The aquatic moth *Acentria ephemerella* as a key species in submerged aquatic vegetation - direct and trait-mediated interactions with predators and food plants

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Table of Contents

General Introduction

Predation and anti-predator responses

Food web interactions vary in space and time and are major forces structuring terrestrial and aquatic ecosystems. Inter- and intraspecific trophic interactions, such as competition, herbivory, parasitism and predation, shape the growth and mortality rates of organisms and thus enable the existence of food webs and the functioning of terrestrial and aquatic ecosystems.

Growth and mortality rates in turn largely determine the population development of a species so that mortality by predation has profound effects on the structure of populations and communities. Mortality by predation often reduces the densities of animal populations and is hence also referred to as a density-mediated interaction (DMI). In contrast to this lethal effect, non-lethal anti-predator responses often are induced in prey organisms, the so-called trait-mediated interactions (TMI) (Lima & Dill 1990; Preisser *et al.* 2005; Beketov & Liess 2007). Induced anti-predator defences are adapted to the current level of predation risk (Lima & Dill 1990) since predation risk in nature strongly varies in ecological time scales. They hence involve plastic phenotypical changes in morphological, physiological and life-history traits to enable the animals to survive and reproduce in a variable environment (Newman 1988; Agrawal 2001; Peckarsky *et al.* 2001).

Although behavioural changes due to a predation risk that is perceived by visual or olfactory cues occur in terrestrial as well as in aquatic animals (Lima & Dill 1990; Kats & Dill 1998; McIntosh & Peckarsky 1999; von Elert & Pohnert 2000), responses of morphological, physiological and life-history traits are often restricted to marine and freshwater habitats (Grostal & Dicke 1999; Dicke & Grostal 2001; Lass & Spaak 2003; Benard 2004) since the perception of predators is easier accomplished there through the higher persistence of predator odours in aquatic environments. Zooplankters (e.g. *Daphnia*), aquatic insects (e.g. damselflies) and other aquatic invertebrates develop spines or other morphological defences under fish predation (Johansson & Samuelsson 1994; Kats & Dill 1998; Lass & Spaak 2003; Mikolajewski & Rolff 2004). Life-history traits, for example size and age at metamorphosis, growth rates and developmental time, are highly flexible in many aquatic species with terrestrial adult stages. They have been shown to be reduced under fish predation in damselflies, mayflies and amphibians in order to decrease the amount of time that is spent in the vulnerable aquatic larval stage (Peckarsky *et al.* 2002; Dahl & Peckarsky 2003; Benard 2004; Mikolajewski *et al.* 2005; Relyea 2007).

An important life-history trait that strongly determines the population dynamics of many insects and can display plastic changes is the choice of developmental strategies, i.e. the decision to develop directly from the larval stage to metamorphosis or indirectly via an additional diapause life-stage. When the diapause life-stage is protected from mortality by predation, the decision for indirect development can be considered as a predator avoidance strategy. Predator-induced changes in diapause strategies occur in certain aquatic and terrestrial species, for example the two-spotted spider mite that reacts to a predatory mite with an increased diapause incidence (Kroon *et al.* 2005) and *Daphnia magna* that produces resting eggs in response to fish chemical cues (Slusarczyk *et al.* 2005). However, the influence of biotic interactions, especially the effect of predation, on voltinism patterns has not yet been studied in detail (but see Martin *et al.* 1991; Johansson *et al.* 2001) and deserves further attention.

In addition, Mikolajewski *et al.* (2005; 2007) highlighted the need to consider sex-specific approaches when plastic changes in life-history traits are studied. Although many animal species show a sexual dimorphism in life-history traits (Crowley 2000; Blanckenhorn 2005), the developmental pathways via which the dimorphism is achieved have however not been studied yet in detail (Mikolajewski *et al.* 2005; Mikolajewski *et al.* 2007). For example many arthropod species show a female-biased sexual size dimorphism (SSD, Blanckenhorn *et al.* 2007) with a weak connection between SSD and sex-specific differences in developmental time, i.e. sexual bimaturism (SBM). Hence the female-biased SSD of the studied taxa was mainly caused by a faster growth of the females, not by a larger developmental time dimorphism (Blanckenhorn *et al.* 2007). As a consequence, the foraging activity rates of females are probably higher than those of males, and hence females are probably more susceptible to predation. Furthermore, since environmental influences such as predation and food limitation have been shown to induce plastic changes in life-history traits (Day & Rowe 2002; Benard 2004), the sex-specificity of these plastic changes deserves further attention.

Indirect trait-mediated interactions

The above-described plastic anti-predator responses of animals can have indirect influences on other organisms: for example a spatial or diurnal change in the feeding behaviour of herbivorous mayfly larvae may result in a decreased grazing of epilithic algae (Culp *et al.* 1991; Peckarsky 1996). Likewise is the predation pressure on benthic macroinvertebrates decreased under a reduced foraging activity of predatory damselfly larvae. Thus for example in food webs involving trophic interactions between primary producers, herbivores and predators not only the direct consumption of herbivores has an effect on the growth and population development of plants and algae, but also behavioural changes in the foraging activity of grazers through predation (Lima & Dill 1990; Schmitz *et al.* 2004; Preisser *et al.* 2005; Bolnick & Preisser 2005). These indirect trait-mediated interactions often positively affect and enhance the growth of primary producers under grazing pressure when the feeding behaviour of the herbivores is changed by anti-predator strategies so that grazing is reduced (Gastreich 1999; Schmitz & Suttle 2001; Schmitz 2003; Rothley & Dutton 2006).

Furthermore, also herbivores, especially in terrestrial ecosystems, cause changes in the traits of their food plants, thereby influencing the population dynamics of other organisms (Ohgushi 2005; Ohgushi 2008). This is analogous to the above-described, indirect effects of the anti-predator responses of animal prey. Contrary to predation, vascular plants rarely suffer from lethal herbivory but can show strong changes in plant allelochemistry, cell structure, growth, physiology, morphology and phenology under grazing (Ohgushi 2005). For example herbivores that are spatially (i.e. feeding on different plant parts) or seasonally (i.e. feeding at different times) separated influence each other via herbivore-induced changes in plant traits (Gange & Brown 1989; Strauss 1991; Ohgushi 2005).

Ecosystem engineering occurs if the structure of plants or plant patches is physically altered, for example by herbivory, and this as a consequence affects the interactions between other species. Ecosystem engineers are defined as "organisms that directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials" (Jones *et al.* 1994). In terrestrial plants, physical modifications by insect ecosystem engineers are often observed, for example through gall makers, leafrollers, case bearers and stem borers, which provide new habitats to herbivores and/or their predators. Many herbivorous insects build shelters that can be subsequently used by other insects and function as refuges against predators (Damman 1987), as protection from unfavourable microclimates (Hunter & Willmer 1989; Larsson *et al.* 1997) and as a foraging habitat (Sagers 1992; Fukui *et al.* 2002). More research is needed to study the impacts of ecosystem engineers on trophic interactions, community organisation and ecosystem processes (Lawton & Jones 1995; Jones *et al.* 1997), especially in aquatic habitats.

Aquatic food webs - the ecological importance of macrophyte patches in lake littoral zones and the influence on fish and macroinvertebrate communities

In aquatic food webs, tri-trophic interactions often occur, involving primary producers, herbivores and predators (Strong 1992; Polis 1999). The relative importance of bottom-up and top-down processes, i.e. trophic effects that are cascading up and down through food webs, has been intensively discussed (Brett & Goldman 1997), and the existence of top-down cascading trophic interactions is well documented in a range of different, mainly aquatic ecosystems (Strong 1992), e.g. freshwater lakes (Carpenter *et al.* 1985; Carpenter & Kitchell 1993), rivers (Power 1990), coastal kelp beds (Estes *et al.* 1998) and pelagic zones in the ocean (Worm & Myers 2003). However, evidence for terrestrial trophic cascades is strongly increasing (Schmitz *et al.* 2000; Hebblewhite *et al.* 2005; Finke & Denno 2006). In many terrestrial ecosystems, tritrophic interactions with three trophic levels - predators, herbivores and primary producers - have been intensively studied (Strong 1992; Preisser 2003; Finke & Denno 2006). In freshwater ecosystems however, tritrophic top-down cascades have been mainly studied in pelagic (Carpenter *et al.* 1985; Carpenter & Kitchell 1993; Persson 1997; Bertolo *et al.* 1999) or lotic food webs (Power 1992). In lentic littoral ecosystems, the research focus has been mainly laid on algal-based food webs, particularly the benthic fish - snail - epiphyton food chain (Martin *et al.* 1992; Brönmark & Vermaat 1998; Jones & Sayer 2003) whereas interactions between vascular water plants, herbivores and their predators received comparably little attention (but see Sutter & Newman 1997; Ward & Newman 2006).

Patches of submerged freshwater macrophytes have an important structuring role in lakes, since they influence nutrient dynamics, sedimentation and resuspension (Barko & James 1998), microbial communities (Söndergaard *et al.* 1998; Wetzel & Söndergaard 1998), carbon dynamics (Wetzel & Söndergaard 1998; Wetzel 2001) and water turbidity (Faafeng & Mjelde 1998; van Donk 1998). Submerged aquatic vegetation furthermore increases the physical complexity of the littoral zones (Crowder & Cooper 1982; Crowder *et al.* 1998; Jeppesen *et al.* 1998) and offers thereby a foraging and refuge habitat for fishes and macroinvertebrates (Diehl & Kornijow 1998; Weaver *et al.* 1998; Warfe & Barmuta 2006). Hence trophic interactions in lake littoral zones should be strongly determined by the population dynamics, life-histories and trophic niches of the animal species inhabiting submerged macrophyte patches.

Macrophyte-associated macroinvertebrates either belong to exclusively epiphytic species or can be assigned to opportunistic taxa, such as *Asellus aquaticus* (Isopoda) or *Helobdella stagnalis* (Hirudinea) that also occur in typically benthic faunas, i.e. organisms on bottom sediments or stony substrates (Diehl & Kornijow 1998). Herbivorous macroinvertebrates use macrophytes as a food resource by grazing on periphyton that grows on macrophyte leaves (Brönmark & Vermaat 1998; Jones *et al.* 1998), feeding on macrophyte leaves and stems (Lodge 1991; Newman 1991; Lodge *et al.* 1998) and ingesting senescent epiphytic algae and plant tissues (Newman 1991; Kornijow *et al.* 1995). Predatory invertebrates, such as damselflies and dragonflies, in turn feed on herbivorous and detritivorous invertebrates (Power 1992; Turner & Chislock 2007) and constitute together with their prey the food organisms of fishes (Baker *et al.* 1999; Johansson & Brodin 2003).

Patches of submerged macrophytes in freshwater ecosystems are inhabited by adult individuals and especially young-of-the-year (Y-O-Y) fishes seeking shelter from predation (Casselman & Lewis 1996; MacRae & Jackson 2001) or searching for macroinvertebrates as food (Whitfield 1984; Dionne & Folt 1991; Diehl & Kornijow 1998). The abundances of epiphytic macroinvertebrates increase with the increasing physical complexity of a macrophyte patch (Cyr & Downing 1988; Savino *et al.* 1992; Warfe & Barmuta 2006), which in turn depends on the shape and density of the plants (Dionne & Folt 1991; Lillie & Budd 1992; Jeffries 1993; Eklöv 1997; Weaver *et al.* 1997). Previous studies suggest a medium density of plant stems, i.e. a medium habitat complexity, to be optimal for the functioning of macrophyte patches as a foraging and refuge habitat for fishes (Crowder & Cooper 1982). Although the abundances of invertebrate prey for fishes increase with increasing macrophyte complexity (Savino *et al.* 1992; Eklöv 1997; Warfe & Barmuta 2006), the foraging efficiency of benthivorous fishes and their piscivorous predators decreases (Crowder & Cooper 1982; MacRae & Jackson 2001), i.e. their visual contact to prey organisms (Savino & Stein 1982) and their manoeuvrability (Manatunge *et al.* 2000). Hence in macrophyte populations of a medium complexity the conflicting effects of habitat structure on prey capture (visibility and manoeuvrability), predation risk and invertebrate abundances are balanced and allow for maximum juvenile fish abundances.

Herbivory on freshwater angiosperms

Although herbivory on submerged freshwater macrophytes has been widely ignored in the past, the particular importance of herbivore damage is now generally accepted (Lodge 1991; Newman 1991; Cyr & Face 1993). Former studies showed that crayfish, fish and water birds caused the majority of feeding damage on freshwater macrophytes. However, there is a striking lack of knowledge since previously insect herbivores have been shown to exert the smallest grazing influence on macrophytes (Lodge *et al.* 1998), and thus herbivory by invertebrates has been previously considered unimportant. However, recent studies provided evidence of the major grazing pressure of insects on aquatic plants. Newman (1991; 2004) for example showed that herbivorous insects are often effective herbivores and can consume large amounts of freshwater macrophyte biomass. Herbivory on aquatic macrophytes is caused mainly by oligophagous insects that phylogenetically descended from terrestrial insect groups and have colonised aquatic ecosystems as secondary invaders (Newman 1991). These taxonomic groups often include typical terrestrial plant pests, such as chrysomelid beetles, curculionid beetles, lepidopterans and dipterans (Newman 1991). For example the milfoil weevil *Euhrychiopsis lecontei* (Coleoptera: Curculionidae), the midge *Cricotopus myriophylli* (Diptera: Chironomidae) and the water moth *Acentria ephemerella* (Lepidoptera: Crambidae) have been reported to be suitable as agents for the biocontrol of the macrophyte Eurasian watermilfoil (*Myriophyllum spicatum*) (Newman 2004). Especially *Acentria ephemerella* is an important insect herbivore for water plants in lakes (Gross *et al.* 2002), displaying strong outbreak dynamics during the summer growth season.

In aquatic and terrestrial plants, defence strategies against herbivore grazing have developed (Lubchenco & Gaines 1981; Stowe *et al.* 2000; Hanley *et al.* 2007). The evolved strategies include resistance that reduces the palatability and suitability of plants as a resource for herbivore population growth (Fritz & Simms 1992; Hanley *et al.* 2007) and tolerance that enables the plants to survive, grow and reproduce under herbivore grazing (Stowe *et al.* 2000). The production and storage of allelochemicals in grazed tissues as a chemical resistance strategy to reduce herbivore grazing frequently occurs in freshwater and marine plants (Newman *et al.* 1996; Bolser *et al.* 1998; Wilson *et al.* 1999; Kubanek *et al.* 2000; Choi *et al.* 2002). Furthermore, terrestrial plants often display morphological resistance strategies, i.e. spinescence (the growth of spines and thorns), pubescence (the growth of hairs), sclerophylly (the development of hard or thick leaves) and the deposit of minerals in tissues (Hanley *et al.* 2007). Such herbivore-defended surface and tissue structures have been found in marine seagrasses (Verges *et al.* 2007; Verges *et al.* 2008) but rarely occur in submerged freshwater aquatic plants (Lodge 1991; Lodge *et al.* 1998; Hanley *et al.* 2007). The transfer of resources, for example nitrogen (N) and phosphorus (P), to ungrazed and protected plant organs is a common tolerance strategy of plants (Sadras & Wilson 1997; Stowe *et al.* 2000; Honkanen & Jormalainen 2002; Fornara & Du Toit 2007; Schooler *et al.* 2007) although it has also been reported that compensatory effects, i.e. an translocation of biomass and nutrients in the opposite direction towards grazed tissues, occur in terrestrial (Mulder & Ruess 1998; Li *et al.* 2004; Throop 2005) and aquatic plants (Verges *et al.* 2008). Tolerance strategies of freshwater angiosperms against insect herbivory have not been studied yet to my present knowledge and could be of major importance for many freshwater macrophytes, for example the genus *Potamogeton*, that lack morphological or chemical resistance strategies.

Potamogeton perfoliatus mainly reproduces clonally, and seeds are only of minor importance for reproduction (Wolfer & Straile 2004 a). The growth period from May to August is followed by a subsequent senescence of the above-ground shoots. During plant growth, biomass is translocated to below-ground overwintering buds, from which new shoots emerge in the following season (Wolfer & Straile 2004 a). Agrawal & Fishbein (2006) suggest for plant species such as *Potamogeton perfoliatus* that show a high plant edibility/digestibility together with low chemical defences and toughness the tolerance/escape syndrome as a tolerance strategy against herbivory: plant growth is high and allows for a rapid and sufficient allocation of biomass from the above-ground shoots to the below-ground overwintering organs. Subsequently, an enhanced senescence of shoot biomass due to herbivory and a below-ground translocation of nutrients (N,P) are likely to occur. Thus the tolerance/escape syndrome could be a potential tolerance strategy for *Potamogeton perfoliatus* and other aquatic macrophyte species against herbivory.

The herbivorous Lepidopteran *Acentria ephemerella* (Denis & Schiffermüller)

The predominantly aquatic moth species *Acentria ephemerella* (Denis & Schiffermüller) is an important herbivore of various submerged macrophyte species, e.g. *Myriophyllum spicatum* (L.), *Elodea canadensis* (Michx.), *Potamogeton perfoliatus* (L.) and *Potamogeton pectinatus*

(L.), and is commonly found in lakes and coastal waters throughout Europe (Berg 1942; Gross *et al.* 2001; Gross *et al.* 2002). *Acentria* belongs to the subfamily Nymphulinae (Lepidoptera: Crambidae), which consists of 716 Lepidopteran species that have an almost exclusively aquatic life-style in their early developmental stages (Heppner 1991; Munroe & Solis 1999). The aquatic larvae of this group can be either found on vascular plants in lakes and ponds or feeding on benthic algae in rapid streams (Stoops *et al.* 1998; Munroe & Solis 1999). Similar to trichopterans larvae of many Nymphulinae build cases and protective domiciles against predation (Williams & Feltmate 1992; Mueller & Dearing 1994; Dorn *et al.* 2001). The winged adult individuals emerge from the aquatic pupal cocoon and leave the water to become terrestrial during their short adult life span and to mate and reproduce (Williams & Feltmate 1992). *Acentria* has the strongest adaptation to the aquatic habitat since the wings of the aquatic adult females are only rudimentary and they have developed swimming bristles on their legs (Berg 1942; Speidel 1984; Speidel 1998). Furthermore, also the structure of the compound eyes differs between the wingless females and the winged males (Lau *et al.* 2007). The life-cycle of *Acentria* includes a seasonal diapause from September to May in the second and third larval stage (Haenni 1980): *Acentria* larvae overwinter in a cocoon, the so-called hibernaculum, inside the stems of macrophytes (Berg 1942). Larvae that emerge from diapause can be found in the field on the first emerging macrophyte shoots at the end of May. *Acentria* has been shown to display annually varying outbreak dynamics and to build up high *in situ* population densities in macrophyte patches during the summer months, from mid-June to August (Gross *et al.* 2002). Gross *et al.* (2002) suggest, in accordance with findings from Kokocinski (1963) and Haenni (1980), a life-cycle of two generations per year for *Acentria*, a directly developing summer generation from June to August and an indirectly developing generation from August to May that includes a winter diapause stage.

In laboratory experiments, a diet consisting of the perfoliate pondweed *Potamogeton perfoliatus* allowed for a comparatively fast growth and development of *Acentria* due to a low content of herbivore-deterrent polyphenols in *Potamogeton perfoliatus* compared to other macrophyte species (Choi *et al.* 2002). Furthermore, *Potamogeton perfoliatus* and many other freshwater macrophytes lack morphological defences (Lodge 1991; Lodge *et al.* 1998). This suggests together with the seasonally occurring outbreak dynamics that *Acentria* could be potentially regarded as a physical ecosystem engineer (*sensu* Jones *et al.* 1997) that could influence the functioning of macrophyte patches as a refuge and foraging habitat for fishes and invertebrates by changing the physical complexity of the patch. The potential ecosystem engineering role of *Acentria* clearly deserves more attention and should be studied under controlled experimental conditions as well as *in situ*. The growth and mortality rates of *Acentria* determine its population growth and as a consequence the grazing influence on submerged macrophytes. Since up to now the population regulation of *Acentria* has remained largely unclear, it is important to study how *Acentria* population dynamics are controlled and which defence strategies against mortality by predation are involved.

Trophic interactions between *Acentria ephemerella* and the three-spined stickleback *Gasterosteus aculeatus* L.

Potentially important sources of mortality for *Acentria* include predation and other influences, such a temperature stress and starvation due to low food quality and/or quantity. Benthivorous fish species and invertebrates, especially damselflies and dragonflies, feeding on herbivorous and detritivorous invertebrates, could cause mortality by predation of *Acentria*. Other aquatic invertebrate herbivores have been shown to suffer strongly from predation pressure by fishes: sunfish (*Lepomis gibbosus*) in North America for example suppress the densities of the herbivores *Euhrychiopsis lecontei* (Coleoptera: Curculionidae) and *Paraponyx* spp. (Sutter & Newman 1997; Ward & Newman 2006). Sticklebacks (*Gasterosteus aculeatus* L.) are likely to be the main fish predators for *Acentria* in *Potamogeton perfoliatus* patches as they are common in *Potamogeton perfoliatus* stands and their main food organisms are zooplankters and macroinvertebrates (Campbell 1991; Wootton 1994; Finlay *et al.* 2002; Norlin *et al.* 2005). They have furthermore been shown in experiments to consume epiphytic macroinvertebrates, including *Acentria* larvae (personal observation, Korn, unpublished data).

Sticklebacks are widely distributed throughout Europe in marine and brackish habitats and in lakes and rivers along coastal regions (Paepke 1983; Mäkinen *et al.* 2006) and have been anthropogenically introduced in many ponds and lakes of Central Europe (Paepke 1983), for example in Lake Constance (Muckle 1972). The breeding of three-spined sticklebacks in Europe and North America from 35 to 65 degrees north latitude occurs during a large period of time from February to August (Hyatt & Ringler 1989; Baker 1994; Poizat *et al.* 2002; Candolin & Voigt 2003). Especially during the breeding season (Whoriskey & FitzGerald 1994; Candolin & Salesto 2006) sticklebacks commonly occur in patches of submerged aquatic vegetation. From August on juvenile individuals can be found in increasing numbers and comprise the largest part of the population (Poizat *et al.* 2002) since many stickleback populations are annual (Baker 1994) and the adults die after the breeding season (Paepke 1983). Hence particularly in late summer large densities of juvenile individuals may exert a strong predation pressure on *Acentria* larvae.

Here I study the food web interactions within macrophyte beds of Lake Constance with a major emphasis on the potential trophic interactions between predators, herbivores and primary producers in the food web sticklebacks - *Acentria ephemerella* - *Potamogeton perfoliatus*.

Of key interest hereby is the

1) *Acentria* influence on macrophytes
2) population regulation of *Acentria* by predation

For both interactions I am interested in the direct and indirect effects of predation and herbivory. Mortality by predation and the costs of induced anti-predator defences of *Acentria* probably influence indirectly the grazing intensity on *Potamogeton perfoliatus* and hence the ecosystem engineering potential of *Acentria*. I used mesocosm experiments to investigate the functioning of the grazing influence of *Acentria* on *Potamogeton perfoliatus* and of stickleback predation on *Acentria* population dynamics. Field studies provided important informations, especially on the indirect ecosystem engineering role of *Acentria* in macrophyte patches and how it is affected by fish predation.

In **Chapter I** I analyse the herbivore defence strategies of submerged macrophytes on the model organism *Potamogeton perfoliatus*. The absence of morphological and chemical defences as well as the high edibility/digestibility of *Potamogeton perfoliatus* suggests the existence of the tolerance/escape syndrome as a defence strategy: A fast shoot growth should allow for a sufficient buildup of below-ground overwintering resources under herbivory and a subsequent increased senescence of the above-ground shoots. More specifically, I investigated the plant-herbivore interactions between *Acentria* and *Potamogeton perfoliatus* in a mesocosm experiment and studied the following questions: Do defence strategies of *Potamogeton* exist ? Is the tolerance/escape syndrome the defence strategy that *Potamogeton* uses ? Are the effects of *Acentria* herbivory on *Potamogeton* longlasting, i.e. are apart from the above-ground shoots also the resting buds affected ?

Chapter II describes the influence of *Acentria* on the habitat quality for the most abundant fish species in *Potamogeton* patches in Lake Constance, perch (*Perca fluviatilis* L.) and sticklebacks (*Gasterosteus aculeatus* L.). The high edibility/digestibility of *Potamogeton* suggests a strong grazing pressure of *Acentria* in the field: a herbivory-caused loss of plant biomass and an induction of an increased senescence as a defence strategy could influence the complexity of macrophyte patches *in situ* and hence the functioning of *Potamogeton* patches as a foraging and refuge habitat for fishes, especially for the macrophyte-inhabiting species perch and stickleback. This would eventually suggest an ecosystem engineering role of *Acentria*.

The ability of *Acentria* to exert a strong grazing pressure in turn is strongly affected by its life-history and population dynamics that are probably heavily influenced through mortality by predation as well as through the costs of induced anti-predator defences. Sticklebacks as the most common benthivorous fish species inhabiting macrophyte patches in Europe probably have the largest predatory influence on *Acentria*. I first analyse the effects of predation by sticklebacks on *Acentria* densities and sex-ratio in a mesocosm study in **chapter III**. Among other things, this study revealed that fish predation seems to influence the sex-ratio of *Acentria* pupae. However, the causes for this sex-ratio bias could not be resolved as we were only able in this study to sex pupae, but not larvae. In **chapter IV** I present the results from another mesocosm experiment, in which we used a histological method to sex also the larvae. This study suggests that males and females differ in their anti-predator strategies and that fish predation influences *Acentria* dynamics by imposing mortality and by the costs of anti-predator strategies. Finally, **chapter V** analyses seasonal and spatial variability in *Acentria* sex-ratios and life-history strategies in Lake Constance and their association with the distribution of fish.

Chapter I

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

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Manuscript in preparation

Abstract

The perfoliate pondweed *Potamogeton perfoliatus* constitutes large mono-specific macrophyte patches in many Central European lakes. Correlative evidence from the field suggests that *Potamogeton* 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*. We used a mesocosm experiment to determine the influence of *Acentria* herbivory on *Potamogeton* shoot development and resting bud production and to study the defence strategies of this macrophyte. Herbivory resulted in a reduction of the *Potamogeton* vegetation period by more than two months, thereby reducing the average resting bud size and the overall resting bud biomass seven-fold. This suggests that, besides its severe immediate effects, herbivory will affect *Potamogeton* growth and dynamics also during the subsequent season. As a response to herbivory *Potamogeton* retranslocated nutrients (phosphorus (P) and nitrogen (N)) from leaves towards resting buds and changed its allocation strategy in the size versus number of resting buds. 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. *Acentria* larvae had a high P content implying P limitation of larval growth especially within the herbivory

treatment. This suggests that at least the P retranslocation from leaves towards resting buds may be viewed as an anti-predator strategy rather than a nutrient conservation strategy.

Introduction

Herbivore 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. The ability of herbivores to outbreak, i.e. to overcome plant defences, and the frequency of outbreaks may depend on the specific defence strategies adopted by plants. Recently Agrawal & Fishbein (2006) suggested that plant defence strategies may be separated into three syndromes. Plants with a low nutritional quality often display the low nutritional defence syndrome (also see Feeny 1976). This syndrome is characterised 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, a low water and a low N content. The tolerance/escape syndrome has been proposed for plants in environments where they can grow fast under a high resource availability (see Coley *et al.* 1985; Kursar & Coley 2003). In these plants, a high N content of the biomass and low chemical and morphological defences (toughness) are accompanied by phenological strategies that allow for a fast growth of the plants under herbivory. The third strategy is the nutrition and defence syndrome that can be found in plants that show a high edibility and digestibility, for example a high N content, a high water content, a high specific leaf area or a low toughness together with toxic chemical defences against grazers.

One group of herbivores notorious for outbreak dynamics are forest lepidopterans (Myers 1993; Daniel & Myers 1995; Myers 1998; Peltonen *et al.* 2002). Several species exhibit a large variety of population cycles with a period from 3 to 20 years (Daniel & Myers 1995; Myers 1998). Peak densities of these species result in a massive defoliation of trees over large spatial ranges. Population build-up of these species can occur continuously over several years. Outbreaks may be regulated by predation on e.g. the pupal (Hara & Higashiura 1995; Liebhold *et al.* 2000; Liebhold *et al.* 2005) or larval stages (Auerbach 1991; Maron *et al.* 2001; Harrison *et al.* 2005).

We study a plant - herbivore system in the littoral of lakes. The macrophyte *Potamogeton perfoliatus* is a dominant macrophyte species in many lakes (Scheffer *et al.* 1992; Lehmann *et al.* 1997; Wolfer & Straile 2004 a; Sandsten & Klaassen 2008) and often forms large monospecific patches. When present it is of vital importance to the aquatic moth *Acentria ephemerella* (Gross *et al.* 2002), which is commonly found in lakes and coastal waters throughout Europe (Berg 1942; Gross *et al.* 2002; see chapter V). The system is a highly seasonal one in which the macrophyte regrows during late spring every year from resting buds and starts to decay after a short growing period of a few months (Wolfer & Straile 2004 a).

Reproduction of *Potamogeton perfoliatus* takes place primarily via vegetative growth of clones, and seeds are only of minor importance for patch persistence and expansion (Wolfer & Straile 2004 a). As *Acentria* depends to a large extent on *Potamogeton* for population development, it is restricted to the rather short vegetation period of the plant. During this period, *Acentria* density increases over several orders of magnitude (Gross *et al.* 2002). The population increase is followed by a long period from autumn to spring in which the larvae diapause and densities decline to pre-outbreak levels. This cycle is repeated annually, however, with spatial and interannual variability in the population growth rates and maximum densities (Gross *et al.* 2002, see chapter V), presumably due to a variability in fish predation pressure (see chapters III and V). The genus *Potamogeton* lacks chemical (Choi *et al.* 2002) or morphological mechanisms of resistance as do many other freshwater macrophyte species (Lodge 1991; Lodge *et al.* 1998). Furthermore, growth experiments showing fast *Acentria* growth and development on a *Potamogeton* diet suggest a high nutritional quality of *Potamogeton* (Choi *et al.* 2002). The combination of high nutritional quality and low concentrations of toxins and feeding deterrents suggests that the herbivory defences of *Potamogeton* might be classified into the tolerance/escape syndrome (Agrawal & Fishbein 2006). However, the components of this syndrome in the case of *Potamogeton* have not been studied yet. In addition, the current evidence for a strong feeding damage on *Potamogeton perfoliatus* by *Acentria in situ* is only correlative: Increasing densities of *Acentria* larvae in macrophyte patches in Lake Constance during the summer months were associated with increasing signs of feeding damage (Gross *et al.* 2002). However, it is not clear how strongly *Acentria* influences the duration of the overall growing season of *Potamogeton* and the establishment 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 *Acentria* and *Potamogeton perfoliatus* to better understand the mechanisms involved in the feeding relationships in macrophyte beds and the defence mechanisms of *Potamogeton perfoliatus* against herbivory.

More specifically, we ask the following questions:

1) Can *Acentria* shorten the growing period of *Potamogeton perfoliatus* ?
2) Does *Acentria* herbivory affect the resting bud production of *Potamogeton perfoliatus* ?
3) Does *Potamogeton perfoliatus* tolerate *Acentria* feeding?
4) Does herbivory influence the allocation and translocation of nutrients in *Potamogeton perfoliatus* ?

Materials and Methods

We analysed the trophic interactions between larvae of the water moth *Acentria ephemerella* and the perfoliate pondweed *Potamogeton 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 (*Acentria* herbivory and control) and three sampling times (25 August, 16 September and 29 November), resulting in four replicates per treatment and sampling.

The experimental units consisted of a tube out of transparent plastic foil (transparent Tricoron; RKW AG Rheinische Kunststoffwerke, Wasserburg, Germany) with a length of approximately 1.5 m that was connected to a styrodur frame with an area of 0.43 m^2 (0.6 m width, 0.715 m length). The styrodur frame had the function of a buoy to hold the plastic tube in a vertical position in the water column. The styrodur frames were placed in the outdoor mesocosm (length = 10.5 m, width = 5 m, height = 1.5 m) that was filled with fine sediment (height ~ 0.35 m) and water from Lake Constance. The sediment was mixed prior to the experiments to provide the same environmental starting conditions for each replicate. In both experiments, the experimental units were separated from the surrounding water in the mesocosm by the transparent Tricoron plastic foil. Water in the experimental units was not changed during the experiments. The lower end of the plastic tube was fixed with ten metal clips on a metal frame (length = 0.8 m, width = 0.6 m, height = 0.05 m) and dug into the mesocosm sediment. The lower end of the plastic tube was dug into a sediment-filled mortar tray (length = 0.75 m, width = 0.5 m, height = 0.3 m). This was done to prevent rhizome growth out of the experimental units and to be able to harvest also all plant below-ground biomass. Wooden frames (length = 0.92 m, width = 0.73 m) covered with metal gauze (mesh size ~ 1620 µm) were placed approximately 0.1 m above the water surface upon the styrodur frames to prevent the adult stages of *Acentria* from dispersal out of the experimental units and to allow the winged *Acentria* males to fly around and search for females, i.e. the experimental setup allowed for *Acentria* reproduction.

Shoots of *Potamogeton perfoliatus* and associated macroinvertebrates were sampled in June 2005 with a toothed sickle in *Potamogeton 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 *Acentria* larvae were removed from the plants prior to planting. Shoots were allowed to root, and 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, *Acentria* larvae collected from the field were introduced into the herbivory treatment to yield a start density of 22 larvae g^{-1} plant dry mass (Ind. g^{-1} dm). In the control treatment, no *Acentria* larvae were introduced. After the three sampling events, *Acentria* larvae associated with *Potamogeton* were washed through a sieve (mesh size 45 µm) and fixed in 70 % Ethanol in 1 l plastic boxes. The remaining above-ground plant material (stems and leaves) was frozen and was searched through for pupae after the experiment since *Acentria* pupae were closely attached to the stems of *Potamogeton* shoots. Macrophytes were dried at 90 °C for three days, and densities of *Acentria* pupae and larvae were calculated as individuals per gram plant dry mass (Ind. g^{-1} dm). A dissecting microscope (Zeiss Stemi 2000-C) was used to collect and count *Acentria* larvae at a 10–50× magnification from samples. *Potamogeton* 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 *Potamogeton perfoliatus* sampled on 25 August and in small (1st instar, headcapsule width (hcw) ~ 245 µm) and large (probably 5th instar, hcw ~ 1000 to 1170 µm) *Acentria* 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 *Acentria* larvae and calculated the C:P ratio of larvae as the molar ratio of the C and P content.

The *Potamogeton* and *Acentria* samples were dried at 90°C and 60 °C, respectively, and were ground to a powder. For the determination of the C and N contents, approximately 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). To determine the content of particulate P approximately 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, U.S.A.) 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 *et al.* 1985).

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Results

POPULATION DYNAMICS OF *ACENTRIA*

Acentria larvae were introduced at the start of the experiment and subsequently developed into pupae. The hatched adult individuals were detected in the experimental units from 3 August on (Fig. 1 A and B). We observed more adult males than females probably because the detection probability of aquatic females is lower than that of males, which emerge from the water column. *Acentria* successfully reproduced during the experimental period in the mesocosms resulting in increasing larval densities. Maximum densities of 84 ± 29 (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 (SE) Ind. g^{-1} dm) since a large part of *Acentria* larvae had already went into hibernation in plant stems or was drifting on the search for food in the water column.

At the end of August, *Potamogeton* shoots in the herbivory treatment started to senesce. First drifting larvae in the experimental units that were probably searching for alternative food resources were found on 29 August (Fig. 1 C), presumably due to an overexploitation of their food plants. The number of drifting larvae rapidly increased to a maximum of 34 ± 14 (SE) larvae on 12 September and subsequently decreased to 0.25 ± 0.18 (SE) larvae on 12 October. Throughout the experiment no *Acentria* larvae were found in the control treatment.

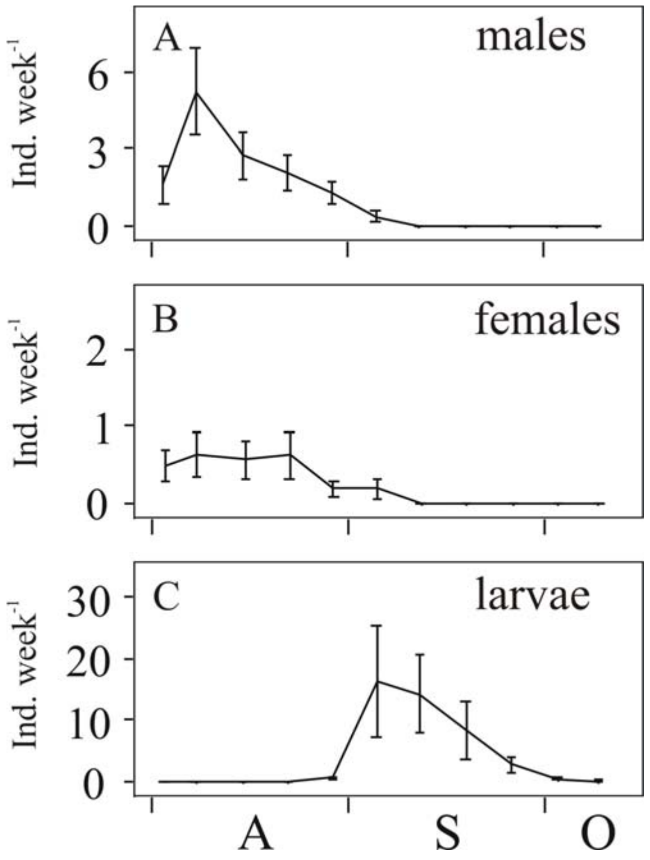

Fig. 1 Number of adult *Acentria* males (A) and females (B) and drifting *Acentria* larvae (C) per week in all experimental units of the herbivory treatment of the mesocosm experiment 2005 in August (A), September (S) and October (O).

GROWTH OF *POTAMOGETON*

Shoot biomass increased during the experiment when *Acentria* was absent and decreased after larvae were introduced in the experimental units of the *Acentria* herbivory treatment (Fig. 2 A). Shoot biomass development in the two treatments differed significantly from each other (ANOVA, factor *Acentria*: $F_{1,35} = 17.87$, $p = 0.0002$, factor month: $F_{3,33} = 1.92$, $p = 0.15$, interaction *Acentria**month: $F_{3,33} = 5.34$, $p = 0.0047$). In the *Acentria* treatment, *Potamogeton* shoots were completely defoliated at the 2nd 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 (see Appendix Fig. A 1). That is *Acentria* reduced the growing season of

Potamogeton in our experiment by more than two months. 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. 2 B).

