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Experimental evidence for changes in submersed macrophyte species composition caused by the herbivore *Acentria ephemerella* (Lepidoptera)

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Abstract. Our field observations on submersed macrophytes in the littoral zone of Cayuga Lake, N.Y., USA indicate that the shift in dominance from *Myriophyllum spicatum* L. to *Elodea canadensis* Michx. may be explained by the high abundance of an aquatic lepidopteran larva, *Acentria ephemerella* Denis & Schiffermüller. Experimental evidence for the preference of *Acentria* for *Myriophyllum* over *Elodea* was obtained from small-scale, short-term laboratory experiments and from a tank experiment that represents a spatial and temporal scale intermediate between that of the small-container laboratory study and whole-lake observations. In laboratory no-choice feeding assays, *Acentria* exhibited higher feeding rates on *Myriophyllum* than on *Elodea*. In choice experiments, the larvae clearly preferred *Myriophyllum* over *Elodea*. Mesocosm tanks were set up with both *Myriophyllum* and *Elodea* planted in patches, and larval densities of 0, 75, 200 and 400 larvae m^{-2}. After 3 weeks, biomass and shoot length of *Myriophyllum* was inversely correlated with larval density, but biomass of *Elodea* was unaffected. In this study, we explore whether a generalist macroinvertebrate herbivore such as *Acentria*, by preference for one macrophyte species over others, may affect the competitive interaction between two rooted plant species and subsequently may change the community composition within submersed macrophyte beds.

Keywords. Herbivory - Aquatic - Lepidoptera - Pyralidae - Diet

Introduction

Macrophyte biomass loss due to herbivory by vertebrate and invertebrate grazers has traditionally been considered low in fresh water (Shelford *1918*; Hutchinson *1975*). Losses of plant biomass from grazing are generally considered to be small among temperate annual macrophytes. Senescence and death both caused by herbivory and other factors during the growing season range between 2% and 10% of the maximum biomass (Wetzel *1983*). However, calculating the deficit only on the maximum biomass underestimates the real losses since many aquatic angiosperms produce multiple cohorts of

photosynthetic tissue during each growing season. Consequently, many authors presented evidence for substantial herbivory of macrophyte tissue in recent years (Lodge *1991*; Newman *1991*; Jacobsen and Sand-Jensen *1992*; Lodge et al. *1998*). If true, however, the value given by Wetzel (*1983*) would put consumption in the littoral zone closer to terrestrial than to pelagic values. In most terrestrial systems leaf herbivory per se has been estimated at 3-18% of net primary productivity (Landsberg and Ohmart *1989*; Cyr and Pace *1993*; Hairston and Hairston *1993*). In aquatic systems in general (including zooplankton-algae trophic interactions), loss of net primary production to herbivory averages between 30% and 33% (Cyr and Pace *1993*; Hairston and Hairston *1993*).

The amount of damage to macrophyte tissue depends on the abundance and feeding preferences of the herbivores, the primary site of herbivory (meristem, leaves, stem, root, buds) and on the life-cycle of both the macrophyte and the herbivore. A careful estimate of herbivore damage must account for plant turnover (multiple leaf cohorts per growing season) and leaf-age dependent consumption (Jacobsen and Sand-Jensen *1994*; Sand-Jensen et al. *1994*). Similarly, herbivores may hinder macrophyte growth in spring after emergence from winter vegetative propagules (Guenzl *1993*) or limit resource allocation to hibernating organs during summer (Newman et al. *1996*).

We have recently observed both preferential feeding of larvae of the generalist herbivore *Acentria ephemerella* (Lepidoptera, see below) on *Myriophyllum spicatum,* Eurasian watermilfoil, and the subsequent increase in relative abundance of *Elodea canadensis* in Cayuga Lake, a glacially scoured mesotrophic lake in upstate New York, USA (Johnson et al. *1998, 2000*). All species involved in this study will be named by their genus name, and *Myriophyllum* also milfoil, in the following. *Myriophyllum* is an invasive exotic species that is currently a major nuisance in many lakes throughout North America (Chambers *1993*; Newman et al. *1996*). *Myriophyllum* and *Elodea* are both highly competitive species which rapidly reproduce vegetatively (Nichols and Shaw *1986*; Smith and Barko *1990*). However, they exhibit distinct growth forms in the water column and react differently to shading and mechanical harvesting with milfoil biomass declining more and shoot length increasing to a greater extent under high shade compared with *Elodea* (Abernethy et al. *1996*). Milfoil has a higher light compensation point than *Elodea*, and may be at a competitive disadvantage with this species at low light levels (Nichols and Shaw *1986*; Madsen et al. *1991*). Shoot elongation and canopy formation in milfoil counteracts light limitation (Nichols and Shaw *1986*; Madsen et al. *1991*), and this canopy formation is especially problematic in lake management. Milfoil has a higher maximum photosynthetic rate than *Elodea,* and when the latter species grows beneath a milfoil canopy, it exhibits a reduced carbon balance that eventually leads to its decline (Madsen et al. *1991*).

For *Myriophyllum* several aquatic macroinvertebrate herbivores have been associated with tissue damage in the field, including the chironomid larva *Cricotopus myriophylli* (Kangasniemi and Oliver *1983*), the lepidopteran larva *Acentria ephemerella* (Painter and McCabe *1988*; Johnson et al. *1998, 2000*), the chrysomelid beetle *Haemonia (Macroplea) appendiculata* (Grillas *1988*) and the weevil *Euhrychiopsis lecontei* (Creed and Sheldon *1995*). All these species are wholly aquatic during their feeding life-history stages and consume only submersed plant tissue.

Acentria ephemerella [formerly *A. nivea* Olivier, see Passoa (*1988*) or *Acentropus niveus* (Berg *1942*)] is a herbivorous Lepidoptera native to Europe. Hedal and Schmidt (*1992*) report densities of 300 *Acentria* larvae per square meter on *Zostera marina* in Danish coastal waters. It is also frequently found in high abundance (50-8000 individuals m^{-2}) in freshwater lakes (Müller-Liebenau *1956*; Sozka *1975*; Johnson et al. *1998*; Gross and Choi, unpublished results). Our observations in many lakes of central New York State suggest that the erect and canopy-like growth of *Myriophyllum* offers a more suitable habitat for *Acentria* than macrophytes growing deeper in the water column (Johnson et al. *1998*).

