COMMUNITY ECOLOGY

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Predator defense along a permanence gradient: roles of case structure, behavior, and developmental phenology in caddisflies

Abstract Species replacements along freshwater permanence gradients are well documented, but underlying mechanisms are poorly understood for most taxa. In subalpine wetlands in Colorado, the relative abundance of caddisfly larvae shifts from temporary to permanent basins. Predators on caddisflies also shift along this gradient; salamanders (*Ambystoma tigrinum nebulosum*) in permanent ponds are replaced by predaceous diving beetles (*Dytiscus dauricus*) in temporary habitats. We conducted laboratory and field experiments to determine the effectiveness of caddisfly cases in reducing vulnerability to these predators. We found that larvae of a temporary-habitat caddisfly (*Asynarchus nigriculus*) were the most vulnerable to salamanders. Two relatively invulnerable species *(Limnephilus externus, L. picturatus)* exhibited behaviors that reduced the likelihood of detection and attack, whereas the least vulnerable species (*Agrypnia deflata*) was frequently detected and attacked, but rarely captured because cases provided an effective refuge. Vulnerability to beetle predation was also affected by cases. The stout cases of *L. externus* larvae frequently deterred beetle larvae, whereas the tubular cases of the other species were relatively ineffective. Two of these vulnerable species (*A. nigriculus* and *L. picturatus*) often co-occur with beetles; thus, case construction alone is insufficient to explain patterns of caddisfly coexistence along the permanence gradient. One explanation for the coexistence of these two species with beetles is that they develop rapidly during early summer and pupate before beetle larvae become abun-
dant. One species (*L. picturatus*) pupates by burying into soft substrates that serve as a refuge. The other (*A. nigriculus*) builds stone pupal cases, which in field experiments, more than doubles survival compared to organic pupal cases. The combined results of these experiments suggest that caddisfly distributions along permanence gradients depend on a suite of primary and secondary predator defenses that include larval and pupal case structure, predator-specific escape behaviors, and the phenology of larval development.

Keywords Permanence gradient · Caddisfly cases · Predator defense · Community structure

Introduction

Shifts in freshwater community composition are often observed along a continuum from relatively temporary to relatively permanent habitats (Batzer and Wissinger 1996; Schneider and Frost 1996; Wellborn et al. 1996; Williams 1996; Schneider 1999; Wissinger 1999). Wellborn et al. (1996) "predator-permanence" model for this phenomenon emphasizes that shifts in lentic community composition often result from species replacements within taxa (typically at the genus or family level) that reflect changes in biotic interactions with top predators, as well as differences in tolerance to drying. Their model provides the framework for both understanding the evolutionary ecology of lineage diversification (e.g., McPeek 1995a, b, 1999, 2000; McPeek and Brown 2000; Richardson 2002; Stoks et al. 2003) and for linking tradeoffs in individual species traits to patterns of lentic community structure. Despite comparative evidence for species replacements in most groups of lentic taxa, nearly all experimental studies on the underlying mechanisms have focused on just two groups—frog tadpoles and damselfly larvae (but see Pierce 1988; Wellborn 2002). In these groups, species replacements reflect tradeoffs between (1) predator avoidance versus. competitive ability or physiological efficiency (e.g., Relyea

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2000; McPeek 2000; McPeek et al. 2001), (2) high activity to facilitate emergence from temporary habitats versus risk-sensitive activity to reduce predator detection in permanent habitats (e.g., Skelly 1995, 1997; Werner and Anholt 1993, 1996; Relyea and Werner 1999; Anholt et al. 2000; Johansson and Suhling 2004), and (3) alternative escape behaviors that are effective against different predators (e.g., McPeek 1990, 1996; Werner and McPeek 1994; Peacor and Werner 1997; Altwegg 2002; Stoks and McPeek 2003a, b). Despite the extensive research on these two groups, relationships among "phenotype, performance, and distribution" are poorly understood for the 25 other lentic taxa for which this phenomenon has been documented (Wellborn et al. 1996).