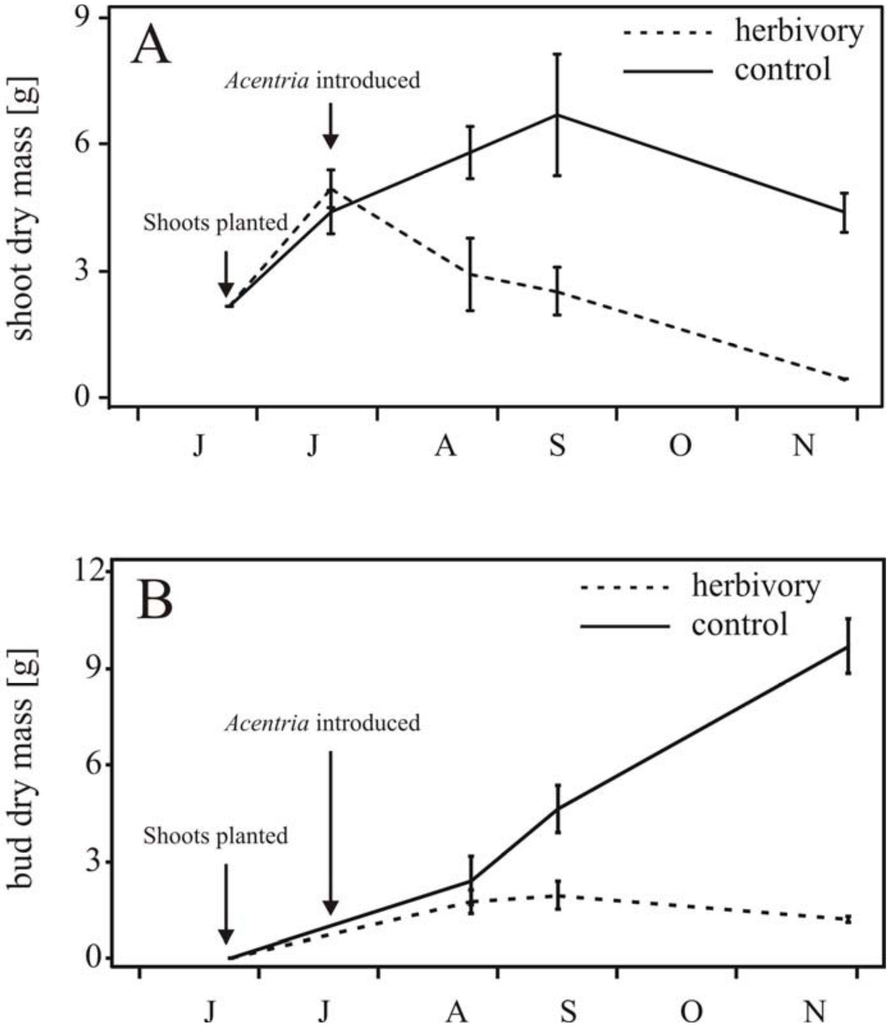

Fig. 2 Influence of *Acentria* herbivory on the development of A) shoot biomass (shoot dry mass [g] experimental unit^{-1}) and B) resting bud biomass (bud dry mass [g] experimental unit^{-1}) in the herbivory treatment of the mesocosm experiment 2005 from June (J) to November (N).

The development of resting bud biomass differed significantly between the two treatments (ANOVA, factor *Acentria*: $F_{1,22} = 65.7$, $p < 0.0001$, factor month: $F_{2,21} = 16.31$, $p < 0.0001$, interaction *Acentria**month: $F_{2,21} = 23.49$, $p < 0.0001$). At the end of the experiment, resting bud biomass produced in the control treatment was seven-fold higher than in the herbivory treatment. Shoots in the control treatment continued to produce resting buds until November whereas after the defoliation of shoots no further production was possible after September in the herbivory treatment.

The mean dry weight of resting buds significantly increased both in the herbivory and the control treatment from 25 August to 16 September (Fig. 3 A, Mixed Model ANOVA, factor sampling, $F_{1,164} = 42.33$, $p < 0.0001$, factor *Acentria*, $F_{1,6} = 0.05$, $p = 0.83$, interaction sampling**Acentria*, $F_{1,164} = 0.02$, $p = 0.895$). However, from 16 September to 29 November the mean resting bud dry weight developed differently in the control and the herbivory treatment (Fig. 3 A, Mixed Model ANOVA, factor sampling, $F_{1,383} = 3.49$, $p = 0.06$, factor *Acentria*, $F_{1,12} = 16.99$, $p = 0.0014$, interaction sampling**Acentria*, $F_{1,383} = 19.69$, $p < 0.0001$): 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.

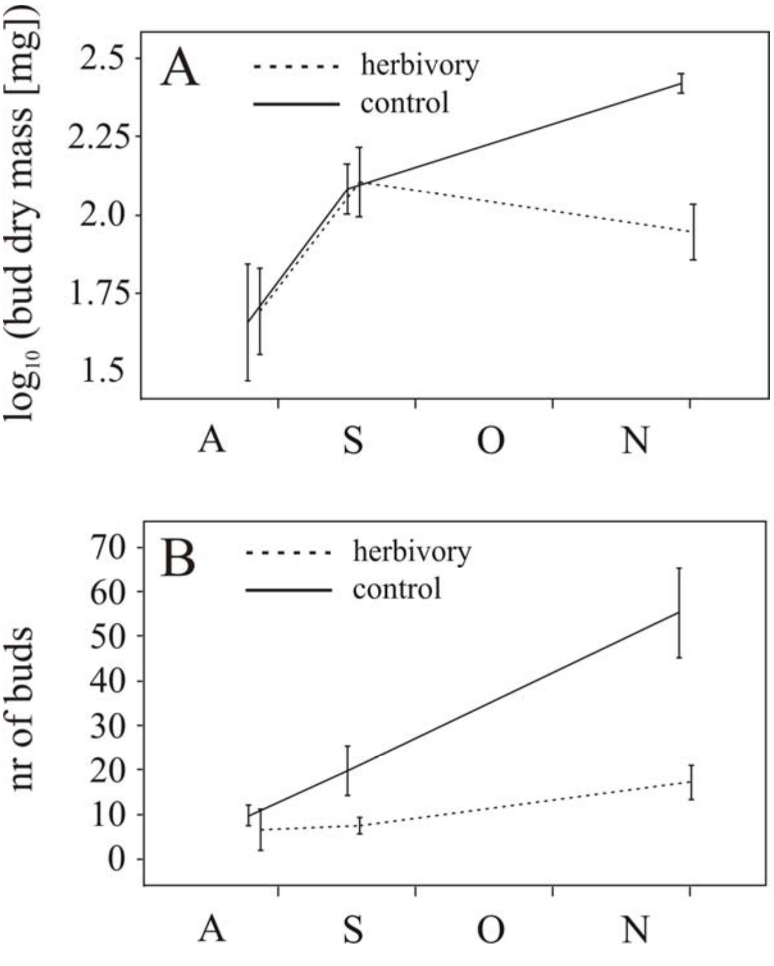

Fig. 3 Influence of *Acentria* herbivory on A) the mean dry weight ($\log_{10}$ (bud dry mass [mg])) and B) the mean number of resting buds in the herbivory treatment compared to the control treatment in the mesocosm experiment 2005 from August (A) to November (N).

The number of resting buds continuously increased in the herbivory and the control treatment (Fig. 3 B). The increase was significantly stronger in the control than in the herbivory treatment (ANOVA, factor sampling, $F_{2,16} = 61.18$, $p < 0.0001$, factor *Acentria*, $F_{1,16} = 65.67$, $p < 0.0001$, interaction sampling**Acentria*, $F_{2,16} = 22.39$, $p < 0.0001$).

ELEMENTAL ANALYSES OF *POTAMOGETON* AND *ACENTRIA*

Acentria herbivory strongly influenced the composition of the shoots and resting buds of *Potamogeton perfoliatus* (Fig. 4). The N content of the shoots was reduced by *Acentria* herbivory and the N content of the resting buds increased with *Acentria* herbivory, suggesting that there was a translocation from leaves to the resting buds for N (Fig. 4 A). 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). Shoot P content was significantly lower than resting bud P content irrespective of the influence of herbivory (Fig. 4 B, ANOVA, factor plant part, $F_{1,12}$ = 131.86, $p < 0.0001$). The P content of the resting buds was not influenced by *Acentria* herbivory whereas the P content of leaves was reduced under grazing (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).

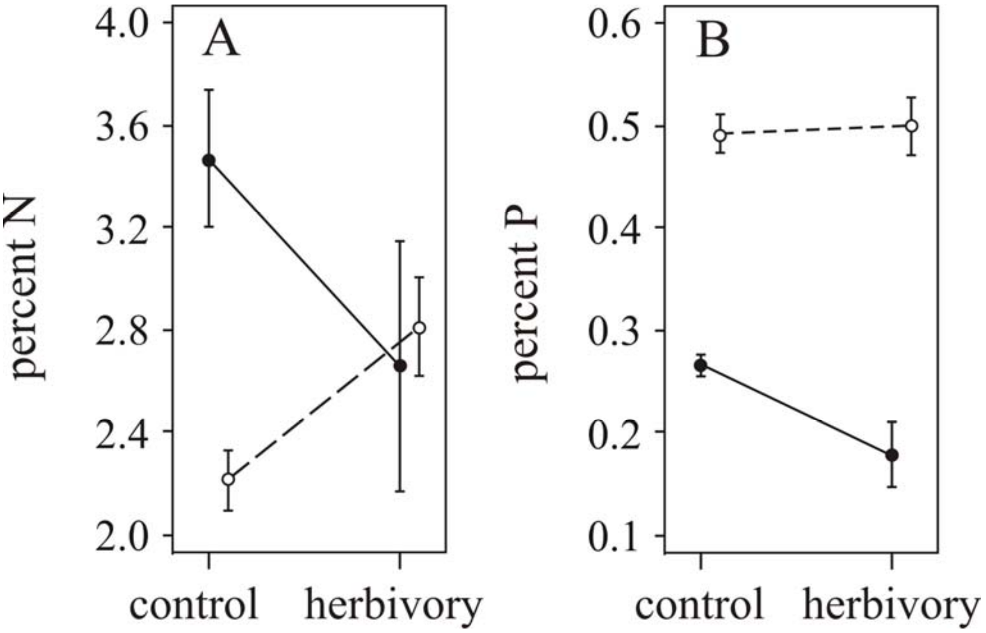

Fig. 4 Percent nitrogen (N, Fig. 4 A) and phosphorus (P, Fig. 4 B) of leaves (filled circles, straight line) and resting buds (empty circles, dotted line) of *Potamogeton perfoliatus* in the control and herbivory treatments in the August sampling of the mesocosm experiment 2005.

The N content was significantly higher for small than for large larvae (Fig. 5 A, 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. 5 B, ANOVA, factor developmental stage, $F_{1,6}$ = 5.34, p = 0.0603). The C:P ratio of large larvae was significantly higher than the C:P ratio of small larvae (Fig. 5 C, ANOVA, factor developmental stage, $F_{2,15}$ = 6.54, p = 0.0091).

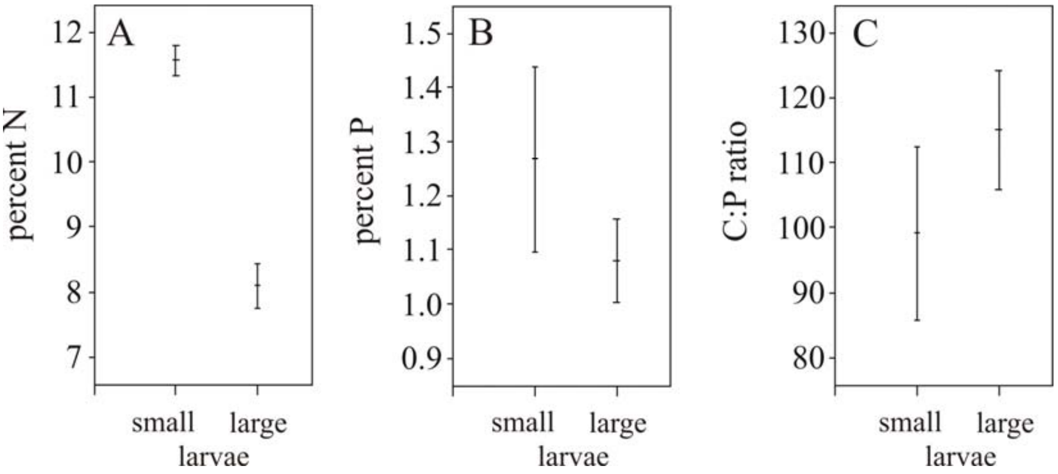

Fig. 5 Percent nitrogen (N, Fig. 5 A), phosphorus (P, Fig. 5 B) and C:P ratio (Fig. 5 C) of small and large *Acentria* larvae from field samples in Lake Constance.

Discussion

This study shows that there is a strong influence of herbivory on *Potamogeton* with a plant defence syndrome adapted to a seasonal outbreak of herbivores. *Acentria* herbivory in our experiment significantly decreased the length of the growing season, the shoot and resting bud biomass as well as 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 seven-fold by *Acentria* herbivory, suggesting that the effects of *Acentria* will not be confined to the actual season but will also influence the shoot development in the following year. Nevertheless, the effect of *Acentria* 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 due to the operation of density - dependent factors, and shoot biomass did not anymore increase from the 1st to the 2nd sampling in the control treatment. This suggests, if our experimental design had allowed for spatial expansion, that the differences in biomass production between the herbivory and control treatment would have been even stronger.

On the other hand, *Acentria* densities during the experiment were higher than *Acentria* densities *in situ*, suggesting that herbivory effects *in situ* might not be as strong and immediate as in our experiment. However, Gross *et al.* (2002) report that 100 % of apical meristems of *Potamogeton* show feeding damage due to *Acentria* herbivory already in September. The growing season of *Potamogeton* 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 *Potamogeton* patches in Lake Constance revealed that the length of their growing season was significantly related to the numbers of *Acentria* present in these patches at the end of July (see chapter II). In patches with a high *Acentria* density, *Potamogeton* patches were completely defoliated due to *Acentria* herbivory within a period of two weeks during August. Hence even with a larval density lower than in our mesocosm there is convincing evidence that *Acentria* is able to exert a strong grazing pressure on *Potamogeton*, finally resulting in defoliation.

The fast growth of *Potamogeton perfoliatus* during the growth season in summer together with a high nutritional quality for *Acentria* due to low tannin concentrations suggests the tolerance/escape syndrome as a defence strategy of *Potamogeton perfoliatus* against herbivory (see Kursar & Coley 2003; Agrawal & Fishbein 2006). A fast growth allows *Potamogeton perfoliatus* to build up a sufficiently high biomass of resting buds, enabling regrowth during the next year before the period of intense *Acentria* herbivory starts. Compensatory responses as a reaction to grazing pressure however, i.e. an allocation of biomass and nutrients towards grazed tissues, have been reported for terrestrial plant species, e.g. for the Yellow Nutsedge (*Cyperus esculentus*, Li *et al.* 2004), the Arrowgrass (*Triglochin palustris*, Mulder & Ruess 1998), the Common Ragweed (*Ambrosia artemisiifolia*, Throop 2005) and also marine seagrasses (*Posidonia oceanica*, Verges *et al.* 2008). In these cases, herbivory stimulated the growth of the leaves as photosynthetically active tissues to grow against the grazing of plant tissue. We found however no evidence for compensatory growth in *Potamogeton*. Clearly, with intense herbivory compensatory growth of plants would be maladaptive as it 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.

In contrast to the pattern expected from compensatory growth, *Potamogeton perfoliatus* seems to translocate nutrients, i.e. phosphorus and nitrogen, under herbivore pressure from grazed tissues to its resting buds. 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 as a defence strategy. P content in resting buds was already rather high in the control treatment, suggesting that the primary adaptive value of the reduction in the P content of the leaves under herbivory pressure is probably not the conservation of nutrients but rather a reduction of the nutrient supply for *Acentria*. The C:P ratio of especially small *Acentria* larvae (80:1) is rather low as compared to the C:P ratio of *Potamogeton* leaves (380:1 and 550:1 in the control and herbivory treatment, respectively), suggesting a P limitation of *Acentria* growth. P limitation of growth has been demonstrated for example for the tobacco hornworm (*Manduca sexta)* 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 ~ 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; de Mott *et al.* 2001). The difference in C:P ratios between *Acentria* and *Potamogeton* is at least of similar magnitude than the differences between herbivore and primary producer in the *Daphnia* and *Manduca* model systems, supporting the possibility for a P limitation of *Acentria* already for *Potamogeton* plants without herbivory. The reduction of the P content of the leaves should further increase *Acentria* 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 1992; Roff 2003). For example many sexually or clonally reproducing plant species trade off the size of seeds (Vaughton & Ramsey 1998; Kery *et al.* 2000; Greenway & Harder 2007; Sadras 2007) and ramets (Johansson 1994; Winkler & Fischer 2002; Aarssen 2008), respectively, against their number. The development of resting bud numbers and mean dry masses in our mesocosms suggests a change in allocation of resting bud number versus size in resting bud production as an accessory strategy to mitigate the effects of herbivory. In the absence of such an allocation change, 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 resting bud size in the herbivory treatment increased as strongly as in the control at the cost of resting bud number: whereas the number of resting buds increased in the control treatment, plants in the *Acentria* treatment did not produce more resting buds within this month. 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). However, despite this change in allocation, mean resting bud size 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 *Potamogeton*.

The influence of *Acentria* 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 (Diehl & Kornijow 1998; Crowder *et al.* 1998; Weaver *et al.* 1998; Warfe & Barmuta 2006). Especially young-of-the-year fishes (Y-O-Y) use patches of submerged aquatic vegetation as a refuge against predation (Casselman & Lewis 1996; MacRae & Jackson 2001) and a foraging habitat, preying on macroinvertebrates (Dionne & Folt 1991; Diehl & Kornijow 1998). The removal of shoot biomass and an earlier senescence reduces the complexity of the macrophyte patch since (1) the density of shoots is reduced and (2) the quality of the shoots and their stability against mechanical destruction by wave action is reduced. Patch structural complexity in turn determines the interactions between predators (piscivorous fish, benthivorous fish, invertebrate predators) and their prey (juvenile fish, macroinvertebrates) via the visibility of prey organisms and the manoeuvrability of predators (Savino & Stein 1989; Eklöv 1997; Manatunge *et al.* 2000; Hornung & Foote 2006; Warfe & Barmuta 2006). *Acentria* thus acts as an ecosystem engineer (Jones *et al.* 1997), influencing indirectly via an earlier plant senescence the fish and invertebrate communities in the littoral zone of lakes.

To conclude, we have shown that *Acentria* has a strong influence on its food plant, which seems to respond to herbivory with a tolerance/escape syndrome associated with a change in biomass allocation in 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 strategy seems to involve a reduction of phosphorus in plant leaves, which most likely increases the P limitation of herbivore growth. The important structuring role of macrophytes, including *Potamogeton perfoliatus*, in lake ecosystems suggests that the observed intense herbivory by *Acentria* but also the indirect effects of herbivory due to the plant tolerance/escape strategy will have striking consequences for ecosystem and food web dynamics.

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Chapter II

Seasonal and spatial distribution of fishes in macrophyte patches in relation to herbivory-induced changes in physical habitat complexity

OLIVER MILER, MICHAEL KORN AND DIETMAR STRAILE

Manuscript in preparation

Abstract

Submerged macrophytes in lakes and rivers are well-known to provide important refuge and foraging habitats, especially for juvenile fishes. The habitat functioning hereby largely depends on the spatially and seasonally varying complexity of the macrophyte patch. This complexity that is determined by the shape and density of the plants is often strongly influenced by consumer damage through herbivorous invertebrates. Naturally occurring seasonal senescence in temperate climates can possibly be enhanced by feeding damage and thus change the available habitat for adult and young-of-the-year (YOY) fishes. In this field study, we could show that in Lake Constance primarily juvenile fishes used patches of the most common macrophyte *Potamogeton perfoliatus*. Fishes were caught with minnow traps, and perch comprised the largest part of the fish catches whereas sticklebacks, ruffe, pike, burbot and cyprinids were only caught in low numbers. Spatial variability in stickleback predation on *Acentria* probably led to spatial differences in *Acentria* densities. Perch catch per unit effort (CPUE) showed strong seasonal and spatial variations that could be attributed to the patch condition, i.e. the degree of patch senescence. In contrast to this, seasonal and spatial variations were present in physical habitat features (water depth, distance from the shore, slope) but did not reasonably explain the distributions of perch. Furthermore, patches with high densities of the most important invertebrate herbivore of *Potamogeton perfoliatus* in Lake Constance, the water moth *Acentria*, showed a strong tendency to be more senescent than patches with lower *Acentria* densities. We hence suggest that *Acentria* can be regarded as an ecosystem engineer, physically changing macrophyte patches as a refuge and foraging habitat especially for perch and sticklebacks. More research is needed to examine the relative influence of *Acentria* on plant senescence and the direct removal of plant material in *Potamogeton* patches and to disentangle the biotic and abiotic effects on the structure and habitat functioning of submerged macrophyte patches.

Introduction

Submerged aquatic vegetation is known to be an important habitat for fish and macroinvertebrates in lakes and rivers (Weaver *et al.* 1997; Diehl & Kornijow 1998; Warfe & Barmuta 2006). Especially juvenile fishes use macrophyte patches in freshwater ecosystems as a refuge against predation (Crowder & Cooper 1982; MacRae & Jackson 2001) and a foraging habitat, feeding on plant-associated invertebrates (Dionne & Folt 1991; Diehl & Kornijow 1998; Hornung & Foote 2006). Food web interactions in submerged aquatic vegetation are complex and show spatial and temporal variations. Much of this variation is due to the differential structural complexity of macrophyte patches as a habitat for fish and macroinvertebrates. The importance of plant density and shape as the parameters determining the complexity of submerged aquatic vegetation on food web interactions has been studied in detail (Dionne & Folt 1991; Eklöv 1997; Weaver *et al.* 1997; Warfe & Barmuta 2006). The degree of habitat structural complexity influences interactions between predators (piscivorous fish, benthivorous fish, invertebrate predators) and their prey (juvenile fish, macroinvertebrates) via the visibility of prey organisms and the manoeuvrability of predators (Savino & Stein 1989; Eklöv 1997; Manatunge *et al.* 2000; Hornung & Foote 2006; Warfe & Barmuta 2006).

Most submerged macrophytes in temperate regions show a seasonal life-cycle: In the short growing season in spring and summer, macrophytes develop from sexually produced seeds and/or from vegetatively produced buds or tubers. Increased senescence of the above-ground plant parts occurs at the end of the growth period when the energy of the plants is transferred to the overwintering stages (the below-ground buds) in the sediment, where macrophytes survive the winter. The effects of macrophyte senescence on the associated aquatic fauna have been rarely studied (but see Whitfield 1984; Caffrey 1993). Caffrey (1993) for example has shown that perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.) and several cyprinid species, including bream (*Abramis brama* L.) and roach (*Rutilus rutilus* L.), prefer channels with plants that are present all the year round over channels where plants are missing seasonally. Likewise, Whitfield *et al.* (1984) showed that a prolonged senescence of aquatic macrophytes and finally the disappearance of the above-ground biomass resulted in a strong decrease of plant-associated macroinvertebrate densities leading to lower densities of two fish species in Southern African lakes. Even if a senescent macrophyte patch has not yet completely disappeared, increasing decay can have effects on the habitat structure by reducing the plant densities, the surface canopy cover and the mechanical stability of stems and leafs (personal observation).

Although senescence in plants usually occurs seasonally, it can be triggered by environmental stress (Nooden & Leopold 1988). Herbivory is known to induce important plant defence and tolerance mechanisms (Mopper *et al.* 1991; Karban & Baldwin 1997; Stowe *et al.* 2000). It has been shown that consumer damage, for example by herbivorous insects, can result in a resource allocation to defended plant parts, e.g. an energy transfer to below-ground plant organs. This transports resources out of the reach of the consumer (Dyer *et al.* 1991; Stowe *et al.* 2000). Resource translocation in combination with the direct removal of plant material by herbivory (Brookshire *et al.* 2002) changes the patch structure and quality by affecting the density and shape of the plants (Mopper *et al.* 1991). Hence herbivory should finally also affect the distribution of other species using plants as a habitat or refuge.

In Lake Constance, submerged aquatic vegetation (SAV) is dominated by the perfoliate pondweed *Potamogeton perfoliatus*, which occurs in large, mostly monospecific patches in the littoral zone during the growth period from May to September. It has been shown in earlier studies that these patches of SAV in Lake Constance are inhabited primarily by young-of-the-year perch and ruffe whereas juvenile cyprinids prefer the shallow-most areas near the shoreline since these probably offer a better protection from piscivorous fishes (Fischer & Eckmann 1997 a; Fischer & Eckmann 1997 b; Stoll *et al.* 2008). The three-spined stickleback also commonly occurs, in addition to perch and ruffe, in macrophyte patches in Lake Constance (personal observation). An important herbivore for SAV that can inflict a strong feeding damage especially on *Potamogeton perfoliatus* and hence change the patch structure is the water moth *Acentria ephemerella*, which frequently occurs in high densities in Lake Constance (see chapter V, Gross *et al.* 2002) and displays annually varying outbreak dynamics. *Acentria* has been shown in a mesocosm experiment to cause severe feeding damage on *Potamogeton perfoliatus* and shorten its vegetation period considerably (see chapter I).

Here we aim at documenting the functioning of *Acentria* as an ecosystem engineer in macrophyte patches of Lake Constance. *Acentria* densities are known to show strong spatial variability (Gross *et al.* 2002; chapter V; Korn, unpublished data). Consequently, our first hypothesis is that spatial variability in patch condition, senescence and/or vegetation period should be related to spatial variability in *Acentria* densities. Second, we want to investigate the relationship between spatial and seasonal variability in patch condition and spatio-temporal variability in fish distribution.

Materials and Methods

STUDY SITE

Lake Constance, with a surface area of 534 km^2 and a maximum depth of 254 m, is the second largest Lake, after Lake Geneva, in the pre-alpine region of Central Europe (47° N; 09° E). The river Rhine is the main tributary and flows into Lake Constance at its eastern end as the “Alpenrhein” (alpine Rhine). The lake consists of two main basins, the Upper Lake Constance (ULC) and the Lower Lake Constance (LLC), that are connected by a four km long section of the river Rhine (Fig. 1). Upper and Lower Lake Constance strongly differ in morphology and macrophyte coverage: In the deep ULC (z_{mean} = 101 m) macrophytes grow only in the littoral zones along the steep slopes whereas they cover wide areas in the shallower LLC (z_{mean} = 13 m). The three sampling sites (Fig. 1) Hagnau (ULC), Güttingen (ULC) and Reichenau (LLC) differed in their morphology (Table 1). We will analyse and discuss these differences in the results and discussion section.

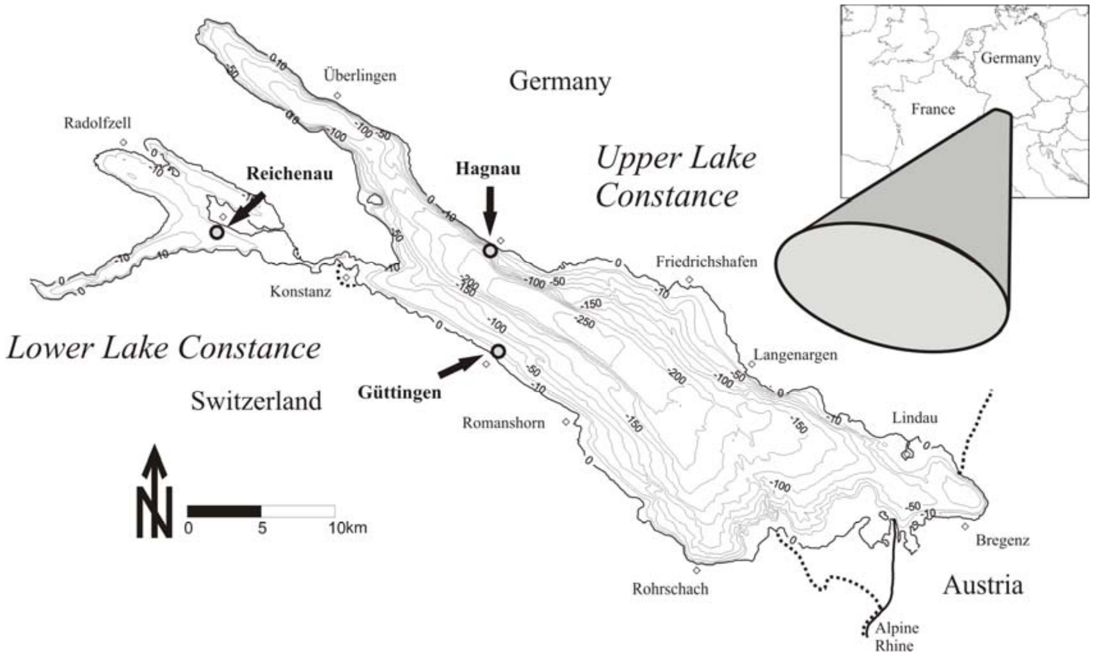

Fig. 1 Map of Lake Constance. Sampling sites of fish and *Acentria* larvae during the field season 2004 are marked with arrows (near the cities Güttingen and Hagnau and the island Reichenau).

Table 1: The mean depth, distance to the shore and slope at the sampling sites Güttingen, Hagnau and Reichenau were analysed statistically with an one-way ANOVA (factor sampling site): A, B and C indicate significant differences between the sampling sites in the field season 2004.

Site	depth: mean ± S.D.	distance to shore: mean ± S.D.	slope: mean ± S.D.
Hagnau	(A) 1.74 ± 0.81	(A) 91.56 ± 32.81	(A) 10.14 ± 11.57
Güttingen	(B) 1.39 ± 0.49	(B) 186.22 ± 43.27	(B) 6.1 ± 4.42
Reichenau	(C) 2.91 ± 0.91	(B) 177.56 ± 162.2	(AB) 8.92 ± 8.24

SAMPLING DESIGN AND PROCEDURES

Fishes in macrophyte patches in the littoral zone of Lake Constance were captured with minnow traps in the field season 2004. Fish traps were baited with beef liver and dog food to attract fishes and set at the sampling sites Hagnau, Güttingen and Reichenau (Fig. 1) in nine, ten and eight *Potamogeton perfoliatus* patches, respectively, and during three sampling periods: (1) = 29.06.04 - 7.07.04, (2) = 20.07.04 - 23.07.04 and (3) = 24.08.04 - 4.09.04. During the last sampling period (3) in August/September 2004, nearly all macrophyte patches at the sampling site Reichenau had vanished due to senescence, and therefore fishes were only sampled in Güttingen and Hagnau. One fish sampling unit consisted of altogether four minnow traps with two different mesh sizes (fine: 3 mm, coarse: 6 mm) and a trap opening of 25 mm. The traps were fixed to a rope and were thus positioned vertically in the water column via a buoy at the upper and two paving stones as a weight (approximately 7.44 kg) at the lower end of the rope (Fig. 2). The order of the traps along the rope (from the upper end) was: fine – coarse – fine – coarse. 24 fish sampling units were set during daytime in the littoral zone of each site within macrophyte patches. On 7 July 2004, during field sampling one sampling unit was lost, and from this date onwards only 23 sampling units were set. The sampling units were hauled inboard and emptied after 24 h. The captured fish were killed with 2 mg liter^{-1} 1,1,1-trichloro-2-methyl-propanol (TCMP), stored on ice and subsequently frozen in a chest-freezer. The catch per unit effort (CPUE) was then calculated as the number of fish that were caught during 24 h in one fish sampling unit. The total length (TL) was measured to the nearest 1 mm.

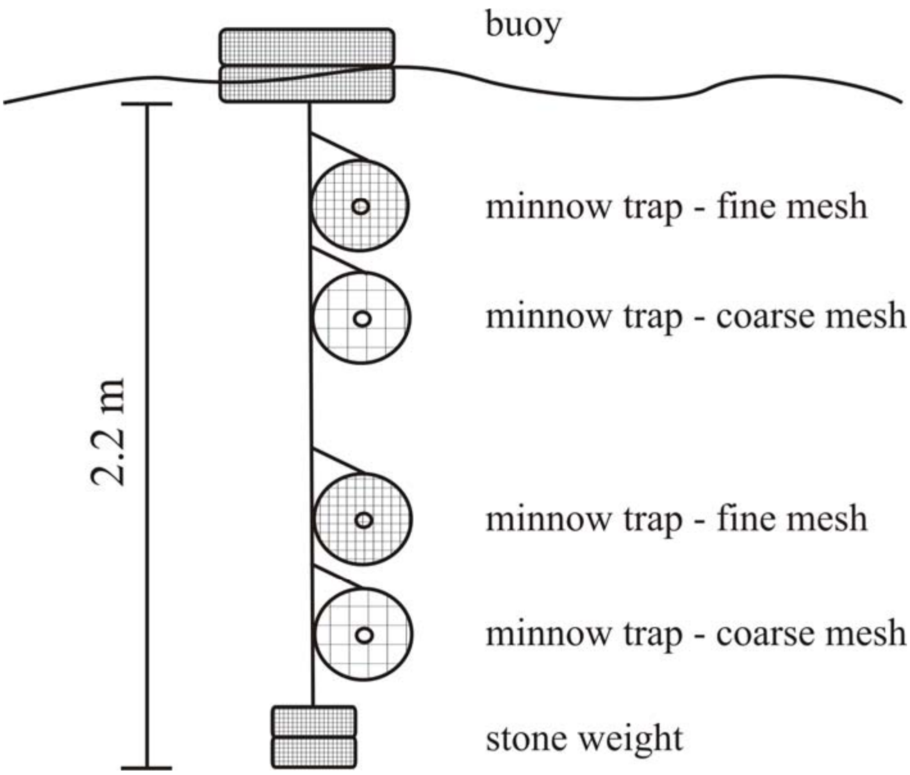

Fig. 2 Sampling unit to estimate the fish catch per unit effort (CPUE) in Lake Constance in the field season 2004, consisting of minnow traps with coarse and fine mesh sizes.

During the fish samplings, the position of the sampling units was determined with a GPS receiver (GPS 72, Garmin International Inc., U.S.A.). We furthermore documented the condition of the macrophyte patches by shooting photos with a camera (EOS 650, Canon, Japan) through the glass bottom of a metal box that was held on the water surface. Subsequently, the condition of the macrophyte patches as a biotic parameter was assessed after the fish sampling by an analysis of the shot photos. We used an index ranging from –1 to 3 to describe the patch condition, and the values were assigned to the growth stage of the patch: -1 = no senescence, plants have not yet reached the surface, 0 = no senescence, plants have reached the surface, 1 = light senescence, 2 = strong senescence (up to 50 % of the plants), 3 = very strong senescence (more than 50 % of the plants), 4 = complete patch senescence with only few senescent plant parts remaining on the ground. Subsequently, no more fish samplings were conducted, but the condition of the analysed patches was continuously determined in a routine monitoring every two weeks in the sampling periods 3 (at Reichenau since no fishes were sampled there), 4 (9.09.04 - 10.09.04), 5 (20.09.04 - 27.09.04), 6 (7.10.04), 7 (22.10.04) and 8 (2.11.04). During the routine monitoring of the patch condition, the additional patch condition index value 4 that describes the complete patch senescence was introduced due to the increasing senescence of the macrophyte patches. The depth, slope and distance perpendicular to the shore line as abiotic parameters were assessed by means of a GIS-based (GIS ArcView 3.2a, ESRI, U.S.A.) digital elevation model (DEM) for each sampling point.

The density of *Acentria* larvae on *Potamogeton* was determined in different patches in July, i.e. in sampling period (2). Macrophytes were sampled with a toothed sickle mounted on a 4 m metal pole. *Acentria* larvae were washed from the plants through a sieve with 200 µm mesh size and preserved in 70 % Ethanol. The remaining plant material was stored in plastic bags in a 5 °C climate chamber. Subsequently, the plants were searched through by the naked eye within one week to collect larvae, prepupae and pupae that were closely attached to the plant stems or leaves and had remained on the plants after washing. The plants were dried for three days at 90 °C in a drying chamber, and the plant dry mass was assessed to calculate the larval *Acentria* densities as individuals per gram plant dry mass (Ind. g^{-1} dm).

Results

The macrophyte patches at the three sampling sites differed in their morphological setting (Table 1). The mean water depth in the littoral zone was highest in macrophyte patches at Reichenau (mean depth = 2.91 ± 0.91 (SD) m) and medium in Hagnau (mean depth = 1.74 ± 0.81 (SD) m). The shallowest patches were found in Güttingen (mean depth = 1.39 ± 0.49 (SD) m). The mean slope followed a similar course, being significantly higher in Hagnau (mean slope = 10.14 ± 11.57 (SD) m) than in Güttingen (mean slope = 6.1 ± 4.42 (SD) m) whereas the mean slope at Reichenau (mean slope = 8.92 ± 8.24 (SD) m) was not significantly different to either of the former and displayed an intermediate value (Table 1). Furthermore, the patches in Güttingen (mean distance to the shore = 186.22 ± 43.27 (SD) m) and Reichenau (mean distance to the shore = 177.56 ± 162.2 (SD) m) were significantly farther away from the shoreline than at Hagnau (mean distance to the shore = 91.56 ± 32.81 (SD) m).

The patch condition in the sampling season 2004 differed strongly with respect to sampling period and sampling site (Fig. 3) as well as *Acentria* densities. Mean *Acentria* densities ranged from 2.6 ± 1.1 Ind. g^{-1} dm in Güttingen, 3.9 ± 1.9 Ind. g^{-1} dm in Hagnau to 4.8 ± 2.8 Ind. g^{-1} dm at Reichenau. We performed a logistic regression to analyse the influence of sampling period, sampling site and *Acentria* densities on the condition index of the sampled *Potamogeton* patches (logistic regression, factor sampling period, Wald $Chi^2 = 23.63$, df = 1, $p < 0.0001$, factor sampling site, Wald $Chi^2 = 15.79$, df = 2, p = 0.0004, factor *Acentria* density, Wald $Chi^2 = 3.72$, df = 1, p = 0.054). Since *Acentria* densities were determined for the sampling period (2) and hence the influence of herbivory could only be analysed for the sampling periods (2) and (3), we excluded the sampling period (1) from the logistic regression analysis.

