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High abundance of herbivorous Lepidoptera larvae (*Acentria ephemerella* DENIS & SCHIFFERMÜLLER) on submersed macrophytes in Lake Constance (Germany)

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With 5 figures and 2 tables

Abstract: Seasonal changes in the abundance of the herbivorous moth larvae *Acentria ephemerella* on submersed macrophytes in Lake Constance were studied between 1998 and 2000. *Acentria* were found feeding on many pondweeds (*Potamogeton lucens, P. pectinatus, P. perfoliatus*), *Ceratophyllum demersum* and *Myriophyllum spicatum*, but not on *Chara* spp., *Najas marina* ssp. *intermedia* or *Elodea nuttallii*. During the vegetated periods of 1998 and 2000 the abundance of larvae on *P. perfoliatus* and *M. spicatum* increased exponentially from approx. 10 ind./m^2 to a maximum of 10,000 ind./m^2 or 20–165 ind./g dm. The much lower abundance in 1999 may be a result of the massive flooding of the lake in spring. Our data suggest that at least part of the *Acentria* population in Lake Constance may have two generations per year. Larvae use predominantly the upper shoot sections during the active feeding period and move to the lower stem sections for winter diapause. Herbivory caused substantial damage especially to the apical meristems of *P. perfoliatus* and *M. spicatum*. This study indicates that aquatic shredder-herbivores may have a stronger impact on submersed macrophytes than previously considered.

Key words: Aquatic lepidopterans, pondweeds, aquatic herbivory, life cycle.

Introduction

Submersed macrophytes are well known to increase settling substrate for bacteria, algae and macroinvertebrates in littoral zones. Among all macroinverte-

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brates colonizing aquatic angiosperms, true shredder herbivores, i.e. those purely feeding on angiosperm tissue but not on epiphytes, have often been ignored (NEWMAN 1991). Recently, the former concept that direct biomass removal of macrophytes due to herbivory is small (SHELFORD 1918, WETZEL 1983) has been questioned and revised (LODGE 1991, NEWMAN 1991, WETZEL 2001). Many studies have revealed the impact of both vertebrate and invertebrate herbivores on standing crop and community changes of submersed macrophytes (e.g., NEWMAN et al. 1996, VAN DONK et al. 1998, GROSS et al. 2001). The impact of aquatic insects on aquatic vascular plants has been considered less significant than damage caused by crayfish, fish, birds or mammals according to calculations by LODGE et al. (1998), whereas insect herbivory in terrestrial systems is well recognized to cause considerable damage (CRAWLEY 1983, ROSENTHAL & BERENBAUM 1992). Both aquatic angiosperms and aquatic herbivorous insects are secondarily aquatic, meaning they have terrestrial ancestors (NEWMAN 1991). Various aquatic insects have been shown to cause significant herbivore damage to the submersed macrophyte *Myriophyllum spicatum*, i.e. *Cricotopus myriophylli* (Chironomidae: Diptera; MACRAE et al. 1990, KANGASNIEMI & OLIVER 1983), *Euhrychiopsis lecontei* (Curculionidae: Coleoptera; CREED & SHELDON 1993, NEWMAN et al. 1996) and *Acentria ephemerella* (Pyralidae: Lepidoptera; PAINTER & MCCABE 1988, GROSS et al. 2001). The latter species is also known as *Acentropus niveus* (see BERG 1941, PASSOA 1988), and will be named *Acentria* in the following. Biomass removal of *M. spicatum* in mesocosms due to natural mid-summer densities of *Acentria* larvae (400 ind./m^2) was 17 % after three weeks (GROSS et al. 2001), a value well within the range of plant losses due to herbivory in terrestrial and other aquatic systems (3–33 %, LANDSBERG & OHMART 1989, CYR & PACE 1993, HAIRSTON & HAIRSTON 1993). Furthermore, high densities of *Acentria* (>0.8 larvae per apical meristem of *M. spicatum*) were considered responsible for the complete disappearance of *M. spicatum* from Canadian lakes (PAINTER & MCCABE 1988).

Data on the abundance of aquatic insect herbivores, especially considering spatial and temporal variation, are rare. So far, reported densities of *Acentria* larvae range from 0 to 300 ind./m^2 or 0 to 6 ind./g plant dry matter (MÜLLER-LIEBENAU 1956, SOSKA 1975, HEDAL & SCHMIDT 1992, NEWMAN & MAHER 1995, GROSS & KORNIJOW, in press). Estimating the system-wide impact of these insect herbivores requires knowledge on host plants, the heterogeneity of distribution and how the life-cycles of the herbivore and their host plants interact. Secondary effects of herbivory may include the loss of buoyancy and/or viability of broken shoots (CREED & SHELDON 1993, CREED & SHELDON 1994), or may interfere with the plant's resource allocation for overwintering (NEWMAN et al. 1996). Furthermore, absolute macrophyte biomass loss due to herbivory has to account for multiple leaf cohorts and the location of primary

damage (SAND-JENSEN et al. 1994). The site of herbivore damage is also closely related to the preferred location of macroinvertebrate herbivores on the plant and their use of the host plant for food, shelter and reproduction. Aquatic moth larvae (e.g., *Acentria, Cataclysma, Nymphula, Parapoynx*) hide in plant tissue while feeding (BERG 1941, MÜLLER & DEARING 1994, PETRISCHAK 2000; own observations). Egg laying, feeding and pupation of *Acentria* occur always close to the water surface on apical shoots. Larvae are positively phototactic and orient towards the water surface (BERG 1941).

At present, data suggest that *Acentria* is univoltine in mid-Europe, although some studies report two or three annual generations (BERG 1941, WESENBERG-LUND 1943, HAENNI 1980). Two generations per year have also been reported for the aquatic pyralid moth larvae *Nymphula nymphaeata* (REICHHOLF 1970) and *Cataclysma lemnata* (PETRISCHAK 2000). Adult emergence of *Acentria* is reported between May and August (HAENNI 1980, BÄNZIGER 2000) and larvae of three or four different instar stages are found throughout the vegetated period (BÄNZIGER 2000). Lepidoptera usually have 5–7 instar stages (WILLIAMS & FELTMATE 1992), and BERG (1941) suggests that the equal distribution of larvae of all sizes in the summer and the uni-modal distribution curve at other times indicate that *Acentria* has only one generation per year. Still, greenhouse experiments point out that under optimal food and temperature conditions, first instar *Acentria* larvae can reach pupal stage in 5–7 weeks (GROSS et al. 2001), allowing for more than one generation per year depending on environmental conditions.