Changes in macrophyte community structure associated with the mass development and the subsequent loss of dominance of milfoil in Cayuga Lake are well documented (Oglesby et al. *1976*; Johnson et al. *1998*, *2000*). *Myriophyllum* contributed almost 100% of the total submersed macrophyte biomass in the early 1970s following severe disturbance of the southern part of Cayuga Lake associated with tropical storm Agnes (Oglesby et al. *1976*; Johnson et al. *1998*). Milfoil biomass declined at the beginning of the 1990s and never again reached its former dominance. At approximately the same time, Johnson and coworkers (*1998*, *2000*) report high densities of the moth *Acentria* on apical meristems of this macrophyte. It seems likely that a major reason why milfoil has failed to build up canopies in Cayuga Lake since 1991 (Johnson et al. *1998*) is herbivory because the primary feeding site of *Acentria* on *Myriophyllum* is the apical meristem where feeding strongly inhibits shoot growth. Thus, we hypothesize that an initial small loss of *Myriophyllum* biomass due to herbivory can be accentuated by competitive interactions with more shade tolerant species such as *Elodea* to result in a shift in macrophyte species dominance.

The aim of this study was to test experimentally whether the preferential feeding of the aquatic lepidopteran larva *Acentria* on Eurasian watermilfoil could explain the change of species dominance from *Myriophyllum* to *Elodea* in Cayuga Lake. *Acentria* is a generalist herbivore that feeds on both of these macrophyte species (Batra *1977*; Buckingham and Ross *1981*; Johnson et al. *1998*). We investigated feeding rate and diet selectivity of this herbivore in short-term laboratory experiments. Then, in order to compare these small-scale experiments with observations from the field, we designed a mesocosm study to determine the effects of different stocking densities of larvae on milfoil and *Elodea* in controlled experimental tanks. Experiments combined with field-studies at different temporal and spatial scales allow us to understand the interaction between this herbivore and the macrophytes on which it feeds, and to document the resulting effects of herbivory on the macrophyte community structure.

Materials and methods

Long-term macrophyte dataset

Studies of submersed macrophytes at the south end of Cayuga Lake occurred at various times during the last century: in the 1920s, early 1940s and 1970s (see Oglesby et al. *1976*; Johnson et al. *1998*). Beginning in 1987, we took annual samples seasonally at the time of maximum plant abundance in August and analyzed for species composition and total biomass. For reference purposes, we divided a map of the littoral zone of the southern end of Cayuga Lake into a grid of 100 × 100-quadrants. We chose 11 representative quadrants that encompass areas that do not become dry during winter water draw-down and have a mean summer water depth of 2-3 m for annual sampling. We sampled submersed macrophytes by hand-harvesting five randomly selected 1-m^2 quadrats within each of the 11 sites during 1987-1992, or 20 randomly selected 0.25-m^2 quadrats within each of these 11 sites during 1993-1998, yielding a constant total sampled area of 55 m^2. This change to sampling more, but smaller, quadrats within each quadrant accounts better for the patchiness of submersed macrophytes. We calculated the mean total biomass and the biomass of individual species from the sampled quadrats for each quadrant separately and used these to statistically account for spatial variation. We then determined the mean biomass value of all 11 quadrants to estimate the biomass of submersed macrophytes in southern Cayuga Lake.

Laboratory experiments

No choice experiments

Larvae pre-fed on *Ceratophyllum demersum* and placed individually in 50-mm-diameter plastic petri dishes filled with 5 ml dechlorinated tap water received a 2-cm shoot cutting from either *Myriophyllum* or *Elodea*. We took plant parts from the upper 10 cm of shoots but not the apical meristem. Culture conditions were 15°C and 15:9 light: dark cycle. The counting of the number of fecal pellets produced per time interval (usually 24 h, values given as fecal pellets h^{-1}) is a good estimate of the feeding rate. Fecal pellets from larvae of the same instar are similar in size. We used this semi-quantitative measure, since the larvae are very small (0.1-10 mg fresh weight depending on instar stage) making alternative measurements difficult. Differential assimilation efficiencies for the tested macrophytes cannot be excluded at the moment and will be tested in future experiments. We tested a direct comparison of feeding rates on *Elodea* and *Myriophyllum* with 20 larvae each per treatment.

Choice experiments

We performed three different choice experiments. In the first experiment we used 100 *Acentria* larvae collected from the stems of over-wintering *C. demersum* in March 1996. They were placed individually in 50-mm-diameter plastic petri dishes, filled with 5 ml dechlorinated tap water and each was offered two pieces of 2-cm-long shoot cuttings, one from *Myriophyllum* and one from *Elodea*. Larvae were kept at 15°C, 15:9 L:D for 24 h, after which the location of each larva, the resulting plant damage and the color of the fecal pellets were recorded. Feeding on milfoil or *Elodea* is distinguished because fecal pellets made up of milfoil tissue turn brown due to high tissue concentrations of hydrolyzable polyphenols (Gross et al. *1996*) while fecal pellets from *Elodea* stay green. Secondly, we tested using *Myriophyllum* whether *Acentria* preferentially feeds on apical meristems and the highest shoots available. We used 35 replicate 200-ml glass jars filled with dechlorinated tap water and 2 cm of washed pebbles as an anchoring material for the macrophyte shoots. In each jar we planted two milfoil shoots, 5 cm apart, one short and one tall (5 and 10 cm above the sediment) and then placed larvae individually in the middle of each jar. We used the same culture conditions and duration of experiment as in the first choice experiment. After 24 h, we recorded the location of the larvae (tall or short stem, apical tip or lower on stem). The third choice experiment was performed in 4-l glass jars in July 1996 using larvae collected from *Myriophyllum* in Cayuga Lake. Five jars were each filled with dechlorinated tap water and 3 cm of sediment. To each jar, we planted three 15-cm apical shoots of milfoil and three of *Elodea* alternately in a circle. After 3 days, we added 15 larvae in the middle of each jar. Five days later, we recorded the location of the larvae.

