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How to cope with a superior enemy? Plant defence strategies in response to annual herbivore outbreaks

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Summary

1. The perfoliate pondweed *Potamogeton perfoliatus* L. constitutes large monospecific macrophyte patches in many Central European lakes. Correlative evidence from the field suggests that *P. perfoliatus* is under an increasing grazing pressure during its short vegetation period from May to September due to seasonal outbreaks of the aquatic moth *Acentria ephemerella* Denis & Schiffermüller. We used a mesocosm experiment to determine the influence of *A. ephemerella* herbivory on *P. perfoliatus* shoot development and resting bud production and to study the defence strategies of this macrophyte.

2. Herbivory resulted in a reduction of the *P. perfoliatus* vegetation period by more than 2 months thereby reducing the average resting bud size and the overall resting bud biomass sevenfold. This suggests that besides its severe immediate effects, herbivory affects *P. perfoliatus* growth and dynamics also during the subsequent season.

3. As a response to herbivory *P. perfoliatus* translocated nutrients (phosphorus (P) and nitrogen (N)) from leaves towards buds. *Acentria ephemerella* larvae had a high P content, implying P limitation of larval growth especially within the herbivory treatment. This suggests that at least the P translocation from leaves towards resting buds may be viewed as an anti-predator strategy rather than as a nutrient conservation strategy.

4. *Acentria ephemerella* herbivory changed the allocation strategy of *P. perfoliatus* in the size versus number of resting buds: only the number, but not the size of resting buds was reduced under shoot grazing by *Acentria ephemerella*. This change in the number versus size trade-off might allow the plant to produce a minimum resting bud size necessary for successful sprouting in the next spring.

5. *Synthesis*. Overall, our results suggest an escape syndrome (after Agrawal & Fishbein, *Ecology*, **87** (2006) S132) as a defence strategy against herbivory for *P. perfoliatus*, consisting of a shortening of the growth period, a translocation of nutrients and a change in allocation strategy. The increased plant senescence that was accompanied by the shortening of the growth period has further implications for the usage of macrophyte patches as a habitat for invertebrates and fishes and for the structure of littoral food webs.

Key-words: *Acentria ephemerella*, defence strategies, herbivory, macrophyte, nutrient translocation, outbreaks, *Potamogeton perfoliatus,* senescence, stoichiometry, tolerance–escape syndrome

Introduction

Herbivory often has a strong impact on the population dynamics, life histories and composition of plant communities. Especially insect herbivores can inflict substantial feeding damage on plants, as their population densities can strongly increase seasonally, resulting in herbivore outbreaks (Wallner 1987; Liebhold *et al.* 2000). One group of herbivores notorious for outbreak dynamics are, for example, forest lepidopterans (Myers 1993, 1998; Daniel & Myers 1995; Peltonen *et al.* 2002), and peak densities of these species result in a massive defoliation of trees over large spatial ranges.

Outbreaks resulting in severe plant defoliations depend on the absence of efficient herbivore control mechanisms either by top-down (predators or parasites) or bottom-up (plant defences) factors (Wallner 1987; Liebhold *et al.* 2000). Outbreaks of forest lepidopterans may be regulated by predation on e.g. the pupal (Hara & Higashiura 1995; Liebhold *et al.*

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2000; Liebhold, Raffa & Diss 2005) or larval stages (Auerbach 1991; Maron, Harrison & Greaves 2001; Harrison, Hastings & Strong 2005). Furthermore, the ability of herbivores to overcome plant defences, and the frequency of outbreaks, may depend on the specific defence strategies adopted by plants. It is well known that many terrestrial plant species have developed morphological or chemical defence mechanisms (Stowe *et al.* 2000; Hanley *et al.* 2007), but the defences of aquatic plants against herbivore outbreaks have been rarely studied. Surface and tissue structures employed in herbivore defence have been shown to occur in marine seagrasses (Verges *et al.* 2007, 2008) but have not yet been found in submerged freshwater plants (Lodge 1991; Lodge *et al.* 1998; Hanley *et al.* 2007). While lacking such morphological defence strategies, some species of freshwater and marine plants are producing and storing allelochemicals in damaged tissues as an anti-herbivore defence (Newman, Kerfoot & Hanscom 1996; Bolser *et al.* 1998; Wilson *et al.* 1999; Kubanek *et al.* 2000; Choi *et al.* 2002). However, for many taxonomic groups of freshwater macrophytes, for example the genus *Potamogeton*, chemical or morphological anti-herbivore defence strategies have not yet been described (Lodge 1991; Lodge *et al.* 1998; Choi *et al.* 2002). *Potamogeton* species, such as *Potamogeton perfoliatus* L., are the dominant macrophytes in many lakes (Scheffer, de Redelijkheid & Noppert 1992; Lehmann, Jaquet & Lachavanne 1997; Wolfer & Straile 2004; Sandsten & Klaassen 2008) and often form large monospecific patches. *Potamogeton perfoliatus* and *P. pectinatus* L., for example, are important host plants of the aquatic moth *Acentria ephemerella* Denis & Schiffermüller (Gross, Feldbaum & Choi 2002), which is commonly found in lakes and coastal waters throughout Europe (Berg 1942; Gross, Feldbaum & Choi 2002; Miler, Korn & Straile 2008). Although *A. ephemerella* is a polyphagous species, it avoids a number of macrophytes (e.g. *Chara* spp. and in some lakes also *Elodea nutalli* (Planch.) St. John and *Najas minor* Allioni, Gross, Feldbaum & Choi 2002) and is less abundant on tannin-rich *Myriophyllum spicatum* L. than on *Potamogeton* spp. (Gross, Feldbaum & Choi 2002). Growth experiments showing fast *A. ephemerella* growth and development on a *P. perfoliatus* diet suggest a high nutritional quality of *P. perfoliatus* (Choi *et al.* 2002). Although the dominating role of *P. perfoliatus* in lakes is in contrast to its high nutritional quality for herbivores, potential anti-herbivore strategies have so far not been studied in detail.