This study was designed (i) to determine the seasonal and spatial abundance of all life-stages of *Acentria* on common submersed angiosperms in Lake Constance, (ii) to investigate the vertical distribution of host plant use by *Acentria*, (iii) to estimate the life-cycle and generation time of this aquatic insect herbivore and (iv) to relate the occurrence of this herbivore with herbivore damage on the respective macrophytes.

Material and methods

Study site

Lake Constance is a large, glacially scored, prealpine lake with three distinct basins (Überlinger See, Obersee, Untersee). Macrophytes occur only in small areas along the steep slopes of Überlinger See and Obersee, but cover wide areas in the much shallower Untersee. Macrophyte development in general depends strongly on the water level at the beginning of and during the vegetated period. Annual water level changes in Lake Constance exhibit an amplitude of 1.5–2.0 m, with low levels in winter and highest levels in June due to snow melt in the Alps (Fig. 1). Whereas the water level in 1998 was below average, the spring of 1999 was characterised by extreme flooding

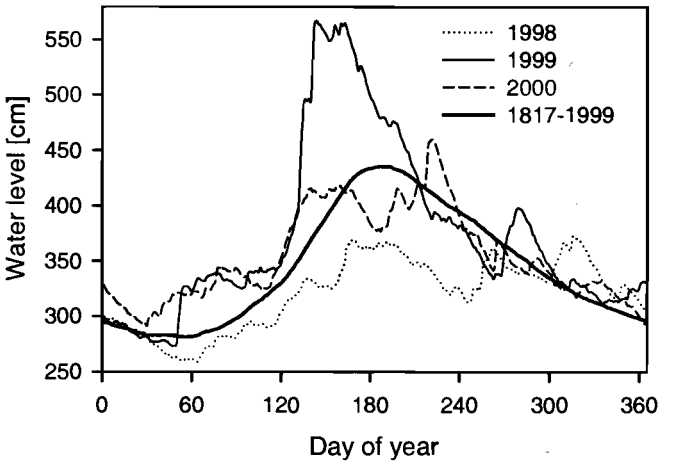

Fig. 1. Water level (Konstanz gauge) 1998–2000 (http://www.wetteronline.de/spezial/pegel.htm) and historic average (1817–1999) at Lake Konstanz measured at Konstanz, Harbour.

Table 1. Abundance of larvae, pupae and eggs of *Acentria ephemerella* on pondweeds *(Potamogeton pectinatus/PC, P. perfoliatus/PF, P. lucens/PL)* at four different locations in Lake Constance (Überlinger See) in 1998. Data are means ± 1 s.e.m, n = number of replicate samples.

Date	Location[a]	Plant	n	Plant biomass [g dm/m^2]	Larvae [ind./m^2]	Pupae [ind./m^2]	Larvae [ind./g dm]	Pupae [ind./g dm]	Egg clutches
25/06/98	1	PC	5	94±25	30±17	4± 2	0.3±0.2	0.1±0.0	
14/07/98	1	PC	5	107±11	132±21	26± 8	1.2±0.2	0.3±0.1	
03/08/98	1	PC	3	40±11	20± 8	20±16	0.5±0.3	0.8±0.6	
07/09/98	1	PC	4	N/A[b]	148±64	18±11	N/A	N/A	
04/06/98	2	PC[c]	19	–	–	–	0.6±0.3	0.3±0.3	
16/06/98	2	PC	11	62±42	0	7± 6	0	0.2±0.2	
05/08/98	3	PC	3	68±13	17± 7	30± 5	0.3±0.2	0.5±0.2	
05/08/98	3	PF	3	105±22	30±16	97±11	0.4±0.2	1.1±0.3	
05/08/98	3	PL	3	88±16	67±21	37± 9	1.0±0.5	0.5±0.2	
25/06/98	4	PF	1	18	40	0	2.2	0	2

[a] Location 1: NNE shore, Egger Hafen; 2: NNE shore, Wassersport Universität, Egg; 3: S shore, Meersburg; 4: NNE shore, Pumpwerk, Staadt.

[b] No biomass measurement possible. *P. pectinatus* was already decaying and it was impossible to retrieve plant parts among other macrophytes (*Chara* and *Najas*) in this sample.

[c] Abundance only based on dry weight, not area.

with water levels up to two meters higher than the long-term annual mean. In 2000, the water level followed more or less the mean curve of the water level gauge for Lake Constance.

We sampled pondweeds and *M. spicatum* from depths of 1.5 to 3 m at five locations in Lake Constance. *P. pectinatus, P. perfoliatus* and *P. lucens* were sampled at four sites in Überlinger See (sites no. 1 to 4; see Table 1). Sites 2 to 4 were checked only once or twice, but site 1 was sampled every three to four weeks in 1998. The macrophyte stands were located by shoreline markers and GPS. Site 1 was dominated by *P. pectinatus* (sago pondweed) emerging in early May, although *Chara* forms an understory in June, and during August the decaying sago pondweed is completely replaced by *Chara* and *Najas marina* ssp. *intermedia*. At all sampling sites only the short-lived form of *P. pectinatus* was present (cf. VAN WIJCK 1988). The main sampling site for this study (no. 5) was located in Untersee at the SSW shore of the island of Reichenau and was a wind-exposed shallow-sloped shore allowing lush macrophyte development. Here we sampled three macrophytes (*P. pectinatus, P. perfoliatus* and *M. spicatum*) at intervals of three to four weeks during their growing period. In 1998, all samples were taken at the sub-location 'NZ' (Niederzell). There, *P. pectinatus* and *M. spicatum* grew adjacent to large stands of *P. perfoliatus*. The next year, pondweeds were sampled at NZ whereas milfoil occurred only approx. 300 m further east at sub-location 'CA' (campground). In 2000, milfoil was sampled at CA and pondweeds at both NZ and CA. Stands of *P. pectinatus* and *P. perfoliatus* remained more or less at the same location during all three years, whereas *M. spicatum* patches exhibited high inter-annual fluctuations both in location and biomass development. In 2000, milfoil grew scattered and less dense than in the years before, when large and uniform stands were present. Due to the extreme flooding in 1999, sampling at site 5 was delayed until the beginning of August, as road and motorboat traffic were unable to gain access. Because in 1998 and 2000 most larvae at site 5 were found after July, we consider this delay not crucial.