Development of *Acentria* larvae

Fifty first-instar larvae, all of which had hatched from a single egg clutch laid on *Myriophyllum*, were divided into two groups. One group received *Elodea*, and the other milfoil to eat. We kept the larvae individually in 100-ml plastic cups at 20-22°C and ambient room light conditions. They received new water and food at weekly intervals. First-instar larvae that died within 24 h after transfer to cups (probably from handling) were replaced with larvae from the same egg clutch reared in batch culture on the appropriate food plant. Survival and date of metamorphosis were recorded for each larva. Although it is possible that maternal effects may influence food choice and development, our preliminary experiments showed that food preferences are little affected by previous food experiences. We had observed, however, that female *Acentria* were very reluctant to lay eggs on *Elodea* and only did so in the absence of other choices after a considerable length of time. In contrast, gravid females laid their eggs readily on milfoil.

Mesocosm experiment

In order to bridge the gap between the temporal and spatial scales used in laboratory bottle and dish experiments and those associated with field data, we carried out a 3-week-long experiment in 18 plastic tanks (volume 95 l; conical form: 50 cm deep, 0.16 m^2 bottom surface area, 0.28 m^2 water surface area.) in a greenhouse using a systematic design (Hurlbert *1984*). Each tank received a 7-cm-thick bottom layer of aquatic sediment, and then was filled with dechlorinated tap water. Tanks were divided into four equal sections to account for macrophyte patchiness found in the lake and diagonally opposite sections were planted with either *Elodea* or *Myriophyllum*. We used stem densities and biomass values of macrophytes and larval densities comparable to field conditions frequently found in Cayuga Lake and its vicinity. We took *Elodea* from a pond at the Cornell Experimental Ponds Facility and milfoil from an herbivore-free site on the northeast shore of Seneca Lake, New York. Apical shoot cuttings of 15 cm length of both macrophytes were planted 5 cm deep into the sediment. We planted *Elodea* 3 days before *Myriophyllum*, because *Elodea* requires more time to root in the sediment. Eight non-branched apical tips of milfoil or 16 bushy shoots of *Elodea* (2-4 side shoots each) were used per quadrant. We calculated the dry weight (DW) of the initial plantings from 10 replicates each of eight milfoil or 16 *Elodea* shoots, respectively (8 milfoil shoots weighed 1.56±0.09 g DW, n =10, mean ±1 SE; 16 *Elodea* shoots weighed 1.13±0.16 g DW, n =10, mean ±1 SE). Two days after milfoil planting, we added *Acentria* larvae (mainly second instar) to the middle of each tank at the intersection of all quadrants. The main experiment ran for 3 weeks from 7 to 28 October 1997. Six tanks remained as control treatments without herbivores. Four tanks received 12 larvae, four received 32 larvae, and four received 64 larvae, resulting in larval densities of 0, 75, 200 and 400 m^{-2}. One of the control tanks was equipped with sensors logging temperature (10 cm below water surface) and light intensity (PAR, surface and 10 cm below surface) at 2-h intervals. On days 3, 15 and 21 after the start of the experiment (i.e., after the addition of *Acentria* larvae) pH and dissolved oxygen (DO) in the tanks were measured. We kept the photoperiod at 15:9 light:dark cycle by using artificial light sources in the morning and evening (metal halide lamps, 1000 W, Metalarc). Greenhouse conditions were set at 22°C day and 10°C night; however, very bright days, especially during the first week resulted in midday temperatures of more than 25°C, exceeding the cooling capacity of the air condition system in this building. Despite this, the temperature in the tanks declined slightly during the experiment from 23 to 20°C (daytime-average). We did not use continuous artificial lighting because excessive heat developed from the lamps. Mean light intensities 10 cm below the water surface were constant during the first 2 weeks (approx. 400 µmol s^{-1} m^{-2}) but declined in week 3 (150 µmol s^{-1} m^{-2}); nevertheless, light conditions were not limiting. All tanks received the same exposure to environmental conditions, so these temporal changes should not have biased our results.

We covered all tanks with a fine white mesh to avoid contamination with other insects and the escape of adult *Acentria* males. Above-sediment shoots of milfoil and *Elodea* were harvested separately at the end of the experiment (day 21). We pooled all *Elodea* shoots in each tank since it is difficult to divide single plants due to adventive rooting in this species. We harvested and placed separately in sealable plastic bags (Ziploc) single milfoil shoots and free-floating shoots. We kept all plant material at 4°C until microscopic examination for the location of *Acentria* larvae or pupae and resulting herbivore damage within 4 days. *Acentria* larvae stop feeding and are inactive below 10°C (Batra *1977*; Buckingham and Ross *1981*; E. M. Gross and R. L. Johnson, personal observations). No movement of larvae could be observed under these conditions. We counted the numbers of side shoots, broken shoots and herbivore-damaged tips and recorded the shoot length of milfoil. For *Elodea*, we divided the harvested plant material into undamaged green shoots, brown shoots and shoots that had obvious marks of herbivory (usually leaves missing along a stem segment, no damage to the apical meristem). Assuming that loss due to herbivory and decay did not change the biomass of the respective shoot part significantly, we calculated the percentage of decaying and grazed material from the total *Elodea* biomass harvested. Finally, we determined the dry weight (105°C, 48 h) of each macrophyte species from every tank.

Data analysis

All statistical analyses were performed with SAS JMP, version 3.2.2. When data were not normally distributed, we used non-parametric statistical tests. For the tank experiment, we based linear regressions on the density of larvae as a continuous variable to account for density dependent effects. Multiple pairwise comparisons were sequentially Bonferroni corrected (Rice *1989*).