The interaction between *A. ephemerella* and *P. perfoliatus* is highly seasonal: *P. perfoliatus* regrows in May every year from resting buds and starts to decay after a short growing period of from June to September (Wolfer & Straile 2004). Reproduction of *P. perfoliatus* takes place primarily via vegetative growth of clones, and seeds are only of minor importance for patch persistence and expansion (Wolfer & Straile 2004). In Lake Constance, a large (476 km^2) and deep ($z_{\max}$ = 252 m, mean depth 100 m) warm monomictic lake situated in the pre-alpine region between Germany, Switzerland and Austria, *P. perfoliatus* is a dominant macrophyte species (Schmieder 1997; Miler, pers. observ.) and an important host for *A. ephemerella*. Hence, *A. ephemerella* in Lake Constance depends to a large extent on *P. perfoliatus* for population development which, consequently, is restricted to the rather short vegetation period of this plant. During this period *A. ephemerella* completes 2–3 life cycles and its density increases over several orders of magnitude (Gross, Feldbaum & Choi 2002; Miler 2009). The population increase is followed by a long period from autumn to spring, in which *A. ephemerella* diapauses in the larval stage inside senescing plant stems. Population density in spring is several orders of magnitude lower than that in late summer suggesting a large mortality during this period (Gross, Feldbaum & Choi 2002; Miler 2009). What causes this mortality is currently unknown, but predation and/or relocation of senescing plant stems to unfavourable habitats are possible explanations. The latter probably results in failure of the larvae to find host plants after emergence from diapause in spring. Surviving larvae continue to grow until metamorphosis. This cycle is repeated annually with spatial and interannual variability in population growth rates and maximum densities (Gross, Feldbaum & Choi 2002; Miler 2009), presumably due to variability in fish predation pressure (Miler, Korn & Straile 2008; Miler 2009). The current evidence for a strong feeding damage on *P. perfoliatus* by *A. ephemerella in situ* is based on field observations: increasing densities of *A. ephemerella* larvae in macrophyte patches in Lake Constance during the summer months were associated with increasing signs of feeding damage, which were observed in up to 100% of the leaves (Gross, Feldbaum & Choi 2002). Despite such strong effects of the herbivore on *P. perfoliatus*, no direct evidence of defence traits or strategies has yet been found in *P. perfoliatus*. Furthermore, it is not clear, how strongly *A. ephemerella* influences the duration of the overall growing season of *P. perfoliatus* and the production of resting buds, which is crucial for successful sprouting in the next spring and hence for patch persistence.

Here, we investigate in a mesocosm experiment the trophic interactions between *A. ephemerella* and *P. perfoliatus* to better understand the mechanisms involved in the feeding relationships in macrophyte beds and the defence mechanisms of *P. perfoliatus* against herbivory.

More specifically we ask the following questions:

1 Can *A. ephemerella* shorten the growing period of *P. perfoliatus*?

2 Does *A. ephemerella* herbivory affect the resting bud production of *P. perfoliatus*?

3 Does herbivory influence the allocation and translocation of nutrients in *P. perfoliatus*?

Materials and methods

We analysed the trophic interactions between larvae of the water moth *A. ephemerella* and the perfoliate pondweed *P. perfoliatus* in a mesocosm experiment during the summer 2005 at the Limnological Institute of the University of Konstanz, Germany. In total, 24 experimental units were randomly assigned to two treatments (*A. ephemerella* herbivory and control) and three sampling dates (25 August, 16

September and 29 November), resulting in four replicates per treatment and sampling date.