Sampling procedure and processing

Sampling was done either by snorkelling or by SCUBA diving. We used a square aluminium frame of 0.1 m^2 which was gently slipped over the macrophyte stands (*Potamogeton* spp., *M. spicatum*) to the bottom. All stems within the frame were removed above the sediment and collected in tight closing plastic bags. We expected no significant loss of *Acentria* larvae or pupae during this sampling procedure because they usually build cases firmly attached to the leaves or stems. The samples were taken randomly within the macrophyte stands omitting the edge of the plant bed. Three to five replicates per macrophyte species and site were taken each time. Although the main objective of this study was to investigate the abundance of *Acentria* on pondweeds and *M. spicatum*, small plant samples from macrophytes growing in the vicinity of the sampled plant patches (*Chara* spp., *Elodea nuttallii, Najas marina* ssp. *intermedia*) were taken at irregular intervals to check for the occurrence of *Acentria*.

Samples were stored in coolers and then transferred to a 4 °C cooling chamber at the institute. They were processed within one or two weeks. Plant shoots from *P. perfoliatus* and *M. spicatum* were sorted and measured. We measured total shoot length, number of nodes and the number of side shoots. The proportion of damaged apical meristems and leaves was calculated by recording undamaged vs. damaged plant organs.

Only damaged meristems and leaves typically caused by *Acentria* were counted. Leaf damage was easier to asses in *P. perfoliatus* with its broad leaves than in *M. spicatum*, where often whole leaves were removed and it was impossible to distinguish whether they had been removed mechanically or due to herbivory. Every shoot was checked on a light table for the presence of *Acentria* larvae and the vertical position of the larvae on the stem was recorded. Larvae are very cryptic and hard to see, especially the first instar stages which usually mine into the leaves. Later samples (summer and autumn 2000) were subdivided immediately after the field trip into apical meristem (internodes <5 mm), upper (25 cm below tip), middle and lower (30 cm above root) shoot to prevent *Acentria* from migrating or detaching from the substrate before the sample was fully analysed. Larvae were kept in separate cultivation jars depending on location and plant type. Later their head capsule width was measured (see below). Pupae, egg clutches and adults were also registered. Plant material was dried for 24 h at 105 °C (or until constant mass was reached) for dry mass determination.

Larval instar stages

The size of the larvae was determined by measuring their head-capsule width using digital imaging (stereomicroscope coupled to a CCD camera and PC equipped with software, either Optimas 5.1 or Wiss. Werkstätten Konstanz). Precision of measurements was ± 3 %. Since *Acentria* instar stages are not distinct in size, DYAR's rule (DYAR 1890) was used to convert the headcapsule measurements into instar size classes. Based on laboratory rearing experiments (GROSS, unpubl. data), mean headcapsule width for first and second instar were determined to be 215 and 291 µm. This indicates a geometric growth factor of 1.35 which is close to the overall factor of 1.4 that DYAR (1890) found for 28 different species of Lepidoptera. With a growth factor of 1.35 between instars, the mean headcapsule sizes for instars III–V were determined as 394, 533 and 722 µm, respectively. Most (aquatic) Lepidoptera have five to seven instar stages (WILLIAMS & FELTMATE 1992). We calculated the following size ranges for the different instars: I: <250 µm, II: 251–350 µm, III: 351–470 µm, IV: 471–630 µm, V: 631–850 µm. Larvae larger than 851 µm were considered to be instar VI. For *Acentria*, mostly five instar stages have been reported so far (BATRA 1977, HAENNI 1980, BÄNZIGER 2000). These studies used larger size intervals for the instars, which may explain the difference to our results.

Statistical analysis

Data were analysed with the statistical packages JMP 3.2.1 or SigmaStat 2.3. Data were tested for normal distribution and homogeneity of variance. Larval densities were log (x+10) transformed and analysed with the Student's t-test (June and August 1998), or the Mann-Whitney U-test (September 1998). Abundances of *Acentria* on *P. perfoliatus* and *M. spicatum* in 2000 at site 5 were compared using a t-test on log (x+10)-transformed means for each sampling date. Multiple comparisons were sequentially Bonferroni-corrected. Distribution of larvae in different shoot sections of *M. spicatum* and *P. perfoliatus* were compared using χ^2 or Fisher's Exact test depending on sample size.

Results

Spatial distribution and seasonal dynamics

Acentria larvae were found at multiple locations in Lake Constance (Überlinger See and Untersee) associated with pondweeds (*Potamogeton lucens, P. pectinatus, P. perfoliatus*), and *M. spicatum*. Larvae were never found associated with or actively feeding on *Chara* spp., *Najas marina* ssp. *intermedia* or *Elodea nuttallii*. Some larvae were found on *Ceratophyllum demersum* sampled at 5 m depth from another site in Untersee, but no quantitative analysis of abundance was performed.

Larvae were found in mean densities of 0–8000 ind./m^2 or 0–120 ind./g dm depending on location, plant and sampling date, with highest abundance seen usually at the end of the vegetated period (Table 1). The density of pupae ranged from 0–160 ind./m^2 or 0–8.2 ind./g dm, with higher numbers reached in late summer before plants started to decay. Eggs were found from the end of June to the end of September, indicating the presence of adults at that time. Egg clutches each consisted of approximately 150 to 400 eggs.

During 1998, site 1 was sampled regularly for *P. pectinatus* and showed peak larval abundance in July when plant biomass was at it's maximum (Table 1). A similar high aerial abundance was observed in September. At this time no exact dry weight of the decaying *P. pectinatus* amidst *Chara* could be determined. At site 5, regular sampling of *P. pectinatus, P. perfoliatus* and *M. spicatum* was undertaken from 1998–2000. In 1998, abundance of *Acentria larvae* on *P. pectinatus* showed a different pattern compared with site 1, exhibiting a maximum in the beginning of August when plants start to decay. However, even in August no significant differences were found between site 1 and 5 for this host plant (Mann-Whitney U-test, $z = 1.77$, $P = 0.1$). Densities in 2000 on this plant were similar on both sub-sites 5 (CA and NZ) with 43–140 larvae/m^2 in mid to late summer while early summer samples yielded neither larvae nor pupae (Table 2).

Both *P. perfoliatus* and *M. spicatum* exhibited substantial species-specific, seasonal and inter-annual variation in biomass development (Fig. 2 A–C). The population dynamics of *Acentria* larvae on *P. perfoliatus* and *M. spicatum* were similar in 1998 and 2000, exhibiting a steady, almost exponential increase during the vegetated period (Fig. 2 D and 2 F). However during 1999, the population dynamics were totally different (Fig. 2 E). The abundance of *Acentria* larvae on *P. perfoliatus* increased from July to October 1998 from approx. 100 to almost 6000 ind./m^2 lake area and on *M. spicatum* the density increased from 15 to 8000 ind./m^2. In June 1998, densities were significantly higher on *P. perfoliatus* compared to *M. spicatum* ($df = 9$, $t = 3.86$, $P = 0.004$), but later in the season no significant differences were found. In 1999, only 50–

Table 2. Abundance of larvae, pupae and eggs of *Acentria ephemerella* on various submersed macrophytes *(Myriophyllum spicatum/MS, Potamogeton pectinatus/PC, P. perfoliatus/PF)* at site 5 in Lake Constance 1998 to 2000. Locations campground (CA) and Niederzell (NZ) differed in the presence of certain species over the years. Data are means ± 1 s.e.m, n = number of replicate samples.

