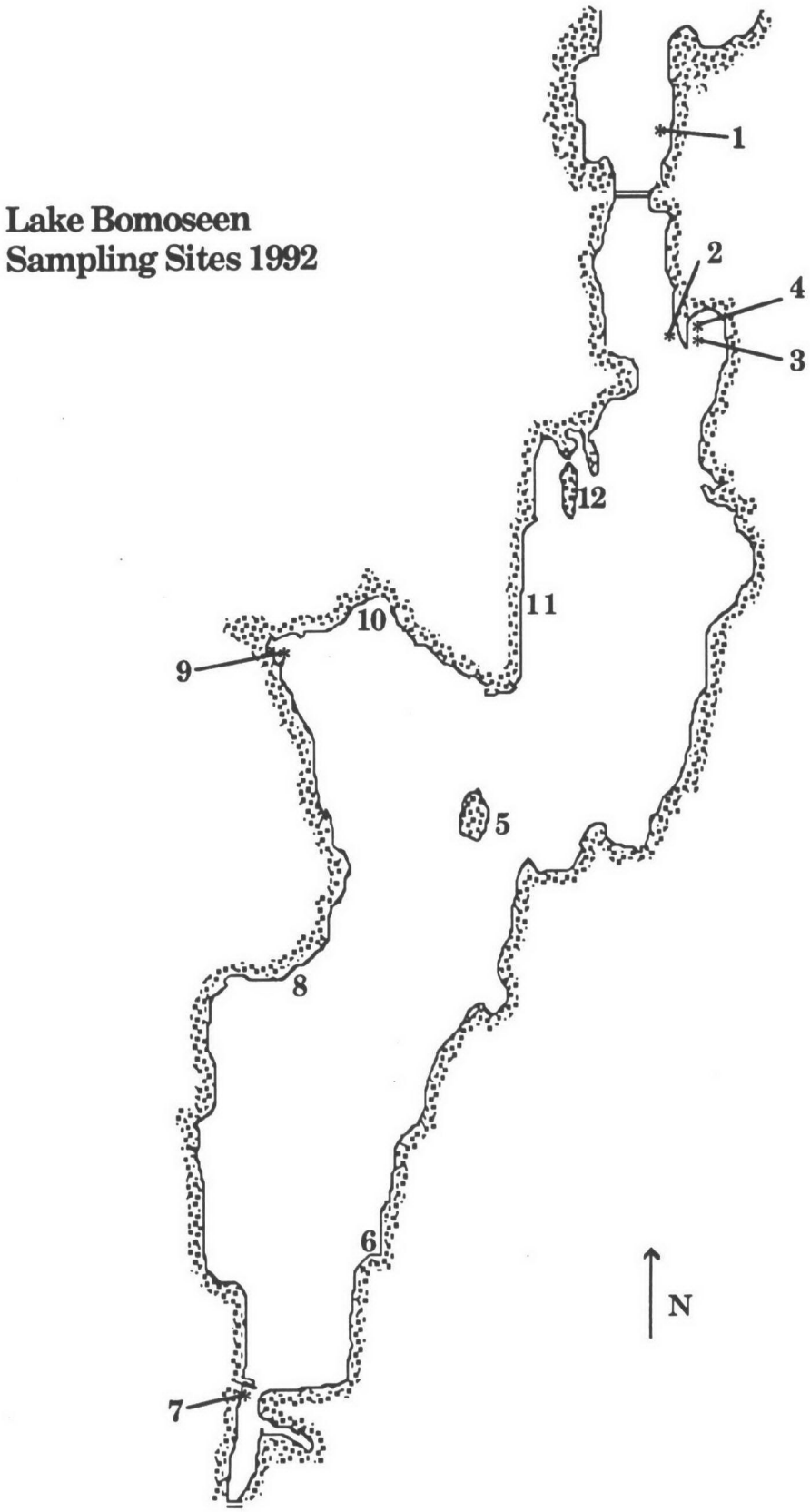


Figure 26.