The 24 experimental units were placed in an outdoor pool (length = 10.5 m, width = 5 m, depth = 1.5 m) with 30 cm of fine sediment from Lake Constance on the bottom and filled with water from Lake Constance. The sediment was mixed before the experiment to provide the same environmental starting conditions for each experimental unit. The experimental units consisted of round, flexible, 1.5 m long plastic tubes (transparent Tricoron foil; RKW AG Rheinische Kunststoffwerke, Wasserburg, Germany) with a diameter of *c.* 0.8 m, each held vertical in the water by a floating polystyrol frame with an area of 0.43 m^2 (0.6 m width, 0.715 m length) and covered on top with a gauze-covered wooden frame (length = 0.92 m, width = 0.73 m, mesh size *c.* 1620 μm). The gauze prevented the *A. ephemerella* imagines from dispersing out of the experimental units and allowed the winged *A. ephemerella* males to fly around and search for females, i.e. the experimental set-up allowed for *A. ephemerella* reproduction. The experimental units were checked daily for imagines to document successful *A. ephemerella* development. In addition, drifting *A. ephemerella* larvae were reported. They indicate dispersal behaviour because of food scarcity. The transparent plastic foil tubes separated the experimental units from the surrounding water in the mesocosm. Water in the experimental units was not changed during the experiments. In the sediment, the units were anchored by digging the lower end of each plastic tube into a sediment-filled mortar tray (length = 0.75 m, width = 0.5 m, height = 0.3 m) to prevent rhizome outgrowth and to be able to harvest also all plant below-ground biomass.

Shoots of *P. perfoliatus* and associated macroinvertebrates were sampled in June 2005 with a toothed sickle in *P. perfoliatus* patches in Upper Lake Constance. Directly after sampling, eight macrophyte shoots (0.2–0.3 m length) were planted into the sediment of each experimental unit. All *A. ephemerella* larvae were removed from the plants prior to planting. Shoots were allowed to produce roots. Shoot length was measured on 20 July to control for shoot growth and to provide an estimate for shoot mass before the start of herbivory.

Between 22 July and 8 August, *A. ephemerella* larvae were collected from *P. perfoliatus* patches in Lake Constance, and were introduced into the herbivory treatment to yield a start density of 22 larvae g^{-1} plant dry mass (Ind. g^{-1} dm) corresponding to 56 ± 33 SD larvae per experimental unit. In the control treatment, no *A. ephemerella* larvae were introduced. After the three sampling events, *A. ephemerella* larvae associated with *P. perfoliatus* were washed through a sieve (mesh size 45 μm) and fixed in 70% ethanol in 1 L plastic boxes. *Acentria ephemerella* pupae that were closely attached to the stems were hand collected from frozen above-ground plant material. Macrophytes were dried at 90 °C for 3 days and densities of *A. ephemerella* pupae and larvae were calculated as individuals per gram plant dry mass (Ind. g^{-1} dm). A dissecting microscope (Zeiss Stemi 2000-C; Zeiss, Oberkochen, Germany) was used to collect and count *A. ephemerella* larvae at a 10–50× magnification from samples. *Potamogeton perfoliatus* resting buds were dug out of the sediment and processed in the same way as shoots.

The contents of particulate phosphorus (P) and particulate nitrogen (N) were measured in leaves and resting buds of *P. perfoliatus* sampled on 25 August and in small (1st instar, headcapsule width (hcw) *c.* 245 μm) and large (probably 5th instar, hcw *c.* 1000–1170 μm) *A. ephemerella* larvae from field samples from Lake Constance. The N and P contents were calculated as the percentage of N and P on the total plant dry weight respectively. We additionally analysed the content of particulate carbon (C) in small and large *A. ephemerella* larvae and calculated the C : P ratio of larvae as the molar ratio of the C and P content.

The *P. perfoliatus* and *A. ephemerella* samples were dried at 90 and 60 °C, respectively, and ground to a powder. For the determination of the C and N content, *c.* 1 mg of the ground sample was placed into a tin cup (HEKAtech, Wegberg, Germany) and analysed with an NCS-2500 analyser (Carlo Erba Instruments/Thermo Scientific, Bonn, Germany). To determine the content of particulate P, *c.* 1 mg of the ground sample was filled in a glass vial and aliquots were filtered through acid-rinsed polysulfone membrane filters (HT-200; Pall, Ann Arbor, MI, USA) and digested with a solution of 10% potassium peroxodisulfate and 1.5% sodium hydroxide at 121 °C for 60 min, before soluble reactive phosphorous was determined using the molybdate–ascorbic acid method (Greenberg, Trussel & Clesceri 1985).