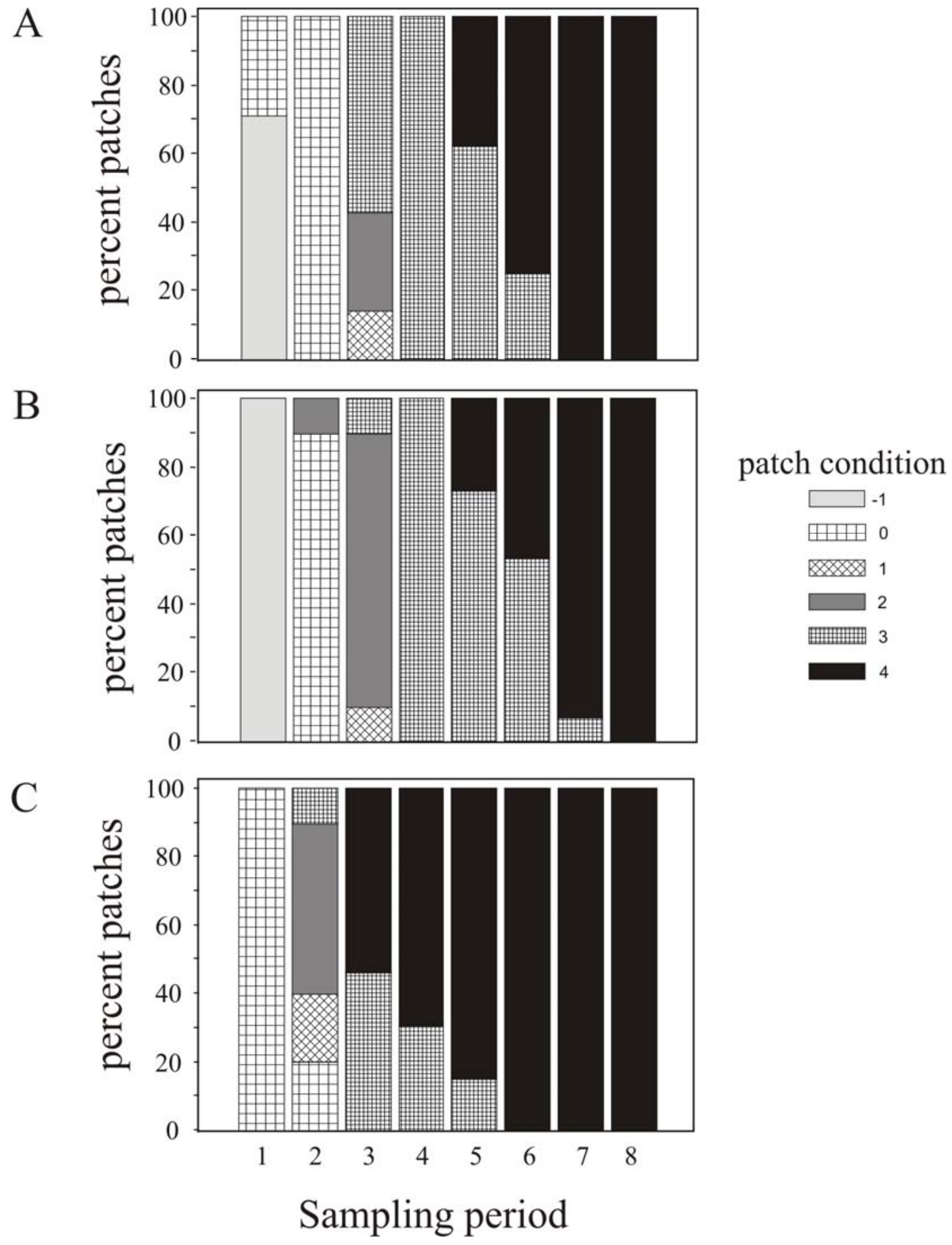

Fig. 3 Distribution of patches with patch conditions ranging from -1 (non-senescent, have not reached the water surface) to 4 (complete senescence) at the sampling sites Güttingen (A), Hagnau (B) and Reichenau (C). The number of patches in each sampling period is defined as 100 %. The patch condition was determined during the fish samplings in the sampling periods 1 (29.06.04 - 7.07.04), 2 (20.07.04 - 23.07.04) and 3 (24.08.04 - 4.09.04) and a routine monitoring of the patch condition was subsequently continued in the periods 4 (9.09.04 - 10.09.04), 5 (20.09.04 - 27.09.04), 6 (7.10.04), 7 (22.10.04) and 8 (2.11.04).

The factors sampling period and sampling site were strongly associated with patch condition. Patch senescence was generally higher at the site Reichenau (i.e. more plants in the studied macrophyte patches were senescent) than in Güttingen and Hagnau, and at Reichenau macrophytes also retreated sooner into overwintering underground stages (Fig. 3). Patches disappeared earlier than in Hagnau and Güttingen, i.e. they reached the patch condition index value 4 (complete senescence) earlier (Fig. 3 and 4). The patch condition in the logistic regression showed a tendency to have higher values of the patch condition index with increasing *Acentria* densities: *Acentria* densities were higher in more senescent patches, i.e. with a greater numerical value of the variable patch condition, especially at the sampling site Reichenau where *Potamogeton perfoliatus* patches disappeared earlier due to senescence than at the sampling sites Güttingen and Hagnau (Fig. 4).

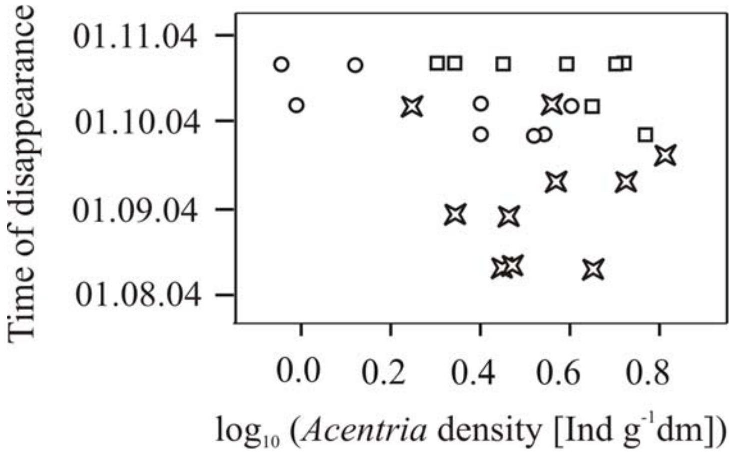

Fig. 4 The time of disappearance of *Potamogeton perfoliatus* patches in Lake Constance in relation to the mean densities of active *Acentria* larvae in the sampling period (2) in 2004, calculated from three replicate samples. Data are shown for *Potamogeton* patches of the three sampling sites Hagnau (squares), Güttingen (circles) and Reichenau (crosses).

This coincides with a significantly shorter occurrence of *Potamogeton perfoliatus* patches (i.e. the time from the main growth period in July until the patches were too senescent to be sampled) at Reichenau with 0.77 ± 0.78 months than at the sites Güttingen and Hagnau with 1.94 ± 0.42 and 1.9 ± 0.91 months, respectively (ANOVA, factor sampling site, $F_{2,33} = 8.91$, $p < 0.0008$).

In total, 293 fishes were caught with minnow traps during the sampling season 2004. The fish species included sticklebacks (*Gasterosteus aculeatus*), burbot (*Lota lota*), ruffe (*Gymnocephalus cernuus*), perch and pike. Additionally, small cyprinids that were partly in the larval stage were not distinguished on the species level and analysed summarised as “Cyprinidae”. Perch constituted the majority of the fish catches with 207 individuals comprising 70.6 % of the total catch (Table 2). Hence we focused in the statistical analyses of the effects of patch morphological setting, sampling period, sampling site and patch condition on the CPUE's and total lengths (TL) of perch. Due to the rather small number of 60 caught individuals, for three-spined sticklebacks only the effect of sampling period and sampling site on the CPUE was analysed. Ruffe, pike, burbot and cyprinids were only caught in low numbers (two, one, 13 and ten individuals, respectively, Table 2).

Table 2: Catches (absolute numbers) of perch, sticklebacks, burbot, cyprinids, ruffe and pike at the sites Güttingen, Hagnau and Reichenau for all three sampling periods in the field season 2004 combined.

site	perch	stickleback	burbot	cyprinids	ruffe	pike
Hagnau	103	47	2	8	0	0
Güttingen	55	13	4	2	1	0
Reichenau	49	0	7	0	1	1
All sites	207	60	13	10	2	1

Sticklebacks, perch and burbot were mainly caught in the two minnow traps near the ground (96.7 %, 65.7 % and 61.5 % of all individuals, respectively). Cyprinids were more evenly distributed with 20 % of all individuals in the two bottom-most minnow traps. Perch, ruffe, pike, burbot and cyprinids were determined as young-of-the-year fish according to their TL (Casselman & Lewis 1996; Fischer & Eckmann 1997 a; Fischer & Eckmann 1997 b; Mehner *et al.* 1998; Table 3; Fig. 5) whereas the sticklebacks also comprised adult specimens (Allen & Wootton 1982).

Table 3: Mean total length (TL) ± S.D. [cm], logarithmic mean TL ± S.D. [cm], minimum TL [cm] and maximum TL [cm] of perch, sticklebacks, burbot, cyprinids, ruffe and pike in the field season 2004.

	perch	stickleback	burbot	cyprinids	ruffe	pike
Mean TL ± S.D. [cm]	4.49 ± 0.71	5.91 ± 1.21	6.32 ± 0.98	5.17 ± 1.09	3.4 ± 0.85	10.8
Log mean TL ± S.D. [cm]	0.65 ± 0.07	0.76 ± 0.11	0.8 ± 0.07	0.7 ± 0.1	0.52 ± 0.11	1.03
Min TL [cm]	2.6	2.6	5.2	3.2	2.8	10.8
Max TL [cm]	9.5	8.7	7.9	6.7	4.0	10.8

Logarithmic stickleback CPUE (Fig. 6 A) showed significant differences between sites, being significantly higher at the sampling site Hagnau compared to Reichenau (ANOVA, factor sampling site, $F_{2,177} = 4.1$, $p = 0.018$). The logarithmic stickleback CPUE was neither significantly different between the sampling periods, nor was the interaction between sampling period and site significant (Fig. 6 A, ANOVA, factor sampling period, $F_{2,177} = 0.68$, $p = 0.51$, interaction sampling site*sampling period, $F_{3,177} = 0.48$, $p = 0.7$).

The influence of patch morphological setting, sampling period and sampling site on the logarithmic CPUE of perch was analysed with an ANOVA (Table 4). The logarithmic CPUE of perch was not significantly influenced by water depth, distance to the shoreline, slope and sampling site whereas the factors patch condition and sampling period had a significant influence (Table 4).

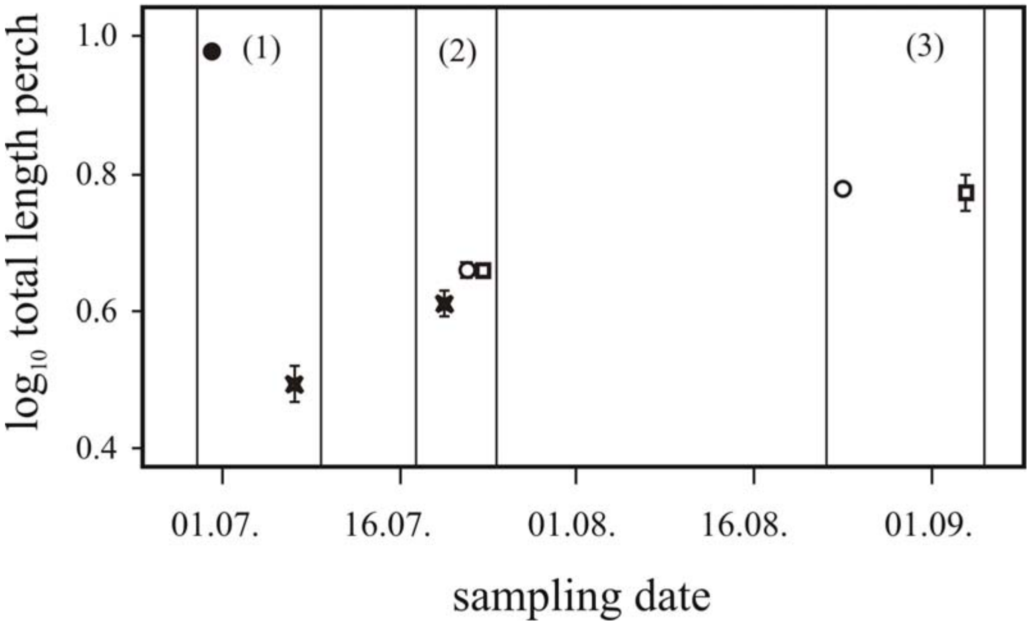

Fig. 5 Total lengths (TL) of perch ($\log_{10}$ (total length [cm]) at the three sites Hagnau (squares), Güttingen (circles) and Reichenau (crosses) in Lake Constance at the three sampling periods (1) = 29.06.04 - 7.07.04, (2) = 20.07.04 - 23.07.04 and (3) = 24.08.04 - 4.09.04.

Furthermore, all two-way interactions between the factors were tested and were not significant, except the interaction between sampling site and sampling period (Table 4). Densities of perch significantly increased at all three sites from sampling period (1) to (2) and subsequently decreased in sampling period (3) in Güttingen and Hagnau (Fig. 6 B). However, since most patches in sampling period (3) had already vanished due to high senescence, no fishes were sampled at Reichenau in the sampling period (3) (Fig. 6 A and B).

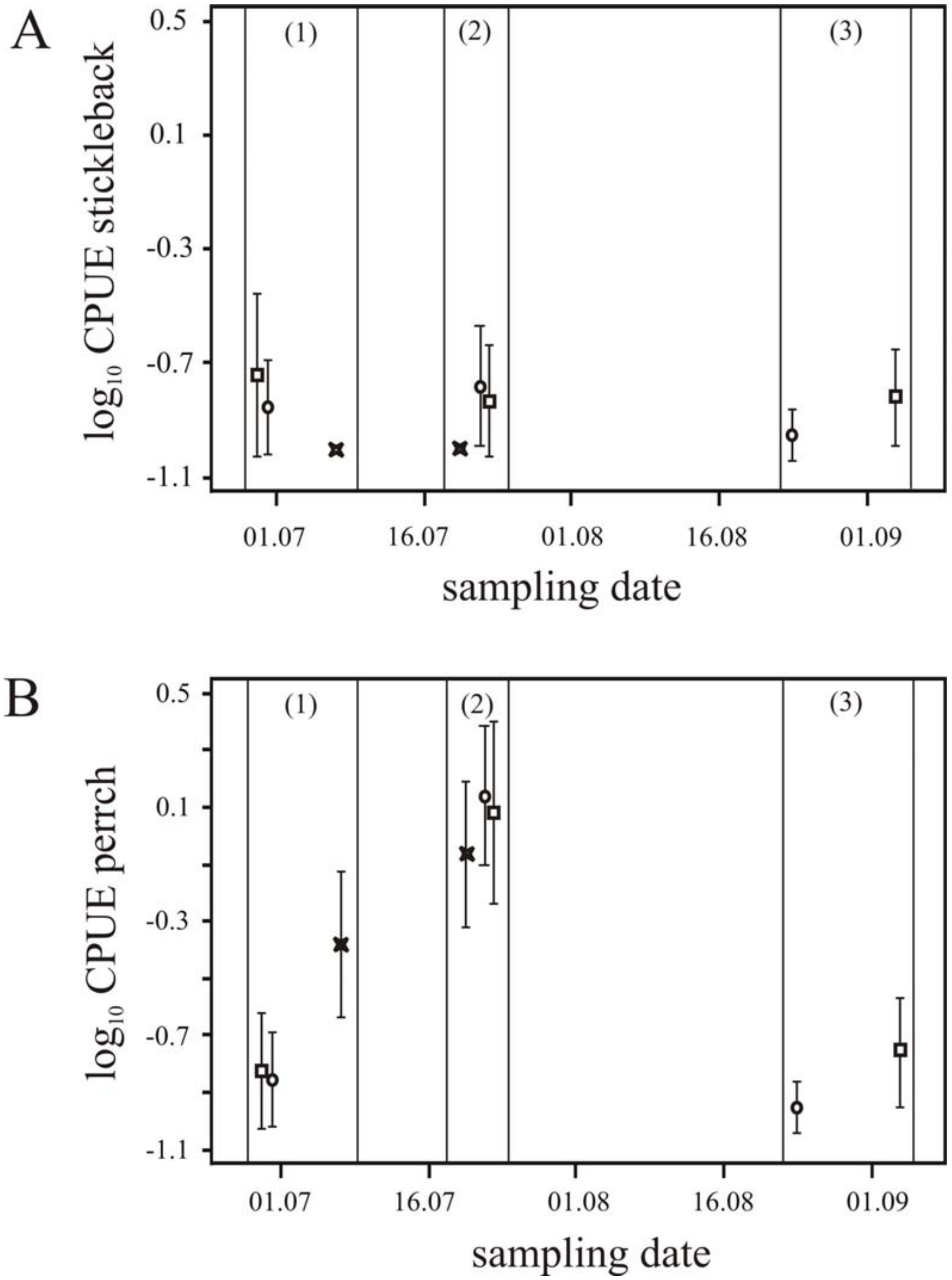

Fig. 6 Logarithmic CPUE of stickleback (A) and perch (B) at the three sampling sites Hagnau (squares), Güttingen (circles) and Reichenau (crosses) in Lake Constance at the three sampling periods (1) = 29.06.04 - 7.07.04, (2) = 20.07.04 - 23.07.04 and (3) = 24.08.04 - 4.09.04.

To visualize the relationship between patch condition and CPUE, in Fig. 7 the residuals of an ANOVA with the significant factors sampling site, sampling period and its interaction, but without patch condition, are shown as a factor of patch condition: The residuals were highest at a patch condition index of 1 (i.e. light senescence), decreasing at higher and at lower index values (Fig. 7).

Table 4: Five-factorial ANOVA analysing the influence of the factors depth, distance to the shore, slope, sampling site and sampling period and the interaction between sampling site and sampling period on the logarithmic CPUE of perch in the field season 2004.

	df	F	p
depth	1	0.49	0.49
distance to the shore	1	0.98	0.32
slope	1	0.26	0.61
patch condition	5	3.46	0.0053
sampling site	2	1.76	0.18
sampling period	2	40.97	< 0.0001
sampling period *sampling site	3	5.89	0.0008

An ANOVA of the logarithmic total lengths of perch showed a highly significant interaction between sampling site and sampling period (ANOVA, factor sampling site, $F_{2,180} = 76.37$, $p < 0.0001$, factor sampling period $F_{2,180} = 21.66$, $p < 0.0001$, interaction sampling site*sampling period, $F_{2,180} = 49.73$, $p < 0.0001$). Total lengths of perch increased at all three sites during the season. The increase was lowest at Reichenau and highest in Güttingen and Hagnau (Fig. 5). The caught individuals were probably juveniles and/or larvae that were steadily growing at the

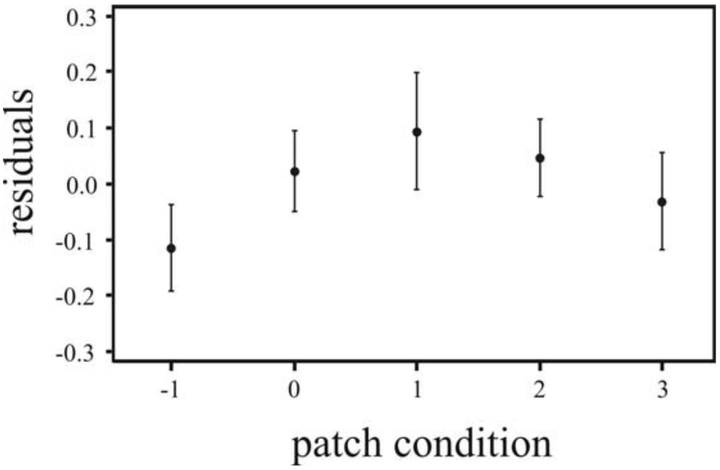

Fig. 7 Residuals of a two-factorial ANOVA of the logarithmic CPUE of perch (factors: sampling period, sampling site) in relation to patch condition. The shown data include CPUE's of all three sampling sites Hagnau, Güttingen and Reichenau combined. Patch condition: -1 = no senescence, plants have not reached yet the surface, 0 = no senescence, plants have reached the surface, 1 = light senescence, 2 = strong senescence (up to 50 % of the plants), 3 = very strong senescence (more than 50 % of the plants).

sites Hagnau, Güttingen and Reichenau during the season (Fig. 5) although in Güttingen in sampling period (1) only one perch specimen could be measured that was considerably larger than the individuals in the sampling periods (2) and (3).

Discussion

Potamogeton perfoliatus patches showed strong seasonal changes in the degree of senescence, i.e. the percentage of senescent shoots in a patch. *Potamogeton* displays a seasonal resource allocation from the above-ground shoots to the below-ground resting buds as overwintering stages at the end of the vegetation period, which lasts from May to September (Wolfer & Straile 2004 b). Senescence starts in August, and in September most of the above-ground plant biomass already has disappeared (personal observation) by decay and removal from the littoral zone through wave action and water currents. A mesocosm experiment suggests that herbivore damage due to *Acentria ephemerella* has a strong influence on *Potamogeton* senescence and vegetation period (see chapter I). This influence is also supported by this field study: *Acentria* densities were associated with the condition of *Potamogeton* patches in a logistic regression even after accounting for spatial differences in patch conditions. However, as *Acentria* densities did also significantly differ between sites, the herbivore might also at least partially affect spatial differences in patch conditions, i.e. the early senescence of *Potamogeton* at the site Reichenau (Fig. 3) might at least be due to the higher *Acentria* densities at this site (Fig. 4).

We found significant seasonal and spatial changes in the distribution and growth of perch and the distribution of sticklebacks in *Potamogeton perfoliatus* patches in the littoral zone of Lake Constance. Perch and sticklebacks dominated the fish community, comprising together more than 90 % of all caught individuals. Sticklebacks showed no significant seasonal but spatial variability in CPUE (Fig. 6 A): Sticklebacks were caught in higher numbers in ULC, at Güttingen and Hagnau, than at Reichenau (LLC).

However, the relation between fish and *Acentria* densities is complex and cannot easily be explained. Despite their low number in our catches, we expect sticklebacks to have a stronger influence on *Acentria* densities than the much more numerous juvenile perch. Sticklebacks can strongly influence the seasonal population growth of *Acentria* (chapters III and IV) and hence might contribute to the lower densities of *Acentria* observed at the ULC sites. If perch have a strong influence on *Acentria*, we should not find such a spatial correlation between stickleback and *Acentria* densities. Furthermore, previous field studies also revealed a spatial association between *Acentria* and stickleback densities (chapter V), and *Acentria* larvae were found in the stomachs of sticklebacks caught in Lake Constance (Korn, unpublished data). In contrast, analyses of the content of 20 juvenile perch stomachs from this field study revealed no ingestion of *Acentria* larvae (Miler, unpublished data). However, we cannot fully exclude the possibility of perch predation on *Acentria* larvae since the juvenile individuals in our minnow traps were of a size in which they should switch from planktonic to benthic food organisms and hence should be capable to feed at least on 1st or 2nd instar *Acentria* larvae. Moreover, our sampling design was not optimal to catch fish for stomach analyses since they continue their digestion inside the traps and could have completed digestion until the traps were emptied after 24 h, resulting in empty stomachs. An analysis of a higher number of perch stomachs from field catches in Lake Constance, caught preferentially by electrofishing, are needed in combination with mesocosm experiments that include juvenile perch as a predator for *Acentria* to solve this question conclusively.

In the previous sampling years 2002 and 2003, densities of *Acentria* larvae in LLC were lower than in ULC (Korn, unpublished data). In these years sticklebacks could be seen in shoals close to the water surface and far more frequently in LLC than in ULC (Korn, unpublished data). In 2002 and 2003, high stickleback densities in LLC corresponded to low densities of *Acentria* larvae at this sampling site. Interestingly, stickleback specimens caught in Lake Constance in 2003 and dissected for gut content analyses were infected without exception with endoparasitic plerocercoid larvae of the cestode *Schistocephalus solidus*, in contrast to individuals from 2004 where no *Schistocephalus solidus* infections could be found (Miler, unpublished data). The large plerocercoid larvae commonly occur in natural stickleback populations and are known to change the anti-predator and foraging behaviour of their hosts: infected specimens are less suspicious against predators, have a reduced manoeuvrability, swim closer to the water surface and have a higher food intake than uninfected ones (Barber 2007). As a consequence of the infection, the high predation pressure on *Acentria* in LLC in 2003 was probably enhanced additionally due to these behavioural effects of parasitisation. Furthermore, parasite infection explains why individuals in 2003 were easily seen in shoals close to the water surface. However, *Schistocephalus solidus* uses sticklebacks only as intermediate hosts and reaches maturity in the stomachs of waterbirds as its definite host when the parasitised fish are eaten. As a consequence of their altered anti-predator and foraging behaviour, we thus expect sticklebacks to be strongly preyed upon by birds in 2003, which explains the low stickleback CPUE in LLC in 2004 as the result of a population breakdown, caused by a high mortality of infected sticklebacks in the year before. This is in accordance with previous research since cyclical population fluctuations are common in sticklebacks and the population densities have been shown in field studies to oscillate with a period of six years (Wootton & Smith 2000; Wootton *et al.* 2005). Furthermore did Pennycuick (1971) associate population fluctuations of sticklebacks with infections by *Schistocephalus solidus*, which supports our explanation of the stickleback population development in Lake Constance in the years 2003 and 2004.

Perch were found throughout the season, with densities reaching a maximum in the sampling period (2) at all three sites. According to their size all individuals were young-of-the-year (YOY) that increased in length from sampling period (1) to (3) (Fischer & Eckmann 1997 b).

The development of perch CPUE from sampling period (1) to (3) (Fig. 6 B) can be related to the condition of the macrophyte patches (Fig. 7). In the sampling period (1), patches were not fully developed and had not reached the water surface yet (Fig. 3). The perch CPUE was highest in patches with a light senescence and macrophytes that have grown to a canopy at the water surface, i.e. a patch condition index value of 1 (Fig. 7). This developmental stage was mostly found in the sampling period (2) and represents a medium patch condition between a high percentage of freshly-grown, non-senescent plants near the sediment that can be found at the start of the season and a high percentage of completely senescent plants at the end of the season that do not any longer form a coherent macrophyte patch. In sampling period (3), CPUE decreased with increasing patch senescence (Fig. 6 B).

However, there are several explanations for the decrease of perch CPUE at the end of the season. First, does the maximum of perch CPUE in patches with a patch condition index value of 1 suggest a preference of perch for habitat structures with a medium patch complexity and density. Previous studies showed that with increasing patch complexity the densities of invertebrate prey for fishes increase (Savino *et al.* 1992; Eklöv 1997; Warfe & Barmuta 2006), but the foraging efficiency decreases, i.e. the visual contact (Savino & Stein 1982) and the manoeuvrability of fishes (Manatunge *et al.* 2000). The reduction in foraging efficiency is also true for piscivorous predators (Crowder & Cooper 1982; MacRae & Jackson 2001). Crowder & Cooper (1982) proposed a medium stem density to be an optimal solution for the conflicting effects of density on prey capture (visibility and manoeuvrability) and invertebrate densities. *Potamogeton* patches in which the plants have reached the water surface offer a high density of macrophyte shoots, and the density is lowered by a moderate percentage of senescent plants. In accordance with this, our field results suggest that fully developed patches with a light senescence (i.e. a patch condition index value of 1) constitute a suitable and optimal habitat for YOY perch by (1) offering an optimum density of invertebrate prey, (2) enabling YOY perch sufficiently to swim in the space between plant shoots and to forage visually on macrophyte-associated invertebrates and (3) providing a refuge against piscivorous fishes, i.e. primarily adult perch that are hindered in their visual orientation and manoeuvrability. A further increasing patch senescence can lead to a rapid reduction of plant biomass in the patch due to physical fragmentation by wave and current action (Odum *et al.* 1972) and through the feeding of detritivorous invertebrates (Webster & Benfield 1986; Newman 1991). As a consequence, plant density and patch complexity is strongly reduced and the overall patch structure changes, affecting the suitability of the patch as a foraging habitat and refuge against predation for juvenile fishes (Crowder & Cooper 1982; Savino & Stein 1982; Dionne & Folt 1991; MacRae & Jackson 2001; Warfe & Barmuta 2006).

Second, perch CPUE in the littoral zone is known to vary seasonally due to ontogenetic habitat shifts during development associated with changes in food size and preference. After hatching in the littoral zone in May, perch larvae migrate into the pelagic zone to feed on zooplankton. In early July then perch return as juveniles to the littoral zone, where they continuously feed and grow in submerged vegetation during the summer in a water depth of 1.5 to 3 m (Wang & Eckmann 1994; Fischer & Eckmann 1997 b) and were caught in the minnow traps used in this study. As perch grow older, they leave the macrophyte patches and prefer open, unvegetated sites, using the littoral as a resting zone during night and foraging in the pelagic zone during daytime (Imbrock *et al.* 1996; Fischer & Eckmann 1997 b) although they have also been shown to feed strongly on juvenile perch in the littoral zone (Schleuter & Eckmann 2008). This is in accordance with results from Fischer & Eckmann (1997 b) who suggested that the use of macrophyte patches by eel and pike as predators of larger, cannibalistic perch prevent them from swimming in macrophyte patches, which can thus be regarded as refuges from predation for juvenile perch. Third, the catchability of the minnow traps for juvenile perch (and other fish species) could decrease during the season as they grow too large to fit through the opening of the trap. In addition, a reduced macrophyte density late in the season could lead to an increased visibility of the minnow traps for fishes.

Other fish species, i.e. cyprinids, ruffe, pike and burbot were caught only in small numbers. Pike were only accidentally caught inside the minnow traps since they were probably not attracted by the trap baits, consisting of beef liver and dog food, but rather by juvenile fishes that were already trapped inside the minnow traps.

The small number of caught ruffe (two individuals) is in contrast to results of Fischer & Eckmann (1997 b) where YOY ruffe occurred in similar densities as YOY perch, but in accordance with recent findings from Lake Constance (Schleuter & Eckmann 2008). Schleuter & Eckmann (2008) could show that the on-going reoligothrophication will lead to a more intense intraspecific competition and as a consequence to decreasing densities of ruffe in Lake Constance. Differences in sampling methods could also explain the small caught number of ruffe in this study. Fischer & Eckmann (1997 a; 1997 b) used electrofishing in submerged aquatic vegetation (SAV) whereas our applied minnow traps could have been more visible for ruffe especially at low light levels since, probably due to a light-reflecting layer in the eye, the so-called “tapetum lucidum”, they are able to orientate and forage at nighttime (Bergman 1988; Schleuter & Eckmann 2006) and thus could have avoided the traps. Furthermore, since we baited the traps with beef liver and dog food that have been found by many fishermen to attract fishes, ruffe may have displayed an avoidance behaviour towards the “strong smelling” traps.

The low CPUE of cyprinids in the macrophyte patches is in accordance with results of Fischer & Eckmann (1997 b) where juvenile bream, dace (*Leuciscus leuciscus*) and chub (*Leuciscus cephalus*), the dominant cyprinids in Lake Constance, preferred shallow areas with a water depth < 50 cm, even over SAV. These very shallow littoral areas probably provide a safer habitat against predatory fishes than macrophyte patches (Fischer & Eckmann 1997 b).

Interestingly, we caught YOY burbot, although with a low CPUE, in macrophyte patches of all three sites (Table 2). Juvenile burbot are bottom-dwelling, night-active fishes in rivers and littoral zones of lakes and hide during daytime under stones (Fischer 2000). Fischer & Eckmann (1997 b) found only low densities of juveniles in SAV, which occurred at water depths between 150 and 300 cm, and higher densities at shallower areas (< 150 cm water depth), especially with gravel and stones as a substrate. However, they did not take samples in LLC where SAV with underlying sediment instead of stones or gravel dominates.

The depth distribution of the four most abundant taxonomic groups, i.e. perch, sticklebacks, burbot and cyprinids, revealed that the former three clearly preferred the two undermost minnow traps whereas cyprinids showed no preference. Since burbot are bottom-dwelling, benthic fishes (Fischer 2000), it was not surprising to find the majority of individuals close to the bottom. The choice of perch and sticklebacks for the two undermost traps can be explained by the deeper sites of macrophyte patches being probably the densest and hence the safest place against predation. Patch zones close to the surface could have been thinner due to plants having not yet reached the surface at the beginning or due to senescence at the end of the summer season. Senescence reduces the plant stability so that macrophyte stems break more easily, and the upper stem parts are lost first due to wave action and water currents. Thus visibility to predators (piscivorous fish, birds) of the two uppermost traps was probably increased during these periods.

To summarise, *Potamogeton* patches could be shown to be a spatially and seasonally restricted habitat for young-of-the-year perch, sticklebacks, burbot, pike, ruffe and cyprinids as well as for adult sticklebacks in Lake Constance. Stickleback predation on *Acentria* probably led to a spatial variability in *Acentria* densities, with highest *Acentria* densities at the site Reichenau where the stickleback CPUE was lowest. We suggest that the observed change in physical structure by *Acentria* feeding damage, i.e. the removal of plant parts, and probably enhanced patch senescence due to consumer damage strongly influenced the availability of suitable foraging and refuge habitats in submerged aquatic vegetation, especially for perch as the most abundant fish species. We thus suggest to refer to *Acentria* as a "physical ecosystem engineer" (*sensu* Jones *et al.* 1997), i.e. "an organism creating, maintaining or modifying physical habitat structures for other organisms". Hence *Acentria* is an important herbivore species in freshwater littoral food webs, structuring macrophyte patches as a habitat for fishes. This study provides important insights into the relationship between macrophyte patch senescence and the spatial and seasonal distribution of small fishes in aquatic vegetation. More research is needed to determine the variations in seasonal abiotic and biotic factors that influence such fish distributions in submerged aquatic vegetation in the littoral zone of Lakes.

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Chapter III

Experimental evidence for a strong influence of stickleback predation on the population dynamics and sex-ratio of an aquatic moth

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Abstract

The larvae of the water moth *Acentria ephemerella* are important herbivores in freshwater macrophyte beds. Due to the considerable damage that *Acentria* can inflict on the plants, the herbivore might influence the important role of macrophytes within littoral ecosystems. It is thus important to know which factors can control *Acentria* populations and consequently its interannual variability. Here we use a mesocosm experiment to test whether fish predation can control *Acentria* population development. We followed *Acentria* population development and size distribution using two different stickleback, *Gasterosteus aculeatus*, densities and one control treatment. Sticklebacks were able to reduce population growth of *Acentria* compared to the control treatment without fishes. Predation of sticklebacks on *Acentria* was confirmed by gut content analyses of sticklebacks caught at the end of the experiment. In addition to a reduction of population growth, stickleback predation resulted in male-biased sex-ratios of *Acentria*, which are typically observed *in situ* in Lake Constance. A comparison of *Acentria* size distributions in the experiment and in stickleback guts suggests that the male bias is not due to size-selective predation of sticklebacks on presumably larger females. In summary, stickleback predation influenced *Acentria* population dynamics and dampened population growth (1)

directly by consuming *Acentria* larvae and (2) indirectly by shifting the sex-ratio via sex-specific mortality towards a stronger male bias. Top-down control by fishes of *Acentria*, a key herbivore in macrophyte beds, suggests the existence of a previously overlooked trophic cascade of major importance in aquatic ecosystems.

Introduction

In many aquatic habitats, food web interactions are strongly determined by the top-down control of herbivore populations (Brett & Goldman 1997; Estes *et al.* 1998; Menge 2000). However, research on top-down controlled trophic effects in freshwater ecosystems is mainly confined to pelagic (Carpenter *et al.* 1985; Persson 1997; Bertolo *et al.* 1999) or lotic food webs (Power 1992). In lentic littoral ecosystems, research has concentrated on algal-based food webs, particularly the benthic fish - snail - epiphyton food chain (Martin *et al.* 1992; Brönmark & Vermaat 1998; Jones & Sayer 2003), and although submerged macrophytes are known to play an important structuring role in the littoral zone (Crowder *et al.* 1998), food webs based on vascular plants and interactions between herbivore macroinvertebrates and their predators in submerged macrophyte beds have up to now received only limited attention (but see Sutter & Newman 1997; Ward & Newman 2006).

The water veneer *Acentria ephemerella* (Denis & Schiffermüller), a herbivorous moth with a predominantly aquatic life-cycle, is commonly found in lakes and brackish coastal waters throughout Central and Northern Europe (Berg 1942; Hedal & Schmidt 1992; Gross *et al.* 2002) and has invaded lakes in North America during the last decades (Batra 1977; Scholtens & Balogh 1996). Seasonal dynamics of *Acentria* densities have been studied in detail in Lake Constance, Germany, (Gross *et al.* 2002) and Lake Neuchâtel, Switzerland, (Haenni 1980) as well as in several smaller lakes in Poland (Kokocinski 1963) and Finland (Palmen 1953). Due to its high densities that can increase over several orders of magnitude during the short summer season, from 0.1 to 100 individuals per gram plant dry mass (Gross *et al.* 2002), *Acentria* is an important herbivore of macrophytes and has been suggested to be responsible for widespread feeding damage on submerged aquatic vegetation (Creed & Sheldon 1994; Gross *et al.* 2001). Consequently, *Acentria* herbivory might interfere with the important structuring role of macrophytes in littoral and shallow lake food webs (e.g. Jeppesen *et al.* 1998) and hence might be of crucial importance for lake functioning. In addition, *Acentria* has been discussed as a possible biocontrol agent for invasive *Myriophyllum* in lakes in North America (Johnson *et al.* 1998; Newman 2004). It is thus important to understand the factors controlling *Acentria* population development.

Macrophyte beds as well as seagrass meadows are well known to provide food and shelter for many fish species (Heck *et al.* 2003; Gillanders 2006) and are regarded to function as important nursery habitats for commercially important fish species (Butler & Jernakoff 1999; Beck *et al.*

2001). Likewise, patches of submerged macrophytes in freshwater ecosystems are inhabited by especially young-of-the-year (Y-O-Y) fishes seeking shelter from predation (Casselman & Lewis 1996; MacRae & Jackson 2001) or searching for macroinvertebrates as food (Whitfield 1984; Dionne & Folt 1991; Diehl & Kornijow 1998). In North American lakes, sunfish use macrophyte patches as a habitat and have been shown to prey on *Acentria* larvae (Ward and Newman 2006). Sticklebacks (*Gasterosteus aculeatus* L.) use macrophyte patches as a habitat as well (e.g. Doucette *et al.* 2004), and they can exert a strong predation pressure on macroinvertebrates (Allen & Wootton 1984; Schluter 1995). This suggests a potential influence of sticklebacks also on the population dynamics of *Acentria*.

Here we use a mesocosm experiment to test the potential of a top-down control of sticklebacks on *Acentria ephemerella*. To increase our understanding of this predator-prey interaction we additionally analyse the influence of stickleback predation on the size distribution and the sex-ratio of *Acentria*.

Methods

The experiment was conducted in July 2003 in a large outdoor mesocosm (length = 10.5 m, width = 5 m, height = 1.5 m), situated at the Limnological Institute of the University of Konstanz. 18 experimental units were placed in the mesocosm that was filled with fine sediment (height ~ 0.35 m) and water from Lake Constance. The sediment was mixed prior to the experiment to provide the same environmental starting conditions for all replicates. An experimental unit consisted of a tube made of transparent Tricoron plastic foil (RKW AG Rheinische Kunststoffwerke, Wasserburg, Germany) that separated the experimental unit from the surrounding water in the mesocosm. Water in the experimental units was not changed during the experiments. A tube had a length of approximately 1.5 m and was connected to a polystyrol ($(C_8H_8)_n$) frame with an area of 0.43 m^2 (0.6 m width, 0.715 m length). The frame had the function of a buoy to hold the plastic tube in a vertical position in the water column. The lower end of the plastic tube was fixed with ten metal clips on a metal frame (length = 0.8 m, width = 0.6 m, height = 0.05 m) and dug into the mesocosm sediment. Wooden frames (length = 0.92 m, width = 0.73 m) covered with plastic (mesh size ~ 1210 µm) gauze were placed approximately 0.1 m above the water surface upon the polystyrol frames to prevent the adult stages of *Acentria* from dispersal out of the experimental units and to allow the winged *Acentria* males to fly around and search for females within experimental units, i.e. the experimental setup allowed for *Acentria* reproduction. Water temperatures during the experiment were 19.7 ± 1.1 (SD) C°.