A. WEEVILS IN HARVESTED AND UNHARVESTED AREAS

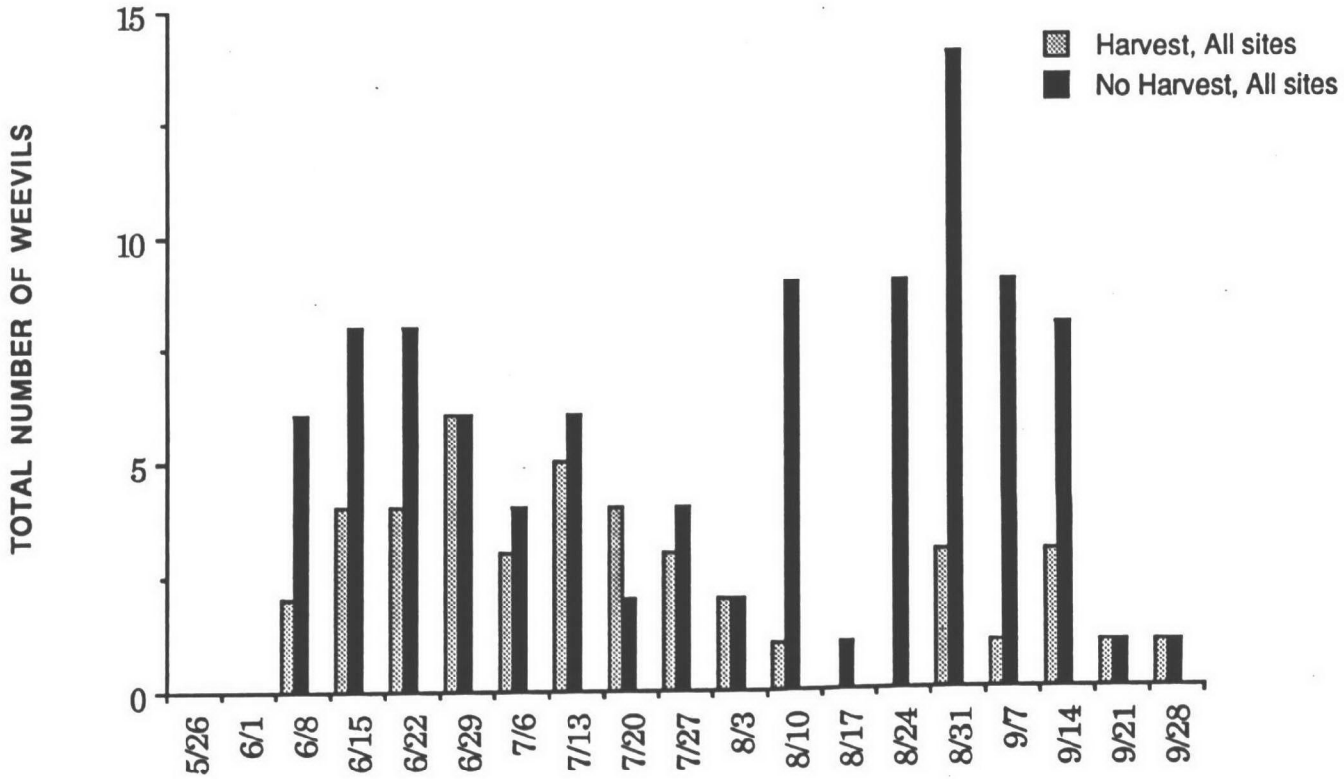


Figure 27.