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

POPULATION DYNAMICS OF *A. EPHEMERELLA*

Acentria ephemerella larvae were introduced at the start of the experiment and they subsequently developed into pupae. A single maximum of imagines was detected in the experimental units developing from 3 August onwards (Fig. 1a, b). The single maximum indicates that only one generation of new larvae was produced in the mesocosms. As we checked only the water surface and not the water column for imagines, we observed more male than female imagines. This is due to a lower detection probability of females which stay aquatic also as imagines – in contrast to males which emerge from the water

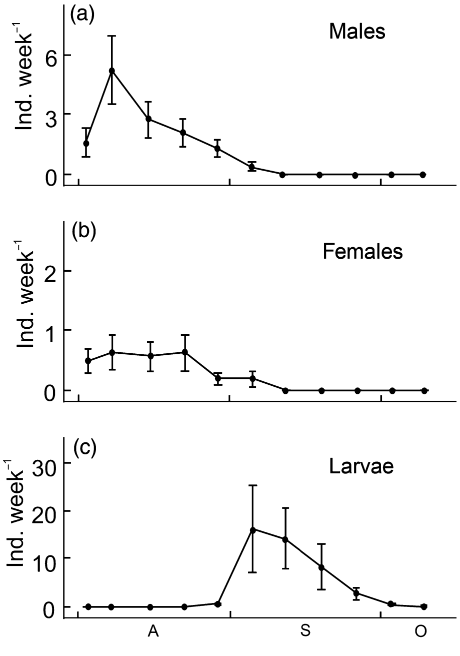

Fig. 1. Number of adult *Acentria ephemerella* males (a) and females (b) and drifting *A. ephemerella* larvae (c) per week in all experimental units of the herbivory treatment of the mesocosm experiment in August (A), September (S) and October (O) 2005.

column after metamorphosis. *Acentria ephemerella* successfully reproduced during the experimental period in the mesocosms resulting in increasing larval densities. Maximum densities of 84 ± 29 (mean ± SE) larvae g^{-1} dm were reached in the August samples. In September, densities of actively feeding larvae were one order of magnitude lower (8.7 ± 3.7 Ind. g^{-1} dm, mean ± SE) as a large part of *A. ephemerella* larvae had already gone into hibernation in plant stems or was drifting in the water column to disperse to other macrophyte patches. At the end of August, *P. perfoliatus* shoots in the herbivory treatment started to senesce and as a consequence first drifting larvae in the experimental units were found on 29 August (Fig. 1c), indicating food scarcity due to an overexploitation of their food plants. The number of drifting larvae rapidly increased to a maximum of 34 ± 14 (mean ± SE) larvae per experimental unit on 12 September and subsequently decreased to 0.25 ± 0.18 (mean ± SE) larvae per experimental unit on 12 October. Throughout the experiment no *A. ephemerella* larvae were found in the control treatment.

GROWTH OF *P. PERFOLIATUS*

Shoot biomass increased during the experiment when *A. ephemerella* was absent and decreased after larvae were introduced in the experimental units of the *A. ephemerella* herbivory treatment (Fig. 2a). Shoot biomass development in the two treatments differed significantly (ANOVA, factor *Acentria*: $F_{1,35} = 17.87$, $P = 0.0002$, factor sampling date: $F_{3,33} = 1.92$, $P = 0.15$, interaction *Acentria* × sampling date: $F_{3,33} = 5.34$, $P = 0.0047$). In the *Acentria* treatment, *P. perfoliatus* shoots were completely defoliated at the second harvest in mid-September, whereas in the control treatment leaves looked still green and healthy, with hardly any signs of senescence at the end of November; *A. ephemerella* thus reduced the growing season of *P. perfoliatus* in our experiment by more than 2 months.