Date	Location	Plant	n	Plant biomass [g dm m^{-2}]	Larvae [ind./m^2]	Pupae [ind./m^2]	Larvae [ind./g dm]	Pupae [ind./g dm]	Egg clutches
23/06/98	NZ	PC	5	85±11	42±32	4±2	0.8±0.7	0.1±0.0	
23/06/98	NZ	PF	3	295±10	97±29	0	0.6±0.1	0	
23/06/98	NZ	MS	5	193±57	14±8	2±2	0.1±0.04	0.01±0.01	
16/07/98	NZ	PC	5	118±15	6±4	80±19	0.1 + 0.0	0.7±0.2	
16/07/98	NZ	PF	5	138±12	0	13±3	0	0.1±0.0	
16/07/98	NZ	MS	5	269±112	12±4	8±4	0	0.01±0.01	2
06/08/98	NZ	PC	3	19±2	580±65	160±36	29.9±0.6	8.2±1.8	
06/08/98	NZ	PF	5	134±36	986±181	76±38	7.2±0.7	0.5±0.1	1
06/08/98	NZ	MS	3	410±196	340±85	7±7	0.8±0.2	0.01±0.01	1
01/09/98	NZ	PF	5	136±54	1322 + 259	8±4	10.0±1.2	0.1±0.0	7
01/09/98	NZ	MS	5	385±128	1538±170	92±13	4.3±0.8	0.3±0.1	6
22/09/98	NZ	PF	5	54±9	5796±920	4±2	119.7±17.5	0.1±0.1	2
22/09/98	NZ	MS	3	336±146	7973±1454	17±9	24.2±2.4	0.1±0.0	
06/08/99	NZ	PF	3	106±12	47±23	43±17	0.4±0.2	0.4±0.2	
06/08/99	CA	MS	3	151±55	80±50	0	0.4±0.1	0	
03/09/99	NZ	PF	3	223±75	80±10	3±3	0.5±0.2	0.01±0.01	
03/09/99	CA	MS	3	125±49	100±45	0	0.8±0.1	0	
22/09/99	NZ	PF	3	149±5	90±65	0	0.6±0.5	0	
22/09/99	CA	MS	3	198±47	87±29	0	0.4±0.1	0	
22/10/99	NZ	PF	3	81±11	57±29	0	0.7±0.4	0	
22/10/99	CA	MS	3	136±26	77±19	3±3	0.5±0.1	0.01±0.01	
26/05/00	CA	PC	3	24±3	0	0	0	0	
26/05/00	CA	PF	3	80±12	17±7	0	0.2±0.1	0	
26/05/00	CA	MS	3	4±0.3	0	0	0	0	
26/05/00	NZ	PC	3	8±2	0	0	0	0	
26/05/00	NZ	PF	3	29±8	0	0	0	0	
16/06/00	CA	PC	3	84±25	0	0	0	0	
16/06/00	CA	PF	3	87±8	0	0	0	0	
16/06/00	CA	MS	3	10±0.2	0	0	0	0	
16/06/00	NZ	PC	3	70±22	0	0	0	0	
16/06/00	NZ	PF	3	35±10	0	0	0	0	
06/07/00	CA	PC	3	153±149	63±13	0	0.4±0.1	0	
06/07/00	CA	PF	3	395±98	80±16	47±19	0.3±0.20	0.1±0.03	
06/07/00	CA	MS	3	55±10	3±3	0	0.1±0.1	0	
06/07/00	NZ	PC	3	54±10	43±5	10±8	0.9±0.1	0.3±0.2	
06/07/00	NZ	PF	3	398±20	0	0	0	0	

Table 2. Continued.

Date	Location	Plant	n	Plant biomass [g dm m^{-2}]	Larvae [ind./m^2]	Pupae [ind./m^2]	Larvae [ind./g dm]	Pupae [ind./g dm]	Egg clutches
03/08/00	CA	PC	3	234±16	50±31	77±36	0.2±0.2	0.4±0.2	5
03/08/00	CA	PF	3	248±25	216±100	3±3	0.8±0.4	0.01±0.01	4
11/08/00	NZ	PF	3	354±99	103±24	20±15	0.3±0.04	0.04±0.03	6
01/09/00	CA	PC	1	78	140	40	1.8	0.5	1
01/09/00	CA	PF	3	221±40	256±111	87±47	1.1±0.5	0.5±0.2	6
01/09/00	CA	MS	3	137±14	17±10	7±7	0.2±0.1	0.04±0.04	
01/09/00	NZ	PF	3	400±68	90±65	33±18	0.3±0.3	0.1±0.1	
27/09/00	CA	PF	3	231±34	2833±673	0	14.1±6.4	0	7
27/09/00	CA	MS	3	175±50	433±101	47±47	2.6±0.3	0.2±0.2	
27/09/00	NZ	PF	3	225±43	2520±342	0	11.4±1.0	0	
27/10/00	CA	PF	3	N/A[d]	2685±452	0	N/A	0	
27/10/00	CA	MS	3	106±23	470±225	0	4.2±0.7	0	

[d] Plant biomass data not available because stems with larvae were kept for later experiments.

100 ind./m^2 were found and densities neither differed between macrophyte species nor during the sampling period, from the beginning of August until end of October. In 2000, densities on *P. perfoliatus* increased from 17 to 2800 ind./m^2 at location CA and from 0 to 2500 ind./m^2 at NZ. Although no larvae were found at NZ during most of the summer, the abundance in late summer and autumn at both sites was basically the same (Fig. 2 F). *Acentria* were first recorded on *M. spicatum* at the beginning of September 2000 and then increased from 26 to 470 ind./m^2. Larval densities at site CA on *M. spicatum* were always lower than on *P. perfoliatus* ($t = 3.89$, $P = 0.01$), whereas no significant difference was found between *P. perfoliatus* at CA and NZ or between *P. perfoliatus* at NZ and *M. spicatum* at CA ($t<2.62$, $P>0.05$).

Densities of pupae usually peaked in mid to late summer with maximum densities of approx. 100 ind./m^2. In autumn, pupae were rarely observed with the exception of 47 pupae/m^2 on *M. spicatum* at site CA. Maximum abundance of pupae on *P. perfoliatus* usually peaked before highest densities were reached on *M. spicatum* (Fig. 2 G and 2 I).