Results

Macrophyte changes in southern Cayuga Lake

We recorded 15 different species of macrophytes at the southern end of Cayuga Lake during the 12 years of study (Johnson et al. *1998*). A more detailed analysis of the macrophyte biomass data was carried out to assess the temporal variation of the major submersed macrophytes in Cayuga Lake. In only 4 out of the 12 years did *Myriophyllum* and *Elodea* together contribute less than 40% to the total biomass of submersed macrophytes (Fig. 1 B). Between 1987 and 1998, biomass of *Elodea* increased strongly over time (Fig. 1A, Spearman rho =0.71, $P<0.001$), whereas biomass of *Myriophyllum* showed no consistent trend over time (Fig. 1A, Spearman rho =-0.009, $P=0.92$). As a result, biomass dominance shifted from *Myriophyllum* to *Elodea*. Total biomass of all submersed macrophytes fluctuated markedly between years (see Johnson et al. *1998*) but increased with time (Spearman rho =0.47, $P<0.001$). *Myriophyllum* as a proportion of all submersed macrophytes strongly declined from 50% in 1987 to an average of about 12% of the biomass of all submersed macrophytes in the lake in the 1990s (Fig. 1, Spearman rho =-0.41, $P<0.001$) while the proportion of *Elodea* increased from 3% to 50-70% of total biomass (Fig. 1, Spearman rho =0.66, $P<0.001$). *Elodea* and milfoil densities are negatively correlated with each other over the period 1987-1998 (Fig. 1B, Spearman rho =-0.281, $P=0.001$). The shift in dominance from *Myriophyllum* to *Elodea* took place in 1990. In 1991 major damage to the apical meristem of milfoil led to the discovery of larvae of the moth *Acentria ephemerella*. Although, due to its cryptic nature, *Acentria* may have been overlooked in previous years, it was not so abundant as to have caused the substantial meristem damage we first observed in 1991. The abundance of *Acentria* ranged from 0.7 to 1.4 individuals per apical meristem on milfoil in southern Cayuga Lake from 1996 to 1998 (Johnson et al. *2000*) resulting in approximate larval densities between 140 and more than 2,000 individuals per square meter, depending on stem density and number of apical shoots. Although still present in the lake, milfoil does not form a canopy any longer and the damage to almost every milfoil tip in the field can obviously be associated with the presence of *Acentria*.

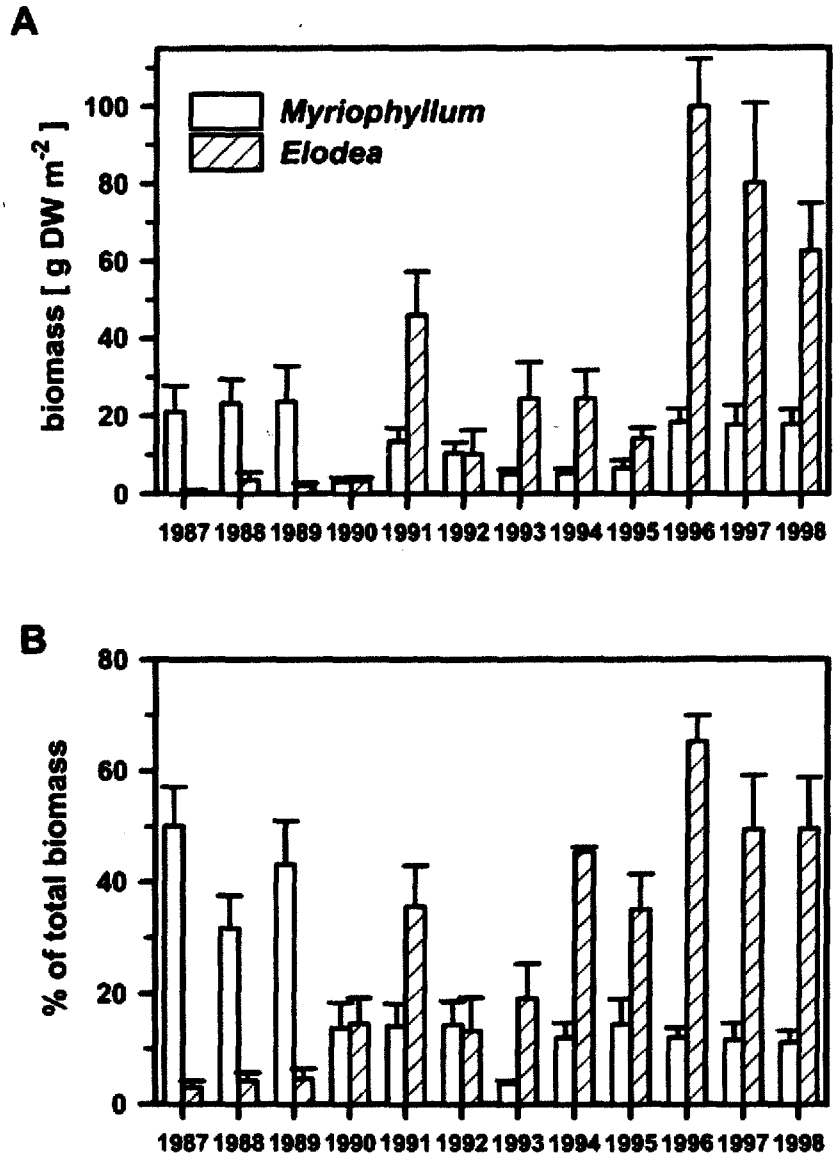

Fig. 1. Changes in absolute (**A**) and relative (**B**) biomass of *Myriophyllum spicatum* (*white bars*) and *Elodea canadensis* (*hatched bars*) from 1987 to 1998. Relative biomass was calculated in percent of the total biomass of submersed macrophytes. Samples were collected at peak annual macrophyte biomass during August of each year in 11 selected, representative quadrants throughout the southern littoral zone of Cayuga Lake. Data represent means ±1 SE, n=11

Laboratory experiments

No-choice feeding experiments

In the no-choice feeding experiments, consumption of macrophyte tissue was estimated by the number of fecal pellets produced. The mean feeding rate (i.e., the number of fecal pellets produced per hour) differed significantly between larvae exposed to the two plant species. Larvae feeding on milfoil produced feces at a rate of 1.34±0.16 pellets h^{-1} (mean ±1 SE), effectively double that of larvae feeding

on *Elodea* at 0.66±0.11 pellets h^{-1} (square-root transformed data, pooled *t*-test, t=-3.07, df=38, P=0.004). These values are comparable to results published previously (Johnson et al. *1998*), where the feeding rate on milfoil was estimated at 1.51±0.08 and on *Elodea* at 0.84±0.08 pellets h^{-1} (pooled *t*-test, t=-2.71, df=39, P=0.01).