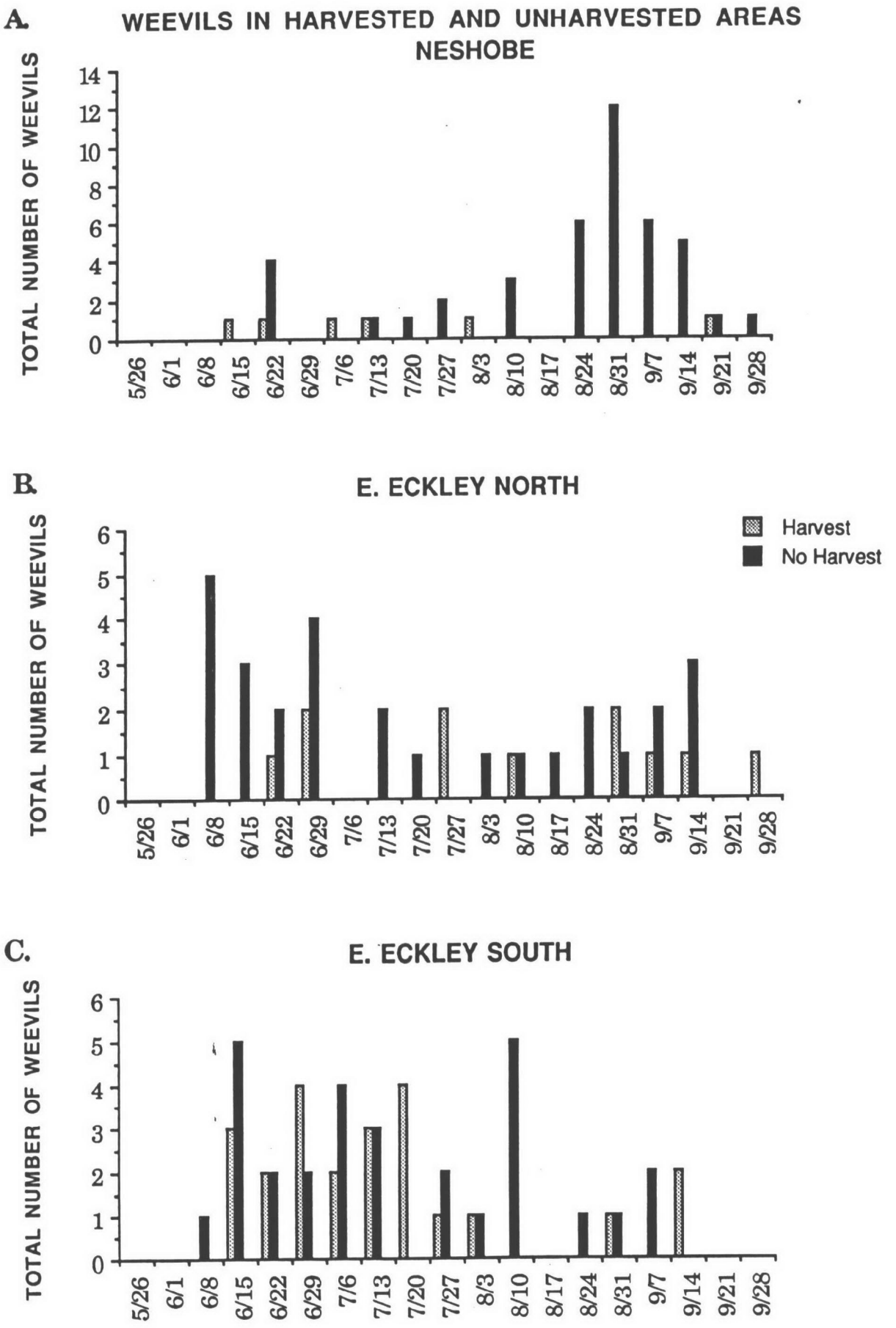

A.
WEEVILS IN HARVESTED AND UNHARVESTED AREAS
NESHOBE
TOTAL NUMBER OF WEEVILS
0
2
4
6
8
10
12
14
5/26
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
8/10
8/17
8/24
8/31
9/7
9/14
9/21
9/28
B.
E. ECKLEY NORTH
Harvest
No Harvest
TOTAL NUMBER OF WEEVILS
0
1
2
3
4
5
6
5/26
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
8/10
8/17
8/24
8/31
9/7
9/14
9/21
9/28
C.
E. ECKLEY SOUTH
TOTAL NUMBER OF WEEVILS
0
1
2
3
4
5
6
5/26
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
8/10
8/17
8/24
8/31
9/7
9/14
9/21
9/28

Figure 28.