Since *Acentria* are closely associated with macrophytes, we also calculated the abundance of *Acentria* based on plant dry weight and shoot number. During 1999, densities stayed low and constant at about 0.4–0.8 ind./g dm. In 1998 and 2000, based on plant dry weight, larval densities were always considerably higher on *P. perfoliatus* (1998: 0.5–110 ind./g dm, 2000: 0.2–34 ind./g dm) than on *M. spicatum* (1998: 0.04–24 ind./g dm, 2000: 0–4.2 ind./g dm, Table 2). We also calculated the number of larvae per apical shoot (main shoot and side shoots longer than 10 cm; tabulated data not shown). In 1998, abundance

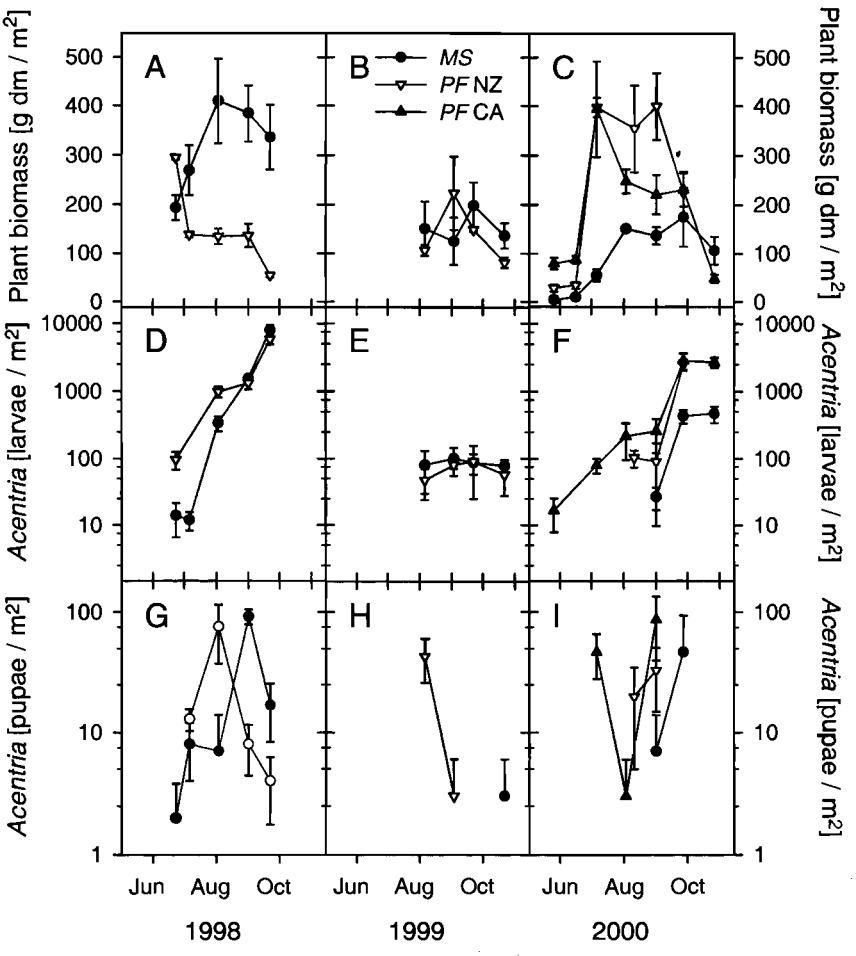

Fig. 2. Seasonal abundance of *Acentria* larvae on *Potamogeton perfoliatus* (*PF*) and *Myriophyllum spicatum* (*MS*) in Lake Constance (Untersee) from 1998–2000. **A–C)** Aboveground biomass of macrophytes. **D–F)** Abundance of larvae based on lake area. **G–I)** Abundance of pupae based on lake area. CA and NZ indicates two sublocations at sampling site 5. *MS* in 1998 originated from site 5-NZ and in 1999 and 2000 from site 5-CA. No *Acentria* larvae or pupae were present at some dates (e.g., summer 2000 for *PF* at NZ, and *MS*). Data are mean values ± 1 s.e.m., n = 3–5 for each data point. Negative error bars were only plotted if they did not exceed the lower x-axis.

of *Acentria* larvae per *P. perfoliatus* main shoot increased from 0.35 to 28.8 ind./shoot (0.13–27.1 ind./shoot, if long side shoots were included). Densities per main shoot on *M. spicatum* in 1998 were 0.1–32 ind./shoot (0.1–8.5 ind./shoot if long side shoots were included). In 2000, abundance on *P. perfoliatus* main shoots increased from 0.08 to 13.3 ind./shoot (May to Sep-