Choice experiments

Larvae of the generalist herbivore *Acentria* probably move in the field between neighboring macrophytes. We frequently observed *Acentria* in aquaria moving between shoots either by crawling down one stem and up another, or between leaves on adjacent shoots. Larvae tend to stay on the highest available shoot. We evaluated the distribution of *Acentria* between *Myriophyllum* and *Elodea* and to test for a preference of apical shoots using three different types of choice experiments (Table 1). Choice tests set up in petri dishes allowed us to estimate feeding on both macrophytes, retreat building and damage to the shoot clippings (experiment I, Table 1). We omitted shoot height and three-dimensional structure as factors influencing food choice in this assay. Preference for milfoil was significantly greater than for *Elodea*, with nearly two-thirds of all larvae found on milfoil after 24 h. Observations during this time revealed that after the initial food selection, larvae seldom switched between offered plants. In 46% of all feeding trials damage was observed only on milfoil whereas in 7% of all trials damage was found exclusively on *Elodea*. In 25% of all cases we observed damage on both shoot cuttings. The remaining cases did not show obvious herbivore damage. This shows that *Acentria* larvae fed preferentially on milfoil. Most actively feeding larvae produced solely (46%) or a majority (41%) of brown (milfoil) fecal pellets, whereas only 4% made solely or a majority of green (*Elodea*) fecal pellets, again indicating higher feeding on the polyphenol-containing milfoil (testing brown excess vs green excess fecal pellets, Likelihood Ratio χ^2-test, df=1, χ^2 =65.80, P<0.0001). In total, larvae produced significantly more brown than green fecal pellets (mean ±1 SE: brown 1.14±0.13, green 0.15±0.03; Wilcoxon two-group test, z=-6.95, P<0.0001).

Table 1. Results of three choice experiments with *Acentria* larvae. Experiment I tested the food choice of 100 larvae for *Myriophyllum spicatum* or *Elodea canadensis* kept individually in petri plates. Larvae that did not make a choice and plates with no obvious feeding damaged were omitted from analysis. Experiment II tested using only *M spicatum* for preference of apical shoots; larvae were offered tall or short shoots in 200 ml jars. Larval choice for tall versus short shoot and apical meristem (tip) versus lower stem was recorded. Experiment III tested the food choice of groups of larvae in 4-l jars

Experiment		*n*	Choice		Test[a]	*P*
I			*M. spicatum*	*Elodea*		
Petri plate	Location	100	62	30	χ^2=11.37	0.0007
	damage exclusively on	100	46	7	χ^2=32.10	<0.0001
II			Tall	Short		
Location	Shoot selection	35	22	9	χ^2=5.62	0.018
			Tip	Lower stem		
		35	29	6	χ^2=28.14	<0.0001
			Tall tip	Not tall tip		
		22	21	1	χ^2=34.18	<0.0001
III			*M. spicatum*	*Elodea*		
4 l glass jar	Ratio of larvae per plant species per jar (15 larvae per jar)	5	0.67±0.04	0.33±0.04	*t*=4.32	0.012

[a]Statistical tests: χ^2/Likelihood ratio χ^2 test; *t* / one sided *t*-test

In the second type of choice experiment (II, Table 1) we tested the preference of *Acentria* for the highest shoots available, because in the field, and especially on milfoil, *Acentria* is usually found at the apical meristem of the main (tallest) shoot. In our experiments, larvae were able to select between a tall and a short shoot and to switch their location on these shoots between the apical meristem and lower shoot parts. Twenty-two out of the 31 larvae (71%) that made a selection we found on the tall shoot; of those, 21 were found on the tip. Furthermore, 8 of the 9 larvae that chose the shorter shoot selected the apical tip for feeding. Our results show a significant preference by *Acentria* for tall shoots, for apical meristems in general, and specifically for the apical tip of the tallest shoot (Table 1).

In the third type of choice experiment (III, Table 1), larvae were offered rooted shoots of both macrophyte species in larger containers (4-l glass jars) in an effort to create more natural three-dimensional conditions. We placed 15 larvae in each of five jars and allowed them to feed for 5 days (120 h). This allowed for larval switching between food plants and for feeding damage to occur without excessive depletion of one food source. Of the 75 larvae included in this experiment, 48 selected *Myriophyllum*, 24 selected *Elodea*, and 3 made no choice. To avoid pseudoreplication when counting the location of each larva, we performed a one-tailed *t*-test on the proportion of larvae found per plant species in each jar. The mean proportion of larvae found on milfoil was 0.67±0.04 while the proportion on *Elodea* was 0.33±0.04 (mean ±1 SE, n=5, t=4.32, P=0.012).

Development of *Acentria* larvae

Of the 50 larvae we isolated from a single clutch, there was substantially better survival to pupation of larvae fed milfoil than for larvae fed *Elodea* (21 for milfoil, 13 for *Elodea*, χ^2-test, χ^2=5.88, P=0.015).

We observed, however, no significant difference in development time (egg to pupation) between the 13 larvae that survived to pupation while feeding on *Elodea* and the 21 larvae that survived to pupation on milfoil. In both treatments it took approximately 7 weeks for first-instar larvae to develop to the pupal stage (milfoil 51.6.±1.3 days, *Elodea* 50.7±1.7 days, *t*-test, df=32, t=-0.42, P=0.68).