Experiments were performed with organisms sampled in Lake Constance, a large, warm monomictic freshwater lake in the pre-alpine region of Southern Germany. The lake is divided into two basins, the deep Upper Lake Constance (z_{mean} = 101 m) and the shallower Lower Lake

Constance (z_{mean} = 13 m). Shoots of *Potamogeton perfoliatus*, a dominant macrophyte species in Lake Constance that forms large, mostly monospecific patches in the littoral zone, and associated macro-invertebrates, i.e. including larvae and pupae from *Acentria*, were sampled on 3 July 2003 in a Lake Constance macrophyte patch. The plants for each replicate were sampled equally from the edge, centre and between edge and centre of a macrophyte patch. They were evenly distributed to separate containers (one for each replicate) during sampling and subsequently planted on 3 July together with the macrophyte-associated macro-invertebrates with a shoot density (n = 18 per experimental unit) which is in the range of densities observed within patches of Lake Constance (Wolfer & Straile 2004 b). Three replicates were sampled at the start of the experiment on 10 July, one week after planting the macrophytes into the sediment of the mesocosm. On the same day, the remaining fifteen experimental units in the mesocosm were randomly assigned to three treatments (no fish (control), low fish density (LF, 12 ind m^{-2}), high fish density (HF, 45 ind m^{-2})) and the fishes were introduced accordingly. The control treatment (no fish) consisted of three replicates, and the LF and HF treatments consisted of six replicates each. Sticklebacks were caught with scoop nets in Lake Constance and introduced into the LF and HF replicates on 10 July. Sticklebacks of two size classes were caught in the lake and consequently used in the experiment: small Y-O-Y sticklebacks (2.6 ± 0.1 (SD) cm total length) and large (5.5 ± 0.5 (SD) cm total length), probably one year old individuals. In each replicate of the LF treatment, five large and one small and in each HF treatment fifteen large and seven small sticklebacks were introduced. Unfortunately, some of the caught sticklebacks were strongly infested by a parasite (*Schistocephalus solidus*). Most probably, this infection resulted in an average fish mortality of 24 % (17 % in the LF treatment and 31 % in the HF treatment, no significant differences between treatments, ANOVA, factor treatment, $F_{1,11}$ = 2.6, p = 0.14), leading to a lower predation pressure than initially intended. Each experimental unit was sampled twice, i.e. approximately 50 % of the plants after ten days and the remaining ones after 20 days of fish predation (on 21 and 31 July, respectively) in order to get more insight into the dynamics of *Acentria* growth and fish predation. The experiment was terminated on 31 July in order to ensure that *Acentria* development was not yet influenced by food limitation due to overexploitation of macrophytes by *Acentria*. After sampling *Acentria* larvae associated with *Potamogeton perfoliatus* were washed through a sieve (mesh size 45 µm) and fixed in 70 % Ethanol in 1-l plastic boxes. Since *Acentria* pupae were closely attached to the stems of *Potamogeton perfoliatus* shoots, the remaining plant material was frozen and was searched through for pupae after the experiment. Macrophytes were dried at 90 °C for three days and densities of *Acentria* pupae and larvae calculated as individuals per gram plant dry mass (Ind. g^{-1} dm). At the end of the mesocosm experiment, the sticklebacks were caught with scoop nets and killed with 2 mg $liter^{-1}$ 1,1,1-trichloro-2-methyl-propanol (TCMP). They were frozen for subsequent gut content analyses. After the experiment, the fishes were weighed and measured, and then their guts were dissected and fixed in 70 % Ethanol. A dissecting microscope (Zeiss Stemi 2000-C) was used to collect and count *Acentria* larvae at a 10–50× magnification from samples (washed samples and stickleback guts). *Acentria* pupae were sexed according to the length of their antennae as published in Berg (1942) (see Appendix, Fig. A 2). Measurements of head capsule widths of *Acentria* larvae found in the experimental units and in ten exemplary stickleback guts, as well as of the pupal lengths of *Acentria* in the experimental units, were made under a microscope equipped with an ocular micrometer. Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Results

Effects of sticklebacks on *Acentria* larval density changed during the course of the experiment (Fig. 1A). During the first ten days of the experiment, sticklebacks did not influence the larval densities (ANOVA, factor treatment, $F_{2,12} = 0.58$, $p = 0.58$) whereas after another ten days of predation densities of *Acentria* larvae differed significantly between treatments (ANOVA, factor treatment, $F_{2,12} = 6.25$, $p = 0.014$). However, despite predation larval densities increased after 21 July in all treatments, showing that *Acentria* was able to reproduce during the experiment. Based on densities in the various treatments at the end of the experiment, *Acentria* mortality rates (d) were calculated for the 2nd period using an exponential model as $d = (\ln(A_C) - \ln(A_F)) / t$ with A_C and A_F = *Acentria* densities in control and fish treatments and t = ten days, i.e. from 21 July to 31 July. Mortality rates were calculated as 0.034 d^{-1} and 0.135 d^{-1} in the LF and HF treatments, respectively. In contrast to *Acentria* larvae, pupal densities declined in all three treatments during the experiment (Fig.1 B). Given a developmental time for pupae between one and two weeks (personal observation) it is unlikely that the pupae found at the end of the experiment were already introduced as pupae at the start of the experiment. Rather all pupae introduced at the start developed further into

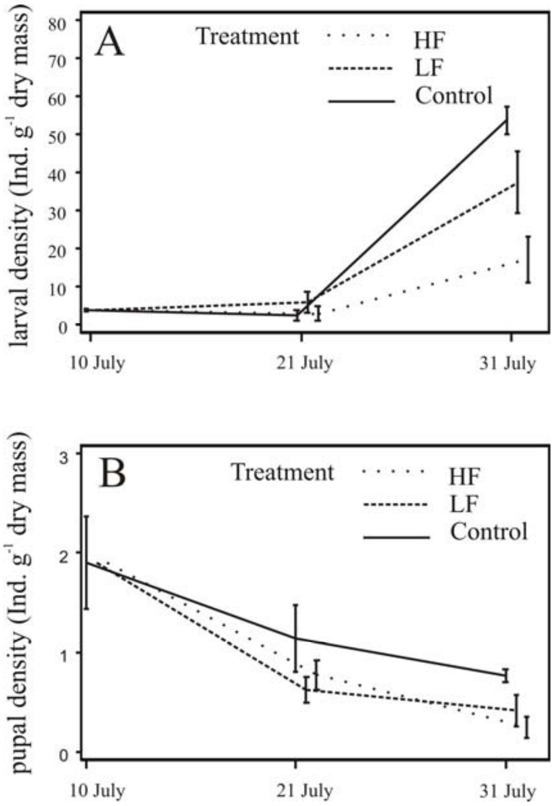

Fig. 1 Density development (Ind. g^{-1} dm ± SE) of *Acentria* larvae (A) and pupae (B) in the high fish density (HF), low fish density (LF) and control treatment during the mesocosm experiment.

adults during the experiment whereas the pupae present at the end of the experiment (31 July) metamorphosed from larvae during the course of the experiment. The general decline of pupae is thus due to a larger number of adults hatching from pupae compared to the number of larvae metamorphosing into pupae and hence is not related to *Acentria* mortality. However, the decline was strongest in the HF treatment suggesting that fewer larvae were able to survive the larval period and metamorphose into pupae when fish were present. Due to the low numbers of pupae present at the end of the experiment and the fact that not all larvae that pupated spent their entire larval phase in the same predation regime, the statistical support of the effect of fish on pupal densities is however not strong (ANOVA, factor treatment, $F_{2,12} = 3.01$, $p = 0.09$).

The general developmental trajectory of *Acentria* larvae during the experiment is shown with frequency distributions of headcapsule widths (HCW) at the start of the experiment and the two following sampling dates within the control treatment (Fig. 2). HCW measurements suggest that we followed the development of three larval cohorts throughout the experiment. On 10 July, only one rather advanced cohort (mean HCW: 921 ± 117 µm (SD), in the following cohort I) of *Acentria* larvae was present (Fig. 2). The samples at 21 July revealed a bimodal size distribution with two cohorts.

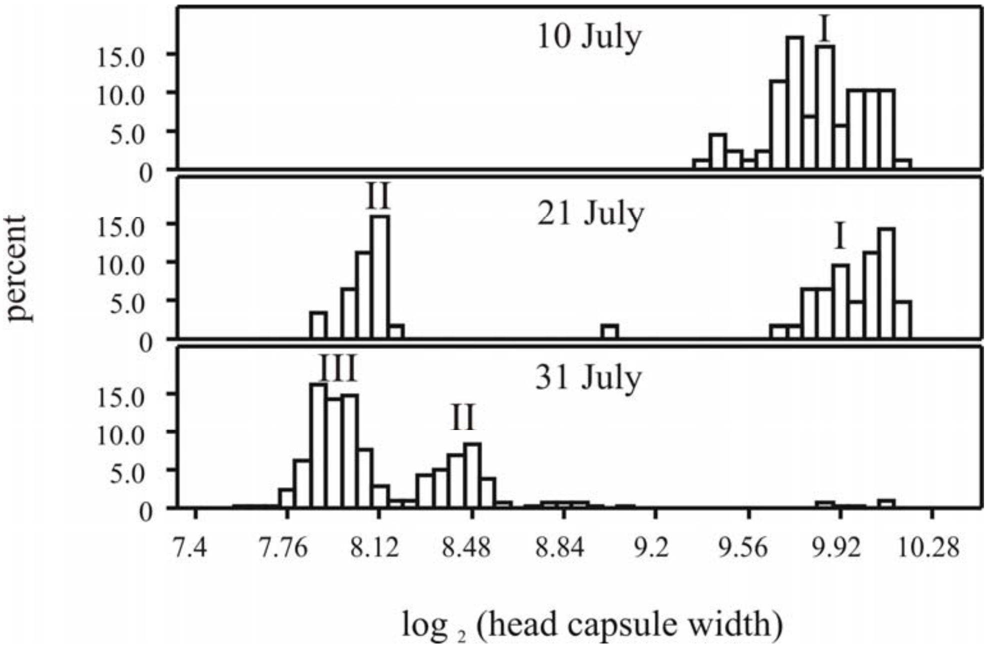

Fig. 2 Frequency distribution of *Acentria* larvae head capsule widths (HCW) at the start of the experiment and in the control treatment at the two sampling dates. Cohorts are represented by roman numerals. Cohorts were distinguished based on HCW: cohort I on 10 July as well as on 21 July consisted of larvae with a HCW larger than 680 µm ($\log_2 = 9.41$). Cohort II on 21 July consisted of larvae with a HCW smaller than 296 µm ($\log_2 = 8.21$). On 31 July, nearly all larvae of the cohort I already had pupated. Cohort II consisted of larvae with a HCW between 315 µm ($\log_2 = 8.30$) and 416 µm ($\log_2 = 8.70$) and cohort III consisted of larvae with a HCW smaller than 315 µm ($\log_2 = 8.30$).

Some individuals from cohort I were still present (mean HCW = 1002 ± 116 µm (SD)), and in addition a cohort of freshly hatched small individuals (mean HCW: 268 ± 13 µm (SD), in the following cohort II) was observed. On 31 July, the size frequency distribution suggests the presence of two cohorts (II and III) of small-sized individuals with a mean HCW of 250 ±17 µm (SD) and 345 ± 20 µm (SD), respectively. The larger ones (cohort II) correspond to the small sized individuals from 21 July (cohort II), which increased in size during the 10-day sampling interval. The smaller ones (cohort III) represent a new cohort of freshly hatched individuals.
A developmental time of *Acentria* eggs of 12 to 13 days at 18 - 20°C (Berg 1942) suggests that

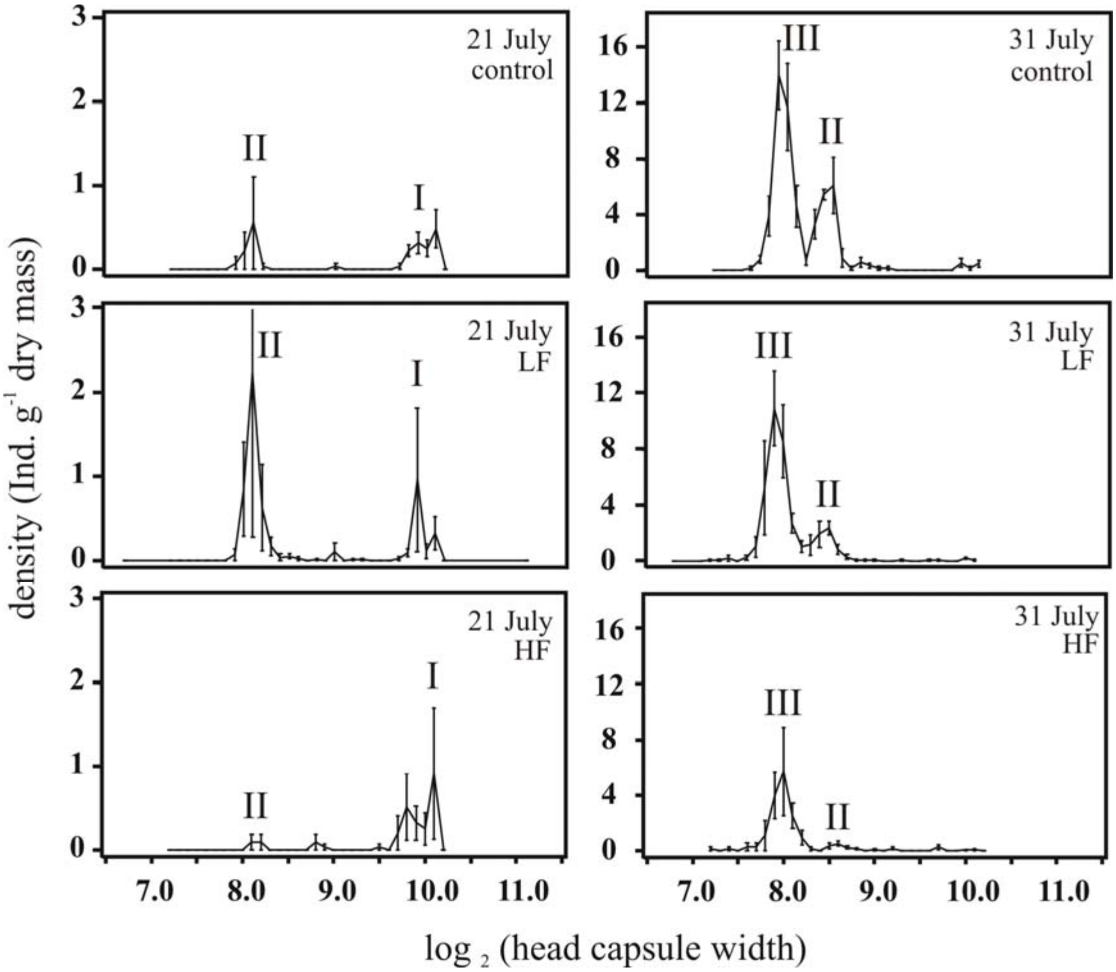

Fig. 3 Density of *Acentria* larvae of different head capsule widths in the control, low fish density (LF) and high fish density (HF) treatments at the two sampling dates. Cohorts are represented by roman numerals. Please note differences in scale on the y–axis between the two sampling dates. Cohorts were distinguished based on HCW: on 21 July cohort I consisted of larvae with a HCW larger than 652 µm (log_2 = 9.41) and cohort II consisted of larvae with a HCW smaller than 296 µm (log_2 = 8.21). On 31 July, nearly all larvae of the cohort I already had pupated. Cohort II consisted of larvae with a HCW between 315 µm (log_2 = 8.30) and 416 µm (log_2 = 8.70), and cohort III consisted of larvae with a HCW smaller than 315 µm (log_2 = 8.30).

the cohorts II and III probably stemmed from reproduction in the mesocosm, i.e. from individuals that were introduced into the experimental units as pupae. Only very few large-sized larvae from cohort I were left on 31 July as this cohort finally metamorphosed to the pupal stage after the 1^{st} sampling.

As expected from the effects of fish predation on overall larval density (Fig. 1A), there was a stronger effect of fish on *Acentria* size distribution on 31 July as compared to 21 July (Fig. 3). Individuals of cohort II were present on 21 July only in 7 out of 15 experimental units indicating that this cohort just started to hatch at the sampling date. Consequently, differences in density of cohort II individuals between experimental units on July 21 stemmed from hatching events of small larvae and were unrelated to predation (21 July, cohort II, ANOVA, factor treatment, $F_{2,12} = 1.19$, $p = 0.34$). In contrast to the few freshly-hatched larvae of cohort II, cohort I larvae already had experienced ten days of predation pressure on 21 July.

However, despite this there were no significant differences in the density of cohort I individuals between treatments (21 July, cohort I, ANOVA, factor treatment, $F_{2,12} = 0.14$, $p = 0.87$). In striking contrast, treatment effects were evident for the two cohorts of small larvae present on 31 July (cohort II, ANOVA, factor treatment, $F_{2,12} = 14.52$, $p = 0.0006$, cohort III, ANOVA, factor treatment, $F_{2,12} = 3.3$, $p = 0.072$; both cohorts: ANOVA, factor treatment, $F_{2,24} = 6.35$, $p = 0.006$, factor cohort, $F_{1,24} = 30.4$, $p < 0.0001$, interaction treatment*cohort, $F_{2,24} = 1.19$, $p = 0.32$). Differences in mean cohort densities between treatments were smaller for cohort III (2.6 : 2 : 1) than for cohort II (11.1 : 4.8 : 1). Assuming that cohort II hatched around 21 July (see above), this suggests that these larvae had been exposed to fish predation on 31 July for ten full days. The size of cohort II on 21 July was similar to the size of cohort III on 31 July implying that the latter cohort just had hatched shortly before 31 July. This implies that on 31 July cohort III larvae had experienced a shorter predation period than cohort II larvae and would explain the larger treatment effects on cohort II relative to

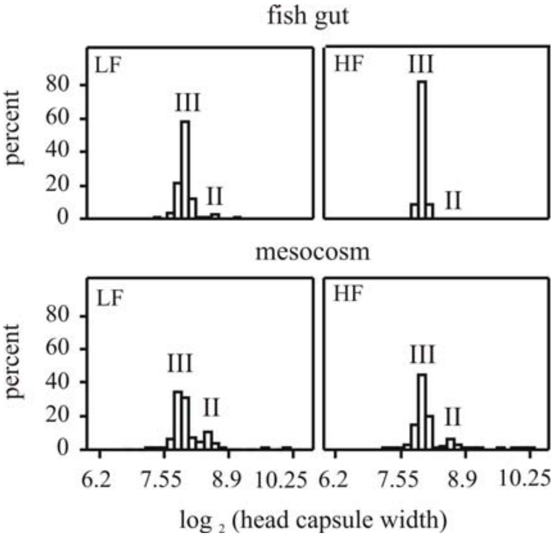

Fig. 4 Frequency distribution of *Acentria* larvae head capsule widths in the experimental units and in stickleback guts at the end of the experiment in the low fish (LF) and high fish (HF) treatments. Cohorts are represented by roman numerals. Cohorts were distinguished based on HCW: Cohort II consisted of larvae with a HCW between 315 µm ($\log_2 = 8.30$) and 416 µm ($\log_2 = 8.70$) and cohort III consisted of larvae with a HCW smaller than 315 µm ($\log_2 = 8.30$).

cohort III larvae. Please note that despite the short predation period also the density of cohort III larvae showed a tendency to differ significantly between treatments (see above). As hardly any individuals of cohort I were still present on 31 July, a statistical analysis of treatment differences for this cohort was not feasible. Gut content analyses of sticklebacks caught at the 2nd sampling date revealed that *Acentria* larvae were an important diet of the fishes. On average sticklebacks had 16.4 ± 35.3 *Acentria* larvae (range 0 - 154 larvae per fish) in their guts. The size distribution of *Acentria* in the guts suggests that sticklebacks did not feed selectively on large larvae (Fig. 4).

Rather the share of large larvae (HCW > 416 µm) and cohort II larvae (HCW between 315 and 416 µm) within the fish guts was lower (0.4 % and 3.5 %, respectively) than the share of large larvae and cohort II larvae in the mesocosms (3.4 % and 14.1 %, respectively) ($Chi^2 = 26.8$, df = 2, p = 0.0001). Female pupae (mean $length_{female} = 6.99 \pm 0.1$ mm (SE)) of *Acentria* in our experiment were significantly larger than male pupae (mean $length_{male} = 6.02 \pm 0.07$ mm (SE), ANOVA, factor sex, $F_{1,130} = 71.38$, $p < 0.0001$). Pupal sizes were neither influenced by fish predation on 21 July (ANOVA, factor treatment, $F_{2,12} = 0.59$, p = 0.57 (male pupae), $F_{2,8} = 0.28$, p = 0.76 (female pupae)) nor on 31 July (ANOVA, factor treatment, $F_{2,9} = 1.81$, p = 0.22 (male pupae), $F_{2,7} = 0.91$, p = 0.45 (female pupae)).

Pupal sex-ratio in the experiment was strongly male-biased until 21 July in all three treatments

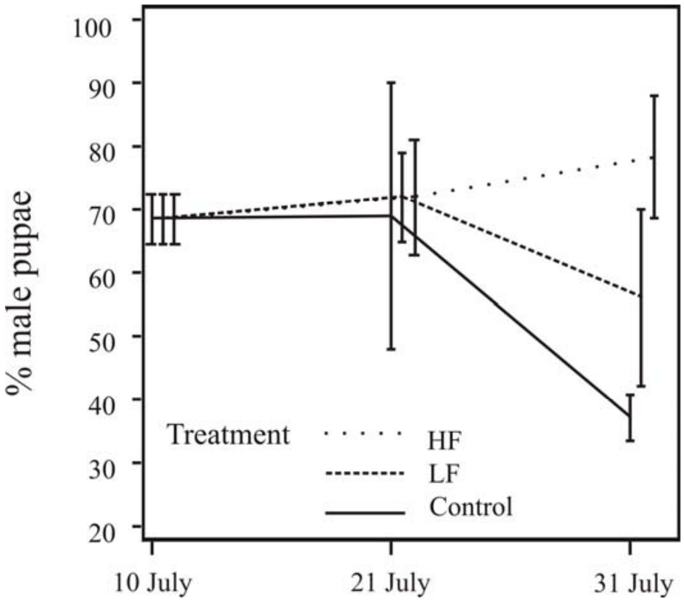

Fig. 5 Sex-ratios of *Acentria* pupae (percent male pupae ± SE) during the mesocosm experiment in the three control, low fish (LF) and high fish (HF) treatments.

(70 to 75 % males) (Fig. 5). On 31 July, the sex-ratio became less male-biased in the treatment with no fishes and with low fish density. However, pupal sex-ratio on 31 July remained highly male-biased in the high fish density treatment (ANOVA, factor treatment, $F_{1,11} = 2.49$, p =

0.03). That is pupae which developed during the experiment from larvae that were exposed to high fish predation showed a strong male bias in sex-ratio whereas pupae developing during the experiment from larvae that were not exposed to fish predation did not show a strong male bias.

Discussion

This study shows that sticklebacks can strongly influence the population dynamics of *Acentria*. We have shown that sticklebacks 1) can dampen population increase of *Acentria*, 2) can shift the sex-ratio towards male bias and 3) consume *Acentria* of different size classes with a tendency towards smaller-sized *Acentria*.

The design of this experiment was carefully chosen in order to show a close correspondence to field conditions: 1) We started the experiment with an *Acentria* size and sex distribution typical for the experimental time period *in situ*, 2) we used *Potamogeton* plants at a density similar to the densities within natural patches to create a complexity of the foraging environment which is encountered also *in situ* by sticklebacks, 3) besides *Acentria* other ephiphytic invertebrates (i.e. Zygoptera, *Simocephalus* sp. (Crustacea, Anomopoda), Chydorinae (Crustacea, Anomopoda), Aloninae (Crustacea, Anomopoda), *Valvata piscinalis* (Gastropoda), *Nais* sp. (Clitellata) and in low densities also *Bithynia tentaculata* (Gastropoda), *Radix* sp. (Gastropoda), *Gyraulus* sp. (Gastropoda), Ostracoda (Crustacea), *Caenis* sp. (Ephemeroptera), Ephydridae (Diptera) and *Bezzia* sp. (Diptera) were available for sticklebacks in the experiment to ensure that they have other dietary choices (see below) and 4) we used the size distribution of sticklebacks usually found at the time of the experiment *in situ*.

The effects of fish predation on *Acentria* might be overestimated by expressing them as individuals per plant dry mass as *Acentria* feeding reduces plant biomass. However, there were no obvious differences in feeding damage on leaves between treatments at the end of the experiment. This is due to two reasons, one is that *Acentria* densities and hence herbivory did not differ significantly during the first ten days of the experiment. Second, treatment differences after July 21 were due to small larvae, which impose a lower feeding damage on plants compared to large larvae. Consequently, there were only small and non-significant differences (≤ 13 %) in sampled plant dry mass between the control and fish treatments at the end of the experiment, which cannot explain the > 100 % differences in *Acentria* density between the control and fish treatments.

Unfortunately, we do not have any data on the density of sticklebacks or generally fish within the macrophyte patches in Lake Constance. However, the fish densities used in our experiment were probably not exceedingly high for the following reasons: 1) Natural stickleback densities may be even higher than those used in our experiment (Ward & FitzGerald 1983). Furthermore, our own observations and those from fishermen suggest that sticklebacks in Lake Constance can reach very high densities within some years. Those years are notorious as sticklebacks then even cause problems for fishermen as they get stuck in large numbers in fisher nets, from which they are difficult to remove. 2) In addition to sticklebacks, other fish species might also contribute to predation pressure on *Acentria*. For example we observed large shoals of young-of-the-year perch swimming within and around the macrophyte patches of Lake Constance. 3) There was still plenty to feed for sticklebacks at the end of the experiment. The most important prey items found in stickleback guts were benthic and planktonic crustaceans, followed by *Acentria* larvae. *Acentria* contributed 19.3 % and 10.6 % to the mean total number of consumed individuals in the LF and HF treatment, respectively. The number of prey items in the gut of LF treatment fishes (150.7 ± 87.0 SD) was higher than that from HF treatment fishes (48 ± 74.6 SD), suggesting that food uptake of sticklebacks was somewhat more limited in the HF treatment as compared to the LF treatment. However, the gut content in both treatments was higher than that (11.9 ± 17.4 SD, n = 49) of sticklebacks caught in the field at 10 July, suggesting that food limitation in the experimental units was not more severe than *in situ*. This is also supported when comparing *Acentria* densities in the experiment with *in situ Acentria* densities: Average maximum densities of *Acentria* in Lake Constance within three study years were found to be around 44.9 ± 65.1 (SD) larvae g^{-1} dm (Gross *et al.* 2002). That is *in situ* maximum density corresponds to maximal densities observed in the LF and control treatments and was approximately 2.5 times higher than the maximum densities observed in our HF treatment. 4) Finally, only within the HF treatment fishes were able to maintain the sex-ratio bias of *Acentria* pupae throughout the experiment (see below). Taken together, there is ample evidence that the fish densities used in our experiment were indeed within the range experienced by *Acentria* larvae also *in situ*.

Gut content analysis as well as the analysis of cohort specific predation impacts suggests that predation risk for *Acentria* larvae seems to decrease with *Acentria* size. Large larvae (cohort I) individuals were hardly present in the stickleback guts. Furthermore, even cohort II larvae were underrepresented in the fish guts as compared to the experimental units. At first sight, this might contradict the fact that the strongest treatment effect was observed for this cohort. However, the strong treatment effect results from up to ten days of predation (from 21 July to 31 July) whereas the *Acentria* size distribution in the fish guts represents a snap shot from July 31. Most likely cohort II larvae suffered from predation throughout most of the ten days but towards the end of the experiment had reached a size which provided them with some protection. The absence of a treatment effect on cohort I larvae on 21 July additionally suggests a reduced predation on larger larvae. Reduced predation on larger larvae is however not due to size per se as sticklebacks are unlikely to be gape-limited with respect to *Acentria*. According to Gill & Hart (1994) the stickleback size groups in our experiment should be able to consume food particles up to 1.8 and 2.8 mm width, respectively. The maximum head-capsule width of *Acentria* in Lake Constance is 1.2 mm, thus their gape-width should in principle allow the small and large sticklebacks in our experiment to consume even the biggest *Acentria* larvae. Hence some attribute of *Acentria* associated with size might yield protection from fish predation. *Acentria* larvae build shelters from plant parts cut off from leaves (Berg 1942). We found that large larvae were especially difficult to remove from these shelters. This might at least have contributed to the reduced predation of fish on larger larvae.

As fish seem to bias *Acentria* sex-ratio towards males, there is a 2nd, indirect effect of fish predation on *Acentria* population dynamics. The lack of fish predation was associated with an increase of the female share of pupae from 25 % to 50 %, which could mean a doubling of the number of offspring. Even if we assume that, due to higher *Acentria* densities in the control treatment, clutch sizes might be lower than in the fish treatments, this indirect effect of fish predation will strongly influence *Acentria* population dynamics. Male-biased pupal sex-ratios of *Acentria* were not only observed in our experiment. Rather a male-biased pupal sex-ratio seems to be a typical phenomenon *in situ* as it was observed within six study years in Lake Constance (Fig. 6). Especially in 2002 and 2003 when sticklebacks were highly abundant in Lake Constance (personal observation), the percentage of male pupae was very high (Fig. 6). The strong male bias *in situ* explains why the pupae that were introduced into the experiment already had a surplus of males. Furthermore, strongly male-biased sex-ratios *in situ* suggest that predation pressure of sticklebacks *in situ* on *Acentria* is as strong as in our fish treatments.

For a better understanding of *Acentria* population dynamics, we need to know more about sex-specific predation of sticklebacks on *Acentria* larvae. Unfortunately, it is not possible to sex *Acentria* larvae morphologically. However, female pupae are considerably larger than male pupae suggesting that also larvae differ in size between the sexes. Hence sex-specific predation might arise when fish would preferentially consume larger *Acentria* larvae over smaller ones. However, our analysis of stickleback gut contents showed that this was not the case, and the size frequency distribution of larvae suggested that predation was important already for rather small larvae. Hence we probably can exclude size-selective predation as the reason for sex-specific mortality differences. Another possible mechanism of sex-specific predation would be differences in activity patterns between males and females, which could make females more

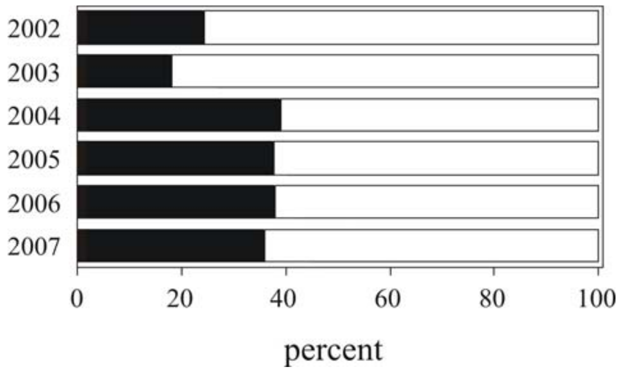

Fig. 6 Percentage distribution of male and female *Acentria* pupae in Lake Constance in the years 2002 to 2007 (black bars: female pupae, open bars: male pupae). The sex-ratios are based on the following sampling sizes: 2002: n = 242, 2003: n = 68, 2004: n= 967, 2005: n = 792, 2006: n = 282, 2007: n = 62.

vulnerable to predation. Higher activity increases the susceptibility to predation as shown e.g. for female Zygopteran larvae, preyed upon by Odonata (Brodin & Johansson 2004; Brodin *et al.* 2006). Assuming no sex-specific differences in growth efficiencies, *Acentria* female larvae have two options to achieve a larger size than males: grow faster or grow over a longer period. Given the short adult period of a few days (Berg 1942) and hence the need to synchronise development, differences in developmental time between sexes are unlikely. In order to grow faster, *Acentria* females might show a higher activity than males, e.g. they might feed more often outside their shelter, thereby increasing predation risk. Behavioural studies are needed to test this hypothesis.

The presence of fish, probably mediated via chemical stimuli, has been shown to result in an earlier metamorphosis at smaller size in insect and amphibian species in order to escape a habitat with a high predation risk (Lardner 2000; Peckarsky *et al.* 2001; Dahl & Peckarsky 2003). Our experimental design did not allow for an analysis of the time at metamorphosis of *Acentria*. We can however not exclude this possibility. If the larvae in our experiment indeed chose an earlier time at metamorphosis under fish predation, this would have affected our results in two ways: an overestimation of the effect of fishes on larval densities and an underestimation of the effect of fishes on pupal densities at the end of our experiment. An earlier metamorphosis in the presence of fish would however not change our general results, i.e. a strong influence of fish on the population dynamics of *Acentria*. Also we could not find an effect of fish on *Acentria* pupal size, which could result from earlier metamorphosis. However, the overall number of pupae at the end of the experiment especially in the replicates of the HF treatment was rather low (n = 8), suggesting a low power of this experiment to detect possible life-history responses of *Acentria* to predators.

Stickleback predation will obviously decrease *Acentria* population growth rates. However, even in our HF treatment predation was not sufficient to actually decrease *Acentria* densities. Although fish may not be able to decrease *Acentria* densities within one season due to the high clutch sizes (up to 400 eggs per clutch, Gross *et al.* 2002) and the reduced predation on larger larvae, our results show that they can dampen *Acentria* population increase. This corresponds to *in situ* observations in which *Acentria* densities in Lake Constance increased during the summer period over several orders of magnitude in all years (Gross *et al.* 2002). However, the achieved maximum densities differed severalfold between years. This interannual variability was suggested by Gross *et al.* (2002) to be due to water level variability influencing the behaviour of *Acentria* larvae. Our study suggests that top-down control by fish might at least contribute to the observed variability. Unfortunately, we do not have any data on stickleback interannual variability in Lake Constance though reports from local fishermen suggest strong population fluctuations and very high densities in some years.

Acentria larvae are important herbivores in many lakes and brackish coastal systems in Europe (Berg 1942; Hedal & Schmidt 1992; Gross *et al.* 2002). In Lake Constance, the growing season of *Potamogeton* usually lasts until September (own observations), and Gross *et al.* (2002) report that 100 % of apical meristems of *Potamogeton* show feeding damage due to *Acentria* herbivory already in September. Our own observations suggest that some *Potamogeton* patches were completely defoliated due to *Acentria* herbivory within a period of two weeks during August in the field season 2004 (see chapter II). Here we show that fish predation can influence the population dynamics of *Acentria* directly and indirectly: directly by consuming *Acentria* larvae and indirectly by decreasing the share of females. Taken together, field observations, documenting a strong influence of *Acentria* on macrophytes (Gross *et al.* 2002), and our experimental results, documenting the effects of fish on *Acentria*, suggest the possibility of a previously overlooked trophic cascade of major importance in the macrophyte beds of many lakes. Furthermore, as *Acentria* herbivory might also change the shape and occurrence of macrophyte beds, this might have also effects on fish which use these patches as a habitat. Clearly, more studies are needed to analyse the consequences of *Acentria* on e.g. fish distribution and show whether this system really represents a trophic cascade.

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We thank Martin Wolf for help with the construction of the experimental setup and the solution of technical problems. A lot of students were helpful by assisting the sampling in the field and the mesocosm experiment and the subsequent analysis of the samples: Petra Eisele, Michaela Fürst, Robert Greither, Judith Hähnlein, Daniela Kassahr, Andrea Kunzmann. We thank Karl-Otto Rothhaupt, Karsten Rinke and two anonymous referees for providing valuable comments on the manuscript. This research project was part of the Collaborative Research Center (CRC) no. 454 “Littoral of Lake Constance” and was financially supported by the Deutsche Forschungsgemeinschaft (DFG).

Chapter IV

Sex-specific life-history decisions of a wing-dimorphic moth in response to predation

Oliver Miler and Dietmar Straile

Manuscript in preparation

Abstract

Predator-induced direct mortality often alters the densities and the structure of populations and communities of many prey organisms. Non-lethal predator-prey interactions are able to significantly influence the adaptive phenotypic plasticity of prey species to minimise predation risk. We used a lethal predator experiment to study the direct and indirect costs of the presence of a predator, the stickleback *Gasterosteus aculeatus*, for an aquatic moth, *Acentria ephemerella.* A previous study had shown that fish predation can reduce the population growth of *Acentria* and shift its pupal sex-ratio towards male bias. Here we show that life-history changes in response to fish predation were sex-specific and consisted of a smaller size at metamorphosis of both sexes and an increased portion of females – but not of males – opting for diapause development instead of direct development (metamorphosis). These differences in developmental pathways in the presence of fish can at least partially explain the male bias of pupae observed *in situ*. We suggest that the difference in developmental pathway is due to a higher mortality risk of the adult wingless, aquatic females compared to the winged, terrestrial males. During the length of our experiment (ten days), fish predation reduced the density of *Acentria* females approximately to one fifth of the control treatment. The indirect effects of fish

predation, i.e. the costs of predator avoidance strategies, caused a reduction of *Acentria* within-season fecundity by a factor of two. That is fish predation resulted in an approximately ten-fold reduction of *Acentria* egg production. Thus finally the found strategies of predator avoidance had important secondary implications for the population dynamics as well as for the operational sex-ratio of *Acentria*. We could show that it is important to study diapause (indirect development) and metamorphosis (direct development) as alternative developmental pathways together with changes in the size at maturity to obtain a complete understanding of predator avoidance strategies in species with a diapause period during their life cycle. Furthermore, our results highlight the importance of analysing predator avoidance behaviour separately for the two sexes.

Introduction

Predators often alter prey densities via the consumption of specific prey types and thereby influence the structure of prey populations and communities. Apart from lethal effects, predation can induce pronounced plastic physiological, morphological, behavioural and life-history changes in prey organisms (Lima & Dill 1990; Schlichting & Pigliucci 1998; Relyea 2001; Werner & Peacor 2003). Such non-lethal predator-prey interactions are able to significantly influence the phenotypic plasticity of prey species but have only recently been studied (Agrawal 2001). Adaptive plasticity in growth and development can enable animals to cope with adverse environmental conditions by minimizing predation risk (Martin *et al.* 1991; Peckarsky *et al.* 1993; Agrawal 2001). In many insect species, female size at metamorphosis directly translates into fitness since the reproductive success, i.e. the number of offspring, depends largely on the size at maturity (McPeek & Peckarsky 1998; Nylin & Gotthard 1998; Taylor *et al.* 1998). Under predation an earlier time and smaller size at metamorphosis enables adult individuals of larvae of some insect and amphibian species to escape a predation-risky habitat earlier (Lardner 2000; Kiesecker *et al.* 2002; Dahl & Peckarsky 2003). Hence body size and the exposure to predation risk are traded off against each other in the presence of predators since small adult female individuals will have a low number of eggs and thus probably a reduced number of offspring, thereby affecting population growth and development.

Population dynamics in insects are furthermore often determined by the choice of developmental strategies. In seasonal environments, individuals of many arthropod species overwinter in the egg, larval or pupal stage and emerge in the beginning of the following season (Nylin *et al.* 1989; Blanckenhorn 1998; Kiss & Samu 2005). Multiple developmental pathways of individuals of the same cohort can for example develop when the pre-diapause size distribution widens and when the growth responses of the larval size classes in such an expanded distribution show differential sensitivities to environmental cues as suggested by Norling (1984). A delay in development to the subsequent season through the diapause stage can result in “cohort splitting” (Martin *et al.* 1991), with a part of the individuals of a certain life-stage delaying their development and entering a diapause stage (indirect development) and the other part developing into adult individuals (direct development). Various studies have analysed the plasticity of voltinism in arthropods in relation to abiotic factors, such as temperature, photoperiod and habitat variability (Johansson & Norling 1994; David *et al.* 1999; Kiss & Samu 2005), but only limited research has dealt with the influence of biotic interactions on voltinism patterns (Martin *et al.* 1991; Johansson *et al.* 2001), especially concerning the effect of predation on the developmental choice and life-history strategies of invertebrates (Martin *et al.* 1991). For some species, diapause can be considered as a predator avoidance strategy. For example the two-spotted spider mite reacts to a predatory mite with increased diapause incidence (Kroon *et al.* 2005), and *Daphnia magna* produces resting eggs in response to fish chemical cues (Slusarczyk *et al.* 2005). Even less is known about differences between the sexes in induced responses due to abiotic and biotic environmental factors. As a result of sexual selection and protandry, there is a male bias to enter diapause in some lepidopteran species (Wiklund *et al.* 1992; Wedell *et al.* 1997). Males and females can also differ in their life-history responses to food availability and predator presence (De Block & Stoks 2003; Mikolajewski *et al.* 2007) and time stress (Shama & Robinson 2006).