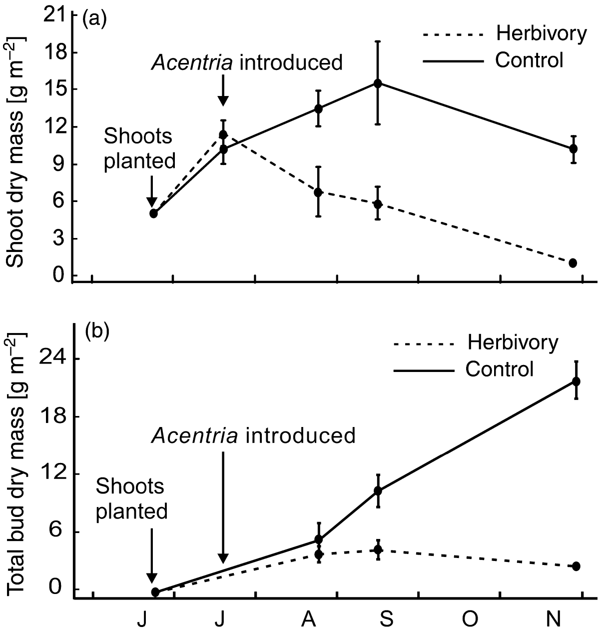

Fig. 2. Influence of *Acentria ephemerella* herbivory on the development of (a) shoot biomass (shoot dry mass [g m^{-2}]) and (b) resting bud biomass (bud dry mass [g m^{-2}]) in the herbivory treatment of the mesocosm experiment from June (J) to November (N) 2005.

Resting buds were found already on 25 August. From August onwards, resting bud biomass strongly increased in the control treatment until November, whereas in the herbivory treatment, maximum resting bud biomass was already reached in September (Fig. 2b). The development of resting bud biomass differed significantly between the two treatments (ANOVA, factor *Acentria*: $F_{1,22} = 65.7$, $P < 0.0001$, factor sampling date: $F_{2,21} = 16.31$, $P < 0.0001$, interaction *Acentria* × sampling date: $F_{2,21} = 23.49$, $P < 0.0001$). At the end of the experiment, resting bud biomass produced in the control treatment was sevenfold higher than in the herbivory treatment. Shoots in the control treatment continued to produce resting buds until November, whereas after the defoliation of shoots in September no further production was possible in the herbivory treatment.

The mean dry weight of resting buds significantly increased both in the herbivory and in the control treatment from 25 August to 16 September (Fig. 3a, ANOVA, factor sampling date: $F_{1,12} = 10.89$, $P = 0.0063$, factor *Acentria*: $F_{1,12} = 0.03$, $P = 0.8610$, interaction sampling date × *Acentria*: $F_{1,12} = 0.01$, $P = 0.9078$). However, from 16 September to 29 November the mean resting bud dry weight developed differently in the control and in the herbivory treatment (Fig. 3a, ANOVA, factor sampling date: $F_{1,12} = 783.68$, $P < 0.0001$, factor *Acentria*: $F_{1,12} = 10.04$, $P = 0.0081$, interaction sampling date × *Acentria*: $F_{1,12} = 11.53$, $P = 0.0053$): whereas there was a strong further increase in mean dry weight of resting buds in the control, there was hardly any change in the herbivory treatment. The number of resting buds strongly increased

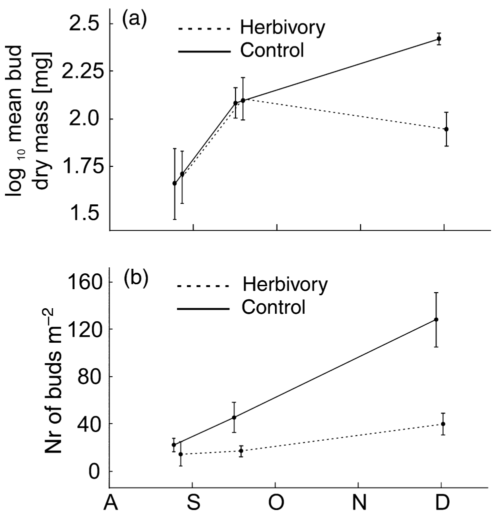

Fig. 3. Influence of *Acentria ephemerella* herbivory on (a) the mean dry weight (log $_{10}$ (bud dry mass [mg])) and (b) the mean number of resting buds m^{-2} in the herbivory treatment compared to the control treatment in the mesocosm experiment from August (A) to November (N) 2005.

in the control but hardly in the herbivory treatment (Fig. 3b, ANOVA, factor sampling date: $F_{2,16} = 61.18, P < 0.0001$, factor *Acentria*: $F_{1,16} = 65.67, P < 0.0001$, interaction sampling date × *Acentria*: $F_{2,16} = 22.39, P < 0.0001$).