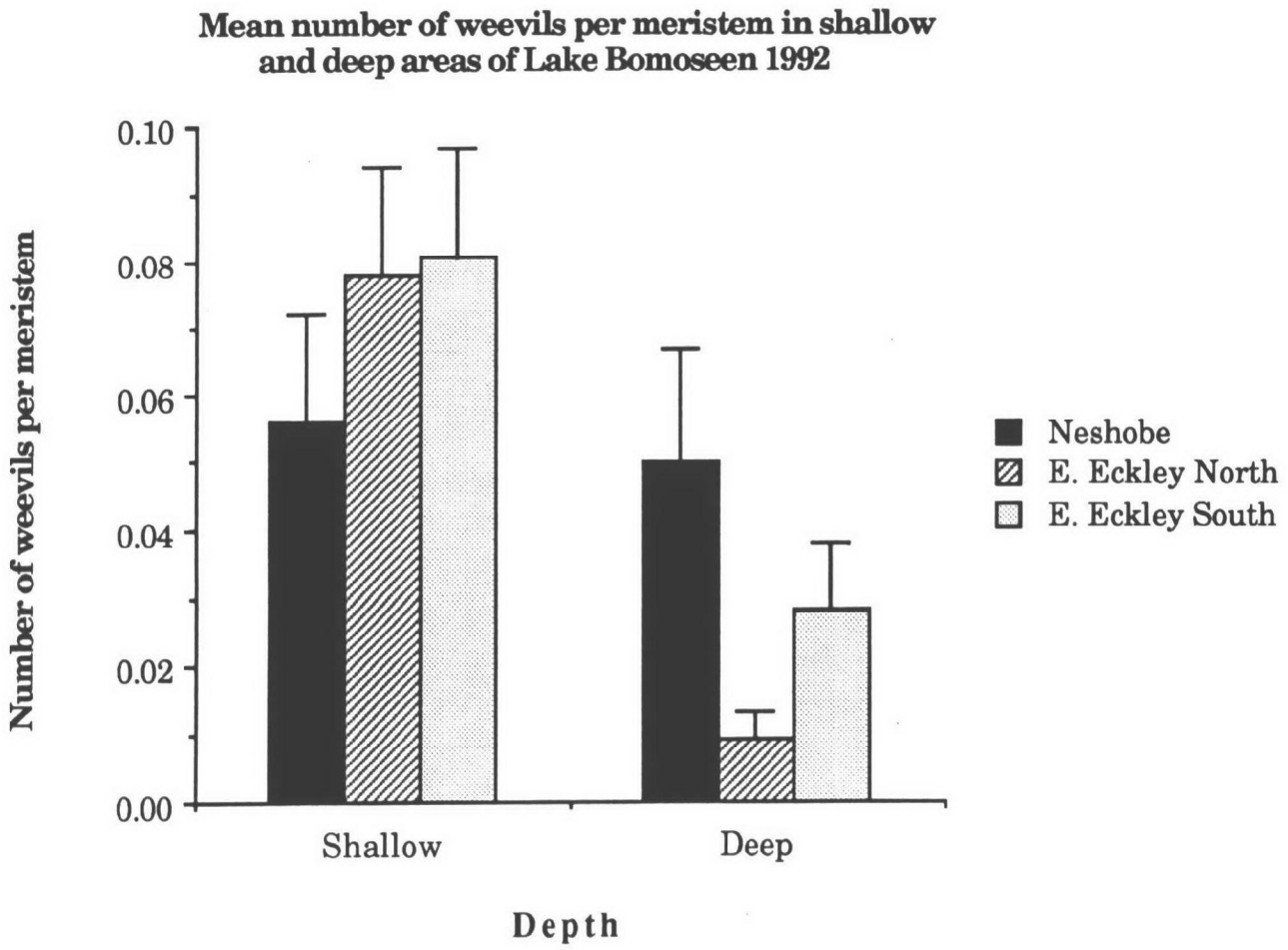
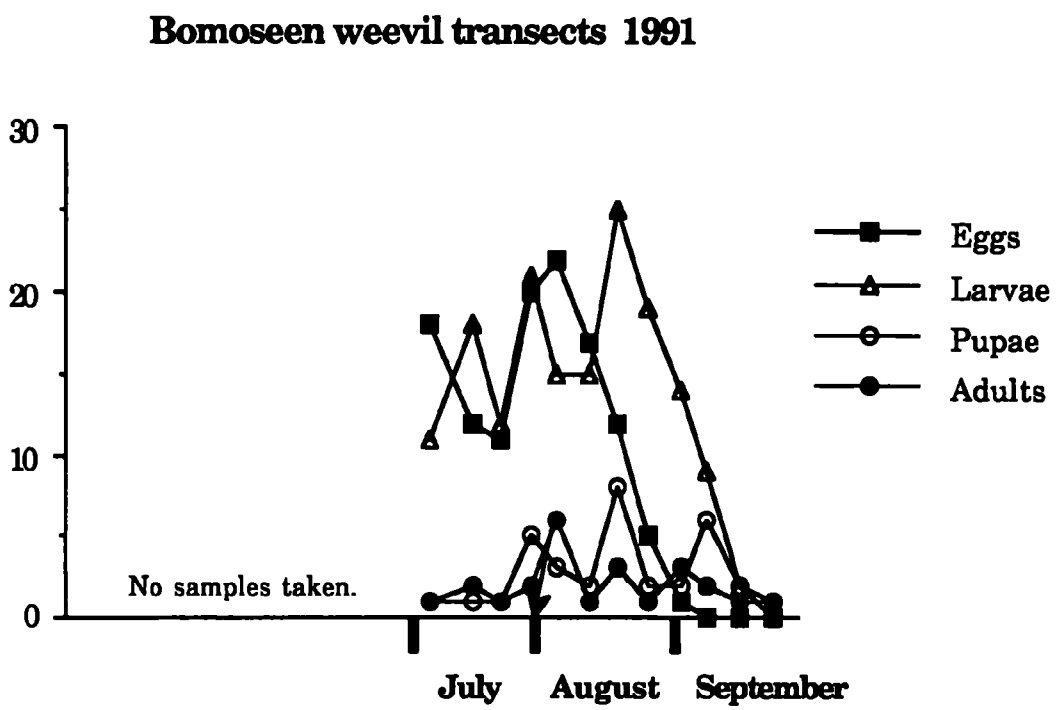