Here we analyse a model system in which females and males have the choice to respond to predator presence either with an earlier time at metamorphosis or with diapause. We use the water moth *Acentria ephemerella* (Lepidoptera: Crambidae) as a model species to study the effects of predation on sex-specific life-history plasticity in both size and age at metamorphosis as well as developmental strategies (metamorphosis or diapause). *Acentria ephemerella* (Lepidoptera: Crambidae) is a moth with a predominantly aquatic life cycle. Recently we have shown that its population dynamics and sex-ratio are influenced by fish predation (see chapter III), suggesting the possibility for a sex-specific plasticity of life-history decisions in response to predation. Furthermore, sexes differ in size, wing morphology and habitat: the brachypterous females spend their short adult life span below the water surface whereas the macropterous males live above the water surface (Berg 1942). These strong differences between the sexes further undermine the necessity to analyse life-history strategies in a sex-specific manner.

In this study we want to address the following questions:

1) Is there an influence of predation on the size at metamorphosis and thus growth plasticity ?
2) Are there sex-specific changes in developmental strategies (diapause vs. metamorphosis) as a response to fish predation and can they provide a possible explanation for the observed highly male-biased sex-ratio of pupae found in the field ?

3) Finally, we want to analyse the costs and consequences of changes in size at metamorphosis and developmental strategies for the fitness and seasonal population growth of *Acentria*.

Materials and methods

For the mesocosm experiment, the following basic experimental setup for all replicates was used. One replicate consisted of a styrodur frame with an area of 0.43 m^2 (length = 0.715 m, width = 0.6 m) connected via a plastic tube (transparent Tricoron; RKW AG Rheinische Kunststoffwerke, Wasserburg, Germany) with a length of approximately 1.5 m to a metal frame (length = 0.8 m, width = 0.6 m, height = 0.05 m) that was fixed with ten metal clips on the Tricoron plastic foil and dug into the sediment. The replicates were placed in a large outdoor mesocosm (length = 10.5 m, width = 5 m, height = 1.5 m) filled with fine sediment (height ~ 0.35 m) and water from Lake Constance. The styrodur frames were used as buoys to keep the plastic tube in a vertical position in the water column. Prior to the experiment the sediment was mixed to provide equal environmental starting conditions for each replicate. The experimental units were separated from the surrounding mesocosm water by the transparent Tricoron plastic foil, and water was not changed during the experiment. Wooden frames (length = 0.92 m, width = 0.73 m) covered with metal gauze (mesh size ~ 1620 µm) were placed upon the styrodur frames approximately 0.1 m above the water surface to prevent adult *Acentria* males from dispersing out of the replicates and to allow them to search for females and to successfully reproduce. The mesocosm experiment was conducted from 14 June to 20 August 2006. The two treatments (control (no fish) and fish) were randomly assigned to the replicates in the mesocosm. Each treatment consisted of five replicates.

Potamogeton shoots were sampled on 14 June 2006 in an Upper Lake Constance macrophyte patch. Macrophyte-associated *Acentria* larvae and pupae were removed, and subsequently eight macrophyte shoots were planted in each of the ten replicates. The shoots were allowed to root and establish new shoots for approximately 5 ½ weeks. From 20 to 22 July altogether 79 *Acentria* pupae from field samples in Lower Lake Constance were introduced in each experimental unit. From these pupae adult individuals hatched, mated and laid egg clutches. The dead adult individuals were collected daily from the water surface and preserved in 70 % Ethanol. On 31 July, after ten days, feeding damage or small, freshly hatched larvae could be observed in all ten experimental units. The control replicates were sampled on 10 August to assess start densities of *Acentria* life stages, and only active larvae, but no hibernating larvae and pupae, were found. Sticklebacks (*Gasterosteus aculeatus*) were caught with minnow traps in a small pond near Lake Constance and subsequently introduced in the fish treatment on 10 August and not on 31 July to prevent the sticklebacks from exerting a too strong predation pressure on active larvae before the onset of hibernation and pupation. Each replicate of the fish treatment contained 12 adult stickleback specimens with a size range from 4.35 to 6.2 cm total length (TL) in a fish density of 24 Ind. m^{-2}. There are no data of stickleback densities from Lake Constance available, but natural densities can vary strongly in the field, showing lower (Thiel *et al.* 1995) or higher (Ward & FitzGerald 1983) densities than in our experiment. After a predation period of ten days, macrophytes including associated larval and pupal life-stages of *Acentria* were sampled on 20 August in the replicates of the fish and the control treatment. We chose to terminate the experiment on 20 August when we observed the first adults. This was necessary to avoid bias in the observed sex-ratio as adult females are much more difficult to detect than males. We distinguished three developmental life stages of *Acentria* during the processing of the samples and in the subsequent statistical data analysis: active larvae (larvae outside macrophyte stems), overwintering larvae (larvae inside macrophyte stems) and pupae. *Acentria* larvae associated with *Potamogeton* were washed through a sieve (mesh size 200 µm), and the *Acentria* larvae visible to the naked eye were fixed in Carnoy's solution (60 % Ethanol, 30 % Chloroform, 10 % Acetic Acid) for subsequent sex determination. The remaining material that was washed from the macrophytes, including also overlooked *Acentria* larvae, was preserved in 70 % Ethanol in 100 ml plastic boxes. Since *Acentria* pupae are closely attached to the stems of *Potamogeton* shoots and larvae overwinter inside the stems, the remaining plant material was stored at 5 °C in plastic bags and searched through for pupae and hibernating larvae within one week after sampling. *Acentria* pupae were fixed in 70 % Ethanol in 100 ml plastic boxes and hibernating larvae in Carnoy's solution.

Macrophytes were dried at 90 °C for three days and densities of *Acentria* pupae and larvae calculated as individuals per gram plant dry mass (Ind. g^{-1} dm). After the macrophyte sampling, the sticklebacks were caught with scoop nets, killed with 2 mg $liter^{-1}$ 1,1,1-trichloro-2-methyl-propanol (TCMP) and frozen in a chest-freezer. A dissecting microscope (Zeiss Stemi 2000-C) was used to collect and count *Acentria* larvae at a 10–50× magnification from samples.

Acentria pupae were sexed morphologically as published in Berg (1942) (see Appendix, Fig. A 2). Active and hibernating *Acentria* larvae were sexed according to the following histological procedure: Larvae stored in Carnoy's solution were dissected, and the internal organs, primarily the Malpighian tubules and the spinning glands, were mounted on a microscope slide, stained red with Lactic Acid – Acetic Acid – Orcein (LAO) and covered with a coverslip. Under the microscope at 1000x magnification, in the cells of female tissues one dark, round spot that consists of densely concentrated sex chromatin is visible in each nucleus (see Appendix, Fig. A 2). The nuclei of cells in male tissues in contrast lack this densely concentrated sex-chromatin so that no dark spot can be observed there. *Acentria* pupal lengths and larval head capsule widths were measured under a microscope equipped with an ocular micrometer.

To convert length measurements into dry mass we established a head-capsule width – dry mass relationship for larvae ($\log_{10}$ (weight [mg]) = - 12.17 + (4.17 * $\log_{10}$ (head capsule width [µm])), n = 45, R^2 = 0.93 , p < 0.0001) and a length – dry mass relationship for pupae ($\log_{10}$ (weight [mg]) = - 1.96 + (2.93 * $\log_{10}$ (body length [mm])), n = 34, R^2 = 0.93 , p < 0.0001). If available 20 individuals per replicate and developmental stage (active larvae: n = 161, diapausing larvae: n = 207) and all individuals in the pupal stage (n = 312) were measured. Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Results

The densities of *Acentria* at the start (10 August) of the mesocosm experiment were 37.1 ± 6.4 (SE) Ind. g^{-1} dm. After ten days of predation, i.e. on 20 August, the *Acentria* density had declined to 9.6 ± 2.9 (SE) Ind. g^{-1} dm in the fish treatment and was 48.7 ± 18.6 (SE) Ind. g^{-1} dm in the control treatment. Stickleback predation thus reduced the density of *Acentria* strongly (Fig. 1, ANOVA, factor fish, $F_{1,8}$ = 6.52, p = 0.034) with a daily mortality rate of 0.14 d^{-1}.

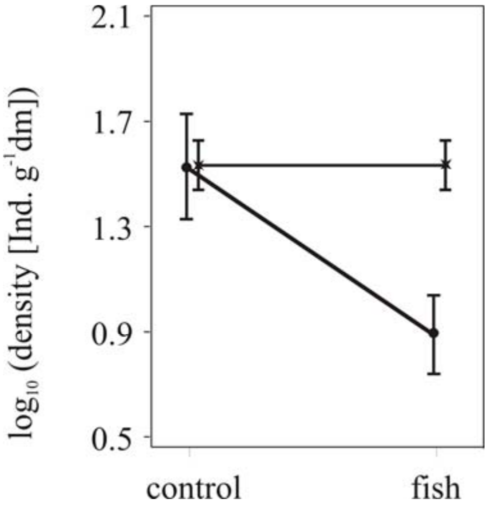

Fig. 1 Densities (logarithmic number of individuals g^{-1} dm) of all *Acentria* life-stages in the fish and control treatment at the start of the experiment (stars) and the end (filled circles).

At the end of the experiment, there were still active larvae present, but some *Acentria* larvae already had developed into the pupal stage whereas others had gone into diapause. Densities of both pupae and diapausing larvae did not differ significantly between the fish versus the control treatment (ANOVA, hibernating larvae, $F_{1,8}$ = 1.55, p = 0.25, pupae, $F_{1,8}$ = 2.6, p = 0.15), resulting in a significantly higher percentage of active larvae on overall *Acentria* density in the control relative to the fish treatment (Fig. 2, ANOVA, factor fish, $F_{1,8}$ = 39.58, p = 0.0002). Thus within the fish treatment the majority of *Acentria* still alive at the end of the experiment already had made their decision of direct (metamorphosis) versus indirect (diapause)

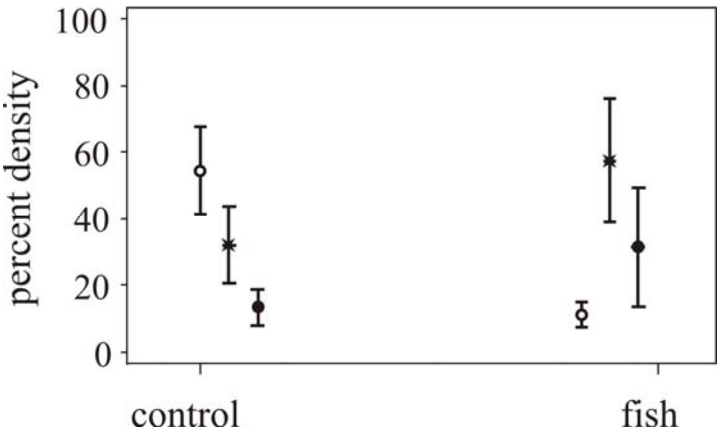

Fig. 2 Mean percentage of the density of the respective developmental stage on the overall density in the fish and control treatment: active larvae (empty circles), hibernating larvae (stars) and pupae (filled circles).

development whereas in the control treatment the majority of larvae still had to face this decision. When considering all life cycle stages together, *Acentria* sex-ratio at the end of the experiment was significantly more male-biased in the fish treatment than in the control (ANOVA, factor fish, $F_{1,8} = 5.5$, $p = 0.047$).

Male and female larvae seemed to differ in their preferences for diapause versus metamorphosis at least in the fish predation treatment: while males were diapausing and metamorphosing in almost equal shares, approximately four times as many females chose diapause instead of metamorphosis (Fig. 3 A, ANOVA, percent, factor sex, $F_{1,28}$ = 0.0, p = 1.0; factor

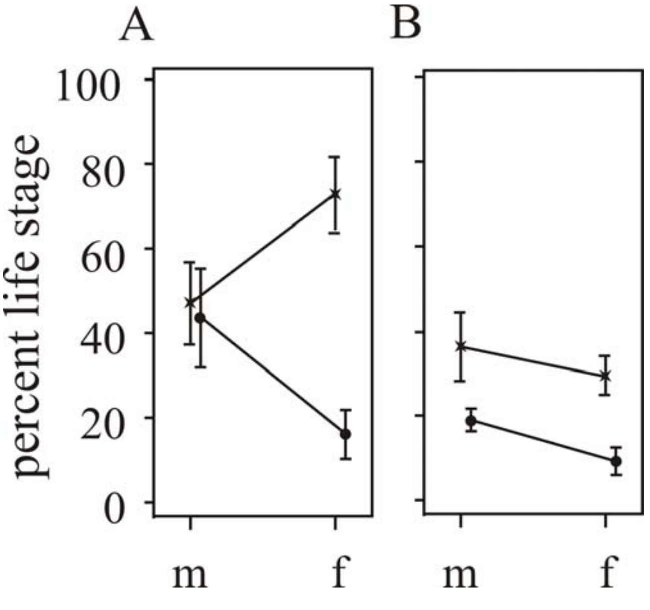

Fig. 3 Sex-specific cohort splitting in *Acentria*. The figure shows the mean percentage of male (m) and female (f) life-stages, i.e. pupating (filled circles) and hibernating (crosses) individuals in the fish (A) and control (B) treatment.

developmental stage, $F_{2,27} = 20.64$, $p < 0.0001$, interaction sex*developmental stage, $F_{2,27} = 5.91$, $p = 0.0082$). In the control treatment, the development status of *Acentria* was not sex-specific (Fig. 3 B, ANOVA, percent life stage, factor sex, $F_{1,28} = 0.0$, $p = 1.0$; factor developmental stage, $F_{2,27} = 20.82$, $p < 0.0001$, interaction sex*developmental stage, $F_{2,27} = 2.8$, $p = 0.081$). Both sexes preferred to enter diapause instead of metamorphosis (females: 36.3 % versus 18.7 %, males: 29.3 % versus 9 %) in the control treatment. However, for both sexes the majority of individuals were still actively feeding larvae (females: 61.6 %, males: 45 %). Differences in the decisions to enter diapause versus metamorphosis between sexes under fish predation should result in biased sex-ratios of distinct developmental stages. While Fig. 4 indeed suggests biased sex-ratios of the pupal and diapausing stages, there was no significant difference in the percentage of male individuals between the treatments when considering all replicates (ANOVA, active larvae, factor fish, $F_{1,8} = 0.95$, $p = 0.36$, hibernating larvae, factor fish, $F_{1,8} = 3.06$, $p = 0.12$, pupae, factor fish, $F_{1,7} = 2.8$, $p = 0.13$).

However, after removing one outlying and influential observation (studentised residual = 2.2, Cook's D = 0.59), the pupal sex-ratio was significantly more male-biased (ANOVA, factor fish, $F_{1,7} = 9.8$, $p = 0.017$) whereas there was a tendency for a stronger female bias within diapausing larvae in the fish treatment (ANOVA, factor fish, $F_{1,7} = 3.93$, $p = 0.088$).

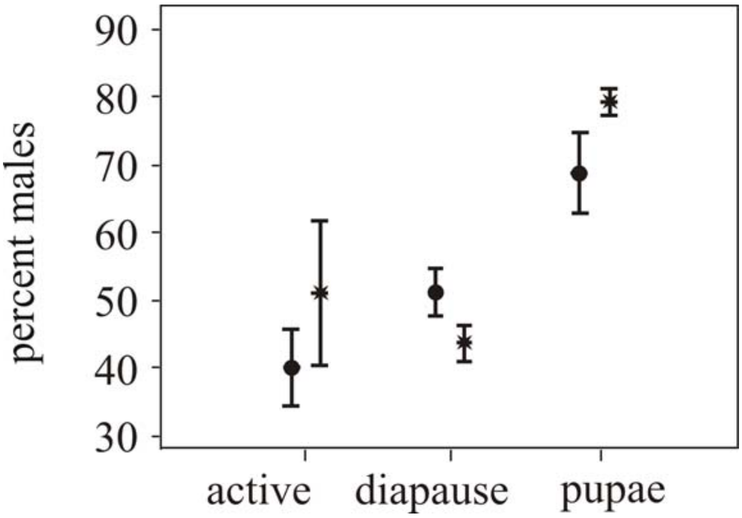

Fig. 4 Percentage of male individuals of active larvae ('active'), hibernating larvae ('diapause') and pupae ('pupae') in the fish (crosses) and control (filled circles) treatment.

Female pupae were on average 66 % larger than male pupae (Fig. 5, Mixed Model ANOVA, factor sex, $F_{1,299} = 603.07$, $p < 0.0001$). The influence of fish predation on pupal size differed between the sexes as indicated by the significant interaction between sex and fish treatment (Mixed Model ANOVA, interaction sex*fish, $F_{1,299} = 7.53$, $p = 0.0064$): Fish predation resulted in a 9.1 % and 18 % reduction in the size of male and female pupae, respectively (Mixed Model ANOVA, male pupae, factor fish, $F_{1,7} = 4.68$, $p = 0.064$, female pupae, factor fish, $F_{1,7} = 18.77$,

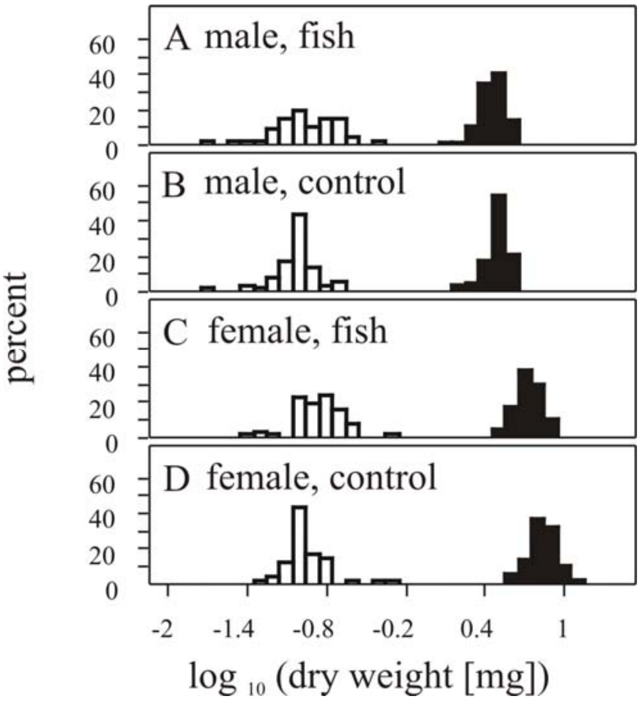

Fig. 5 Distribution of logarithmic dry weights of male (A,B) and female (C, D) hibernating larvae (open bars) and pupae (black bars) in the fish treatment (A, C) and the control (B, D).

p = 0.0025). Female diapausing larvae were also larger than their male counterparts (Mixed Model ANOVA, factor sex, $F_{1,193}$ = 10.29, p = 0.0016), but in contrast to pupal size there was no effect of fish on the size of diapausing larvae (Mixed Model ANOVA, factor fish, $F_{1,7}$ = 3.13, p = 0.11, interaction sex*fish, $F_{1,193}$ = 0.06, p = 0.8).

Fish predation seemed to broaden the size distribution of diapausing larvae: in the presence of fish comparatively small but also comparatively large larvae went into diapause whereas

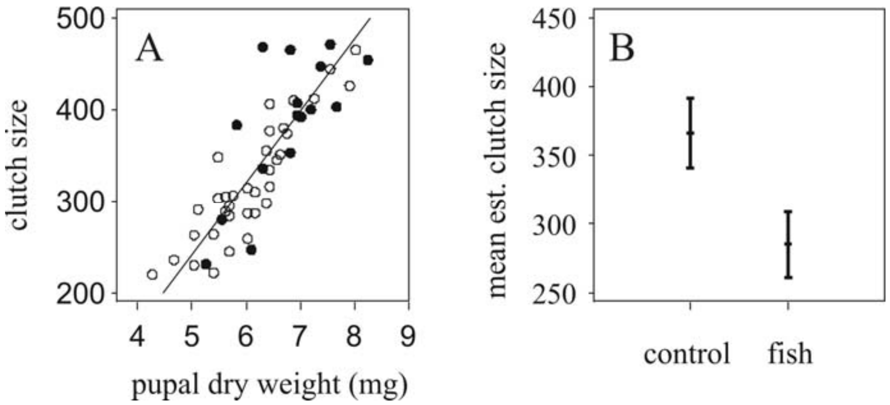

Fig. 6 A Correlation between clutch size (number of eggs) and pupal size (dry weight in mg) of *Acentria* pupae in the fish (empty circles) and control (filled circles) treatment. **B** Mean estimated clutch size (mean number of eggs) in the fish and control treatment, including egg clutches calculated from the dry weight of female pupae via the dry weight – clutch size regression in A.

without fish predation the size of diapausing larvae was more restricted (Fig. 5). In contrast, the width of the size distribution of pupae was hardly affected by fish predation (Fig. 5). Clutch size of *Acentria* is tightly linked to *Acentria* body mass (Fig. 6 A, Correlation, $R^2 = 0.75$, $F_{1,51} = 156.88$, $p < 0.0001$). Consequently, fish predation resulted in a 22 % reduction of *Acentria* clutch size (Fig. 6 B, Mixed Model ANOVA, clutch size, factor fish, $F_{1,7} = 18.77$, $p = 0.0025$).

Discussion

Using a lethal-predator experiment, we show strong effects of stickleback predation on the density and life-history of *Acentria*. Predators influence the population dynamics as well as the life-history of their prey via several mechanisms. Prey life-history will be influenced by the combined effects of induction (i.e. the induction of changes in prey behaviour and morphology by predation via alarm cues and predator kairomones), thinning and selection (Relyea 2007) whereas prey population dynamics will be influenced by mortality and the costs of anti-predator strategies. A lethal-predator experiment allows studying the total effect of the predator on the prey albeit at the cost of an increased difficulty to disentangle the importance of individual processes.

The within-season population dynamics of *Acentria* in this experiment was influenced by sticklebacks severalfold: Besides the direct effect of mortality (0.14 d^{-1}), there are indirect effects due to the predators' influence on *Acentria* sex-ratio, its shift from direct towards indirect development (see below) and a reduced fecundity. We probably did slightly overestimate the *Acentria* mortality rate by expressing *Acentria* numbers relative to plant dry mass. As *Acentria* is feeding on the plants, higher *Acentria* densities in the control treatments are most likely associated with a lower plant dry mass, thus increasing *Acentria* densities. However, as grazing loss at the end of the experiment was small relative to the overall plant mass, a strong overestimation of *Acentria* mortality is unlikely.

Sticklebacks were not limited to *Acentria* larvae in their prey consumption and were not obliged to primarily feed on *Acentria* larvae as they were not the only available food source in the experiment. Sticklebacks could choose between various planktonic and benthic food organisms, and fish stomachs and guts were always filled with prey organisms. Hence the observed mortality rate is likely to be of a magnitude comparable to the field situation since the stickleback densities used in our experiment were within the range of natural stickleback densities (Ward & FitzGerald 1983; Thiel *et al.* 1995). An analysis of three fishes from three mesocosms each revealed that the most common food items were benthic cladocerans (307 ± 372 Ind. $fish^{-1}$), cyclopoid copepods (8.1 ± 6.2 Ind. $fish^{-1}$), harpacticoid copepods (4.1 ± 10.1 Ind. $fish^{-1}$), Ostracoda (53 ± 85 Ind. $fish^{-1}$) and snails (3.5 ± 3.6 Ind. $fish^{-1}$). *Acentria* larvae were only found in low numbers (0.7 ± 0.9 Ind. $fish^{-1}$), which is probably due to the low number of

Acentria in the fish mesocosms at the end of the experiment. Furthermore, large larvae may achieve some protection from predation by sticklebacks (see chapter III). A previous experiment revealed that sticklebacks consume large numbers of smaller-sized larvae when they are abundant (see chapter III).

The distribution of developmental stages and of *Acentria* pupal size suggests an induction effect of fish on *Acentria* life-history. Pupae as well as diapausing individuals (see below) are most probably safe from fish predation as the pupae are firmly attached to the plants and diapausing individuals reside inside the plant stems. Hence both metamorphosis as well as diapause can be regarded as a refuge from predation. The high contribution of pupae to the overall density combined with a reduced size at metamorphosis in the fish treatment suggests that in the presence of fish *Acentria* metamorphoses earlier at a reduced body size. This combination of effects cannot be easily explained or is contrary to the predictions resulting from the effects of thinning and/or selection alone. Thinning should lead to a faster growth of *Acentria*. Thinning could lead to the observed higher contribution of pupae due to an earlier metamorphosis in the fish treatment but should entail no difference in pupal size between the fish and control treatment. Furthermore, even in the control treatment food plant resources were high at the end of the experiment so that an increase in the availability of food due to thinning in the fish treatment most probably would not have strongly influenced the response of *Acentria*. In contrast to the predicted effects of thinning, stickleback selection of the more active and hence probably fastest growing individuals should result in a decreased proportion of pupae in the fish treatment, which is contrary to the observed pattern. Hence the observed response of *Acentria* in the size and timing of metamorphosis is most likely due to induction whereas thinning and selection did not have a strong effect on this life-history trait. Earlier metamorphosis at a smaller size in response to predators has been observed also for the mayfly species *Baetis* and *Ephemerella* (Peckarsky *et al.* 2001; Dahl & Peckarsky 2003). However, a reduced development time associated with a smaller size at metamorphosis in response to predators is not an often observed strategy for prey populations (see reviews by Relya (2007) and Benard (2004)).

The high percentage of diapausing individuals in the fish predation treatment suggests that in addition to metamorphosis diapause can be also considered as a predator avoidance strategy for *Acentria*. Furthermore, our results suggest strong differences between the sexes to enter direct versus indirect developmental pathways, which result in sex-specific cohort splitting. We are only aware of one mechanism resulting in sex-specific cohort splitting reported previously in the literature: selection pressures associated with protandry (Wiklund *et al.* 1992; Wedell *et al.* 1997) result in a male bias within diapausing individuals as it has been shown e.g. for the green-veined white butterfly *Pieris napi* L. (Wiklund *et al.* 1992). Since in *Acentria* more females than males enter diapause, the sex-ratio-bias of diapausing individuals is not due to selection associated with protandry.

Sex-specific cohort splitting clearly occurred within the fish treatment. This strongly suggests that it is indeed a result of the presence of fish. Unfortunately, we cannot fully exclude the possibility that also in the control treatment sex-specific cohort splitting finally would have occurred, e.g. when all remaining active male larvae would have gone into metamorphosis and all remaining active female larvae would have gone into diapause. However, at the time when we had to stop the experiment (see method section) there was no indication of sex-specific differences in the decision of direct versus indirect development in the control treatment. Furthermore, we are not aware of any other mechanistic explanation which should result in a female bias to enter diapause. Hence we consider the presence of predator avoidance as the most likely explanation for the observed differences between the sexes.

Fish predation might result in a female bias to enter diapause because the fitness costs associated with earlier metamorphosis in response to fish predation are larger than the fitness costs for males and/or because of differences in the vulnerability of the adult stages to fish predation. A smaller size at metamorphosis due to fish predation has strong fitness costs for *Acentria* females due to reduced clutch sizes (Fig. 6). The cost of a small size for males is unclear and depends on the strength of male competition for females. The size range and standard deviation of male pupae is similar to the size range and standard deviation of females, which might suggest that selection pressures to obtain a specific size do not differ between males and females, i.e. fitness costs of a reduced size might be as severe for males as for females. There is however most probably a large difference between the sexes in the vulnerability of adults to fish predation. In contrast to males, females spend their short adult phase in the water column (Berg 1942), i.e. in the reach of fish. Hence for females metamorphosis is only a temporary refuge from predation whereas after emergence males are finally out of the reach of fish predation. Females opting for diapause delay metamorphosis to the beginning of the next season when the mortality risk is probably lower. Sticklebacks use macrophyte patches as a habitat mainly later in the season, in July and August, when the *Potamogeton* shoots have grown to the surface and form dense, canopy-like underwater habitats for fishes (personal observation). Furthermore, sticklebacks reproduce and breed during a large period of time from February to August (Hyatt & Ringler 1989; Baker 1994; Poizat *et al.* 2002; Candolin & Voigt 2003; Östlund-Nilsson 2007). From August on juvenile individuals can be found in increasing numbers and constitute the majority of the stickleback population (Poizat *et al.* 2002) since many populations are annual (Baker 1994) and the adults often die after the breeding season (Paepke 1983). We hence can expect the densities of sticklebacks, especially young-of-the-year, as predators of *Acentria* to increase during the summer season. Predation pressure on adult females should hence be lower in spring for females originating from diapausing larvae than in high summer, i.e. from directly developing larvae.

Sex-specific cohort splitting hence might strongly contribute to the sex-ratio bias observed *in situ* (see chapter III). However, overall sex-ratio in our experiment did also differ between treatments suggesting that sex-specific cohort splitting is not the only cause for the observed male bias. This is supported by field observations which show that during specific sampling periods already small larvae can show a strong male bias (see chapter V). Nevertheless, sex-specific cohort splitting will obviously contribute to the male bias frequently observed in the pupal stage. In addition, our experiment predicts that the sex-ratio of larvae during spring, i.e. from larvae emerging from diapause, should be female-biased. This prediction should be tested with further field studies. Furthermore, sex-specific cohort splitting will result in seasonal shifts in operational sex-ratio, i.e. the ratio of adult males and females that are ready to mate in a population at a given time (Kvarnemo & Ahnesjö 1996), which might finally result in seasonal changes in the strength of sexual selection. As *Acentria* is a key herbivore (chapter I), it is important to estimate how strongly fish predation would have reduced within-season progeny numbers and consequently herbivore pressure due to the combined effects of mortality and costs of anti-predator strategies. Starting with a population of 1000 larvae a mortality rate of 0.14 d^{-1} combined with the observed sex-ratio bias under predation would reduce within-season progeny to 21.65 % during a period of ten days (Table 1). The costs of anti-predator-strategies, i.e. the change of developmental pathway and the reduction in size at metamorphosis corresponding to a reduced clutch size, will finally reduce overall progeny size to 9 %. Hence both direct and indirect effects of predation will have a strong influence on *Acentria* population growth as well as on the effects of *Acentria* on its food plants (Johnson *et al.* 1998, see chapters I and II). This estimate of the reduction of within-season progeny numbers should not be confused with the reduction of *Acentria* fitness as diapausing females contribute to the latter, but not to within-season progeny. However, the change in developmental pathway will also incur large fitness costs due to mortality during diapause and the long delay in the timing of reproduction by at least eight months.

So far most studies analysing life-history plasticity have focused either on size and age at maturity/metamorphosis (Peckarsky *et al.* 2001; Kiesecker *et al.* 2002; Dahl & Peckarsky 2003) or on developmental strategies (Martin *et al.* 1991; Johansson *et al.* 2001). Our study shows that it is important to combine these two avenues of research. Especially for females we identified two alternative anti-predator strategies: direct development at a reduced size at metamorphosis versus indirect development with a chance to achieve a large size at metamorphosis in the next season. Finally, our results suggest the necessity to consider the life-history response including their fitness benefits and costs for males and females separately (Mikolajewski *et al.* 2007).

Table 1 Calculation of the direct (mortality) and indirect (anti-predator costs) effects of fish predation on *Acentria* egg production in comparison to the control treatment (without predation, i.e. without mortality and anti-predator costs) within the experiment. Please note that a longer experimental duration would have increased the differences between control and fish treatments as more larvae finally would have developed into adults and produced eggs.

	Control	Fish treatment	
	without predation	mortality	mortality + anti-predator costs
starting point: 1000 individuals in the larval stage	1000 larvae	1000 larvae	
remaining numbers of individuals after 10 days of predation (mortality rate = 0.14 d^{-1})	1000 larvae	250 larvae	
number of females after accounting for enhanced mortality of females compared to males	500 female larvae	108 female larvae	
choice of developmental pathway (diapause or pupation)	167 female pupae	36 female pupae	20 female pupae
total number of eggs of directly developing females	61 109 eggs	13 209 eggs	5 559 eggs
percentage of the number of eggs compared to the control		21.65 %	9.1 %

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Chapter V

Seasonal and spatial variation in sex-ratio dynamics and size at metamorphosis of an aquatic insect - direct and induced influences of predation

OLIVER MILER, ELISABETH GROSS AND DIETMAR STRAILE

Manuscript in preparation

Abstract

Plasticities in growth rate, developmental time and size and age at metamorphosis in response to predators have been shown in damselflies, mayflies and amphibians, but the sex-specificity of these responses has been rarely studied. We studied the water moth *Acentria ephemerella* that has been experimentally shown to strongly suffer from mortality caused by stickleback (*Gasterosteus aculeatus*) predation with field samplings in Lake Constance to analyse the sex-specific life-history plasticity of an aquatic moth in response to predation *in situ*.

Acentria exhibited a strong spatial and seasonal life-history variability concerning the size at metamorphosis and developmental strategy (i.e. the choice for diapause or metamorphosis). The size at metamorphosis decreased during the season and increased spatially from the sampling site Reichenau over Hagnau to the sampling site Güttingen. *Acentria* larvae at the end of the growth season opted increasingly for diapause instead of metamorphosis. The life-history changes of *Acentria* were sex-specific. *Acentria* displays a sexual wing and size dimorphism in the adult stages: females are wingless and aquatic in contrast to the smaller winged and terrestrial adult males. The seasonal decrease in the size at metamorphosis and the spatial

differences were far more pronounced for the larger females than for the males. Male larvae chose in almost equal shares to pupate or to develop indirectly via a diapause stage whereas female larvae strongly preferred diapause over metamorphosis. This resulted in an increased male bias of the pupal sex-ratio. Stickleback predation strongly reduced *Acentria* densities, and we suggest mortality to be higher for the more active female larvae, which further shifted the pupal sex-ratio towards male bias.

The sex-specific life-history changes of *Acentria* were probably induced by fish predation. A high predation pressure of juvenile sticklebacks at the end of the growth season probably lead to a decrease in developmental time that reduced the size and age at metamorphosis, especially for the entirely aquatic female individuals. *Acentria* pupae were spatially different in size, i.e. they were large under a high and small under a low stickleback predation pressure. The preference for a development via a diapausing larval stage inside macrophyte stems that function as a refuge over a direct development to metamorphosis probably also led to a reduction of the mortality risk by fish predation primarily for the exclusively aquatic females. To summarise, stickleback predation had lethal (mortality) as well as non-lethal (changes in the size at metamorphosis and the developmental strategies) effects on *Acentria in situ*. Due to the sexual wing dimorphism of *Acentria* were the non-lethal, trait-mediated interactions sex-specific, affecting the exclusively aquatic females stronger than the terrestrial adult males.

Introduction

Predation is a major force influencing the growth and mortality rates of organisms, thus regulating their population dynamics and eventually enabling the existence of food webs and the functioning of ecosystems (Johnson *et al.* 1995; Nyström *et al.* 2001; Bolnick & Preisser 2005). Direct mortality by predators reduces population densities and changes the structure of populations via density-mediated predator-prey interactions (DMI) (Murdoch *et al.* 2003; Preisser *et al.* 2005). In addition, non-lethal predatory interactions (trait-mediated interactions (TMI) (Preisser *et al.* 2005; Beketov & Liess 2007) can substantially influence the behavioural decisions of animal species (Lima & Dill 1990) and furthermore often involve plastic responses in morphological, physiological and life-history traits (Schlichting & Pigliucci 1998; Relyea 2001). Plasticities in growth rate, developmental time and size and age at metamorphosis in response to predators for example have been shown in damselflies (Johansson *et al.* 2001; Brodin *et al.* 2006), mayflies (Peckarsky *et al.* 2002; Tseng 2003) and amphibians (Benard 2004; Relyea 2007).

Although induced anti-predator responses will decrease the mortality risk of an organism and enhance its survival (Lima & Dill 1990; Relyea 2007), these induced responses are often associated with costs (Peckarsky *et al.* 1993; Benard 2004; Preisser *et al.* 2005). While the induction of anti-predator responses and associated costs have been studied intensively for a number of species in the laboratory (Relyea 2007), much less is known about their importance *in situ*. Many species display sex-specific trait differences: for example are sex-specific differences in the adult body size of insects caused by differential growth rates of the sexes (Blanckenhorn 2005; Blanckenhorn *et al.* 2007). This might result in sex-specific susceptibilities to predators and/or sex-specific differences in the cost-benefit considerations of induced responses (Mikolajewski *et al.* 2007). Sex-specific differences in induced anti-predator responses have been shown for vertebrate prey organisms that display behavioural changes, for example lizards (Radder *et al.* 2006) and squirrels (Clucas *et al.* 2008). However, respective field studies of sex-specific anti-predator responses of invertebrates are missing: apart from the experimental study of Mikolajewski *et al.* (2007), sex-specific differences in plastic changes of the life-history and behaviour of invertebrates have up to now been mainly studied as responses to the environmental factors food availability (De Block & Stoks 2003) and time stress (Shama & Robinson 2006).

The aquatic moth *Acentria ephemerella*, a key herbivore in macrophyte beds in many temperate lakes that displays annually varying outbreak dynamics (Gross *et al.* 2002, see chapter II), exhibits sex-specific differences in adult body size, wing morphology and habitat (Berg 1942, see chapter IV). *Acentria* builds up high *in situ* population densities during the summer months, from mid-June to August (Gross *et al.* 2002), and overwinters in the second and third larval diapause stage from September to May (Haenni 1980). A mesocosm experiment showed a male-biased sex-ratio, an earlier age and smaller size at metamorphosis and a sex-specific decision of larvae for direct (metamorphosis) versus indirect (diapause) development under fish predation (see chapter IV). The latter resulted in highly male-biased pupal sex-ratios and a female-biased sex-ratio of diapausing larvae. Pupal sex-ratios, ranging from 60 % to 80 % male dominance, are typically observed in Lake Constance (see chapters III and IV). However, as we were only able to sex pupae, but not larvae, in previous field studies, our ability to develop a mechanistic understanding for the observed male bias was limited. Here we use a histological method, the staining of the W-chromatin in female Lepidopteran larvae that is used for cytogenetic analyses (Traut & Scholz 1978; Traut *et al.* 2007), for the first time in an ecological context. This method allows us to study the larval sex-ratio and sex-specific cohort dynamics and to increase our insight into the possible effect of predation on the *in situ* sex-ratio bias and cohort dynamics of *Acentria*.