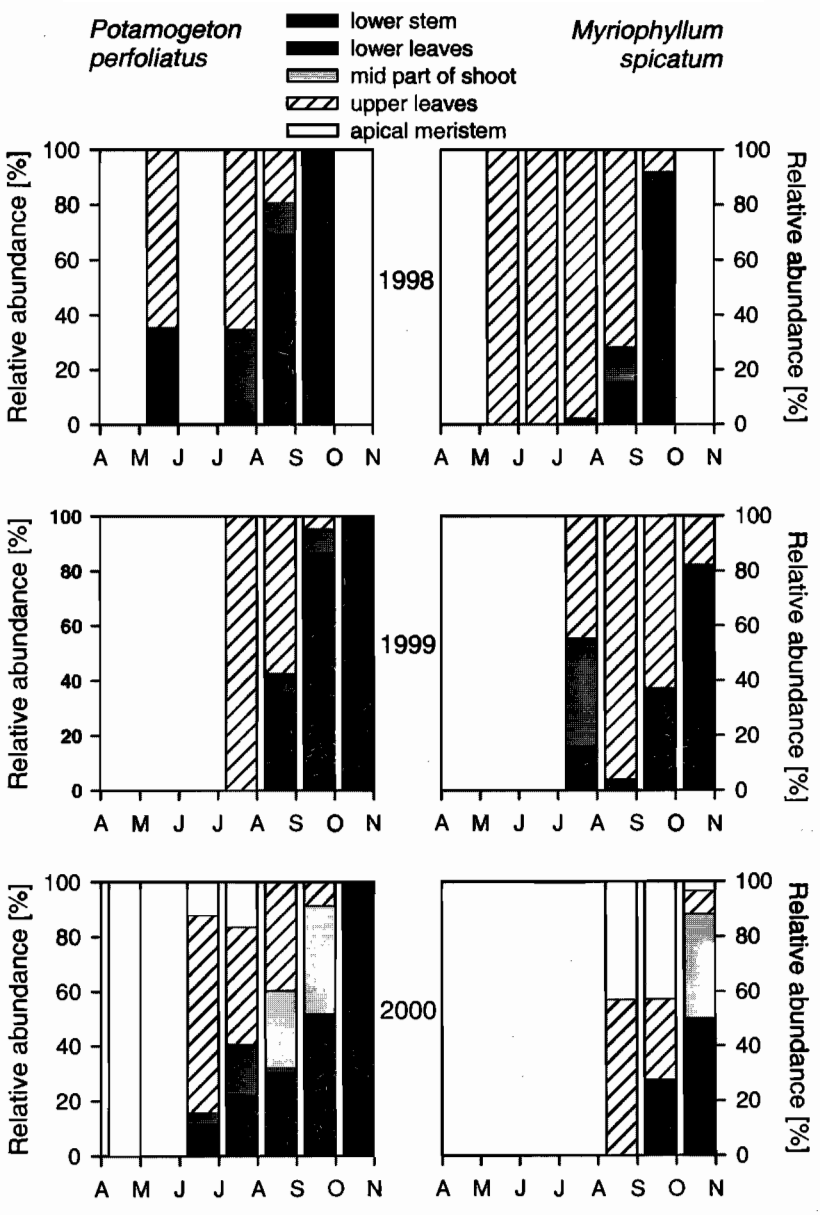

Fig. 3. Vertical distribution of *Acentria* larvae on shoots of *Potamogeton perfoliatus* (*PF*) and *Myriophyllum spicatum* (*MS*) from 1998 to 2000. Dates are according to Fig. 2 but were equidistantly distributed for better visibility. Calculations of relative abundance is based on the following absolute numbers: *PF* 1998 (from left to right) n = 18, 226, 212, 2699; *MS* 1998 n = 7, 4, 46, 510, 2180; *PF* 1999 n = 12, 14, 23, 10; *MS* 1999 n = 18, 24, 16, 23; *PF* 2000 n = 3, 25, 49, 84, 650, 790; *MS* 2000 n = 7, 122, 129.

tember) whereas only 0.1 to 2.1 ind./shoot were observed on *M. spicatum* from the beginning of September until end of October.

Vertical distribution on host plant

Acentria were found predominantly on upper leaves and apical meristems of both *P. perfoliatus* and *M. spicatum* during summer (Fig. 3). During the active feeding period, significantly more larvae were found on upper leaves of both macrophytes (upper 25 cm) than on lower shoot sections (e.g., *P. perfoliatus* May 26 and July 6, 2000: Fisher's Exact Test, $P = 0.02$). In autumn, *Acentria* moved to the lower shoots and mined into the stems. At the end of the summer, hibernating *Acentria* were lined side by side in the lower stems of both macrophytes. More larvae used the lower leaves (1998) or mined in the stems (2000) of *P. perfoliatus* compared to *M. spicatum*. In general, fewer larvae moved into *M. spicatum* stems than *P. perfoliatus* stems and this movement was later on milfoil than on the pondweed (e.g., Sep 3 or 22, 1998: $\chi^2 > 196.7$, $P < 0.0001$). In all years of our research, we observed a shift in vertical habitat preference of *Acentria* larvae from apical leaves in spring and summer to lower stems in autumn.

Larval size

In late spring, only large larvae of instar V or higher were found. Larvae found on *P. pectinatus* at site 1 in May 1999 had a mean head capsule width of 835 ± 31 µm ($n = 19$). The mean size increased slightly from May to June (924 ± 25 µm; $n = 17$). In July 1999, larvae were significantly smaller than in May of June (461 ± 46 µm, $n = 12$; one-way ANOVA, $df = 2$, $F = 46.88$, $P < 0.0001$). A post-hoc test revealed that head-capsule width did not differ significantly between May and June (Tukey-Kramer HSD, $P > 0.05$). Similar observations were made with larvae on *P. perfoliatus* in 2000. A detailed measurement of instar stages was performed for larvae found on *P. perfoliatus* and *M. spicatum* in summer and early autumn of 1998 and 2000 (Fig. 4). In August 1998 the frequency of larvae in instars II–V is very similar (9–33 %) and similar distribution was observed for larvae found in August 2000 on *P. perfoliatus* (no larvae were found on *M. spicatum* at that time). At the end of September most larvae were found in instar IV (41–76 %) with a mean head capsule width of approx. 550 µm.

Feeding damage

Feeding damage by *Acentria* on apical meristems and leaves was cumulative over the vegetated period (Fig. 5). In 1998, herbivory caused damage to apical meristems of *P. perfoliatus* ranging from 46 % (June) to 100 % (September/Oc-

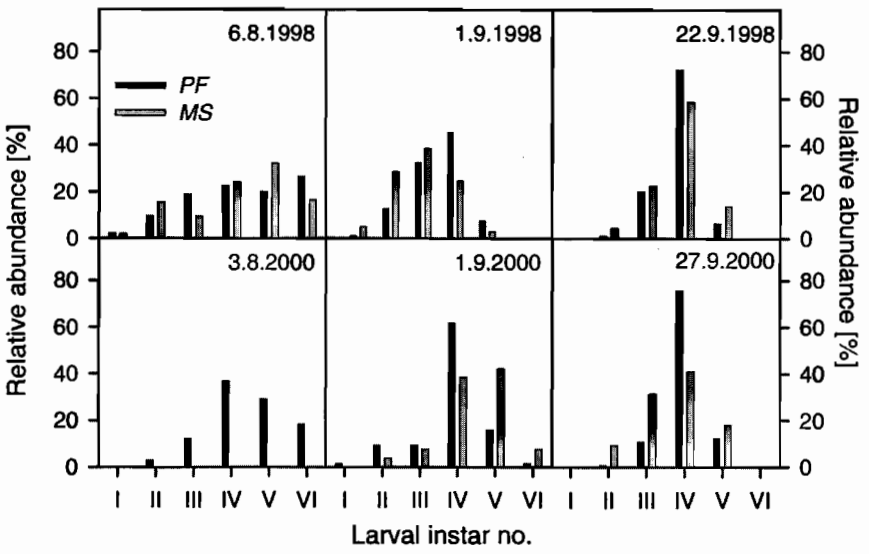

Fig. 4. Size-distribution of *Acentria* larvae found on *Potamogeton perfoliatus* (*PF*) and *Myriophyllum spicatum* (*MS*) in mid to late summer of 1998 and 2000. Larvae were grouped into instar stages according to size: I: <250 µm, II: 251–350 µm, III: 351–470 µm, IV: 471–630 µm, V: 631–850 µm. Larvae bigger than 851 µm were considered to be in the VI instar. Relative abundance is based on the following absolute numbers: 6.8.1998 *PF* = 472, *MS* = 96; 1.9.1998 *PF* = 710, *MS* = 566; 22.9.1998 *PF* = 1714, *MS* = 1772, 3.8.2000 *PF* = 65; 1.9.2000 *PF* = 63, *MS* = 26; 27.9.2000 *PF* = 808, *MS* = 127.