Mean number of weevils per meristem in shallow and deep areas of Lake Bomoseen 1992
Number of weevils per meristem
0.10
0.08
0.06
0.04
0.02
0.00
Shallow
Deep
Depth
Neshobe
E. Eckley North
E. Eckley South

Figure 29.

A.


Bomoseen weevil transects 1991
30
20
10
0
No samples taken.
July
August
September
Eggs
Larvae
Pupae
Adults

B.

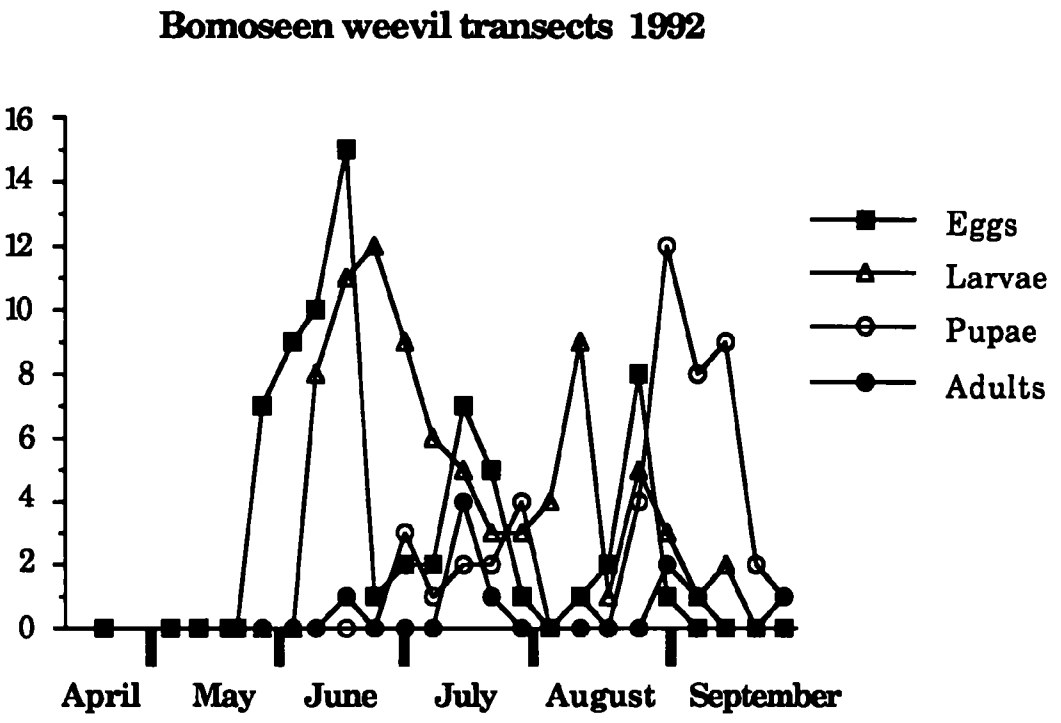

Bomoseen weevil transects 1992
16
14
12
10
8
6
4
2
0
April
May
June
July
August
September
Eggs
Larvae
Pupae
Adults

Figure 30.