In this study, we analyse the seasonal dynamics of *Acentria* at three sampling sites in a large pre-alpine lake, Lake Constance. We chose these three sites as we expected from previous studies that they differ in the densities of sticklebacks, which have been shown to be important predators of *Acentria* (see chapter III). Sticklebacks are a common fish species in macrophyte patches in Lake Constance (Miler, unpublished data), with benthic and planktonic invertebrates as their main prey organisms (Allen & Wootton 1984; Campbell 1991). Sticklebacks in Europe and North America from 35 to 65 degrees north latitude reproduce during a large period of time from February to August (Hyatt & Ringler 1989; Baker 1994; Poizat *et al.* 2002; Candolin & Voigt 2003). Young-of-the-year sticklebacks should increase the predation pressure on *Acentria* from late mid summer onwards: many stickleback populations are annual (Baker 1994), and the adults die after the breeding season (Paepke 1983) so that from August on juvenile individuals can be found in increasing numbers and comprise the largest part of the population (Poizat *et al.* 2002). Hence we expect *Acentria* sex-ratios and size at metamorphosis to differ both seasonally and spatially in our field study.

More specifically, we want to test the following hypotheses:

1) There is a seasonal and spatial variability of *Acentria* sex-ratio and size at metamorphosis
2) Male sex-ratio bias will increase and the size at metamorphosis will decrease with ongoing season
3) Male sex-ratio bias will increase and the size at metamorphosis will decrease with spatially increasing stickleback density
4) There is not only a sex-ratio bias of pupae but also of active larvae

Materials and methods

Lake Constance, with a surface area of 534 km^2 and a maximum depth of 254 m, is the second largest lake in the pre-alpine region of Central Europe (47° N; 09° E). The river Rhine is the main tributary and flows into Lake Constance at its eastern end as the “Alpenrhein” (alpine Rhine). The lake consists of two main basins, the Upper Lake Constance (ULC) and the Lower Lake Constance (LLC), that are connected by a 4 km long section of the river Rhine (Fig. 1). Upper and Lower Lake Constance strongly differ in morphology and macrophyte coverage: In the deep ULC (z_{mean} = 101 m) macrophytes grow only in the littoral zones along the steep slopes whereas they cover wide areas in the shallower LLC (z_{mean} = 13 m). The three sampling sites (Fig. 1) Hagnau (ULC), Güttingen (ULC) and Reichenau (LLC) differed in their morphology: The littoral zone at the island Reichenau had a steeper slope and a greater depth than in Güttingen and Hagnau at the southern and northern shore of ULC, respectively. The shallowest littoral areas with the smallest slope were found in Güttingen.

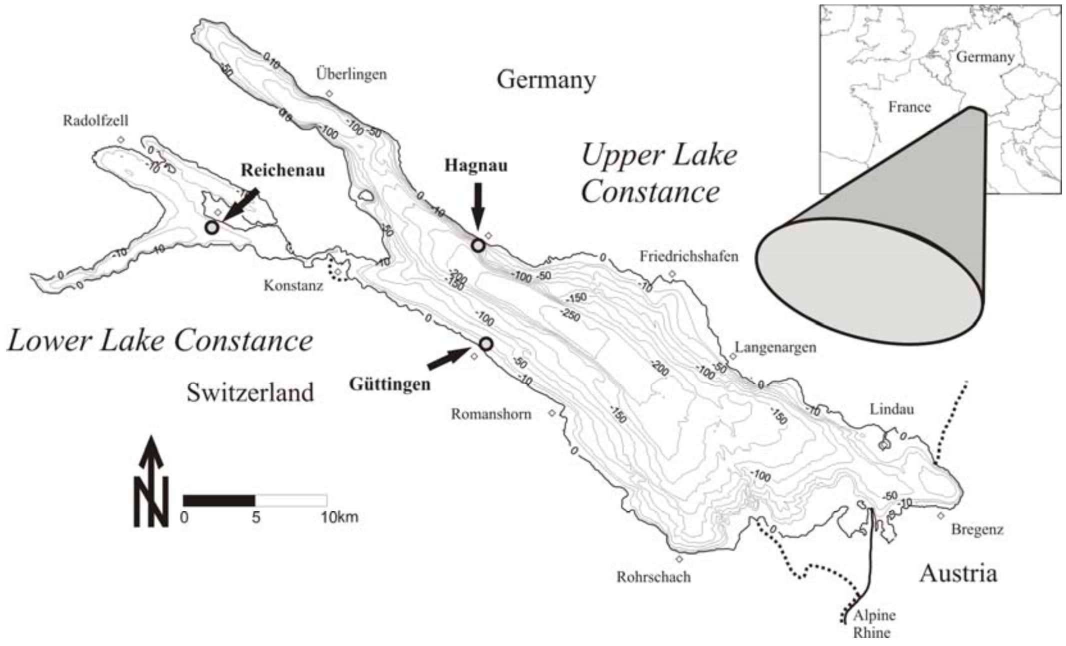

Fig. 1 Sites for the sampling of sticklebacks and *Acentria* larvae and pupae in Lake Constance, Germany, during the field season 2005, marked with arrows (near the cities Güttingen and Hagnau and the island Reichenau).

SAMPLING DESIGN AND PROCEDURES

In Lake Constance, macrophyte patches at three different sampling sites, in the littoral zone near Güttingen, Hagnau and the island Reichenau, were sampled (Fig. 1, black dots). Field samples for the analysis of sex-ratios and headcapsule widths were taken in six sampling periods: (1) (12 to 14 June), (2) (28 to 30 June), (3) (13 to 20 July), (4) (26 July to 3 August), (5) (29 August to 1 September) and (6) (24 to 25 September). From the field samples the larval densities of *Acentria* as individuals per gram plant dry mass (Ind. g^{-1} dm) in macrophyte patches were calculated for all sampling periods from three replicates per patch, except for the sampling period (3) where one large sample per sampling site in the main growth period of *Acentria* was taken in order to sample randomly from a large range of larval size classes. However, since in the sampling periods (1) and (2) only 109 and 226 larvae, respectively, were available for sex determination, we merged the larvae from the sampling periods (1) and (2) for the statistical sex-ratio analyses to enhance the number of observations. Furthermore, since the larval sex-ratios were not significantly different between the sampling periods (3) and (4), we also merged the larvae from these sampling periods. Hence for the statistical analyses of sex-ratios the sampling periods were merged into four main periods: (1/2) (12 to 14 June + 28 to 30 June), (3/4) (13 to 20 July + 26 July to 3 August), (5) (29 August to 1 September) and (6) (24 to 25 September). Submerged macrophytes were sampled with a toothed sickle, mounted on a 4 m metal pole. The plant samples were stored in plastic bags in a 5 °C climate chamber and processed within one week. Macroinvertebrates, including *Acentria* larvae, were washed from the plants through a sieve with a 200 µm mesh size. All *Acentria* larvae that were visible to the naked eye were stored in a fixation fluid, consisting of Carnoy's solution (60 % technical Ethanol, 30 % Chloroform p.a., 10 % Acetic Acid p.a.) for the subsequent sex determination of the larvae. The remaining larvae and macroinvertebrates were preserved in 70 % technical Ethanol. The macrophytes were searched for active larvae and pupae that were closely attached to the stem and for hibernating larvae inside the stems under a stereo microscope (Zeiss Stemi 2000-C) at a 10–50× magnification, and macrophytes were subsequently dried at 90 °C for at least 72 h to calculate the *Acentria* densities (Ind. g^{-1} dm). A stereo microscope was used to collect and count *Acentria* larvae and pupae at a 10–50× magnification from preserved field samples and to sex *Acentria* pupae morphologically as published in (Berg 1942) (see Appendix, Fig. A 2). The histological sex determination of *Acentria* larvae was performed according to the following protocol: The larvae were dissected under a stereo microscope at a 10–50× magnification, and the tissue of the internal organs, preferentially of the Malpighian tubules and the spinning glands, was stained with Lactic Acid – Acetic Acid – Orcein (LAO). Under 1000x magnification (microscope: Zeiss Axioskop 40), in female individuals the sex chromatin (w-chromatin) can be seen as a densely concentrated spot inside the cell nucleus whereas males lack this feature (see Appendix, Fig. A 2). The pupal lengths and larval head capsule widths were measured under a Zeiss Stemi 2000-C stereo microscope. To analyse the primary sex-ratio we sampled 14 egg clutches in Lake Constance and incubated them at 20°C in the laboratory. From each clutch we analysed 30 freshly hatched larvae. 60 *Acentria* pupae from the sampling site Güttingen in the sampling periods 4 (end of July) and 5 (end of August) in an advanced developmental stage where the eggs could already be observed were randomly picked and dissected after measuring the pupal length under a Zeiss Stemi 2000-C stereo microscope. The number of eggs was counted and correlated with the pupal size.

Sticklebacks were captured in the littoral zone of the three sampling sites Güttingen, Hagnau and Reichenau. One fish sampling unit consisted of altogether four minnow traps with two different mesh sizes (fine: 3 mm, coarse: 6 mm) and a trap opening of 25 mm that were fixed to a rope and thus positioned vertically in the water column via a buoy at the upper and two paving stones as a weight at the lower end of the rope (see chapter II). The traps were placed in the order (from the upper end) fine – coarse – fine – coarse along the rope. From 23 to 28 July 2005 23 sampling units were set during daytime in the littoral zone of each sampling site within macrophyte patches and were emptied after 24 h. The catch per unit effort (CPUE) was then calculated as the number of fish that were caught during 24 h in one sampling unit. The captured fish were killed with 2 mg $liter^{-1}$ 1,1,1-trichloro-2-methyl-propanol (TCMP) and frozen in a chest-freezer.

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, U.S.A.). Based on a previously established correlation between the dry weight - length of pupae ($\log_{10}$ (weight [mg]) = - 1.96 + (2.93 * $\log_{10}$ (body length [mm])), n = 34, R^2 = 0.93 , $p < 0.0001$) and the dry weight - head capsule width of prepupae and larvae ($\log_{10}$ (weight [mg]) = -12.17 + (4.17 * $\log_{10}$ (head capsule width [µm])), n = 45, R^2 = 0.93 , $p < 0.0001$), we calculated the dry weights of pupae, prepupae and larvae to compare the sex-specific size differences between the ontogenetic stages pupae, prepupae and larvae.

We used an analysis of variance (ANOVA) to test for the effect of sampling site on stickleback CPUE and for the effect of sampling period and sampling site on *Acentria* pupal size. The goodness of fit of the model was described by the coefficient of determination R^2 that ranges between 0 and 1. An R^2 of 1.0 indicates a perfect fit of the model to the data. We used a multifactorial logistic regression analysis with the complete model method in the model selection to test for the effects of sampling site and sampling period on *Acentria* sex-ratio. The predictor variables used in the regression were sampling site and sampling period. Wald's statistic (Wald Chi²) permitted to assess the significance of each predictor variable and the interactions between them. The logistic regression was performed separately for *Acentria* pupae, active larvae and diapausing larvae. The –2 log likelihood described the total fit of the model: the lower the –2 log likelihood is, the better is the model adapted to the data.

Results

The stickleback catch per unit effort (CPUE) differed significantly between sampling sites (Fig. 2, ANOVA, factor sampling site, $F_{2,66} = 7.61$, $p = 0.0011$). A post-hoc analysis revealed that the CPUE was significantly higher in Güttingen as compared to Hagnau and Reichenau.

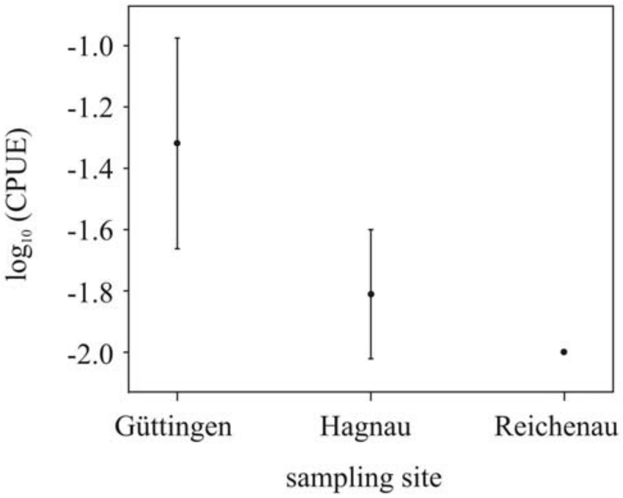

Fig. 2 Logarithmic CPUE (Catch per unit effort) of sticklebacks in Lake Constance in July 2005 (sampling period (3), 13 to 20 July).

The CPUE calculations were however based on a low number of caught sticklebacks, especially at Reichenau and Hagnau with zero and four sticklebacks, respectively, as compared to 19 sticklebacks at Güttingen. The densities of active *Acentria* larvae increased significantly from June to August in all sampling sites (Fig. 3 A).

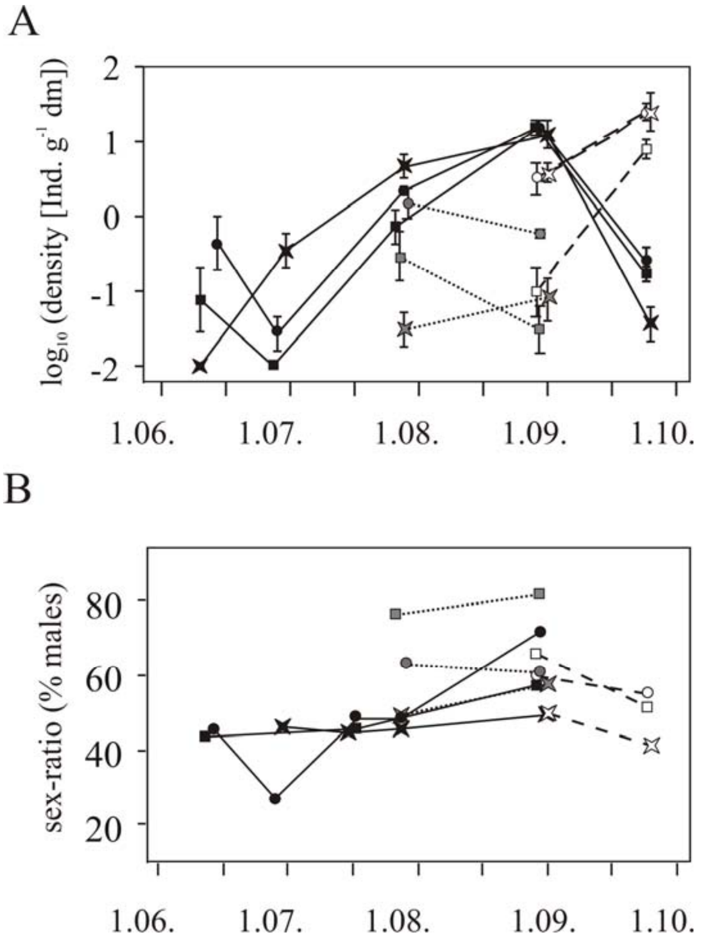

Fig. 3 Densities (Ind. g^{-1} dm, A) and sex-ratios (percentage of males, B) of *Acentria* in Lake Constance at the sampling sites Güttingen (G, circles), Hagnau (H, squares) and Reichenau (R, crosses). Presented are the densities and sex-ratios of active larvae (black symbols, solid line), pupae (grey symbols, dotted line) and hibernating larvae (white symbols, dashed line) in six sampling periods: (1) (12 to 14 June), (2) (28 to 30 June), (3) (13 to 20 July), (4) (26 July to 3 August), (5) (29 August to 1 September) and (6) (24 to 25 September) in 2005.

The low density of *Acentria* at the start of June (sampling period (1)), measured as the number of *Acentria* larvae per gram plant dry mass, declined until the end of June (sampling period (2)), which is most likely due to a fast biomass increase of *Potamogeton* before the first reproduction of *Acentria*, resulting in a dilution of *Acentria* numbers. In late June and late July, i.e. in the sampling periods (2) and (4), respectively, larval densities tended to differ spatially: in the sampling period (4) the density was higher at Reichenau compared to Güttingen and Hagnau

(Mixed Model ANOVA, factor sampling site, $F_{2,1} = 5.41$, $p = 0.1$), suggesting a faster population growth at the site with low fish densities.

Pupae were present in the sampling periods (4) and (5), i.e. in the late July and late August samples (Fig. 3 A). The spatial differences in pupal densities did not correspond to spatial differences in larval densities, i.e. the pupal density at Reichenau was lowest despite high larval densities at this site. This might be caused by subtle differences in cohort dynamics between sites, e.g. Reichenau larvae may have been late in pupation or pupae early in eclosion. Diapausing larvae were first detected in the sampling period (5) in late August and increased in density strongly towards the sampling period (6) at the end of September.

Table 1 Logistic regression of the sex-ratios of *Acentria* active larvae, hibernating larvae and pupae in the field season 2005. For each developmental stage, the effects of sampling site, sampling period and the interaction was analysed.

active larvae (n = 1311, -2 log likelihood (constant & variables) = 1730.597)			
	df	Wald Chi^2	p
sampling site	2	6.35	0.04
sampling period	3	19.48	< 0.0001
sampling site*sampling period	6	9.62	0.047
hibernating larvae (n = 352, -2 log likelihood (constant & variables) = 480.085)			
	df	Wald Chi^2	p
sampling site	2	4.48	0.11
sampling period	1	2.58	0.11
sampling site*sampling period	2	0.46	0.79
pupae (n = 513, -2 log likelihood (constant & variables) = 636.224)			
	df	Wald Chi^2	p
sampling site	2	13.36	0.0013
sampling period	1	5.85	0.016
sampling site*sampling period	2	3.15	0.21

The sex-ratios of freshly hatched larvae, i.e. the primary sex-ratio, was determined for 14 egg clutches and showed no evidence for male bias with a male share of 47.5 ± 7.62 (SD) %. The sex-ratio of active larvae and pupae showed strong seasonal and spatial patterns (Fig. 3 B and

Table 1). The percentage of male larvae increased seasonally from below 50 % in June (sampling periods (1/2)) towards a strong male bias at the end of August (sampling period (5)). The latter one was especially pronounced at the sampling site Güttingen whereas an unbiased sex-ratio was observed at Reichenau. Pupal sex-ratio also tended to be the least male-biased at Reichenau whereas a strong male bias was observed at Hagnau (81.8 % male pupae, Fig. 3 B). The overall pupal sex-ratio in August showed a stronger male bias (63.3 % males) than that of active larvae (59.3 % males). In contrast, the sex-ratio of diapausing larvae in August tended to be more unbiased (56.8 % males) with an equal share of males and females at Reichenau (50 % males, Fig. 3 B). The size distributions of larvae did not show evidence for site-specific cohort dynamics (data not shown). Combining larvae from all three sites revealed the simultaneous presence of at least three cohorts during the different sampling periods (Fig. 4). The presence of three cohorts is clearly evident from the size distributions in the sampling periods (1) to (4).

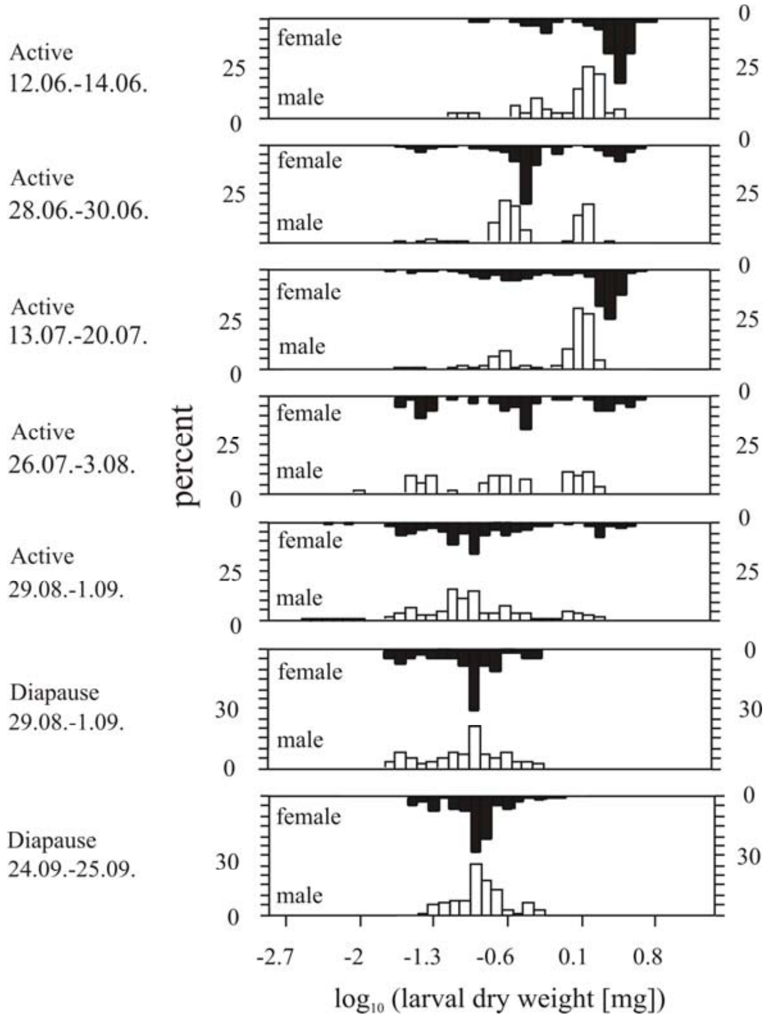

Fig. 4 Frequency distribution of the logarithmic dry weights of active and diapausing larvae in 2005, shown for male and female larvae in white and black bars, respectively. Field samples for the analysis of headcapsule widths were taken in six sampling periods: (1) (12 to 14 June), (2) (28 to 30 June), (3) (13 to 20 July), (4) (26 July to 3 August), (5) (29 August to 1 September) and (6) (24 to 25 September).

In addition, we should expect at least in the sampling periods (3) and (4) also *Acentria* present in the egg or first instar stages. First instar larvae that have a head capsule width between ~ 180 to ~ 260 µm, which equals a dry weight below approximately 0.01 mg, are clearly underrepresented in our samples. This is because early larvae mine inside *Potamogeton* stems (Berg 1942), and we did not survey the stems for mining larvae. In the sampling period (5), the cohort structure is not anymore evident in the data, and the size structure of diapausing larvae seems not to differ much from that of active larvae (Fig. 4). However, since *Acentria* larvae overwinter in the second and third larval diapause stage from September to May (Haenni 1980), late instar larvae were not found in the diapause stage. When larvae have reached the fourth or a later larval instar stage, they continue direct development, i.e. they undergo metamorphosis. The size distribution also shows that female larvae in the different cohorts are larger than male larvae (Fig. 4), which corresponds to the sexual size dimorphism of pupae (Fig. 5).

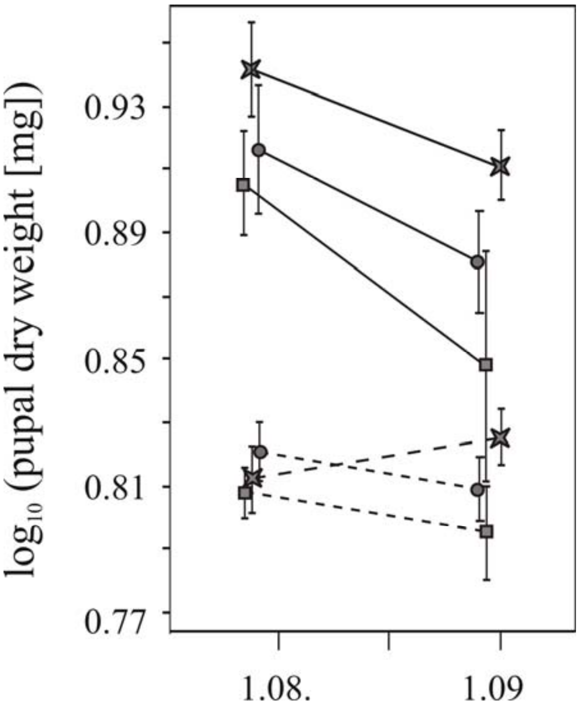

Fig. 5 Female (solid line) and male (dashed line) logarithmic pupal dry weights in the sampling periods: (4) (26 July to 3 August) and (5) (29 August to 1 September) in 2005 at the sampling sites Güttingen (circles), Hagnau (squares) and Reichenau (crosses).

In addition to the size differences between sexes, there were also strong seasonal and spatial differences in pupal size (Fig. 5 and Table 2). However, a decline in pupal size from the end of July (sampling period (4)) towards the end of August (sampling period (5)) was only evident for females and not for males (Table 2: significant sex*sampling period interaction). Likewise spatial differences in pupal size differed between sexes (Table 2: significant sex*sampling site interaction), being more pronounced for female pupae (Fig. 5). In addition, the seasonal change in pupal size differed between sampling sites (Table 2: significant sampling site*sampling period interaction).

Table 2 Three-factorial ANOVA analysing the influence of the factors sampling site, sampling period and sex on the size at metamorphosis of *Acentria* in the field season 2005 (n = 513, $R^2 = 0.58$).

factor	df	F	p
sampling site	2	20.58	< 0.0001
sampling period	1	27.46	< 0.0001
sex	1	431.24	< 0.0001
sampling period*sampling site	2	3.46	0.032
sampling site*sex	2	5.69	0.0036
sampling period*sex	1	19.2	< 0.0001
sex*sampling period*sampling site	2	0.69	0.5

The seasonal and spatial reduction of female body size has a strong effect on female fitness as clutch sizes were strongly related to body size (Fig. 6, clutch size = - 237.39 + 676.93 * $\log_{10}$ (pupal dry weight [mg]), $R^2 = 0.58$, n = 70). At the end of July (sampling period (4)), pupae had on average a 31 % larger clutch size than pupae at the end of August (sampling period (5)), with 347.9 ± 21.1 (SE) versus 239.2 ± 18.75 (SE) eggs, respectively.

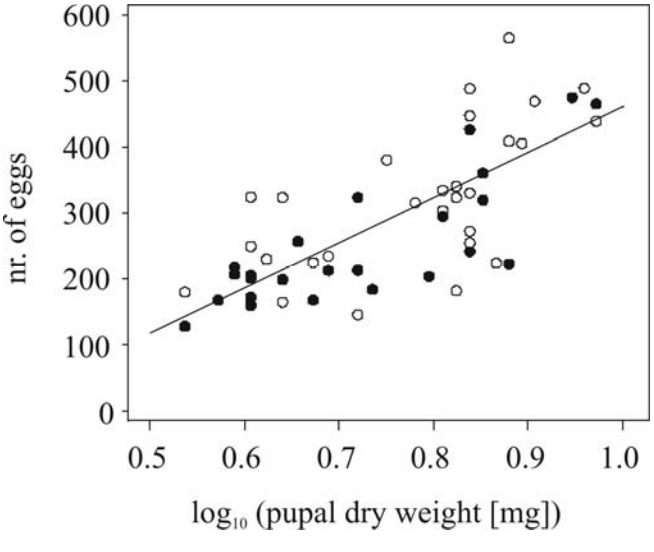

Fig. 6 Clutch Sizes (number of eggs) of *Acentria* larvae in July (empty circles) and August (filled circles) 2005 in relation to the logarithmic pupal weight.

Discussion

This study provided strong field evidence for sex-specific seasonal and spatial variability in the population density, sex-ratio, phenotypic plasticity and life-history decisions of moth larvae. We show furthermore that the variabilities of these different traits were linked to each other, which strongly suggests at least a partial association of these patterns with a variability in fish predation pressure.

Seasonal variability – The *Acentria* density in 2005 showed a strong increase over several orders of magnitude as it was previously observed in other study years (Gross *et al.* 2002). Additionally, we observed a decrease in the sizes of pupae and an increasingly male-biased larval sex-ratio. Both changes possibly result from a seasonal increase in fish predation. Sticklebacks reproduce during a large period of time from February to August (Hyatt & Ringler 1989; Baker 1994; Poizat *et al.* 2002; Candolin & Voigt 2003), with a major part of the adults dying after the breeding season (Paepke 1983). Since from August on juvenile individuals can be found in increasing numbers and comprise the largest part of the population (Poizat *et al.* 2002), young-of-the-year sticklebacks likely enhance the predation pressure after overcoming gape limitation in mid summer. The seasonal decline in female pupal size was of a similar magnitude as the reduction of female pupal size due to an earlier metamorphosis in the presence of fish in a mesocosm experiment (see chapter IV). Predator chemicals are well known to induce an earlier time and smaller size at metamorphosis in several insect and amphibian larvae (Lardner 2000; Kiesecker *et al.* 2002; Dahl & Peckarsky 2003) to enable adult individuals to escape a predation-risky habitat earlier. This suggests an important role of fish also for causing the seasonal variability of *Acentria* pupal sizes. However, in the mesocosm experiment fish predation resulted in a reduction of the pupal sizes of both sexes (9.1 % for males, 18 % for females) whereas we observed *in situ* only a seasonal decline in pupal size by 11 % for females. Hence, additional factors may also contribute to the phenotypic plasticity in size at metamorphosis. For example the seasonal increase of *Acentria* density results into an increasing feeding damage on food plants, plant senescence and consequently reduced food quality for *Acentria* (see chapters I and II). As female adults, in contrast to male adults, have reduced dispersal abilities due to their rudimentary wings, they may react more sensitively to a deteriorating food quantity and quality. Females seem to be more sensitive to a change of environmental factors and as a consequence often show a relatively larger decrease in size than males (Teder & Tammaru 2005). Additionally, as differences in pupal sex-ratios most likely lead to differences in the operational sex-ratio, the role of sexual selection, i.e. male competition for females, may differ both seasonally and spatially. This might also influence the male size at metamorphosis.

Pupae and diapausing larvae are not susceptible to fish predation as they are firmly attached to or mine inside *Potamogeton* stems (Berg 1942). In the absence of induced sex-specific developmental pathways, the sex-ratios of pupae and diapausing larvae should not differ from those of active larvae. This is clearly not the case: pupal sex-ratios showed a stronger male bias and diapausing larvae a less biased sex-ratio as compared to active larvae, suggesting that female larvae prefer to diapause whereas male larvae prefer to metamorphose. Hence the strong male bias of pupal sex-ratio observed in this study and also in other study years (see chapter III) seems to result from the additive effects of lethal and induced responses to predation. On the other side, the female preference for diapause may result - depending on the sex-ratio of the active population - in a female-biased sex-ratio of the diapausing individuals and consequently of the population emerging from diapause in the subsequent late spring/early summer period.

The seasonal increase in male bias of active larval sex-ratio most likely also resulted from a seasonal increase in fish predation. At the end of August, active larvae showed a strong male bias, suggesting a higher mortality of female larvae. The larger size of female larvae and pupae (Fig. 4 and 5) may suggest higher female growth rates and foraging activities. The latter might result in an increased predation risk as it was observed for example in Zygoptera (Brodin & Johansson 2004). This male bias in August was already evident for rather small larvae and did not increase with larval size. This supports mesocosm results which suggest that early larval instars are more susceptible to fish predation than later ones (see chapter III).

The significant seasonal increase in the male bias of the active larval sex-ratio was however partially also due to non - lethal, i.e. induced predator effects. In June (sampling periods (1/2)), no male bias was observed, rather there was a tendency for a female bias ($Chi^2 = 2.84$, df = 1, $p = 0.092$). The population sampled in June (sampling periods (1/2)) consisted to a large extent most probably of specimens emerging from diapause. This supports mesocosm results showing that in the presence of fish females preferentially opt for indirect development, i.e. diapause, whereas males opt for direct development, i.e. metamorphosis (see chapter IV). The actual sex-ratio of the diapausing specimens and of those emerging from diapause in the next year will result from the male bias of the active larval sex-ratio, i.e. a direct effect of predation, and the difference in developmental decisions between the sexes, i.e. an induced effect of predation.

Spatial variability - Our study suggests a link between population growth, sex-ratios and pupal sizes of *Acentria*. The *Acentria* population at the Lower Lake Constance sampling site (Reichenau) was characterised by higher summer densities, the largest pupae and the least biased pupal sex-ratios. *Acentria* population characteristics at the 2nd Upper Lake Constance site Güttingen were intermediate whereas *Acentria* at the Upper Lake Constance site Hagnau showed the lowest densities, the smallest pupae and a highly male-biased pupal sex-ratio. Exactly this combination of characteristics was altered by the induced and lethal effects of stickleback predation in mesocosm experiments (see chapters III and IV), suggesting that the observed spatial differences might be also due to fish predation.

The presumed spatial differences in fish predation pressure were partially supported by stickleback catches done within a short period in mid-July (sampling period (3)) at the three sampling sites: our minnow traps did not catch a single stickleback at Lower Lake Constance, suggesting that the low predation pressure at this site resulted in a high *Acentria* population growth, a large size at metamorphosis and a largely unbiased sex-ratio. We were in contrast able to catch sticklebacks at the two Upper Lake Constance sites albeit the ranking of stickleback catches was opposite to those expected from *Acentria* characteristics. However, it should be kept in mind that although we used 92 minnow traps distributed across 23 sites per sampling site, the ranking of sites based on stickleback catches is based on a small overall number of sticklebacks (only four and 19 sticklebacks were caught in three patches each at the sampling sites Hagnau and Güttingen, respectively). Furthermore, there is likely a seasonal and small-scale spatial variability in stickleback density within sites. The scale of spatial stickleback variability is currently not well understood. However, in a fish sampling campaign covering several sampling dates during the summer 2004 the stickleback CPUE was highest at Hagnau, intermediate at Güttingen and lowest at the Reichenau site (see chapter II). This corresponds to stickleback predation pressures inferred from *Acentria* characteristics in 2005 and supports a lower stickleback density at the Reichenau site.

Although the earlier metamorphosis at a smaller size and the female preference for hibernation as anti-predator strategies probably reduce the mortality risk of *Acentria* larvae and increase their survival, there are certain costs involved. A reduced female body size has to be traded off against the number of offspring. The clutch size of female *Acentria* is highly positively correlated with body size (Fig. 6) as this is often the case in arthropods (Honek 1993; McPeek & Peckarsky 1998; Nylin & Gotthard 1998; Taylor *et al.* 1998). Consequently, with decreasing size at metamorphosis the number of eggs is reduced. As the result of the male-biased pupal sex-ratio and the reduced clutch size under fish predation, the operational sex-ratio (OSR), defined as the ratio of males and females that are ready to mate in a population at a given time (Kvarnemo & Ahnesjö 1996), was probably skewed towards male dominance (see above). Since either highly female- (Juchault *et al.* 1993; Hatcher *et al.* 1999) or male-biased sex-ratios (Heppell *et al.* 2006; Steifetten & Dale 2006; Lenz *et al.* 2007) can potentially lead to a reduction in population growth and in extreme cases to population extinction, we can therefore conclude that also in this field study the observed male-biased *Acentria* OSR as a result of smaller clutch sizes and higher male-biased sex-ratios due to the presence of fishes probably reduced the population growth of *Acentria*.

Stickleback predation influenced the population densities and the life-history of the aquatic moth *Acentria ephemerella* both seasonally and spatially. The strong effects of stickleback predation on *Acentria* were lethal (mortality by predation) as well as non-lethal (induced anti-predator defences, i.e. a reduction of the size and age at metamorphosis and a sex-specific change in developmental strategies) and eventually resulted in a reduction of *Acentria* densities and pupal sizes and an increase of the male bias at metamorphosis. Seasonal time stress, influencing the size and age at metamorphosis of many insects (Gotthard 1998; Johansson & Rowe 1999; De Block & Stoks 2003; Shama & Robinson 2006), might be an alternative explanation for the seasonal and spatial life-history plasticity of *Acentria*. For example in damselflies it has been shown that a shorter photoperiod, simulating time stress late in the season, can lead to an earlier time and smaller size at metamorphosis (Johansson & Rowe 1999; De Block & Stoks 2003). The potential effect of seasonal time stress needs to be studied in future experiments and field samplings. However, our results show that the spatial variability was as strong as the observed seasonal one, and this is unlikely due to time stress. To conclude, fish predation has strong seasonal and spatial influences on the life-history and population dynamics of *Acentria*, an important aquatic herbivore on submerged macrophytes. Predation-induced life-history changes can be regarded as trait-mediated interactions (Preisser *et al.* 2005; Beketov & Liess 2007) and will have consequences for the grazing pressure of *Acentria* and eventually for the structure and habitat functioning of macrophyte patches in littoral zones.

Acknowledgements

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Concluding remarks and perspectives for future research

I studied in this thesis the trophic interactions between sticklebacks (*Gasterosteus aculeatus*), the water moth *Acentria ephemerella* and the submerged macrophyte *Potamogeton perfoliatus* with mesocosm experiments and field data from Lake Constance, Germany. The outcomes of this thesis are the following:

- *Acentria* herbivory strongly damages the above-ground shoots of *Potamogeton perfoliatus*. *Potamogeton perfoliatus* defends itself against herbivory by an increased senescence of shoots and an increased below-ground transfer of nutrients after a sufficient biomass allocation towards the resting buds. These plant responses suggest that *Potamogeton perfoliatus* displays the tolerance/escape syndrome as an anti-herbivore defence strategy.
- The condition of *Potamogeton perfoliatus* patches rapidly deteriorates under strong *Acentria* herbivory, i.e. an increased proportion of shoots becomes senescent. Fishes that frequently inhabit submerged aquatic vegetation (SAV), especially juvenile perch (*Perca fluviatilis*), reduce their use of SAV in macrophyte patches that contain a high proportion of senescent shoots.
- The growth and mortality rates and thus the population dynamics of *Acentria* are strongly influenced by three main processes, i.e. by mortality (1.) and by fish-induced anti-predator strategies and their costs (2., 3.):
    1. Mortality through predation strongly reduces the larval densities of *Acentria*. Mortality is probably sex-specific, being higher for females and thus contributing to a high male bias under stickleback predation in experiments and in the field.
    2. Under stickleback predation, the size at metamorphosis of *Acentria* is reduced to decrease the total developmental time in the larval stage and to reproduce earlier. However, a small female size at metamorphosis has to be traded off against a decreased clutch size.
    3. Stickleback predation induces a sex-specific change in developmental strategies of *Acentria*, i.e. a female preference for hibernation over pupation at the onset of winter diapause whereas male larvae choose hibernation and pupation in equal shares. This leads together with sex-specific mortality to the found high male bias of *Acentria* at metamorphosis.

  A reduced size at metamorphosis and sex-specific changes in developmental strategies are probably life-history strategies to escape fish predation. Since *Acentria* displays a sexual wing-dimorphism, this holds especially true for the wingless adult females that cannot leave the aquatic habitat in contrast to the winged adult males.

The changes in macrophyte patches due to *Acentria* herbivory and the resulting habitat suitability of macrophyte patches for fishes showed that *Acentria* can be regarded as an ecosystem engineer (Jones *et al.* 1997). Physical ecosystem engineers are defined as "organisms that directly or indirectly control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials" (Jones *et al.* 1997). More closely regarded cause ecosystem engineers physical state changes in two basic ways: Autogenic physical engineers influence their environment via endogenous processes that alter the structure of the engineer itself, which remains a part of the engineered environment (e.g. the growth of trees and other plants). However, allogenic engineers are not necessarily part of the environment that is physically changed by them. Autogenic engineers are often plants, e.g. trees (Bertness & Leonard 1997; Callaway & Walker 1997; Hacker & Gaines 1997), and animals often allogenic engineers, e.g. beavers (Naiman *et al.* 1988; Johnston & Naiman 1990). However, the roles can be reversed, for instance function corals as autogenic engineers, and trees can function as allogenic engineers since e.g. the understory of tree canopies does not include the tree engineer and nevertheless is often influenced by it (Jones *et al.* 1994). In the light of these differentiations, the grazing effect of *Acentria* belongs to allogenic ecosystem engineering: *Acentria* influences via herbivory the senescence and the density of *Potamogeton* shoots, i.e. the physical state of *Potamogeton perfoliatus* patches and consequently the suitability as a foraging and refuge habitat for fishes.