Mesocosm experiment

Distribution of larva. All control tanks remained herbivore-free during the experiment. Of the 432 larvae added to all the treatments, 303 (70%) were retrieved at the end of the experiment, or earlier as adults matured. At the end of the experiment, 21% of all retrieved *Acentria* were in the larval stage, 70% had reached the pupal stage and 9% were adults. Because larvae were added mainly as second instars, the generation time of these animals was approximately 6-7 weeks (assuming 8-10 days for eggs to hatch and 9-11 days after hatching to reach the second instar stage, Gross, personal observation). Although the numbers of larvae found on both macrophyte species were very similar, more pupae were found on *Elodea* than on milfoil.

Damage of the apical meristem and resulting lateral branching. A major competitive trait of milfoil is the ability of its rapidly growing shoots to form canopies. Apical dominance in *Myriophyllum* is high with side shoots growing generally only after the main shoot has reached the water surface. As a result, selective herbivory on the apical meristems of this plant should result in decreased length of the main shoots and enhanced lateral branching in the form of increased numbers of side shoots. We observed extensive damage to the apical meristems of *Myriophyllum* in tanks containing larvae. Depending on the stocked larval density, damage to the apical tips of the main shoots varied between 39% and 87%, and to the side shoots between 20% and 72% (Fig. 2E). Tip damage of main and side shoots increased significantly as a function of larval density (main shoots: R^2=0.80, P<0.0001; side shoots: R^2=0.75, P<0.0001, Fig. 2E). Whereas plants in the control tanks reached the water surface and formed a canopy, this was never observed for plants damaged by herbivory even at the lowest stocking density. No herbivore damage of apical meristems was observed for *Elodea* because *Acentria* feeding on this species removed leaves below the apical tips.

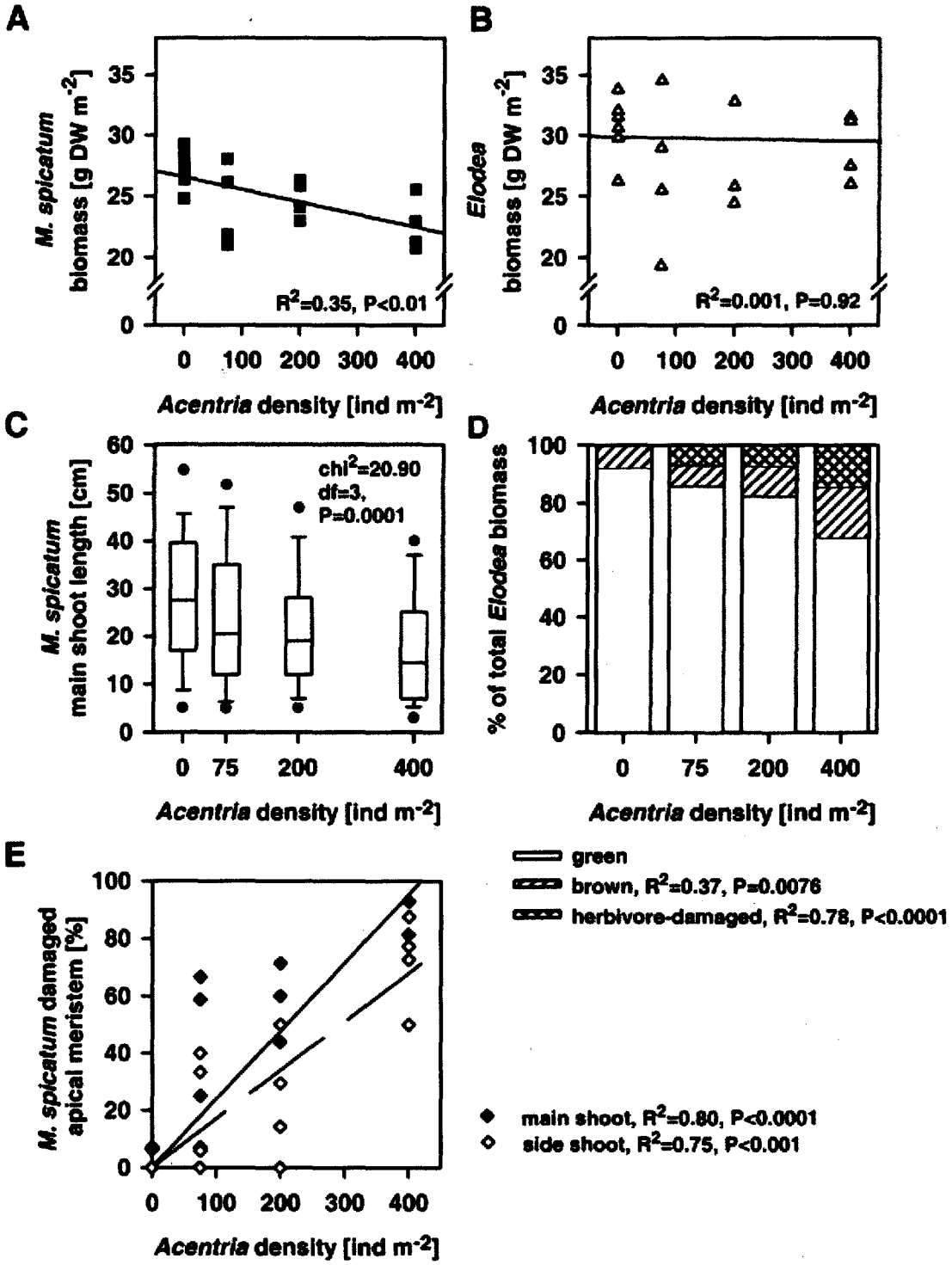

Fig. 2. The effect of increasing *Acentria* stocking densities on the total biomass of *M. spicatum* (**A**) and *E. canadensis* (**B**). The length of the main shoots of *Myriophyllum* are given in **C**. Statistical information in **C** refers to a one-tailed Wilcoxon rank sum test. The box plot presents median, 25 and 75 percentiles (*box*) and 5th / 95th percentiles (*dots*). n=92 for controls, and 63, 61 and 60 for 75, 200 and 400 larvae per square meter, respectively. The percentages of green, brown and herbivore-damaged tissue of *Elodea* are shown in panel **D**. Both brown and damaged tissue produced new side shoots. Damage to the apical meristem of *Myriophyllum* caused by *Acentria* on main and side shoots is given in panel **E**