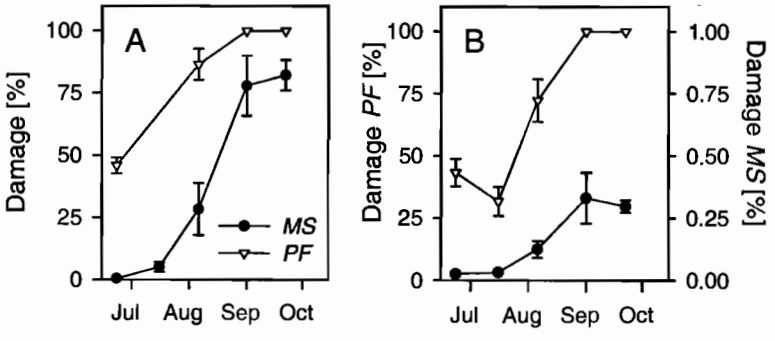

Fig. 5. *Acentria* feeding damage to apical meristems (**A**) and leaves (**B**) of *P. perfoliatus* (*PF*) and *M. spicatum* (*MS*) in 1998. Note the different y-axis for leave damage for *M. spicatum* (B, right axis).

tober, Fig. 5 A). Damage to apical meristems of *M. spicatum* was always lower (0.5–82 %, Fig. 5 A) and followed the colonization of this macrophyte by *Acentria*. Damage to leaves of *P. perfoliatus* was similar to apical meristems, accumulating from 43 to 100 % (Fig. 5 B). Herbivore damage on *M. spicatum*

leaves was much lower (less than 0.5 %; Fig. 5 B). In 1998, only damaged leaves were included in the damage rating and missing leaves were omitted. Therefore, the damage rating was repeated in 2000, at times of highest abundance of *Acentria*. Damage to milfoil leaves was 13 and 18 % at the end of September and October, respectively. The apical meristems exhibited damage of 21 and 12 %. Missing plant parts accounted for 32 and 62 % (leaves) and 28 and 65 % (apical shoots) at these dates. At the end of September 2000, site 5 CA exhibited 85 % missing and 9 % feeding damaged apical meristems of *P. perfoliatus*. Similar values were found for site 5 NZ, with 81 % missing and 18 % damaged tips.

Discussion

This study reveals that a true shredder-herbivore, *Acentria ephemerella*, occurs regularly in high abundance feeding on several submersed macrophytes. The occurrence on *Potamogeton* spp., *Myriophyllum spicatum* and *Ceratophyllum demersum* and the avoidance of *Chara* spp. is in agreement with other investigations of the autecology of this herbivorous aquatic moth (BATRA 1977, BUCKINGHAM & ROSS 1981, JOHNSON et al. 1998). However, *Acentria* has been observed feeding or case building on *Elodea canadensis* and *Najas minor* (BERG 1941, BUCKINGHAM & ROSS 1981, GROSS et al. 2001), whereas we never found *Acentria* associated with *Elodea nuttallii* and *Najas marina* ssp. *intermedia* in Lake Constance. Whether the pattern we observe in Lake Constance is based on larval choice for milfoil and pondweeds, or the avoidance of certain species of *Elodea* and *Najas* is based on chemical deterrents, needs to be addressed in further preference and no-choice tests.

Acentria exhibited an exponential increase in abundance on both *P. perfoliatus* and *M. spicatum* in 1998 and 2000. On the short-lived *P. pectinatus*, more larvae and pupae were detected at the end of the growing period of this macrophyte. Especially in late summer, variation in *Acentria* abundance between different patches of the same macrophyte (*Potamogeton pectinatus, P. perfoliatus*) is low and indicates that larvae spread evenly over host plants in a lake. Higher abundance on the tannin-rich *M. spicatum* (based on lake area, but not on plant biomass) occurred only in 1998 after the more palatable *P. perfoliatus* started to decline. Otherwise, more larvae were associated with *P. perfoliatus* than *M. spicatum*. Further laboratory investigations are needed to test host plant choice of *Acentria* between *P. perfoliatus* and *M. spicatum* with special regard to the potential defensive polyphenolic compounds in *M. spicatum*. Preliminary results indicate a slower growth on *M. spicatum* compared to *P. perfoliatus*, which might be due to the polyphenols present in milfoil (CHOI et al., in press).

Abundances of several thousand individuals per square meter observed in autumn in Lake Constance is about 1 to 2 orders of magnitude higher than previously reported in Europe (MÜLLER-LIEBENAU 1956, SOSKA 1975, HEDAL & SCHMIDT 1992). Densities per apical shoot (longer than 10 cm) were comparable to, or higher, than those found in the Finger Lakes, USA by JOHNSON et al. (2000). From August onwards, densities of *Acentria* larvae per shoot were in most cases higher than 0.8 ind./shoot on both *M. spicatum* and *P. perfoliatus*. Abundances higher than 0.8 ind/shoot were considered to cause a decline of *M. spicatum* (PAINTER & MCCABE 1988). Our data also indicate that *Acentria* can recover rapidly from adverse years, such as 1999, when densities were much lower, probably due to the spring flooding of Lake Constance.

The strong decline in larval abundance during winter may be due to dislocation of plant remains by wind and wave action, and mortality (BAREISS & GROSS, unpubl. data). Only 1 to 5 % of the larvae were found in spring. Contrary to the Finger Lakes, no palatable plants (*M. spicatum, C. demersum*) remain green in Lake Constance, providing refuge for larvae during winter. All decaying plant debris at the wind-exposed shore at site 5 were gone by January (BAREISS & GROSS, unpubl. results). At present, we neither know where larvae stay over winter nor how they locate regrowing plant patches in spring. Larvae may survive winter in dislodged macrophyte stems in deeper water and use phototaxis to migrate into macrophyte stands at shallower depth in spring. Positive phototropism was already observed by BERG (1941). During the spring flooding of 1999 in Lake Constance, lower light intensity at the site of emergence of macrophytes may have interfered with such migration patterns of *Acentria*, and subsequently have resulted in the overall low abundance that year.