ELEMENTAL ANALYSES OF *P. PERFOLIATUS* AND *A. EPHEMERELLA*

Acentria ephemerella herbivory influenced the elemental composition of the shoots and resting buds of *P. perfoliatus* (Fig. 4). The N content of the shoots was reduced by *A. ephemerella* herbivory and the N content of the resting buds increased with *A. ephemerella* herbivory, suggesting that there was a translocation of N from leaves to the resting buds (Fig. 4a). This is indicated by the significant interaction between the factors plant part and *Acentria* treatment (ANOVA, factor *Acentria*: $F_{1,12} = 0.13$, $P = 0.7$, factor plant part: $F_{1,12} = 3.4$, $P = 0.09$, interaction *Acentria* × plant part: $F_{1,12} = 5{,}56$, $P = 0.036$). By contrast, shoot P content was significantly lower than resting bud P content, irrespective of the influence of herbivory (Fig. 4b, ANOVA, factor plant part: $F_{1,12} = 131.86, P < 0.0001$). A tendency for nutrient translocation was, however, also observed regarding P as the interaction *Acentria* × plant part was marginally significant (ANOVA, factor *Acentria*: $F_{1,12} = 2.92, P = 0.11, P < 0.0001$, interaction *Acentria* × plant part: $F_{1,12} = 3.9, P = 0.072$).

The N content was significantly higher for small than for large larvae (Fig. 5a, ANOVA, factor developmental stage: $F_{1,6} = 212.26$, $P < 0.0001$). Furthermore, small larvae showed a tendency for a significantly higher P content than large larvae (Fig. 5b, ANOVA, factor developmental stage: $F_{1,6} = 5.34$, $P = 0.0603$). The C : P ratio of large larvae was significantly higher than that of small larvae (Fig. 5c, ANOVA, factor developmental stage: $F_{2,15} = 6.54$, $P = 0.0091$).

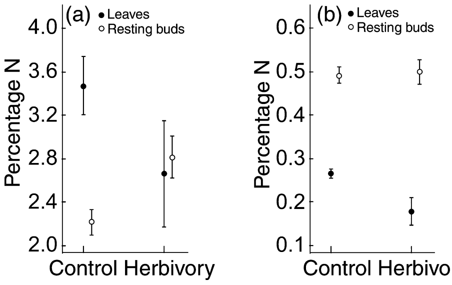

Fig. 4. Percentage (a) nitrogen (N) and (b) phosphorus (P) of leaves and resting buds of *Potamogeton perfoliatus* in the control and herbivory treatments in the August 2005 sampling of the mesocosm experiment.

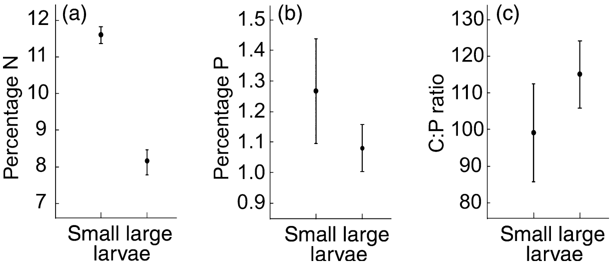

Fig. 5. Percentage (a) nitrogen (N), (b) phosphorus (P) and (c) C:P ratio of small and large *Acentria ephemerella* larvae from field samples in Lake Constance.

Discussion

This study shows a strong influence of larvae of the aquatic lepidopteran *A. ephemerella* on the biomass development of the macrophyte *P. perfoliatus*. This influence is due to density-dependent interactions, i.e. feeding pressure and defoliation by a high density of moth larvae, but most likely also due to the response of the plant to herbivory, i.e. its defence strategy. The latter consists of a translocation of biomass and nutrients to the herbivory-protected overwintering buds, thereby simultaneously reducing the herbivore's habitat and food resource.

Acentria ephemerella herbivory in our experiment significantly decreased the length of the growing season, the shoot and resting bud biomass, and the number and the average biomass of individual resting buds suggesting a strong influence on plant fitness, plant growth and plant ecosystem function. The resting bud biomass was reduced sevenfold by *A. ephemerella* herbivory suggesting that the effects of *A. ephemerella* will not be confined to the actual season but will also influence the shoot development in the following year. Nevertheless, the effect of *A. ephemerella* was probably underestimated as our experimental design did not allow for patch expansion of macrophytes. Consequently, biomass production in the experimental units of the control treatment was reduced because of the operation of density-dependent factors and shoot biomass did not increase further from the first to the second sampling in the control treatment. This suggests that if our experimental design had allowed for spatial expansion, the differences in biomass production between the herbivory and control treatment would have been even stronger.