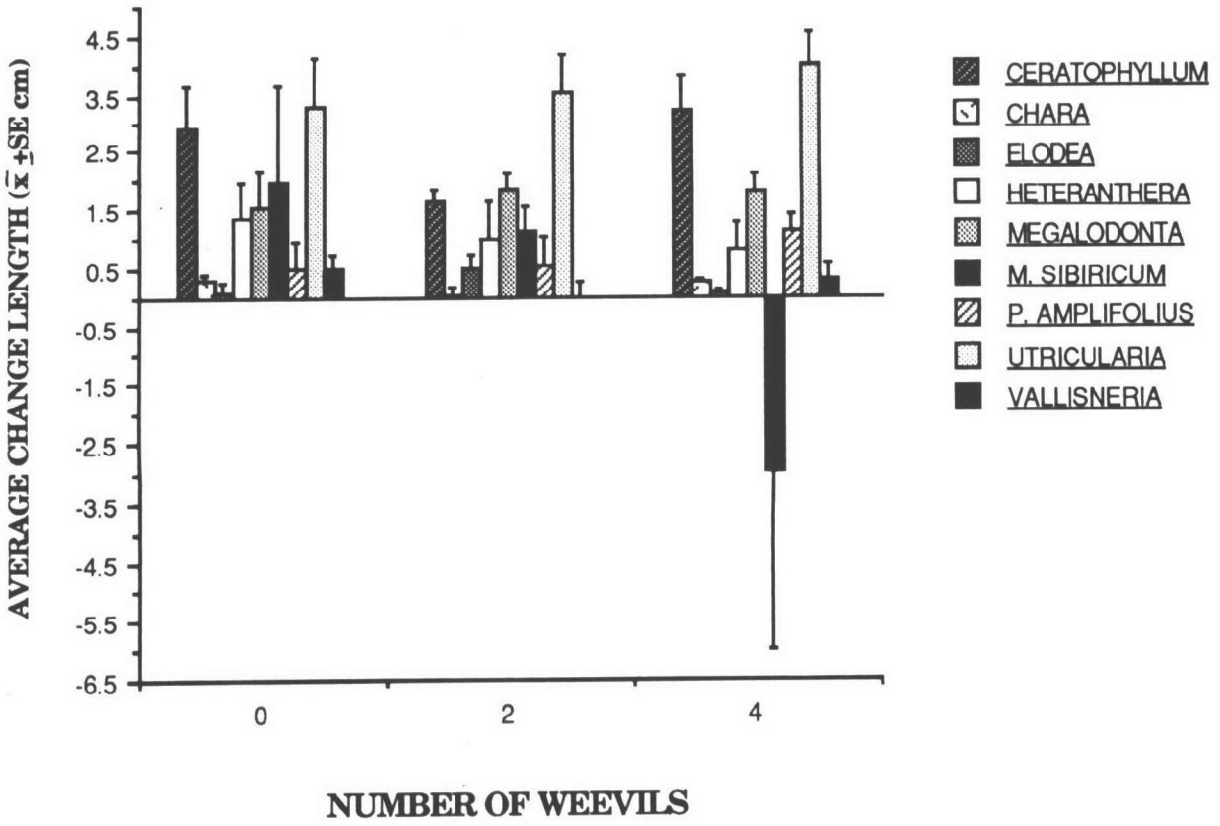

AVERAGE CHANGE LENGTH (x̄ ±SE cm)
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
-2.5
-3.5
-4.5
-5.5
-6.5
0
2
4
NUMBER OF WEEVILS
CERATOPHYLLUM
CHARA
ELODEA
HETERANTHERA
MEGALODONTA
M. SIBIRICUM
P. AMPLIFOLIUS
UTRICULARIA
VALLISNERIA

Figure 31.