Acentria grazing modified the availability of foraging and refuge resources that were connected with macrophyte senescence and shoot density since juvenile perch preferred a medium patch complexity as it has also been shown for other fish species. With decreasing macrophyte senescence and shoot density, the patch complexity and concomitantly the abundances of macrophyte-associated invertebrate prey for fishes is reduced (Savino *et al.* 1992; Eklöv 1997; Warfe & Barmuta 2006). The foraging efficiency of fishes follows the opposite development since the ability to orientate visually (Savino & Stein 1982) and the manoeuvrability of fishes increases with decreasing macrophyte senescence and shoot density (Manatunge *et al.* 2000). However, this holds also true for piscivorous predators (Crowder & Cooper 1982; MacRae & Jackson 2001). Hence a medium stem density and patch complexity is an optimal solution for the conflicting effects of density on prey capture (visibility and manoeuvrability), refuge use and invertebrate abundances (Crowder & Cooper 1982; Wiley *et al.* 1984; Savino *et al.* 1992). However, populations of macroinvertebrates are probably differently influenced by *Acentria* induced feeding damage and plant senescence: With increasing patch senescence the abundances of herbivorous macroinvertebrates that graze on periphyton that grows on macrophyte leaves (Brönmark & Vermaat 1998; Jones *et al.* 1998) or feed on macrophyte leaves and stems (Lodge 1991; Newman 1991; Lodge *et al.* 1998) will decrease. In contrast, the densities of species ingesting senescent epiphytic algae and plant tissues (Newman 1991;

Kornijow *et al.* 1995) will probably increase. Damselflies and dragonflies as predatory invertebrates that feed on herbivorous and detritivorous invertebrates (Power 1992; Turner & Chislock 2007) and constitute together with their prey the food organisms of fishes (Baker *et al.* 1999; Johansson & Brodin 2003) often show complex reactions to the presence of fishes. Many species show morphological defences in combination with behavioural anti-predator responses (Brodin & Johansson 2002; Brodin & Johansson 2004; Mikolajewski & Johansson 2004) and strong species-specific differences in activity, which result in differential vulnerabilities to fish predation. Hence complex food webs could emerge from such indirect trophic interactions when *Acentria* modifies via herbivory the macrophyte patch condition and the densities of fishes and plant feeding macroinvertebrates, and this in turn has effects on the densities of predatory damselflies and dragonflies. These indirect interactions, especially trait-mediated interactions, have up to now been primarily studied in terrestrial and marine habitats (Schmitz *et al.* 1997; Menge 2000; Schmitz *et al.* 2004). Research on indirect trophic interactions in freshwater habitats has mainly focused on effects on plankton or periphyton (Schmitz *et al.* 2004) whereas patches of submerged aquatic vegetation have however only little been studied and deserve further attention.

The ecosystem engineering role of *Acentria* is influenced by its mortality and growth rates: Anti-predation strategies, i.e. a reduced size at metamorphosis and a sex-specific change in developmental strategies (indirect vs. direct development) are displayed by *Acentria* and affect its mortality and population development. A reduction in the size at metamorphosis is probably caused by an earlier pupation at a smaller size, which is a strategy found also for the mayfly species *Baetis* and *Ephemerella* (Peckarsky *et al.* 2001; Dahl & Peckarsky 2003). However, such an anti-predator response in developmental plasticity, i.e. a reduced development time associated with a smaller size at metamorphosis, is in general a rarely observed strategy for amphibian and aquatic invertebrate prey species (but see reviews by Benard (2004) and Relyea (2007)). A reduction in the size at metamorphosis reduces the larval developmental period where *Acentria* are highly vulnerable to stickleback predation as could be shown in this thesis by the strong reduction of larval densities under stickleback predation.

Furthermore, *Acentria* displays sex-specific developmental strategies. *Acentria* population dynamics are determined by several generations, at least two per year: a directly developing summer generation and an indirectly developing winter generation that includes a diapause stage. As a consequence, before the onset of winter *Acentria* larvae can choose to diapause and to undergo indirect development or to develop until metamorphosis and form an additional directly developing autumn generation. As an anti-predator strategy, female larvae prefer diapause over metamorphosis whereas male larvae show no preference. To explain the developmental strategies of predator avoidance, they have to be discussed particularly with regard to sex-specific life-history and size differences. Adult males are winged and leave the water after emergence from the aquatic pupal cocoon to become terrestrial during their short adult life span and to mate and reproduce (Williams & Feltmate 1992). However, the adult females are strongly adapted to the aquatic habitat since their wings are only rudimentary, and they have developed swimming bristles on their legs (Berg 1942; Speidel 1984; Speidel 1998). Adult females stay in the water column to lay their eggs on submerged plant leaves after they have emerged and have mated at the water surface and are in the aquatic vegetation still vulnerable to fish predation in contrast to the males. The risk of mortality through stickleback predation for adult female *Acentria* can be reduced by undergoing indirect development and delaying reproduction to the following season. Diapause takes place inside macrophyte stems that offer a strongly protected refuge for *Acentria*. This is furthermore reasonable since we expect stickleback predation to be highest in August at the onset of *Acentria* diapause: juvenile sticklebacks can be found in increasing numbers in August and comprise the largest part of the population (Poizat *et al.* 2002) since many adult sticklebacks die after the breeding season (Paepke 1983).

Sex-specific life-history changes have nevertheless received only limited attention, and Mikolajewski et al. (2005; 2007) highlighted the need for sex-specific approaches in life-history research. Blanckenhorn et al. (2007) could show in a review that a weak connection between sexual size dimorphism (SSD) and sexual bimaturism (SBM) is a generally found pattern in many arthropod species. Since all studied taxa displayed a female-biased SSD, the size difference between the sexes was primarily caused by a faster growth and hence probably a higher foraging activity of females compared to males. As a consequence, females are probably more susceptible to predation, and this should lead to male-biased sex-ratios at metamorphosis in the field as found for *Acentria* in this thesis. More experimental research is needed to study the bias and dynamics of the sex-ratio at metamorphosis of arthropods and the interacting factors that influence male-biased sex-ratios in the field. Based on the results of the mesocosm experiment discussed in chapter IV, a complete separation of the lethal and non-lethal influences of predation that probably determine *Acentria* sex-ratio was not possible. Hence especially experiments with caged predators which are able to release kairomones but cannot feed on *Acentria* could reveal highly interesting results on the relative contribution of sex-specific mortality and kairomone-induced changes in life-history strategies on *Acentria* sex-ratio.

To sex Lepidoptera larvae, until now mainly a sex determination by the external morphology was applied to individuals of a body length larger than 5 mm through the identification of sex-specific abdominal pits (Underwood 1994). An important, innovative tool in entomological research is the histological sex determination method for Lepidoptera larvae that was used in this thesis and that was developed by Prof. František Marec (České Budějovice, Czech Republic). This method is based on the staining of the sex chromatin of the heterozygotic female adults that occurs as a darkly stained spot in the cell nucleus (Traut & Scholz 1978; Clarke 1984) of larval individuals of the clade Ditrysia that comprises 98 % of all known Lepidoptera species. It has to my knowledge previously not been applied to investigate ecological research questions, especially with respect to the study of sex-specific aspects of Lepidoptera life-histories. The use of sex chromatin staining hence offers a powerful tool to study the sex-ratio dynamics and sex-specific life-history strategies of *Acentria* and other Lepidoptera.

Although mesocosm experiments revealed fundamental and important insights into the influence of stickleback predation on *Acentria* population dynamics and life-history, detailed informations on the larval development of *Acentria* until pupation and the factors controlling growth rate and development time as important life-history traits are still lacking. Many ectotherm animals in a seasonal environment are influenced by changes of the day-length and are subject to seasonal time constraints that affect their growth and development (Johansson & Rowe 1999; Gotthard 2001; Laurila *et al.* 2001; Danks 2006). Aquatic insects often show strong and interacting influences of photoperiod and other environmental factors on their development and life-history, for example between photoperiod and hydroperiod (Shama & Robinson 2006) and between photoperiod and predation risk (Johansson *et al.* 2001; Tseng 2003). *Acentria* development takes place in a strongly seasonal environment with a short growth season from May to August and a long diapause during the remaining months. Hence we expect also *Acentria* to exhibit seasonally differing growth patterns that interact with other influences, especially predation risk. Such interactions are important issues for future life-history research and should be studied in aquarium experiments investigating the developmental responses of *Acentria* to variations in photoperiod, imitating early and late season environments, with and without predation.

An interesting aspect to study in the interaction between *Acentria* and *Potamogeton perfoliatus* is the potential existence of clonal differences of *Potamogeton perfoliatus* in the ability to react with an induced senescence to grazing as part of the suggested tolerance/escape syndrome. If controlled senescence is a part of this evolved anti-herbivore strategy, then we should expect clonal variability in this trait: a clone that has the ability to allocate biomass faster and senesce earlier when grazed upon contributes less to *Acentria* population growth, cohort size of the *Acentria* diapause stage and hence grazing pressure in the following season. To investigate if this potential selective advantage leads to clonal differences is of major importance for the understanding of macrophyte defence strategies and should be studied with an experimental approach, using plant shoots from different sampling sites and exposing them to a range of *Acentria* grazing intensities.

Trophic cascades, including predators, herbivores and primary producers as trophic levels, have been intensively studied in many terrestrial ecosystems (Strong 1992; Preisser 2003; Finke &

Denno 2006), but research in freshwater ecosystems is mainly confined to pelagic (Carpenter *et al.* 1985; Persson 1997; Bertolo *et al.* 1999) or lotic food webs (Power 1992). Interactions in submerged macrophyte patches between water plants, herbivores and their predators in the littoral zone have received comparably little attention (but see Sutter & Newman 1997; Ward & Newman 2006) and have been often focused on algal-based food webs, such as the benthic fish - snail - epiphyton food chain (Martin *et al.* 1992; Brönmark & Vermaat 1998; Jones & Sayer 2003). In this thesis, strong trophic interactions between *Acentria* and *Potamogeton perfoliatus* and between sticklebacks and *Acentria* were found. This suggests the potential existence of a tritrophic cascade sticklebacks - *Acentria* - *Potamogeton perfoliatus* in submerged macrophyte patches in the littoral zones of lakes and ponds, which should be tested with sticklebacks, *Acentria* and *Potamogeton perfoliatus* included together in the very same mesocosm experiment. Since *Acentria ephemerella* (Denis & Schiffermüller) is widely distributed in freshwater lakes and brackish water ecosystems throughout Europe (Berg 1942; Gross *et al.* 2001; Gross *et al.* 2002), this cascade should be of major importance in structuring the shorelines of many temperate lakes and coastal regions in the Northern Hemisphere.

Zusammenfassung

Makrophytenbestände sind bedeutende Habitate für Fische und Makroinvertebrate und wichtige, strukturgebende Bestandteile des Uferbereichs von Stillgewässern und marinen Ökosystemen. Trophische Interaktionen zwischen Primärproduzenten (submersen Makrophyten), Herbivoren und Prädatoren beeinflussen littorale Nahrungsnetze, die strukturelle Komplexizität von Makrophytenpatches und deren Funktion als Versteck und Nahrungshabitat für Fische und Makroinvertebrate. Die Wassermotte *Acentria ephemerella* ist eine wichtige herbivore Insektenart, die starke Fraßschäden an submersen Wasserpflanzen verursacht und häufig in Flüssen, Seen und Küstenregionen der nördlichen Hemisphäre vorkommt. Eine wichtige Futterpflanze für *Acentria ephemerella* ist das Durchwachsene Laichkraut *Potamogeton perfoliatus*, das die dominante Wasserpflanzenart im Bodensee und vielen anderen europäischen Seen ist und große, mono-spezifische Wasserpflanzenbestände ausbildet. Stichlinge (*Gasterosteus aculeatus*) kommen häufig in Makrophytenbeständen vor und ernähren sich von epiphytischen Makroinvertebraten. In dieser Arbeit habe ich die trophischen Interaktionen zwischen Stichlingen (*Gasterosteus aculeatus*), der herbivoren, aquatischen Lepidoptere *Acentria ephemerella* und der Wasserpflanzenart *Potamogeton perfoliatus* mit Mesokosmosexperimenten und Freilandstudien untersucht, um Einblick in den Einfluss von *Acentria* auf *Potamogeton* durch Herbivorie und die Populationsregulation von *Acentria* durch Prädation zu bekommen.

Acentria übte einen starken Frassdruck auf *Potamogeton perfoliatus* aus: Mesokosmosexperimente zeigten, dass Herbivorie durch *Acentria* (1) die Biomasse von *Potamogeton perfoliatus* reduzierte und (2) die Seneszenz von *Potamogeton perfoliatus* Sprossen verstärkte und (3), dass *Potamogeton perfoliatus* Nährstoffe (Phosphor und Stickstoff) in Überwinterungsknollen im Boden verlagerte, die vor Herbivorie geschützt sind. Daher sind eine erhöhte Sprossseneszenz und eine Verlagerung der Nährstoffe Teil des „tolerance/escape syndromes“ als Toleranzstrategie von *Potamogeton perfoliatus* gegen *Acentria*-Frass als Folge des Fehlens morphologischer und chemischer Resistenzstrategien. Einem schnellen Pflanzenwachstum und dem Aufbau einer ausreichend großen unterirdischen Überwinterungsbiomasse folgt eine verstärkte Seneszenz der oberirdischen Biomasse. Diese Reaktionen von *Potamogeton* dienen zusammen mit einer Verlagerung von Nährstoffen in die Überwinterungsorgane als Verteidigungsstrategie gegen Herbivorie.

Da *Acentria* einen starken Frassdruck auf *Potamogeton perfoliatus* Bestände ausübt und saisonale Massenvermehrungen stattfinden, kann *Acentria* als „ecosystem engineer“ angesehen werden, der die Struktur und Qualität von Wasserpflanzenbeständen beeinflusst, die als Habitat für andere Organismen dienen. Freilandprobenahmen haben gezeigt, dass Frass und Seneszenz die Sprossdichte und somit die strukturelle Komplexität von submerser aquatischer Vegetation

verringerten: Benthivore Fische, insbesondere Stichlinge und juvenile Flussbarsche (*Perca fluviatilis*), bevorzugten Makrophytenbestände mit einem mittleren Anteil seneszenter Sprosse und damit einer optimalen, mittleren strukturellen Komplexität, da eine hohe Sprossdichte/geringe Seneszenz die Bewegungsmöglichkeiten der Fische einschränkt, wohingegen eine niedrige Sprossdichte/starke Seneszenz die verfügbare Menge an wirbellosen Futterorganismen und die Funktionsfähigkeit des Wasserpflanzenbestandes als Schutz vor piscivoren Räubern verringert.

Die Rolle als „ecosystem engineer“ wird direkt von den Mortalitäts- und Wachstumsraten von *Acentria* beeinflusst: Mesokosmosexperimente zeigten, dass Stichlingsprädation die Dichten an *Acentria* Larven stark reduzierte (lethaler Prädationseffekt) und einen starken Einfluss auf die Life-History von *Acentria* hatte (nicht-lethaler Prädationseffekt). Unter dem Prädationsdruck von Stichlingen verpuppten sich die *Acentria* Larven bei einer geringeren Größe und eine Veränderung der Entwicklungsstrategien fand statt: gegen Ende der Wachstumssaison zogen weibliche Larven die Überwinterung der Verpuppung vor, wohingegen männliche Larven zu gleichen Teilen überwinterten und sich verpuppten. Dies führte zu einem stark männchen-dominierten Geschlechterverhältnis der Puppen. Die Reduktion der Puppengröße und die Präferenz der Weibchen zu überwintern sind wahrscheinlich Life-History Strategien, um der Fischprädation zu entkommen, insbesondere für die flügellosen adulten Weibchen, die den aquatischen Lebensraum im Gegensatz zu den geflügelten Männchen nicht verlassen können. Zusammenfassend führen sowohl lethale (d.h. eine Reduktion der Populationsdichten) als auch nicht-lethale Prädationseffekte (d.h. ein stark männchen-dominiertes Geschlechterverhältnis der Puppen und eine verringerte Puppengröße) zu einer Verringerung des Populationswachstums von *Acentria*, indem sich (1) die Gesamtanzahl der adulten Individuen verringerte, (2) die Anzahl der für die Reproduktion verfügbaren adulten Weibchen durch ein stark männchen-dominiertes Geschlechterverhältnis verringerte und (3) die mittlere Gelegegröße durch eine geringere Größe der adulten Weibchen verkleinerte.

Die starken Interaktionen zwischen Stichlingen und *Acentria* und zwischen *Acentria* und *Potamogeton* legen schließlich die mögliche Existenz einer trophischen Kaskade in submersen Pflanzenbeständen des Littorals von Seen und Teichen nahe, bestehend aus den trophischen Ebenen Stichlinge - *Acentria* - *Potamogeton perfoliatus*. Durch die weite Verbreitung der beteiligten Organismen ist diese trophische Kaskade wahrscheinlich von großer Bedeutung bei der Strukturierung der Uferzonen vieler Süsswasserseen und Brackwasserökosysteme der nördlichen Breiten.

Summary

Macrophyte patches are important habitats for fishes and macroinvertebrates and major components structuring the littoral zones of lacustrine and marine ecosystems. Trophic interactions between primary producers (submerged macrophytes), herbivores and predators shape the littoral food webs, the structural complexity of macrophyte patches and their functioning as a refuge and foraging habitat for fishes and macroinvertebrates. The water moth *Acentria ephemerella* is an important insect herbivore for submerged macrophytes and commonly occurs in lakes, rivers and coastal regions of the Northern Hemisphere. A major food source for *Acentria ephemerella* is the perfoliate pondweed *Potamogeton perfoliatus* as the dominant water plant species in Lake Constance and many other European lakes, forming large, mostly mono-specific patches. Sticklebacks (*Gasterosteus aculeatus*) commonly occur in macrophyte patches and prey on macrophyte-associated macroinvertebrates. In this thesis, I studied the trophic interactions between sticklebacks, *Acentria ephemerella* and *Potamogeton perfoliatus* with mesocosm experiments and field studies to get insight into the grazing influence of *Acentria* on *Potamogeton perfoliatus* and the population regulation of *Acentria* through predation.

Acentria had a strong grazing effect on *Potamogeton perfoliatus*: Mesocosm experiments revealed that (1) *Acentria* herbivory reduced the biomass of *Potamogeton perfoliatus*, (2) *Acentria* herbivory increased the senescence of *Potamogeton perfoliatus* shoots and (3) *Potamogeton perfoliatus* translocated nutrients (phosphorus and nitrogen) to the herbivore-protected below-ground overwintering buds. Thus an increased shoot senescence and nutrient allocation might be a part of the tolerance/escape syndrome as a tolerance strategy of *Potamogeton perfoliatus* against *Acentria* grazing due to the absence of morphological and chemical resistance strategies. A fast growth accompanied by a sufficient buildup of below-ground overwintering biomass is followed by an enhanced above-ground shoot senescence. These responses of *Potamogeton* serve together with an increased translocation of nutrients to the overwintering organs as a defence strategy against herbivory.

Since *Acentria* exerts a strong grazing influence on *Potamogeton perfoliatus* patches and displays seasonally occurring outbreak dynamics, it can potentially be regarded as an ecosystem engineer, shaping the structure and quality of macrophyte patches that function as a habitat for other organisms. Field samplings in Lake Constance have shown that grazing and senescence decrease the shoot density and thus the structural complexity of submerged aquatic vegetation. Benthivorous fishes, especially sticklebacks and juvenile perch (*Perca fluviatilis*), preferred *Potamogeton* patches with a medium share of senescent shoots and hence an optimal medium structural complexity since a high shoot density/low senescence impairs the capacity of fishes to swim and forage visually for food organisms whereas a low shoot density/high senescence

presumably decreases the available macroinvertebrate food sources and the functioning of the patch as a refuge against piscivorous predators.

However, *Acentria*'s ecosystem engineering role is influenced by the mortality and growth rates of *Acentria*: Mesocosm experiments and a field sampling in Lake Constance revealed that stickleback predation strongly reduced the abundances of *Acentria* larvae (lethal effect of predation) and had a large influence on the life-history of *Acentria* (non-lethal effect of predation). Under stickleback predation pressure, the size at metamorphosis of *Acentria* was smaller and a change in the developmental strategies occurred: late in the growth season female larvae preferred hibernation over pupation whereas male larvae chose hibernation and pupation in equal shares. This led to a high male bias of *Acentria* at metamorphosis. The reduction in size at metamorphosis and a female preference for diapause are probably life-history strategies to escape fish predation, especially for the wingless adult females that cannot leave the aquatic habitat in contrast to the winged adult males. Hence in conclusion both lethal (i.e. a reduction of population densities) and non-lethal effects of predation (i.e. a male-biased sex-ratio at metamorphosis and a reduced size at maturity) dampened *Acentria* population growth by (1) reducing the overall number of adults, (2) reducing the number of adult females that are available for reproduction due to a male-biased sex-ratio at metamorphosis and (3) a decreased clutch size due to a decreased female size at metamorphosis.

Eventually, the interactions between *Acentria* and *Potamogeton perfoliatus* and between sticklebacks and *Acentria* suggest the potential existence of a tritrophic cascade sticklebacks - *Acentria* - *Potamogeton perfoliatus* in submerged macrophyte patches in the littoral zones of lakes and ponds. Due to the wide distribution of the involved organisms, this cascade is probably of major importance in structuring the shorelines of many freshwater lakes and brackish water ecosystems in the Northern Hemisphere.

References

Aarssen, L.W. 2008. Death without sex - the 'problem of the small' and selection for reproductive economy in flowering plants. *Evolutionary Ecology* **22,** 279-298.

Agrawal, A.A. & Fishbein, M. 2006. Plant defense syndromes. *Ecology* **87,** 132-149.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* **294,** 321-326.

Allen, J.R.M. & Wootton, R.J. 1982. Age, growth and rate of food consumption in an upland population of the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology* **21,** 95-105.

Allen, J.R.M. & Wootton, R.J. 1984. Temporal patterns in diet and rate of food consumption of the three-spined stickleback (*Gasterosteus aculeatus* L.) in Llyn Frongoch, an upland Welsh lake. *Freshwater Biology* **14,** 335-346.

Auerbach, M. 1991. Relative impact of interactions within and between trophic levels during an insect outbreak. *Ecology* **72,** 1599-1608.

Baker, J.A. 1994. Life history variation in female threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M.A. Bell & S.A. Foster), pp. 144-187. New York, Oxford University Press Inc.

Baker, R.L., Elkin, C.M. & Brennan, H.A. 1999. Aggressive interactions and risk of fish predation for larval damselflies. *Journal of Insect Behavior* **12,** 213-223.

Barber, I. 2007. Host-parasite interactions of the three-spined stickleback. In: *Biology of the Three-Spined Stickleback* (Ed. by S. Östlund-Nilsson, I. Mayer & F.A. Huntingford), pp. 271-317. Boca Raton, CRC Press.

Barko, J.W. & James, W.F. 1998. Effects of submerged aquatic macrophytes on nutrient dynamics, sedimentation, and resuspension. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 197-214. Berlin, Springer.

Batra, S.W.T. 1977. Bionomics of the aquatic moth, *Acentropus niveus* (Olivier), a potential biological control agent for Eurasian watermilfoil and *Hydrilla*. *Journal of the New York Entomological Society* **85,** 143-152.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F. & Weinstein, M.P. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* **51,** 633-641.

Beketov, M.A. & Liess, M. 2007. Predation risk perception and food scarcity induce alterations of life-cycle traits of the mosquito *Culex pipiens*. *Ecological Entomology* **32,** 405-410.

Benard, M.F. 2004. Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology Evolution and Systematics* **35,** 651-673.

Berg, K. 1942. Contributions to the biology of the aquatic moth *Acentropus niveus* (Oliv.). *Videnskabelige meddelser fra Dansk Naturhistoriska Forening* **105,** 59-139.

Bergman, E. 1988. Foraging abilities and niche breadths of two percids, *Perca fluviatilis* and *Gymnocephalus cernua*, under different environmental conditions. *Journal of Animal Ecology* **57,** 443-453.

Bertness, M.D. & Leonard, G.H. 1997. The role of positive interactions in communities: lessons from intertidal habitats. *Ecology* **78,** 1976-1989.

Bertolo, A., Lacroix, G. & Lescher-Moutoue, F. 1999. Scaling food chains in aquatic mesocosms: do the effects of depth override the effects of planktivory ? *Oecologia* **121,** 55-65.

Blanckenhorn, W.U. 1998. Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. *Evolution* **52,** 1394-1407.

Blanckenhorn, W.U. 2005. Behavioral causes and consequences of sexual size dimorphism. *Ethology* **111,** 977-1016.

Blanckenhorn, W.U., Dixon, A.F.G., Fairbairn, D.J., Foellmer, M.W., Gibert, P., van der Linde, K., Meier, R., Nylin, S., Pitnick, S., Schoff, C., Signorelli, M., Teder, T. & Wiklund, C. 2007. Proximate causes of Rensch's rule: Does sexual size dimorphism in arthropods result from sex differences in development time ? *American Naturalist* **169,** 245-257.

Bolnick, D.I. & Preisser, E.L. 2005. Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta-analysis. *Ecology* **86,** 2771-2779.

Bolser, R.C., Hay, M.E., Lindquist, N., Fenical, W. & Wilson, D. 1998. Chemical defenses of freshwater macrophytes against crayfish herbivory. *Journal of Chemical Ecology* **24,** 1639-1658.

Brett, M.T. & Goldman, C.R. 1997. Consumer versus resource control in freshwater pelagic food webs. *Science* **275,** 384-386.

Brodin, T. & Johansson, F. 2002. Effects of predator-induced thinning and activity changes on life history in a damselfly. *Oecologia* **132,** 316-322.

Brodin, T. & Johansson, F. 2004. Conflicting selection pressures on the growth/predation risk trade-off in a damselfly. *Ecology* **85,** 2927-2932.

Brodin, T., Mikolajewski, D.J. & Johansson, F. 2006. Behavioural and life history effects of predator diet cues during ontogeny in damselfly larvae. *Oecologia* **148,** 162-169.

Brönmark, C. & Vermaat, J. 1998. Complex fish-snail-epiphyton interactions and their effects on submerged freshwater macrophytes. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 47-68. Berlin, Springer.

Brookshire, E.N.J., Kauffman, J.B., Lytjen, D. & Otting, N. 2002. Cumulative effects of wild ungulate and livestock herbivory on riparian willows. *Oecologia* **132,** 559-566.

Butler, A.J. & Jernakoff, P. 1999. *Seagrass in Australia: Strategic Review and Development of an R & D Plan*. Collingwood: CSIRO Publishing.

Caffrey, J.M. 1993. Aquatic plant management in relation to Irish recreational fisheries development. *Journal of Aquatic Plant Management* **31,** 162-168.

Callaway, R.M. & Walker, L.R. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78,** 1958-1965.

Campbell, C.E. 1991. Prey selectivities of threespine sticklebacks (*Gasterosteus aculeatus*) and phantom midge larvae (*Chaoborus* spp.) in Newfoundland lakes. *Freshwater Biology* **25,** 155-167.

Candolin, U. & Salesto, T. 2006. Effects of increased vegetation cover on nesting behavior of sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* **59,** 689-693.

Candolin, U. & Voigt, H.R. 2003. Do changes in risk-taking affect habitat shifts of sticklebacks ? *Behavioral Ecology and Sociobiology* **55,** 42-49.

Carpenter, S.R. & Kitchell, J.F. 1993. The trophic cascade in lakes: synthesis of ecosystem experiments. *Bulletin of the Ecological Society of America* **74,** 186-187.

Carpenter, S.R., Kitchell, J.F. & Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. *BioScience* **35,** 634-639.

Casselman, J.M. & Lewis, C.A. 1996. Habitat requirements of northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 161-174.

Choi, C., Bareiss, C., Walenciak, O. & Gross, E.M. 2002. Impact of polyphenols on growth of the aquatic herbivore *Acentria ephemerella*. *Journal of Chemical Ecology* **28,** 2245-2256.

Clarke, C. 1984. Upsets in the sex-ratio of some Lepidoptera. In: *The Biology of Butterflies* (Ed. by R.I. Vane-Wright & P.R. Ackery), pp. 255-258. London, Academic Press.

Clucas, B., Rowe, M.P., Owings, D.H. & Arrowood, P.C. 2008. Snake scent application in ground squirrels, *Spermophilus* spp.: a novel form of antipredator behaviour ? *Animal Behaviour* **75,** 299-307.

Coley, P.D., Bryant, J.P. & Chapin, F.S. 1985. Resource availability and plant antiherbivore defense. *Science* **230,** 895-899.

Creed, R.P. & Sheldon, S.P. 1994. The effect of two herbivorous insect larvae on Eurasian watermilfoil. *Journal of Aquatic Plant Management* **32,** 21-26.

Crowder, L.B. & Cooper, W.E. 1982. Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* **63,** 1802-1813.

Crowder, L.B., McCollum, E.W. & Martin, T.H. 1998. Changing perspectives on food web interactions in lake littoral zones. In: *The Structuring Role of Submerged Macrophytes in*

Lakes (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 240-249. Berlin, Springer.

Crowley, P.H. 2000. Sexual dimorphism with female demographic dominance: age, size, and sex ratio at maturation. *Ecology* **81,** 2592-2605.

Culp, J.M., Glozier, N.E. & Scrimgeour, G.J. 1991. Reduction of predation risk under the cover of darkness: avoidance responses of mayfly larvae to a benthic fish. *Oecologia* **86,** 163-169.

Cyr, H. & Downing, J.A. 1988. The abundance of phytophilous invertebrates on different species of submerged macrophytes. *Freshwater Biology* **20,** 365-374.

Cyr, H. & Face, M.L. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* **361,** 148-150.

Dahl, J. & Peckarsky, B.L. 2003. Developmental responses to predation risk in morphologically defended mayflies. *Oecologia* **137,** 188-194.

Damman, H. 1987. Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology* **68,** 88-97.

Daniel, C.J. & Myers, J.H. 1995. Climate and outbreaks of the forest tent caterpillar. *Ecography* **18,** 353-362.

Danks, H.V. 2006. Key themes in the study of seasonal adaptations in insects II. Life-cycle patterns. *Applied Entomology and Zoology* **41,** 1-13.

David, J.F., Celerier, M.L. & Geoffroy, J.J. 1999. Periods of dormancy and cohort-splitting in the millipede *Polydesmus angustus* (Diplopoda: Polydesmidae). *European Journal of Entomology* **96,** 111-116.

Day, T. & Rowe, L. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *American Naturalist* **159,** 338-350.

De Block, M. & Stoks, R. 2003. Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *Journal of Evolutionary Biology* **16,** 986-995.

De Mott, W.R., Gulati, R.D. & van Donk, E. 2001. Effects of dietary phosphorus deficiency on the abundance, phosphorus balance, and growth of *Daphnia cucullata* in three hypereutrophic Dutch lakes. *Limnology and Oceanography* **46,** 1871-1880.

Dicke, M. & Grostal, P. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics* **32,** 1-23.

Diehl, S. & Kornijow, R. 1998. Influence of submerged macrophytes on trophic interactions among fish and macroinvertebrates. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 24-46. Berlin, Springer.

Dionne, M. & Folt, C.L. 1991. An experimental analysis of macrophyte growth forms as fish foraging habitat. *Canadian Journal of Fisheries and Aquatic Sciences* **48,** 123-131.

Dorn, N.J., Cronin, G. & Lodge, D.M. 2001. Feeding preferences and performance of an aquatic lepidopteran on macrophytes: plant hosts as food and habitat. *Oecologia* **128,** 406-415.

Doucette, L.I., Skulason, S. & Snorrason, S.S. 2004. Risk of predation as a promoting factor of species divergence in threespine sticklebacks (*Gasterosteus aculeatus* L.). *Biological Journal of the Linnean Society* **82,** 189-203.

Dyer, M.I., Acra, M.A., Wang, G.M., Coleman, D.C., Freckman, D.W., McNaughton, S.J. & Strain, B.R. 1991. Source-sink carbon relations in two *Panicum coloratum* ecotypes in response to herbivory. *Ecology* **72,** 1472-1483.

Eklöv, P. 1997. Effects of habitat complexity and prey abundance on the spatial and temporal distributions of perch (*Perca fluviatilis*) and pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences* **54,** 1520-1531.

Estes, J.A., Tinker, M.T., Williams, T.M. & Doak, D.F. 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* **282,** 473-476.

Faafeng, B.A. & Mjelde, M. 1998. Clear and turbid water in shallow Norwegian lakes related to submerged vegetation. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 361-368. Berlin, Springer.

Feeny, P. 1976. Plant apparancy and chemical defense. In: *Biochemical Interaction Between Plants and Insects* (Ed. by J.W. Wallace & R.L. Mansell), pp. 1-40. New York, Plenum.

Finke, D.L. & Denno, R.F. 2006. Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. *Oecologia* **149,** 265-275.

Finlay, J.C., Khandwala, S. & Power, M.E. 2002. Spatial scales of carbon flow in a river food web. *Ecology* **83,** 1845-1859.

Fischer, P. 2000. Test of competitive interactions for space between two benthic fish species, burbot *Lota lota*, and stone loach *Barbatula barbatula*. *Environmental Biology of Fishes* **58,** 439-446.

Fischer, P. & Eckmann, R. 1997 a. Seasonal changes in fish abundance, biomass and species richness in the littoral zone of a large European lake, Lake Constance, Germany. *Archiv für Hydrobiologie* **139,** 433-448.

Fischer, P. & Eckmann, R. 1997 b. Spatial distribution of littoral fish species in a large European lake, Lake Constance, Germany. *Archiv für Hydrobiologie* **140,** 91-116.

Fornara, D.A. & Du Toit, J.T. 2007. Browsing lawns ? Responses of *Acacia nigrescens* to ungulate browsing in an African savanna. *Ecology* **88,** 200-209.

Fritz, R. & Simms, E. 1992. *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics*. Chicago: University of Chicago Press.

Fukui, A., Murakami, M., Konno, K., Nakamura, M. & Ohgushi, T. 2002. A leaf-rolling caterpillar improves leaf quality. *Entomological Science* **5,** 263-266.

Gange, A.C. & Brown, V.K. 1989. Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia* **81,** 38-42.

Gastreich, K.R. 1999. Trait-mediated indirect effects of a theridiid spider on an ant-plant mutualism. *Ecology* **80,** 1066-1070.

Gill, A.B. & Hart, P.J.B. 1994. Feeding behaviour and prey choice of the threespine stickleback: the interacting effects of prey size, fish size and stomach fullness. *Animal Behaviour* **47,** 921-932.

Gillanders, B.M. 2006. Seagrasses, fish and fisheries. In: *Seagrasses: Biology, Ecology and Conservation* (Ed. by A.W.D. Larkum, R.J. Orth & C.M. Duarte), pp. 503-536. Berlin, Springer.

Gotthard, K. 1998. Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: investigating an adaptive reaction norm. *Journal of Evolutionary Biology* **11,** 21-39.

Gotthard, K. 2001. Growth strategies of ectothermic animals in temperate environments. In: *Animal Developmental Ecology* (Ed. by D. Atkinson & M. Thorndyke), pp. 1-18. Oxford, BIOS Scientific Publishers Ltd.

Greenberg, A.E., Trussel, R.R. & Clesceri, L.S. 1985. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association (APHA).

Greenway, C.A. & Harder, L.D. 2007. Variation in ovule and seed size and associated size-number trade-offs in angiosperms. *American Journal of Botany* **94,** 840-846.

Gross, E.M., Feldbaum, C. & Choi, C. 2002. High abundance of herbivorous Lepidoptera larvae (*Acentria ephemerella* DENIS & SCHIFFERMULLER) on submersed macrophytes in Lake Constance (Germany). *Archiv für Hydrobiologie* **155,** 1-21.

Gross, E.M., Johnson, R.L. & Hairston, N.G. Jr. 2001. Experimental evidence for changes in submersed macrophyte species composition caused by the herbivore *Acentria ephemerella* (Lepidoptera). *Oecologia* **127,** 105-114.

Grostal, P. & Dicke, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology* **10,** 422-427.

Hacker, S.D. & Gaines, S.D. 1997. Some implications of direct positive interactions for community species diversity. *Ecology* **78,** 1990-2003.

Haenni, J.-P. 1980. Contribution à la connaissance de la biologie des papillons aquatiques (Lepidoptera, Pyraloidea) sur la rive sud du Lac de Neuchâtel. *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **103,** 29-43.

Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology Evolution and Systematics* **8,** 157-178.

Hara, H. & Higashiura, Y. 1995. Factors causing outbreak decline of larch geometrid moth, *Zethenia rufescentraria*. *Japanese Journal of Applied Entomology and Zoology* **39,** 15-23.

Harrison, S., Hastings, A. & Strong, D.R. 2005. Spatial and temporal dynamics of insect outbreaks in a complex multitrophic system: tussock moths, ghost moths, and their natural enemies on bush lupines. *Annales Zoologici Fennici* **42,** 409-419.

Hatcher, M.J., Taneyhill, D.E., Dunn, A.M. & Tofts, C. 1999. Population dynamics under parasitic sex ratio distortion. *Theoretical Population Biology* **56,** 11-28.

Hebblewhite, M., White, C.A., Nietvelt, C.G., McKenzie, J.A., Hurd, T.E., Fryxell, J.M., Bayley, S.E. & Paquet, P.C. 2005. Human activity mediates a trophic cascade caused by wolves. *Ecology* **86,** 2135-2144.

Heck, K.L., Hays, G. & Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology-Progress Series* **253,** 123-136.

Hedal, S. & Schmidt, S.C. 1992. On the occurrence of *Acentria ephemerella* Den. and Schiff. *Entomologiske Meddelser* **60,** 17-20.

Heppell, S.S., Heppell, S.A., Coleman, F.C. & Koenig, C.C. 2006. Models to compare management options for a protogynous fish. *Ecological Applications* **16,** 238-249.

Heppner, J.B. 1991. Faunal regions and the diversity of Lepidoptera. *Tropical Lepidoptera Research* **2,** 1-85.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66,** 483-492.

Honkanen, T. & Jormalainen, V. 2002. Within-alga integration and compensation: effects of simulated herbivory on growth and reproduction of the brown alga, *Fucus vesiculosus*. *International Journal of Plant Sciences* **163,** 815-823.

Hornung, J.P. & Foote, A.L. 2006. Aquatic invertebrate responses to fish presence and vegetation complexity in western boreal wetlands, with implications for waterbird productivity. *Wetlands* **26,** 1-12.

Hunter, M.D. & Willmer, P.G. 1989. The potential for interspecific competition between two abundant defoliators on oak: leaf damage and habitat quality. *Ecological Entomology* **14,** 267-277.

Hyatt, K.D. & Ringler, N.H. 1989. Role of nest raiding and egg predation in regulating population density of threespine sticklebacks (*Gasterosteus aculeatus*) in a coastal British Columbia lake. *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 372-383.

Imbrock, F., Appenzeller, A. & Eckmann, R. 1996. Diel and seasonal distribution of perch in Lake Constance: a hydroacoustic study and in situ observations. *Journal of Fish Biology* **49,** 1-13.

Jeffries, M. 1993. Invertebrate colonization of artificial pondweeds of differing fractal dimension. *Oikos* **67,** 142-148.