Biomass development

Increasing stocking densities of *Acentria* hindered biomass development in *Myriophyllum* (Fig. 2A, linear regression, R^2=0.35, P<0.01). Although side shoot development measured as the total length of side and broken shoots did not differ between treatments (data not shown), side shoots from treatments with larvae seemed more fragile and had thinner stems than those from controls. This, in addition to biomass differences of the main shoots, probably caused the overall lower biomass yield in the presence of larvae. The mean length of the main shoots was affected in a similar way, declining from a median of 27.5 cm in the control tanks to 14.5 at the highest stocking density (Fig. 2C, one-tailed Wilcoxon rank sum test, χ^2-approximation, χ^2=20.9, df=3, P<0.0001).

In contrast, *Elodea* biomass (referring to the total biomass retrieved in one tank, including green, brown and grazed plant parts) was not affected by the stocking density of *Acentria* (Fig. 2B, linear regression, R^2=0.001, P=0.92). An effect of *Acentria* could only be observed for proportions of the plant biomass that were brownish or subjected to herbivory indicated by missing leaves (linear regression, brown parts: R^2 =0.37, P<0.01; herbivore-damage: R^2=0.78, P<0.0001; Fig. 2D). Brownish plant parts were present in 8% of control *Elodea* plants as well as in 7-18% of the plants in the treatments with larvae added. In the latter, shoot parts with obvious *Acentria* damage ranged between 7% and 14% (Fig. 2D), no such damage was observed in control tanks. Nevertheless, both brownish and grazed shoots exhibited new side shoot development, indicating that herbivory had no lasting negative impact on the growth potential of this species.

Discussion

Our study indicates that herbivory by the aquatic moth, *Acentria ephemerella* is directed to a significantly greater extent at the exotic nuisance aquatic plant *Myriophyllum* than at native *Elodea*. This in turn can affect canopy formation and biomass development of *Myriophyllum*. In consequence it may indirectly change the community structure of submersed macrophytes. Field observations and a thorough analysis of our long-term data set on submersed macrophytes in Cayuga Lake revealed a shift in dominance from milfoil to *Elodea* during the 1990s (Fig. 1B). Direct evidence for herbivore-related damage to the apical meristems of *Myriophyllum* and for an *Acentria*-related decline in milfoil biomass come from our mesocosm study involving the simultaneous culture of these two dominant submersed macrophytes, and from the preference of this generalist macroinvertebrate herbivore for milfoil over other macrophytes in short-term laboratory experiments. We argue that losses in biomass, when directed at reproductive tissue such as apical meristems, weaken the competitive ability of milfoil and ultimately may cause milfoil to lose dominance over other submersed macrophytes in the presence of *Elodea*. One reason might be that *Elodea* profits from the higher light availability when milfoil cannot form a canopy (Abernethy et al. *1996*; Madsen et al. *1991*).

Is *Acentria* an important herbivore?

Our data show that *Acentria* causes major damage to milfoil under laboratory conditions. The high abundance of larvae found on apical meristems and associated damage suggest that this herbivore also has a substantial impact in the field (see also Johnson et al. *1998*, *2000*). The multiple sampling sites of our long-term study on submersed macrophytes in Cayuga Lake provide a high-resolution picture of changes in individual macrophyte biomass (Johnson et al. *1998*, *2000*). *Acentria* has also been

implicated as a cause of a *Myriophyllum* decline in the Kawartha Lakes (Painter and McCabe *1988*). In Brownington Pond, Vermont (Creed and Sheldon *1994*) and Lake Memphremagog, Vermont (Creed and Sheldon *1993*) milfoil declines were ascribed to herbivory by the true aquatic weevil *Euhrychiopsis lecontei*. However, *Acentria* was present in both water bodies at densities of about one larvae per apical meristem (Creed and Sheldon *1993*) that are comparable to those in the Kawartha Lakes (Painter and McCabe *1988*) and Cayuga Lake (Johnson et al. *2000*). Further, Creed and Sheldon (*1994*) found in a laboratory study that *Acentria* can cause more damage to Eurasian water milfoil than the weevil, and it seems likely that herbivore damage in the Vermont lakes was at least in part caused by *Acentria*. At the same time, it is very unlikely that the weevil caused significant damage to milfoil in Cayuga Lake because *E. lecontei* is present only in very low densities (0.03 individuals per apical meristem or ca. 6-10 individuals m^{-2}, Johnson et al. *2000*), whereas significant biomass losses have only been reported at weevil densities of 250 individuals m^{-2} in field studies (Creed and Sheldon *1995*) or approximately 350 individuals m^{-2} in tank studies (Newman et al. *1996*). Finally, the damage to milfoil caused by the two herbivores can clearly be distinguished and was predominantly caused by *Acentria* in Cayuga Lake, visible as removed tips and the remains of feeding cocoons made out of milfoil leaves. *Acentria* actively feeds on milfoil when water temperatures exceed 10°C, as would be the case from May to November in Cayuga Lake. Thus, it is present during most of the active growing period of milfoil.

The total amount of milfoil biomass removed by *Acentria* in our mesocosm experiment was small. The tanks with highest larval stocking densities had biomass losses due to herbivory of only 17% after 3 weeks. This value lies, however, well within the range of plant losses due to herbivory in terrestrial and other aquatic systems (Landsberg and Ohmart *1989*; Cyr and Pace *1993*; Hairston and Hairston *1993*), and the effect would have been more pronounced if we had distinguished between decaying and freshly green milfoil tissue. *Acentria*-damaged and broken milfoil shoots seldom produced side shoots or adventive roots, making regrowth from this tissue unlikely. In our tanks with the highest stocking density the combined main shoot length was reduced by almost 40%, and nearly 90% of the apical meristems were completely damaged by herbivory. The average lengths of the main shoots were reduced by almost 30%, which was sufficient to prevent canopy formation in these treatments. Although lateral branching had started by the end of the experiment, new side shoots also had substantial damage to their apical tips (72%).