On the other hand, *A. ephemerella* densities during the experiment were higher than *A. ephemerella* densities *in situ*, suggesting that herbivory effects *in situ* might not be as strong and immediate as in our experiment. However, Gross, Feldbaum & Choi (2002) report that 100% of apical meristems of *P. perfoliatus* show feeding damage due to *A. ephemerella* herbivory already in September. The growing season of *P. perfoliatus* in Lake Constance usually lasts until September, which is consistent with the herbivory treatment, but is considerably shorter than in the control treatment. Furthermore, a survey of the senescence status of several *P. perfoliatus* patches in Lake Constance revealed that the length of their growing season was significantly related to the numbers of *A. ephemerella* present in these patches at the end of July (Miler 2009). In patches with high *A. ephemerella* density, *P. perfoliatus* plants were completely defoliated due to *A. ephemerella* herbivory within a period of 2 weeks during August. Hence, even with a larval density lower than in our mesocosm there is convincing evidence that *A. ephemerella* is able to exert a strong grazing pressure on *P. perfoliatus*, eventually resulting in defoliation.

The fast growth of *P. perfoliatus* during the growing season in early summer, together with a high nutritional quality for *A. ephemerella* due to low tannin concentrations (Choi *et al.* 2002), suggests the tolerance–escape syndrome as a defence strategy of *P. perfoliatus* against herbivory (see Kursar & Coley 2003; Agrawal & Fishbein 2006). Agrawal & Fishbein (2006) suggested that plant defence strategies may be separated into three syndromes: The 'low nutritional quality' syndrome is characterized by plant traits that reduce the nutritional value and render the plants unattractive for herbivores, for example high physical plant defences (a high toughness of plant organs), digestibility-reducing compounds or a low water and nutrient content (Agrawal & Fishbein 2006). The second strategy is the 'nutrition and defence syndrome' that can be found in plants that show high edibility and digestibility, for example a high N content, high water content, high specific leaf area or a low toughness, together with toxic chemical defences against grazers (Agrawal & Fishbein 2006). Finally, the tolerance–escape syndrome has been proposed for plants in high-resource environments where they can grow fast (see Coley, Bryant & Chapin 1985; Kursar & Coley 2003). In these plants, a high nutrient content of the biomass and low chemical and morphological defences (toughness) allow for fast plant growth and consequently tolerance of herbivory and/or a seasonal escape from herbivory. The latter strategy seems to be especially important when there are seasonal fluctuations of herbivore abundance and/or activity (Hanley 1998). In *P. perfoliatus*, fast growth allows for building up a sufficiently high biomass of resting buds, enabling regrowth during the next year before the period of intense *A. ephemerella* herbivory starts, i.e. to escape from herbivory.

Compensatory responses as a reaction to grazing pressure, i.e. an allocation of biomass and nutrients towards grazed tissues, have been reported for terrestrial plant species, e.g. for Yellow Nutsedge (*Cyperus esculentus* L., Li *et al.* 2004), Arrowgrass (*Triglochin palustris* L., Mulder & Ruess 1998), Common Ragweed (*Ambrosia artemisiifolia* L., Throop 2005) and also marine seagrasses (*Posidonia oceanica* L., Verges *et al.* 2008) and macroalgae (Cerda *et al.* 2009). Likewise goldenrod (*Solidago altissima* L.), a clonal rhizomatous perennial, compensates defoliation by a univoltine leaf-chewing beetle (*Trirhabda* sp.). Partially, compensation in this system is achieved by delayed leaf senescence after the damage period (Meyer 1998). By contrast, we found no evidence for compensatory growth in *P. perfoliatus* and plant senescence seems to be rather enhanced during herbivory. With intense herbivory, which is the result of large within-season population increase of the multivoltine *A. ephemerella*, compensatory growth would provide herbivores with additional food resources, possibly resulting in further increased herbivory. Moreover, compensatory growth, i.e. the translocation of nutrients towards grazed tissues, would reduce the nutrient content in resting buds, thereby compromising shoot regrowth in the next spring. Consequently, compensatory growth in macrophytes and macroalgae has been observed at low or moderate herbivore pressure (Verges *et al.* 2008; Cerda *et al.* 2009), but not at high herbivore pressure (Steinberg 1995). This suggests that also in our system compensatory growth would be maladaptive. Hence, in contrast to the pattern expected from compensatory growth, *P. perfoliatus* seems to translocate nutrients, i.e. phosphorus and nitrogen, under herbivore pressure from grazed tissues to its resting buds (see below). This does not exclude the possibility of compensatory growth of *P. perfoliatus* at lower levels of herbivory but suggests that at a given herbivory pressure compensatory growth, i.e. tolerance, and escape should be regarded as two alternative strategies. Whether there is a possible switch from compensatory growth to an escape strategy with increasing herbivore pressure in the *A. ephemerella*–*P. perfoliatus* system should be a topic of future studies.