Jeppesen, E., Söndergaard, M., Söndergaard, M. & Christoffersen, K. 1998. *The Structuring Role of Submerged Macrophytes in Lakes*. Berlin: Springer.

Johansson, F. & Brodin, T. 2003. Effects of fish predators and abiotic factors on dragonfly community structure. *Journal of Freshwater Ecology* **18,** 415-423.

Johansson, F. & Norling, U. 1994. A five year study of the larval life history of *Coenagrion hastulatum* (Charpentier) and *C. armatum* (Charpentier) in northern Sweden (Zygoptera: Coenagrionidae). *Odonatologica* **23,** 355-364.

Johansson, F. & Rowe, L. 1999. Life history and behavioral responses to time constraints in a damselfly. *Ecology* **80,** 1242-1252.

Johansson, F. & Samuelsson, L. 1994. Fish-induced variation in abdominal spine length of *Leucorrhinia dubia* (Odonata) larvae ? *Oecologia* **100,** 74-79.

Johansson, F., Stoks, R., Rowe, L. & De Block, M. 2001. Life history plasticity in a damselfly: effects of combined time and biotic constraints. *Ecology* **82,** 1857-1869.

Johansson, M.E. 1994. Life history differences between central and marginal populations of the clonal aquatic plant *Ranunculus lingua*: a reciprocal transplant experiment. *Oikos* **70,** 65-72.

Johnson, D.M., Martin, T.H., Mahato, M., Crowder, L.B. & Crowley, P.H. 1995. Predation, density dependence, and life histories of dragonflies: a field experiment in a freshwater community. *Journal of the North American Benthological Society* **14,** 547-562.

Johnson, R.L., Gross, E.M. & Hairston, N.G. 1998. Decline of the invasive submerged macrophyte *Myriophyllum spicatum* (Haloragaceae) associated with herbivory by larvae of *Acentria ephemerella* (Lepidoptera). *Aquatic Ecology* **31,** 273-282.

Johnston, C.A. & Naiman, R.J. 1990. Aquatic patch creation in relation to beaver population trends. *Ecology* **71,** 1617-1621.

Jones, C.G., Lawton, J.H. & Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos* **69,** 373-386.

Jones, C.G., Lawton, J.H. & Shachak, M. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* **78,** 1946-1957.

Jones, J.I. & Sayer, C.D. 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes ? *Ecology* **84,** 2155-2167.

Jones, J.I., Moss, B. & Young, J.O. 1998. Interactions between periphyton, nonmolluscan invertebrates, and fish in standing freshwaters. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 69-90. Berlin, Springer.

Juchault, P., Rigaud, T. & Mocquard, J.P. 1993. Evolution of sex determination and sex ratio variability in wild populations of *Armadillidium vulgare* (Latr.) (Crustacea, Isopoda): a case study in conflict resolution. *Acta Oecologica-International Journal of Ecology* **14,** 547-562.

Karban, R. & Baldwin, I.T. 1997. *Induced Responses to Herbivory*. Chicago: Chicago University Press.

Kats, L.B. & Dill, L.M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5,** 361-394.

Kery, M., Matthies, D. & Spillmann, H.H. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology* **88,** 17-30.

Kiesecker, J.M., Chivers, D.P., Anderson, M. & Blaustein, A.R. 2002. Effect of predator diet on life history shifts of red-legged frogs, *Rana aurora*. *Journal of Chemical Ecology* **28,** 1007-1015.

Kiss, B. & Samu, F. 2005. Life history adaptation to changeable agricultural habitats: developmental plasticity leads to cohort splitting in an agrobiont wolf spider. *Environmental Entomology* **34,** 619-626.

Kokocinski, W. 1963. Remarks on biology of *Acentropus niveus* Oliv. (Lepidoptera, Pyralidae). *Polskie Pismo Entomologiczne* **33,** 233-243.

Kornijow, R., Gulati, R.D. & Ozimek, T. 1995. Food preference of freshwater invertebrates: comparing fresh and decomposed angiosperm and a filamentous alga. *Freshwater Biology* **33,** 205-212.

Kroon, A., Veenendaal, R.L., Egas, M., Bruin, J. & Sabelis, M.W. 2005. Diapause incidence in the two-spotted spider mite increases due to predator presence, not due to selective predation. *Experimental and Applied Acarology* **35,** 73-81.

Kubanek, J., Fenical, W., Hay, M.E., Brown, P.J. & Lindquist, N. 2000. Two antifeedant lignans from the freshwater macrophyte *Saururus cernuus*. *Phytochemistry* **54,** 281-287.

Kursar, T.A. & Coley, P.D. 2003. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology* **31,** 929-949.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution* **11,** 404-408.

Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. *Oikos* **88,** 169-180.

Larsson, S., Häggström, H.E. & Denno, R.F. 1997. Preference for protected feeding sites by larvae of the willow-feeding leaf beetle *Galerucella lineola*. *Ecological Entomology* **22,** 445-452.

Lass, S. & Spaak, P. 2003. Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia* **491,** 221-239.

Lau, T.F., Gross, E.M. & Meyer-Rochow, V.B. 2007. Sexual dimorphism and light/dark adaptation in the compound eyes of male and female *Acentria ephemerella* (Lepidoptera: Pyraloidea: Crambidae). *European Journal of Entomology* **104,** 459-470.

Laurila, A., Pakkasmaa, S. & Merilä, J. 2001. Influence of seasonal time constraints on growth and development of common frog tadpoles: a photoperiod experiment. *Oikos* **95,** 451-460.

Lawton, J.H. & Jones, C.G. 1995. Linking species and ecosystems: organisms as ecosystem engineers. In: *Linking Species and Ecosystems* (Ed. by C.G. Jones & J.H. Lawton), pp. 141-150. New York, Chapman & Hall.

Lehmann, A., Jaquet, J.M. & Lachavanne, J.B. 1997. A GIS approach of aquatic plant spatial heterogeneity in relation to sediment and depth gradients, Lake Geneva, Switzerland. *Aquatic Botany* **58,** 347-361.

Lenz, T.L., Jacob, A. & Wedekind, C. 2007. Manipulating sex ratio to increase population growth: the example of the lesser kestrel. *Animal Conservation* **10,** 236-244.

Li, B., Shibuya, T., Yogo, Y. & Hara, T. 2004. Effects of ramet clipping and nutrient availability on growth and biomass allocation of yellow nutsedge. *Ecological Research* **19,** 603-612.

Liebhold, A., Elkinton, J., Williams, D. & Muzika, R.M. 2000. What causes outbreaks of the gypsy moth in North America ? *Population Ecology* **42,** 257-266.

Liebhold, A.M., Raffa, K.F. & Diss, A.L. 2005. Forest type affects predation on gypsy moth pupae. *Agricultural and Forest Entomology* **7,** 179-185.

Lillie, R.A. & Budd, J. 1992. Habitat architecture of *Myriophyllum spicatum* L. as an index to habitat quality for fish and macroinvertebrates. *Journal of Freshwater Ecology* **7,** 113-125.

Lima, S.L. & Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **68,** 619-640.

Lodge, D.M. 1991. Herbivory on freshwater macrophytes. *Aquatic Botany* **41,** 195-224.

Lodge, D.M., Cronin, G., van Donk, E. & Froelich, A.J. 1998. Impact of herbivory on plant standing crop: comparisons among biomes, between vascular and nonvascular plants, and among freshwater herbivore taxa. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 149-174. Berlin, Springer.

Lubchenco, J. & Gaines, S.D. 1981. A unified approach to marine plant-herbivore interactions. 1. populations and communities. *Annual Review of Ecology and Systematics* **12,** 405-437.

MacRae, P.S.D. & Jackson, D.A. 2001. The influence of smallmouth bass (*Micropterus dolomieu*) predation and habitat complexity on the structure of littoral zone fish assemblages. *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 342-351.

Mäkinen, H.S., Cano, J.M. & Merilä, J. 2006. Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Molecular Ecology* **15,** 1519-1534.

Manatunge, J., Asaeda, T. & Priyadarshana, T. 2000. The influence of structural complexity on fish-zooplankton interactions: a study using artificial submerged macrophytes. *Environmental Biology of Fishes* **58,** 425-438.

Maron, J.L., Harrison, S. & Greaves, M. 2001. Origin of an insect outbreak: escape in space or time from natural enemies ? *Oecologia* **126,** 595-602.

Martin, T.H., Crowder, L.B., Dumas, C.F. & Burkholder, J.M. 1992. Indirect effects of fish on macrophytes in Bays Mountain lake: evidence for a littoral trophic cascade. *Oecologia* **89,** 476-481.

Martin, T.H., Johnson, D.M. & Moore, R.D. 1991. Fish-mediated alternative life-history strategies in the dragonfly *Epitheca cynosura*. *Journal of the North American Benthological Society* **10,** 271-279.

McIntosh, A.R. & Peckarsky, B.L. 1999. Criteria determining behavioural responses to multiple predators by a stream mayfly. *Oikos* **85,** 554-564.

McPeek, M.A. & Peckarsky, B.L. 1998. Life histories and the strengths of species interactions: combining mortality, growth, and fecundity effects. *Ecology* **79,** 867-879.

Mehner, T., Plewa, M., Hülsmann, S. & Worischka, S. 1998. Gape-size dependent feeding of age-0 perch (*Perca fluviatilis*) and age-0 zander (*Stizostedion lucioperca*) on *Daphnia galeata*. *Archiv für Hydrobiologie* **142,** 191-207.

Menge, B.A. 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology* **250,** 257-289.

Mikolajewski, D.J. & Johansson, F. 2004. Morphological and behavioral defenses in dragonfly larvae: trait compensation and cospecialization. *Behavioral Ecology* **15,** 614-620.

Mikolajewski, D.J. & Rolff, J. 2004. Benefits of morphological defence demonstrated by direct manipulation in larval dragonflies. *Evolutionary Ecology Research* **6,** 619-626.

Mikolajewski, D.J., Brodin, T., Johansson, F. & Joop, G. 2005. Phenotypic plasticity in gender specific life-history: effects of food availability and predation. *Oikos* **110,** 91-100.

Mikolajewski, D.J., Joop, G. & Wohlfahrt, B. 2007. Coping with predators and food limitation: testing life history theory for sex-specific larval development. *Oikos* **116,** 642-649.

Mopper, S., Maschinski, J., Cobb, N. & Whitham, T.G. 1991. A new look at habitat structure: consequences of herbivore-modified plant architecture. In: *Habitat Structure - the Physical Arrangement of Objects in Space* (Ed. by S.S. Bell, E.D. McCoy & H.R. Muschinsky), pp. 260-280. London, Chapman & Hall.

Muckle, R. 1972. Der dreistachlige Stichling im Bodensee. *Schriften des Vereins für Geschichte des Bodensees und seiner Umgebung* **90,** 249-258.

Mueller, U.G. & Dearing, M.D. 1994. Predation and avoidance of tough leaves by aquatic larvae of the moth *Parapoynx rugosalis* (Lepidoptera, Pyralidae). *Ecological Entomology* **19,** 155-158.

Mulder, C.P.H. & Ruess, R.W. 1998. Effects of herbivory on arrowgrass: interactions between geese, neighboring plants, and abiotic factors. *Ecological Monographs* **68,** 275-293.

Munroe, E. & Solis, M.A. 1999. The Pyraloidea. In: *Lepidoptera, Moths and Butterflies* (Ed. by N.P. Kristensen), pp. 233-256. Berlin, de Gruyter.

Murdoch, W.W., Briggs, C.J. & Nisbet, R.M. 2003. *Consumer-Resource Dynamics*. Princeton: Princeton University Press.

Myers, J.H. 1993. Population outbreaks in forest Lepidoptera. *American Scientist* **81,** 240-251.

Myers, J.H. 1998. Synchrony in outbreaks of forest Lepidoptera: a possible example of the Moran effect. *Ecology* **79,** 1111-1117.

Naiman, R.J., Johnston, C.A. & Kelley, J.C. 1988. Alteration of North American streams by beaver. *BioScience* **38,** 753-762.

Newman, R.A. 1988. Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. *Evolution* **42,** 774-783.

Newman, R.M. 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. *Journal of the North American Benthological Society* **10,** 89-114.

Newman, R.M. 2004. Invited review - Biological control of Eurasian watermilfoil by aquatic insects: basic insights from an applied problem. *Archiv für Hydrobiologie* **159,** 145-184.

Newman, R.M., Kerfoot, W.C. & Hanscom, Z. 1996. Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology* **77,** 2312-2323.

Nooden, L.D. & Leopold, A.C. 1988. *Senescence and Aging in Plants*. Burlington: Academic Press.

Norlin, J.I., Bayley, S.E. & Ross, L.C.M. 2005. Submerged macrophytes, zooplankton and the predominance of low- over high-chlorophyll states in western boreal, shallow-water wetlands. *Freshwater Biology* **50,** 868-881.

Norling, U. 1984. Life history patterns in the northern expansion of dragonflies. *Advances in Odonatology* **2,** 127-156.

Nylin, S. & Gotthard, K. 1998. Plasticity in life-history traits. *Annual Review of Entomology* **43,** 63-83.

Nylin, S., Wickman, P.O. & Wiklund, C. 1989. Seasonal plasticity in growth and development of the speckled wood butterfly, *Pararge aegeria* (Satyrinae). *Biological Journal of the Linnean Society* **38,** 155-171.

Nyström, P., Svensson, O., Lardner, B., Brönmark, C. & Graneli, W. 2001. The influence of multiple introduced predators on a littoral pond community. *Ecology* **82,** 1023-1039.

Odum, W.E., Zieman, J.C. & Heald, E.J. 1972. The importance of vascular plant detritus to estuaries. In: *Coastal Marsh and Estuary Management* (Ed. by R.H. Chabrech), pp. 91-114. Baton Rouge, La. State University Press.

Ohgushi, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology Evolution and Systematics* **36,** 81-105.

Ohgushi, T. 2008. Herbivore-induced indirect interaction webs on terrestrial plants: the importance of non-trophic, indirect, and facilitative interactions. *Entomologia Experimentalis et Applicata* **128,** 217-229.

Östlund-Nilsson, S. 2007. Reproductive behaviour in the three-spined stickleback. In: *Biology of the Three-Spined Stickleback* (Ed. by S. Östlund-Nilsson, I. Mayer & F.A. Huntingford), pp. 157-178. Boca Raton, CRC Press.

Paepke, H.-J. 1983. *Die Stichlinge*. Wittenberg: A. Ziemsen Verlag.

Palmen, E. 1953. Hatching of *Acentropus niveus* (Oliv.) (Lep., Pyralidae) in the brackish waters of Tvärminne, S. Finland. *Annales Entomologici Fennici* **19,** 181-186.

Peckarsky, B.L. 1996. Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. *Ecology* **77,** 1888-1905.

Peckarsky, B.L., Cowan, C.A., Penton, M.A. & Anderson, C. 1993. Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. *Ecology* **74,** 1836-1846.

Peckarsky, B.L., McIntosh, A.R., Taylor, B.W. & Dahl, J. 2002. Predator chemicals induce changes in mayfly life history traits: a whole-stream manipulation. *Ecology* **83,** 612-618.

Peckarsky, B.L., Taylor, B.W., McIntosh, A.R., McPeek, M.A. & Lytle, D.A. 2001. Variation in mayfly size at metamorphosis as a developmental response to risk of predation. *Ecology* **82,** 740-757.

Peltonen, M., Liebhold, A.M., Bjornstad, O.N. & Williams, D.W. 2002. Spatial synchrony in forest insect outbreaks: roles of regional stochasticity and dispersal. *Ecology* **83,** 3120-3129.

Pennycuick, L. 1971. Quantitative effects of three species of parasites on a population of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Zoology* **65,** 143-162.

Perkins, M.C., Woods, H.A., Harrison, J.F. & Elser, J.J. 2004. Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology* **55,** 153-168.

Persson, A. 1997. Effects of fish predation and excretion on the configuration of aquatic food webs. *Oikos* **79,** 137-146.

Piqueras, J. 1999. Herbivory and ramet performance in the clonal herb *Trientalis europaea* L. *Journal of Ecology* **87,** 450-460.

Poizat, G., Rosecchi, E. & Crivelli, A.J. 2002. Life-history variation within a three-spined stickleback population in the Camargue. *Journal of Fish Biology* **60,** 1296-1307.

Polis, G.A. 1999. Why are parts of the world green ? Multiple factors control productivity and the distribution of biomass. *Oikos* **86,** 3-15.

Power, M.E. 1990. Effects of fish in river food webs. *Science* **250,** 811-814.

Power, M.E. 1992. Habitat heterogeneity and the functional significance of fish in river food webs. *Ecology* **73,** 1675-1688.

Preisser, E.L. 2003. Field evidence for a rapidly cascading underground food web. *Ecology* **84,** 869-874.

Preisser, E.L., Bolnick, D.I. & Benard, M.F. 2005. Scared to death ? The effects of intimidation and consumption in predator-prey interactions. *Ecology* **86,** 501-509.

Radder, R.S., Saidapur, S.K. & Shanbhag, B.A. 2006. Big boys on top: effects of body size, sex and reproductive state on perching behaviour in the tropical rock dragon, *Psammophilus dorsalis*. *Animal Biology* **56,** 311-321.

Relyea, R.A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82,** 523-540.

Relyea, R.A. 2007. Getting out alive: how predators affect the decision to metamorphose. *Oecologia* **152,** 389-400.

Roff, D.A. 2003. *Life History Evolution*. Sunderland: Sinauer.

Rothley, K.D. & Dutton, G. 2006. Behavioral responses to environmental change alter direct and indirect trait-mediated interactions. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **84,** 1053-1058.

Sadras, V.O. & Wilson, L.J. 1997. Nitrogen accumulation and partitioning in shoots of cotton plants infested with two-spotted spider mites. *Australian Journal of Agricultural Research* **48,** 525-533.

Sadras, V.O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research* **100,** 125-138.

Sagers, C.L. 1992. Manipulation of host plant-quality: herbivores keep leaves in the dark. *Functional Ecology* **6,** 741-743.

Sandsten, H. & Klaassen, M. 2008. Swan foraging shapes spatial distribution of two submerged plants, favouring the preferred prey species. *Oecologia* **156,** 569-576.

Savino, J.F. & Stein, R.A. 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submersed vegetation. *Transactions of the American Fisheries Society* **111,** 255-266.

Savino, J.F. & Stein, R.A. 1989. Behavioural interactions between fish predators and their prey: effects of plant density. *Animal Behaviour* **37,** 311-321.

Savino, J.F., Marschall, E.A. & Stein, R.A. 1992. Bluegill growth as modified by plant density: an exploration of underlying mechanisms. *Oecologia* **89,** 153-160.

Scheffer, M., de Redelijkheid, M.R. & Noppert, F. 1992. Distribution and dynamics of submerged vegetation in a chain of shallow eutrophic lakes. *Aquatic Botany* **42,** 199-216.

Schleuter, D. & Eckmann, R. 2006. Competition between perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernuus*): the advantage of turning night into day. *Freshwater Biology* **51,** 287-297.

Schleuter, D. & Eckmann, R. 2008. Generalist versus specialist: the performances of perch and ruffe in a lake of low productivity. *Ecology of Freshwater Fish* **17,** 86-99.

Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland: Sinauer.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* **76,** 82-90.

Schmitz, O.J. 2003. Top predator control of plant biodiversity and productivity in an old-field ecosystem. *Ecology Letters* **6,** 156-163.

Schmitz, O.J. & Suttle, K.B. 2001. Effects of top predator species on direct and indirect interactions in a food web. *Ecology* **82,** 2072-2081.

Schmitz, O.J., Beckerman, A.P. & O'Brien, K.M. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* **78,** 1388-1399.

Schmitz, O.J., Hambäck, P.A. & Beckerman, A.P. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist* **155,** 141-153.

Schmitz, O.J., Krivan, V. & Ovadia, O. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters* **7,** 153-163.

Scholtens, B.G. & Balogh, G.J. 1996. Spread of *Acentria ephemerella* (Lepidoptera: Pyralidae) in central North America. *Great Lakes Entomologist* **29,** 21-24.

Schooler, S.S., Yeates, A.G., Wilson, J.R.U. & Julien, M.H. 2007. Herbivory, mowing, and herbicides differently affect production and nutrient allocation of *Alternanthera philoxeroides*. *Aquatic Botany* **86,** 62-68.

Shama, L.N.S. & Robinson, C.T. 2006. Sex-specific life-history responses to seasonal time constraints in an alpine caddisfly. *Evolutionary Ecology Research* **8,** 169-180.

Slusarczyk, M., Dawidowicz, P. & Rygielska, E. 2005. Hide, rest or die: a light-mediated diapause response in *Daphnia magna* to the threat of fish predation. *Freshwater Biology* **50,** 141-146.

Söndergaard, M., Theil-Nielsen, J., Christoffersen, K., Schlüter, L., Jeppesen, E. & Söndergaard, M. 1998. Bacterioplankton and carbon turnover in a dense macrophyte canopy. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 250-261. Berlin, Springer.

Speidel, W. 1984. Revision der Acentropinae des paläarktischen Faunengebietes (Lepidoptera, Crambidae). PhD Fakultät für Bio- und Geowissenschaften; Universität Karlsruhe.

Speidel, W. 1998. Studies on the phylogeny of the Acentropinae (Lepidoptera, Crambidae). *Memoires de la Societe Royale BeIge d'Entomologie* **38,** 25-30.

Spencer, D.F. 1987. Tuber size and planting depth influence growth of *Potamogeton pectinatus* L. *American Midland Naturalist* **118,** 77-84.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford: Oxford University Press.

Steifetten, O. & Dale, S. 2006. Viability of an endangered population of ortolan buntings: the effect of a skewed operational sex ratio. *Biological Conservation* **132,** 88-97.

Sterner, R.W. & Hessen, D.O. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics* **25,** 1-29.

Stoll, S., Fischer, P., Klahold, P., Scheifhacken, N., Hofmann, H. & Rothhaupt, K.O. 2008. Effects of water depth and hydrodynamics on the growth and distribution of juvenile cyprinids in the littoral zone of a large pre-alpine lake. *Journal of Fish Biology* **72,** 1001-1022.

Stoops, C.A., Adler, P.H. & McCreadie, J.W. 1998. Ecology of aquatic Lepidoptera (Crambidae: Nymphulinae) in South Carolina, USA. *Hydrobiologia* **379,** 33-40.

Stowe, K.A., Marquis, R.J., Hochwender, C.G. & Simms, E.L. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics* **31,** 565-595.

Strauss, S.Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* **72,** 543-558.

Strong, D.R. 1992. Are trophic cascades all wet ? Differentiation and donor-control in speciose ecosystems. *Ecology* **73,** 747-754.

Sutter, T.J. & Newman, R.M. 1997. Is predation by sunfish (*Lepomis* spp.) an important source of mortality for the Eurasian watermilfoil biocontrol agent *Euhrychiopsis lecontei* ? *Journal of Freshwater Ecology* **12,** 225-234.

Taylor, B.W., Anderson, C.R. & Peckarsky, B.L. 1998. Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. *Oecologia* **114,** 494-502.

Teder, T. & Tammaru, T. 2005. Sexual size dimorphism within species increases with body size in insects. *Oikos* **108,** 321-334.

Thiel, R., Sepulveda, A., Kafemann, R. & Nellen, W. 1995. Environmental factors as forces structuring the fish community of the Elbe estuary. *Journal of Fish Biology* **46,** 47-69.

Throop, H.L. 2005. Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. *Oikos* **111,** 91-100.

Traut, W. & Scholz, D. 1978. Structure, replication and transcriptional activity of the sex-specific heterochromatin in a moth. *Experimental Cell Research* **113,** 85-94.

Traut, W., Sahara, K. & Marec, F. 2007. Sex chromosomes and sex determination in Lepidoptera. *Sexual Development* **1,** 332-346.

Tseng, M. 2003. Life-history responses of a mayfly to seasonal constraints and predation risk. *Ecological Entomology* **28,** 119-123.

Turner, A.M. & Chislock, M.F. 2007. Dragonfly predators influence biomass and density of pond snails. *Oecologia* **153,** 407-415.

Underwood, D.L.A. 1994. Methods for sexing Lepidoptera larvae using external morphology. *Journal of the Lepidopterists' Society* **48,** 258-263.

van Donk, E. 1998. Switches between clear and turbid water states in a biomanipulated lake (1986-1996): the role of herbivory on macrophytes. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 290-297. Berlin, Springer.

Vaughton, G. & Ramsey, M. 1998. Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). *Journal of Ecology* **86,** 563-573.

Verges, A., Becerro, M.A., Alcoverro, T. & Romero, J. 2007. Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant-herbivore interactions. *Oecologia* **151,** 675-686.

Verges, A., Perez, M., Alcoverro, T. & Romero, J. 2008. Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish. *Oecologia* **155,** 751-760.

von Elert, E. & Pohnert, G. 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. *Oikos* **88,** 119-128.

Wang, N. & Eckmann, R. 1994. Distribution of perch (*Perca fluviatilis* L.) during their first year of life in Lake Constance. *Hydrobiologia* **277,** 135-143.

Ward, D.M. & Newman, R.M. 2006. Fish predation on Eurasian watermilfoil (*Myriophyllum spicatum*) herbivores and indirect effects on macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences* **63,** 1049-1057.

Ward, G. & FitzGerald, G.J. 1983. Fish predation on the macrobenthos of tidal salt marsh pools. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **61,** 1358-1361.

Warfe, D.M. & Barmuta, L.A. 2006. Habitat structural complexity mediates food web dynamics in a freshwater macrophyte community. *Oecologia* **150,** 141-154.

Weaver, M.J., Magnuson, J.J. & Clayton, M.K. 1997. Distribution of littoral fishes in structurally complex macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences* **54,** 2277-2289.

Weaver, M.J., Magnuson, J.J. & Clayton, M.K. 1998. Habitat heterogeneity and fish community structure: inferences from north temperate lakes. In: *Multidimensional Approaches to Reservoir Fisheries Management: a National Symposium on Managing Reservoir Fisheries* (Ed. by D.R. De Vries & L.E. Miranda), pp. 335-346. Bethesda, American Fisheries Society.

Webster, J.R. & Benfield, E.F. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* **17,** 567-594.

Wedell, N., Nylin, S. & Janz, N. 1997. Effects of larval host plant and sex on the propensity to enter diapause in the comma butterfly. *Oikos* **78,** 569-575.

Werner, E.E. & Peacor, S.D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84,** 1083-1100.

Wetzel, R.G. 2001. *Limnology - Lake and River Ecosystems*. San Diego: Academic Press.

Wetzel, R.G. & Söndergaard, M. 1998. Role of submerged macrophytes for the microbial community and dynamics of dissolved organic carbon in aquatic ecosystems. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Ed. by E. Jeppesen, M. Söndergaard, M. Söndergaard & K. Christoffersen), pp. 133-148. Berlin, Springer.

Whitfield, A.K. 1984. The effects of prolonged aquatic macrophyte senescense on the biology of the dominant fish species in a Southern African coastal lake. *Estuarine, Coastal and Shelf Science* **18,** 315-329.

Whoriskey, F.G. & FitzGerald, G.J. 1994. Ecology of the threespine stickleback on the breeding grounds. In: *The Evolutionary Biology of the Three-Spine Stickleback* (Ed. by M.A. Bell & S.A. Foster), pp. 189-206. Oxford, Oxford University Press.

Wiklund, C., Wickman, P.O. & Nylin, S. 1992. A sex difference in the propensity to enter direct/diapause development: a result of selection for protandry. *Evolution* **46,** 519-528.

Wiley, M.J., Gorden, R.W., Waite, S.W. & Powless, T. 1984. The relationship between aquatic macrophytes and sport fish production in Illinois ponds: a simple model. *North American Journal of Fisheries Management* **4,** 111-119.

Williams, D.D. & Feltmate, B.W. 1992. *Aquatic Insects*. Wallingford: CABI.

Wilson, D.M., Fenical, W., Hay, M., Lindquist, N. & Bolser, R. 1999. Habenariol, a freshwater feeding deterrent from the aquatic orchid *Habenaria repens* (Orchidaceae). *Phytochemistry* **50,** 1333-1336.

Winkler, E. & Fischer, M. 2002. The role of vegetative spread and seed dispersal for optimal life histories of clonal plants: a simulation study. *Evolutionary Ecology* **15,** 281-301.

Wolfer, S.R. & Straile, D. 2004 a. Density control in *Potamogeton perfoliatus* L. and *Potamogeton pectinatus* L. *Limnologica* **34,** 98-104.

Wolfer, S.R. & Straile, D. 2004 b. Spatio-temporal dynamics and plasticity of clonal architecture in *Potamogeton perfoliatus*. *Aquatic Botany* **78,** 307-318.

Wootton, R.J. 1994. Energy allocation in the threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by A.M. Bell & S.A. Foster), pp. 114-143. New York, Oxford University Press Inc.

Wootton, R.J. & Smith, C. 2000. A long-term study of a short-lived fish: the demography of *Gasterosteus aculeatus*. *Behaviour* **137,** 981-997.

Wootton, R.J., Adams, C.E. & Attrill, M.J. 2005. Empirical modelling of the population dynamics of a small population of the threespine stickleback, *Gasterosteus aculeatus*. *Environmental Biology of Fishes* **74,** 151-161.

Worm, B. & Myers, R.A. 2003. Meta-analysis of cod-shrimp interactions reveals top-down control in oceanic food webs. *Ecology* **84,** 162-173.

Appendix

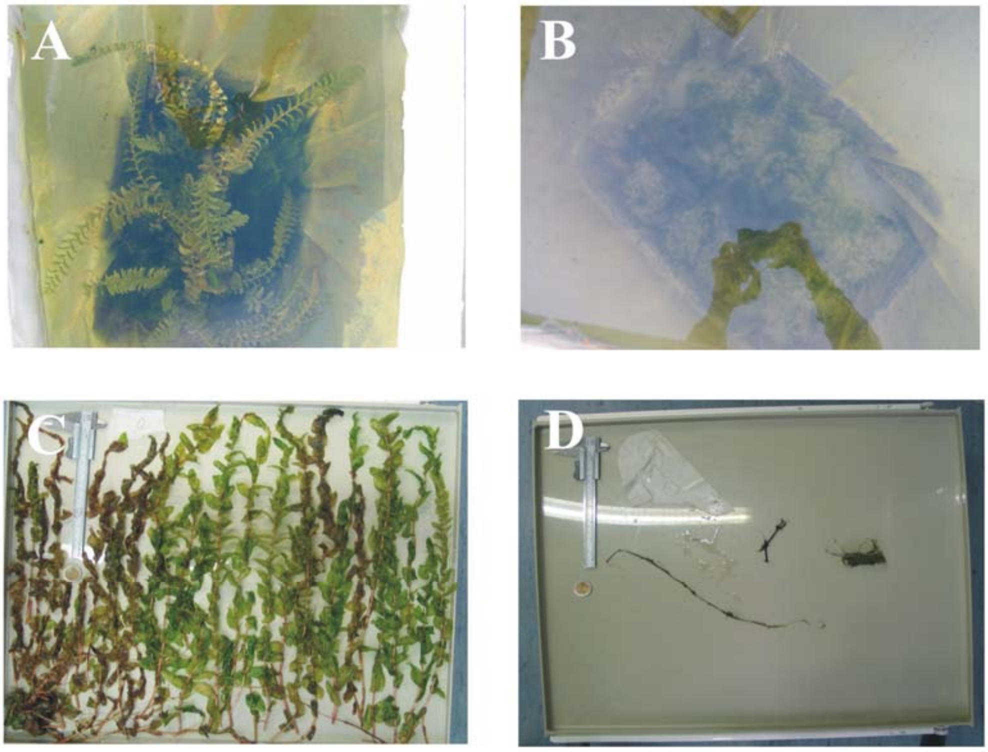

Fig. A 1 Pictures of plant shoots and leaves from two sampling units of the mesocosm experiment in November 2005 (see chapter I). Shown are plant shoots and leaves from a control (A, C) and a herbivory treatment (B, D) in the sampling units (A, B) and spread out on a table in the laboratory (C, D). Please note the strong differences in appearance (leaf and shoot colour in A, B, C, D) and amount of above-ground biomass (C, D) between the control and the herbivory treatment.

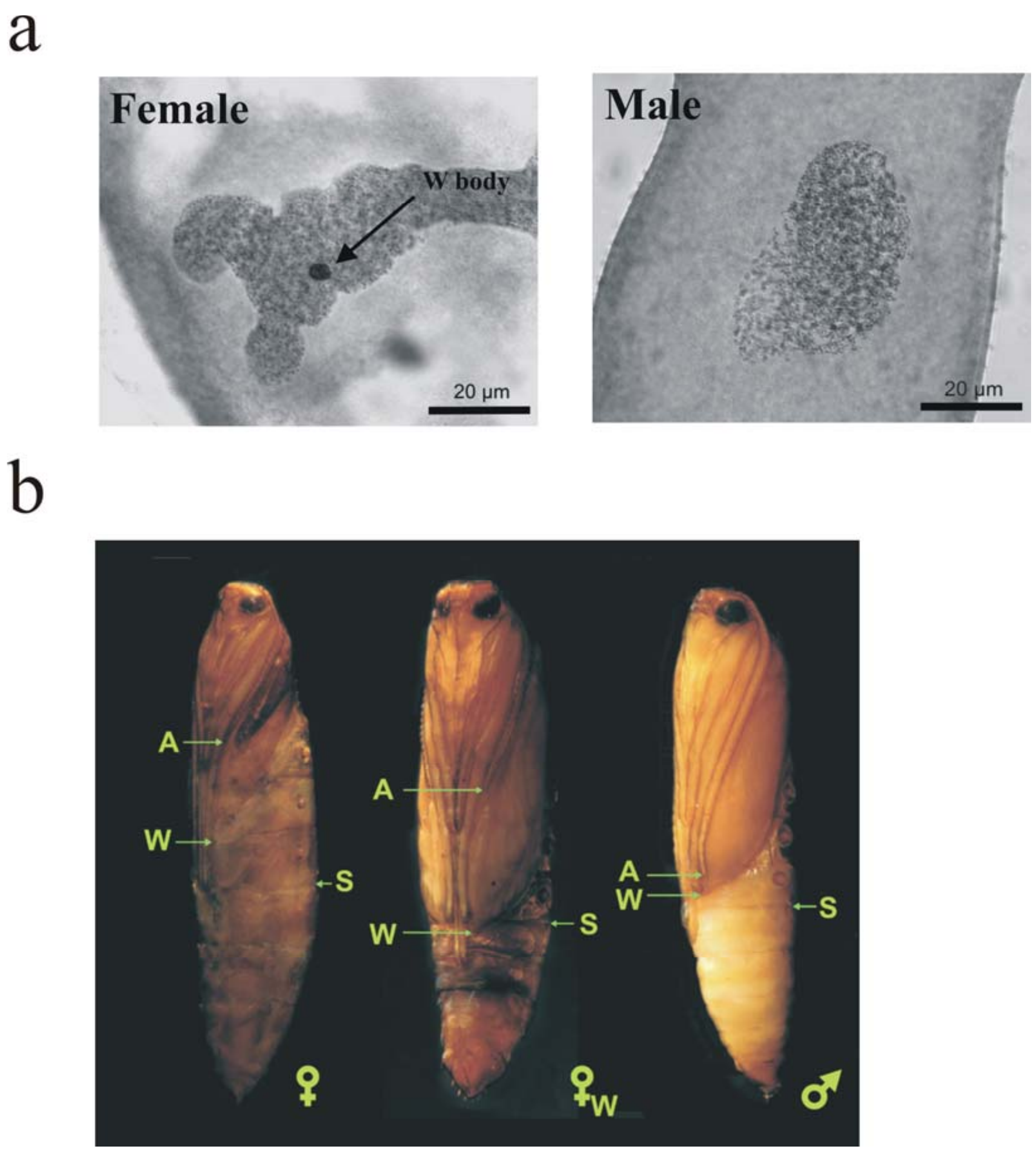

Fig. A 2 Sex determination of *Acentria* larvae (a) and pupae (b).

(a) The sex of *Acentria* larvae is histologically determined via a staining of the sex chromatin (i.e. the w body, see arrow) that appears concentrated in the cell nucleus of body tissues exclusively in female individuals.

(b) To sex *Acentria* pupae morphologically the wings (W), the antennae (A) and the stigmata of the abdomen are used. The 2nd, 3rd and 4th abdominal segments contain closed stigmata, and the posterior end of the 4th abdominal segment (i.e. the beginning of the 5th abdominal segment) is marked with (S). Wingless female pupae have short antennae and only rudimentary wing remains that do not extend over half of the length of the 5th abdominal segment (♀). Rarely winged females occur, with short antennae but wings that are longer than half of the length of the 5th abdominal segment (♀$_W$). Male pupae have always long antennae and wings extending over half of the length of the 5th abdominal segment (♂).

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Abgrenzung der Eigenleistung: Erklärung zum eigenen Beitrag in Arbeiten mit mehreren Autoren

Die hier präsentierten Arbeiten basieren auf eigenen Versuchsplanungen, -durchführungen, -anleitungen, Daten und Auswertungen, mit folgenden Ausnahmen:

Chapter II – Die Daten, die der Analyse des Seneszenzzustandes der Pflanzenpatches und der Verteilungen von Fischen und *Acentria* Larven aus den Freilandproben zugrunde liegen, wurden in Zusammenarbeit mit Michael Korn nach gemeinsamer Planung der Probenahmen erhoben.
Chapter III – Die Daten, die der Auswertung des Mesokosmosversuches zugrunde liegen, basieren auf der Versuchsplanung und -durchführung von Michael Korn.
Chapter IV – Die Methode der Geschlechtsbestimmung, die der Analyse des Geschlechts der *Acentria* Larven aus dem Mesokosmosversuch zugrunde liegt, wurde von František Marec entwickelt.
Chapter V – Die Methode der Geschlechtsbestimmung, die der Analyse des Geschlechts der *Acentria* Larven aus den Freilandproben zugrunde liegt, wurde von František Marec entwickelt.

Ich erkläre hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet. Weitere Personen, insbesondere Promotionsberater, waren an der inhaltlich materiellen Erstellung dieser Arbeit nicht beteiligt. Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Konstanz, im August 2008

Oliver Miler

Curriculum Vitae

Name:	Oliver Miler
Date of birth:	11.09.1975
Place of birth:	Offenbach am Main
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Scientific career

July 2004 - August 2008	PhD thesis at the Limnological Institute / University of Konstanz, research group Limnology of Lakes Title of thesis: The aquatic moth *Acentria ephemerella* as a key species in submerged aquatic vegetation - direct and trait-mediated interactions with predators and food plants. Supervisor: Dr. Dietmar Straile (assistant professor, University of Konstanz)
Oct. 2003 - June 2004	Scientific assistant at the Limnological Institute of the University of Konstanz, research group Limnology of Lakes
April 2002 - Sept. 2003	Technical assistant at the Limnological Institute of the University of Konstanz, research group Fish Ecology

University education

March 2001 - March 2002	Diploma thesis at the Limnological Institute / University of Konstanz, research group Fish Ecology Title of thesis: Distribution and migration behaviour of burbot larvae (*Lota lota* L.) during the littoral migration in Lake Constance. Supervisor: Dr. Philipp Fischer (professor, Alfred Wegener Institute (AWI) Helgoland)
Oct. 1996 - March 2002	Biological studies at the University of Konstanz: University Degree: Diploma