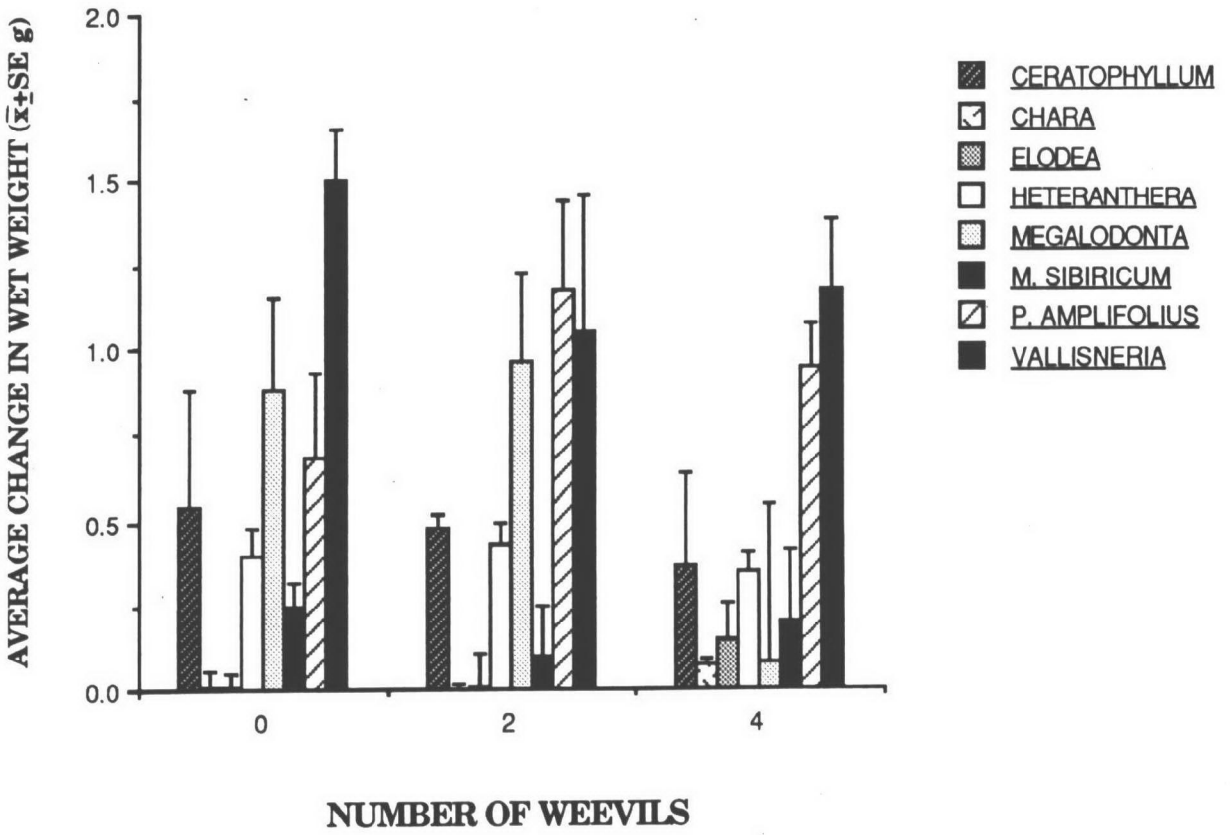

AVERAGE CHANGE IN WET WEIGHT (x̄±SE g)
2.0
1.5
1.0
0.5
0.0
0
2
4
NUMBER OF WEEVILS
CERATOPHYLLUM
CHARA
ELODEA
HETERANTHERA
MEGALODONTA
M. SIBIRICUM
P. AMPLIFOLIUS
VALLISNERIA

Figure 32.