Acentria damages predominantly the apical meristem of *M. spicatum*, the site of highest nutrient content, but also the highest concentration of the algicidal polyphenols, especially tellimagrandin II (GROSS 2000). The loss of allelopathically active compounds was considered responsible for the higher epiphyte density on *M. spicatum* shoots subjected to herbivory (GROSS et al. 2001). Additionally, compact apical shoots offer a better protection from visually oriented predators, such as fish. Insect herbivores are not immune to fish predation (SUTTER & NEWMAN 1997). Further, positioning pupae close to the water surface is advantageous for emerging adults of *Acentria* (BERG 1941). Our data show that larvae start to migrate into the lower stems of all host plants when these start to decay. Downward-migration starts earlier in *P. perfoliatus* than in *M. spicatum*, whose growing period starts and ends later in Lake Constance. Although not many shoot fragments of macrophytes were found during winter, we assume that burrowing into the lower stems offers the best survival chances for hibernating larvae. Further studies are needed to quantify losses during the winter (see above). So far, we have only little infor-

mation about vertical host plant use of aquatic insect herbivores. Comparable distributions of *Acentria* in apical tips and later in stems have been observed by BERG (1941). GRILLAS (1988) described that *Haemonia* (*Macroplea*) *appendiculata* (Coleoptera: Chrysomelidae), another herbivore on *M. spicatum*, uses lower stems and roots during pupal stage and upper shoots for the imagos. *Euhrychiopsis lecontei* uses lower stems of *M. spicatum* for pupation (NEWMAN et al. 1996), probably because those offer better stability and structural integrity than upper shoot sections.

Laboratory studies and field observations indicate that under optimal conditions, *Acentria* may have more than one generation per year (HAENNI 1980, BUCKINGHAM & ROSS 1981, GROSS et al. 2001). Size classes of larvae on *P. perfoliatus* and *M. spicatum* in September 1998 and 2000 exhibit a uni-modal distribution curve similar to the one observed by BERG (1941). He interpreted this distribution as evidence for only one generation of *Acentria* per year. Theoretically, hatched larvae from early egg clutches in June may complete a full life-cycle by September, when the last egg clutches were found (BERG 1941, GROSS et al. 2001). Pupae in Lake Constance were found from June through September, allowing for a second generation of *Acentria* for at least part of the population. Although we have no proof of two generations, our data suggest that at least some *Acentria* complete a full generation cycle during the summer. We conclude this according to the wide distribution of larval size in August, the occurrence of eggs until the end of September, and the exponentially increasing abundance of larvae from spring to autumn. On average, perhaps there are 1.5 generations per year.

Apart from the question of how many generations are present, many other aspects of the life-cycle remain unclear. For example, how do *Acentria* larvae diapause at locations, where the only palatable food is the short-lived *P. pectinatus*, as found at some locations in Überlinger See. During August, none of the macrophytes replacing *P. pectinatus* (*Chara* spp., *Najas marina* ssp. *intermedia*) are palatable to *Acentria* (JOHNSON et al. 1998, GROSS, unpubl. results) which means the larvae have to survive for eight to nine months partly at temperatures above 10 °C before the next *P. pectinatus* shoots emerge. Larvae are actively feeding at temperatures above 10 °C (GROSS, unpubl. results).

In autumn 1998, every apical meristem of *P. perfoliatus* and 82 % of the apical meristems of *M. spicatum* were damaged or missing; similar values were observed for herbivore damage to the leaves of *P. perfoliatus*. The extremely low visible damage to *M. spicatum* leaves (less than 0.5 % when missing leaves are not included) can either be explained by the complete removal of leaves when *Acentria* feeds on this macrophyte, as observed in laboratory studies, or by sloughing during leave turnover. Since in 1998 we did not distinguish between missing and damaged meristems or leaves, a similar qualitative damage screening was performed in 2000. At the end of September, at

both sampling sites (CA and NZ), more than 80 % of apical meristems of *P. perfoliatus* were missing and up to 20 % showed typical feeding damage caused by *Acentria*. Damage to *M. spicatum* leaves of 10 to 20 % was comparable to that of *P. perfoliatus* in 2000, suggesting we underestimated damage in 1998. Based on laboratory studies, we can expect that some of the missing leaves were also removed by this herbivore and not by mechanical damage. Yet, the higher percentage of removed leaves in October is probably due to the onset of senescence. These results corroborate earlier laboratory, mesocosm and field studies indicating substantial herbivore damage of *Acentria* on *M. spicatum* (JOHNSON et al. 1998, JOHNSON et al. 2000, GROSS et al. 2001). JOHNSON et al. (2000) found a strong negative correlation between numbers of larvae per apical meristem and the percentage of *M. spicatum* in late summer. In Cayuga Lake, NY, the decline of *M. spicatum* was associated with the high abundance of *Acentria* (JOHNSON et al. 1998).

The impact of this specialist herbivore on submersed macrophytes in Lake Constance is rather strong. According to a recent review, insect herbivory caused on average only 10 % reduction in standing crop of vascular aquatic plants (LODGE et al. 1998). However, in the particular graph, two studies exhibited reduction rates of 50 and 100 %, respectively. The latter is probably the study by PAINTER & MCCABE (1988) on *Acentria* herbivory in Canada. The low mean value in Lodge's study may result from generalist herbivores that only occasionally feed on vascular plants. The majority of herbivores are not obligate phytophages but generalist shredders, facultative herbivores or detrivores (NEWMAN 1991, KORNIJOW 1996). Damage caused by generalist insect herbivores is usually small, accounting for less than 10 % of the total food consumed (KORNIJOW 1996). Cumulative damage by *Acentria* on *P. perfoliatus* observed in our study, was much higher than that by Trichoptera on the same macrophyte larvae in Danish streams (JACOBSEN & SAND-JENSEN 1994, 1995). Based on our findings, we consider specialist insect herbivores much more effective in damaging freshwater macrophytes than generalists.

Even with low absolute biomass loss, herbivore damage to apical meristems may have systemic effects on *M. spicatum*. The meristems are the primary site of the allelopathically active tellimagrandin II and loss of meristem tissue may therefore interfere with the effect of this allelochemical against epiphytes and phytoplankton (GROSS 1999, GROSS 2000). Herbivore damaged milfoil plants apparently have higher epiphyte densities than undamaged control plants (NEWMAN et al. 1996, GROSS et al. 2001). Open wounds caused by herbivory may boost pathogen growth on milfoil (e.g., SMITH et al. 1989) and pondweeds. Further, apical shoots of *M. spicatum* are used for dispersal in autumn and herbivore damage to these organs may impair coloniziation of new patches in the next season. On the whole, the high abundance of *Acentria* at times of maximum macrophyte biomass (August to September) and observed

plant damage, confirm that aquatic insects are important herbivores in littoral zones.

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