The allocation of resources to ungrazed and protected plant organs as a mechanism of grazing tolerance has been widely reported in the literature (Dyer *et al.* 1991; Stowe *et al.* 2000), with a major emphasis on N translocation (Honkanen & Jormalainen 2002; Fornara & Du Toit 2007; Schooler *et al.* 2007). This process has been mostly discussed within the paradigm of nutrient conservation. However, our data suggest that especially changes in P content might also be considered a defence strategy. Phosphorous content in resting buds was already rather high in the control treatment, suggesting that the primary adaptive value of the reduction in P content of the leaves under herbivory pressure is probably not the conservation of nutrients, but rather a reduction of the nutrient supply for *A. ephemerella*. The C:P ratio of especially small *A. ephemerella* larvae (80:1) is rather low compared to the C:P ratio of *P. perfoliatus* leaves (380:1 and 550:1 in the control and herbivory treatment respectively) suggesting a P limitation of *A. ephemerella* growth. Phosphorous limitation of growth has been demonstrated for tobacco hornworm (*Manduca sexta* L.) with a body C:P ratio of 160:1 growing on plants with a C:P ratio of 628:1 (Perkins *et al.* 2004). Likewise, the cladoceran *Daphnia* (C:P ratio *c.* 80:1) has been shown to be P limited at a threshold algal molar C:P ratio of 200:1 to 300:1 (Sterner & Hessen 1994; Demott, Gulati & van Donk 2001). The difference in C:P ratios between *A. ephemerella* and *P. perfoliatus* is at least of similar magnitude than the differences between herbivore and primary producer in the *Daphnia* and *Manduca* model systems, supporting the possibility of a P limitation for *A. ephemerella* even in *P. perfoliatus* plants without herbivory. The reduction of the P content of the leaves should further increase *A. ephemerella* P limitation and consequently decrease the growth rate of this herbivore.

Plant and animal species usually exhibit phenotypic plasticity in their allocation strategies regarding the number versus the size of their offspring (Stearns 1995; Roff 2003). For example, many sexually or clonally reproducing plant species trade-off the size of seeds (Vaughton & Ramsey 1998; Kery, Matthies & Spillmann 2000; Greenway & Harder 2007; Sadras 2007) and ramets (Johansson 1994; Winkler & Fischer 2001; Aarssen 2008), respectively, against their number. The changes in resting bud numbers and mean dry masses in our mesocosms suggest a resting bud dry mass – number trade-off under grazing and can be regarded as an accessory strategy to mitigate the effects of herbivory. In the absence of such a trade-off, a reduced primary production due to herbivory is expected to reduce both the size and numbers of resting buds. However, from August to September, the mean size of resting buds in the herbivory treatment increased as strongly as in the control and it did so at the cost of resting bud number: whereas in the control treatment the number of resting buds increased, plants in the *A. ephemerella* treatment did not produce more resting buds during this time. This change in biomass allocation may be important for plant success under intense herbivory, as presumably a minimum resting bud size is necessary for successful sprouting in the next spring (see e.g. Spencer 1987; Piqueras 1999). Likewise, the sensitivity of newly sprouted plants to herbivory decreases with increasing initial resting bud size (Elger, de Boer & Hanley 2007). By contrast, the number of buds produced is not necessarily important for plant fitness in established beds because of the density-dependent growth of resting buds during the subsequent growing season (Hidding *et al.* 2009). However, despite this change in allocation, mean size of resting buds at the end of the experiment was higher in the control than in the herbivory treatment, as from September onwards photosynthesis could have taken place only in the control treatment. This reduction in resting bud size may have important fitness consequences for *P. perfoliatus*.

The influence of *A. ephemerella* on the length of the growth period and the senescence status of the patches will affect fish and invertebrate communities in the lake littoral. Macrophytes provide habitat structure for fishes and macroinvertebrates and a refuge for young fishes (e.g. Diehl & Kornijow 1998; Warfe & Barmuta 2006). The removal of shoot biomass and an earlier senescence hence will interfere with these ecosystem functions of macrophytes. *Acentria ephemerella* thus acts as a key species influencing indirectly via earlier plant senescence, the fish and invertebrate communities in the littoral zone of lakes.

To conclude, we have shown that *A. ephemerella* has a strong influence on its food plant, which seems to respond to herbivory with an escape strategy associated with a change in biomass allocation towards the number versus size of resting buds. This defence syndrome may incorporate a strategy of controlled senescence, thereby reducing the habitat, energy and nutrients of the herbivore. The senescence seems to involve a reduction of P in plant leaves, which most likely increases the P limitation of herbivore growth. The important structuring role of macrophytes, including *P. perfoliatus*, in lake ecosystems suggests that the observed intense herbivory by *A. ephemerella*, but also the indirect effects of herbivory due to the plant's escape strategy will have striking consequences for ecosystem and food web dynamics.

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