Continuous removal of meristematic tissue in *Myriophyllum* should have pronounced long-term effects beyond the 3 weeks of our study because the competitive advantage of milfoil relies heavily on the formation of a canopy (Smith and Barko *1990*; Madsen et al. *1991*). Recurrent tip damage to milfoil should reduce resource allocation to the roots and thus inhibit regrowth in succeeding years (Newman et al. *1996*). In contrast, herbivore damage to *Elodea* in our study was minor and did not significantly reduce plant biomass. Due to its highly plastic growth form, *Elodea* is able to form new side shoots at all nodes even when leaves are missing or when the usually green stem turns brown as a result of herbivory. We did not observe increased *Elodea* biomass in our tanks with highest larval stocking densities, but the long-term impact of *Acentria* herbivory on plant competition could not be followed in our experiment because the maximum time we could run the mesocosm experiment was constrained by the developmental rate of *Acentria*. Further, since our tanks were only 50 cm deep we had already observed canopy formation by milfoil in the control tanks by the end of the second week. Nevertheless, despite the small size of our tanks and the relatively short time span of the experiment, we observed significant effects of *Acentria* on the performance of *Myriophyllum* and not on *Elodea*. With the effective removal of the apical meristem and a continuous presence of *Acentria* a vigorous regrowth of milfoil would not occur. Our field observations show that in Cayuga Lake *Myriophyllum* has failed to form a canopy since early in the 1990s. Most apical meristems are damaged by *Acentria* herbivory. We conclude that the prevention of canopy formation in milfoil due to *Acentria* very likely caused the shift of dominance from

milfoil to *Elodea* in Cayuga Lake. Selective herbivory by vertebrates (fish and waterfowl) in lakes in The Netherlands has been shown to change macrophyte community structure due to selective feeding (Van Donk *1998*).

Effect of spatial heterogeneity on the herbivory of *Acentria* on macrophytes

Littoral zones offer heterogeneous habitats due to different sediment characteristics, slope, wind exposure and other factors. This often results in a patchy distribution of macrophytes. We observed this in Cayuga Lake for *Elodea* and *Myriophyllum*. In 1996 minimum and maximum biomass found in the individual small quadrates sampled (0.25 m^2) in the 11 large quadrants varied between 0 and 172 g DW m^{-2} for *Myriophyllum* (mean ±1 SE: 16.5±3.8 g DW m^{-2}) and between 1 and 270 g DW m^{-2} for *Elodea* (mean ±1 SE: 94.8±14.0 g DW m^{-2}). Thus, the patches varied in dominant species and community structure. This heterogeneity is difficult to address in tank experiments of the size we used. At present, we have no detailed data about the heterogeneous distribution of *Acentria* larvae in the field. In most cases, only dense milfoil stands were sampled for *Acentria* for the purpose of relating larval abundance to damage of apical meristems (Johnson and Gross, unpublished data). We did not quantify densities on other macrophytes. In dense patches of milfoil we usually find 0.7-1.4 larvae per apical meristem (Johnson et al. *2000*), yielding between 140 and over 2,000 larvae per square meter. Since most females of *Acentria* are wingless, dispersal of this species should be restricted to the vicinity where the female had emerged. This would result in higher densities of *Acentria* in suitable macrophyte patches (e.g., dense *Myriophyllum* areas). These moth larvae may not be able to control any further their density because milfoil patches are not evenly distributed. This is similar to Kouki's (*1991a*), observation that females of *Galerucella nymphaea* (Chrysomelidae, Coleoptera) feeding on waterlily leaves fly close to the water surface in a random search and are not able to locate dense host plant patches from long distance. Life-cycle dependent redistribution of *G. nymphaeae* may strongly affect the damage experienced by the host plant *Nuphar lutea* (Otto and Wallace *1989*; Kouki *1991b*). In Cayuga Lake, however, *Elodea* and other macrophytes offer alternative food sources when milfoil abundance is low. From these refuge plant species *Acentria* may recolonize milfoil when it again increases in dominance.

Herbivory and competition among macrophytes

Many studies have shown that grazing by zooplankton and macroinvertebrates can strongly influence phytoplankton and epiphytes, leading to grazing-resistant or grazing-tolerant species and to changes in species composition (e.g., Gulati et al. *1992*; Lampert *1987*, *1994*). Herbivory can affect plant competition in two ways. First, herbivory often changes plant growth or morphology, thus changing access to resources, and second, it alters the relative abundance of some species, thereby changing resource availability for the competitors (Louda et al. *1990*). In Cayuga Lake, *Elodea* tolerates herbivory because *Acentria* larvae avoid feeding on its apical meristems. The meristems of *Elodea* seem to be more compact and impenetrable than those in *Myriophyllum* for the larvae. *Myriophyllum* suffers directly and indirectly from herbivory. The direct effect is the removal of biomass, although as we observed in our mesocosm experiment, this effect might not be very pronounced. Secondary effects of herbivory, including the removal of apical meristems can prevent the formation of a canopy. This, in turn, requires milfoil to invest in secondary growth. Further, by losing its canopy it surrenders its competitive dominance of the resource light and as a result suffers enhanced competition with other submersed macrophytes such as *Elodea*. Finally, with the loss of its apical tips, *Myriophyllum* loses a substantial amount of the allelochemical tellimagrandin II, which inhibits epiphytic algal growth (Gross *1999*, *2000*). In fact, the milfoil shoots in the tanks with the highest larval densities exhibited more epiphyte cover than those in the controls. These indirect effects ultimately dictate the long-term success or failure of the milfoil population.

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