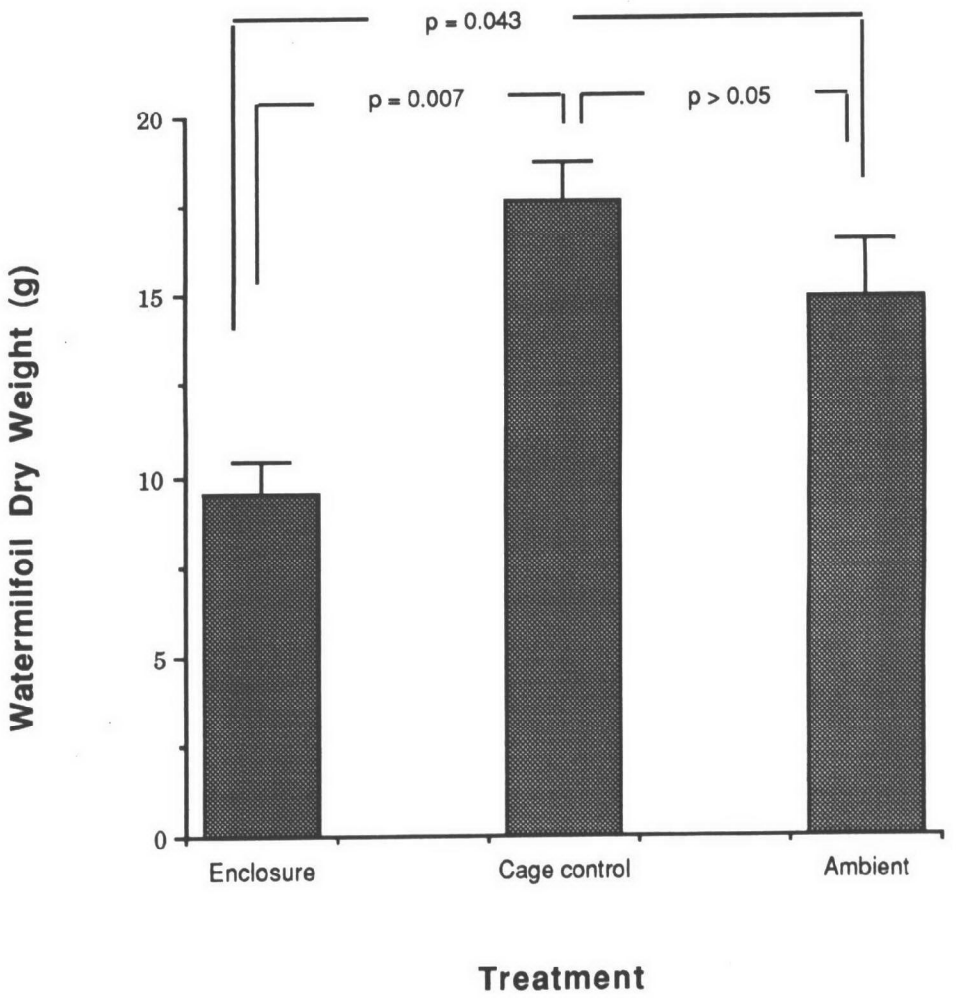
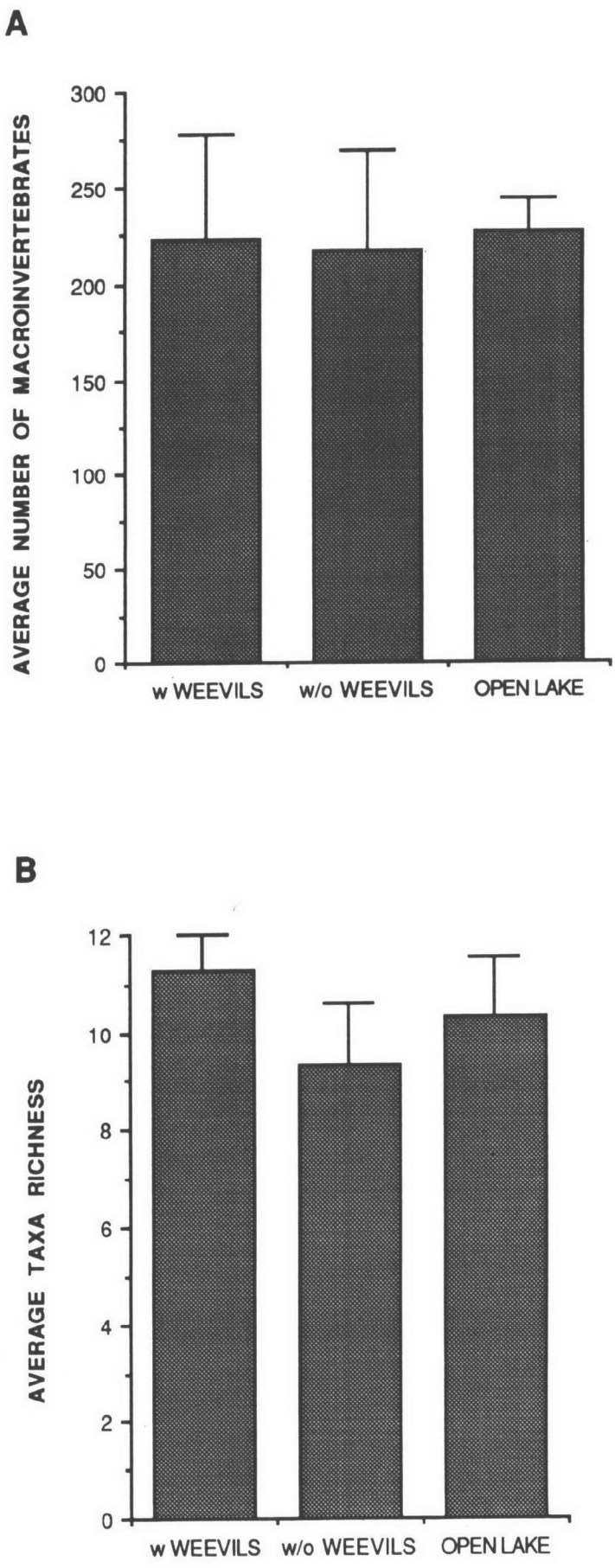

p = 0.043
p = 0.007
p > 0.05
20
15
10
5
0
Watermilfoil Dry Weight (g)
Enclosure
Cage control
Ambient
Treatment

Figure 33.


A
AVERAGE NUMBER OF MACROINVERTEBRATES
300
250
200
150
100
50
0
w WEEVILS
w/o WEEVILS
OPEN LAKE
B
AVERAGE TAXA RICHNESS
12
10
8
6
4
2
0
w WEEVILS
w/o WEEVILS
OPEN LAKE