Cased caddisflies (Trichoptera: Limnephilidae and Phryganeidae) exhibit species replacements along permanence gradients in a variety of types of lentic habitats (e.g., Otto 1976; Zamora-Munoz and Svensson 1996; Wissinger and Gallagher 1999; Wissinger et al. 2003). These caddisfly larvae use silk to construct portable cases of mineral and vegetation fragments, and although there are several potential functions (see Williams et al. 1987; Otto and Johansson 1995), the cases of many species reduce predation (Otto 1976, 1982; Otto and Svensson 1980; Johansson 1991; Johansson and Johansson 1992; Nislow and Molles 1993; Johansson and Englund 1995; Otto and Johansson 1995). In this study, we tested the prediction that the case morphologies of different species of caddisflies should be most effective against the predators with which they typically co-occur. This prediction follows from the observation that antipredator traits in prey are often specifically effective against predators with sympatric distributions, but confer only partial or no protection from other types of predators (Matsuda et al. 1993, 1996). As in previous studies with cased aquatic insects, the experimental removal of cases provided comparative data on their efficacy in reducing vulnerability to predation (Power et al. 1992; Wissinger et al. 2004b). Detailed observations during the trials with cased larvae revealed interspecific differences in pre- and post-attack behaviors at different steps in the predator foraging sequence (detection, attack, capture). Thus, we could compare the importance of caddisfly behavior before attack and vis-à-vis the use of cases versus case structure per se in avoiding capture by salamander and beetle predators. The experiments also provided evidence for the degree to which cases act as a primary level of defense (i.e., a cryptic role in reducing the probability of attack) versus a secondary level of defense (reducing the probability of capture after attack; after Edmunds 1974).

Caddisfly and predator distributions along permanence gradient

Larvae of the four species of caddisflies that we studied inhabit subalpine ponds and wetlands in central Colorado, USA, where they shift in dominance along a continuum from vernal (dry by early summer) to autumnal (dry in late summer in some years) to permanent wetlands (Fig. 1). In vernal wetlands, caddisfly intraguild predation and cannibalism is the main predatory threat to larvae, and elsewhere we report on the importance of cases in deterring these interactions (Wissinger et al. 2004b). In autumnal wetlands, larvae of a predaceous diving beetle, *Dytiscus dauricus*, are the top predators on caddisflies (Wissinger et al. 1999b). *D. dauricus* can complete development in permanent ponds, but this large beetle is absent or rare in those with larval and paedomorphic salamanders (*Ambystoma tigrinum nebulosum*), both of which prey on the beetles. Because of the protracted development rates of salamanders at high elevations, the populations in subalpine permanent ponds include several year classes of larvae and paedomorphic adults that prey on a variety of invertebrates (>100 taxa) including caddisflies (Wissinger et al. 1999b; Whiteman et al. 1994; Whiteman and Wissinger 2005).

One of the caddisflies (*Agrypnia deflata* Milne) has a life cycle that restricts it to permanent ponds, and
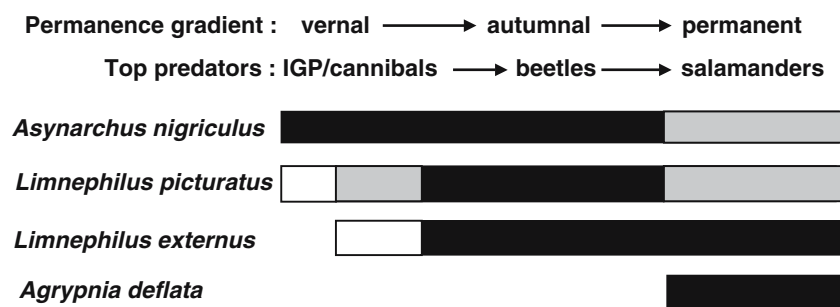

Fig. 1 Distribution of caddisfly larvae [*Asynarchus nigriculus* Banks (Limnephilidae), *Limnephilus picturatus* McLachlan (Limnephilidae), *Limnephilus externus* Hagen (Limnephilidae), *Agrypnia deflata* Milne (Phryganeidae)] in high-elevation lentic habitats in central Colorado, USA. The fundamental niche of each species along the permanence gradient is indicated by the total length of the *bars*, and is based on life history and desiccation tolerance (Wissinger et al. 2003). Realized distributions at subalpine elevations are indicated by the *black bars* (common), *gray bars* (occasional) and *open bars* (not present). *IGP* Intraguild predators

larvae therefore always co-occur with salamanders. Adults of the other three species deposit desiccation-tolerant egg masses in or adjacent to dried basins in autumn so that their fundamental niches include both temporary and permanent basins (Fig. 1). The ability to exploit early-drying vernal habitats differs among these species depending on larval development rates in spring and early summer (Wissinger et al. 2003). Each of these species realizes only a subset of the habitats that it can exploit (Fig. 1). One (*Limnephilus externus*) is broadly distributed across permanent and autumnal habitats, whereas a congener (*Limnephilus picturatus*) is most abundant in autumnal habitats (Fig. 1). The fourth (*Asynarchus nigriculus*) is an aggressive species that typically dominates in autumnal and vernal habitats. Although *A. nigriculus* and *L. picturatus* can complete development in permanent ponds, they are rare or absent in those with salamanders (Wissinger et al. 2003).

Caddisfly cases

Case morphology and body size vary considerably among the four species of caddisflies (Fig. 2; detailed metrics of each larval instar in Wissinger et al. 2003). The larval case of *L. externus* (final instar body length 16–19 mm) is a stout, "hedge-hog" structure that is only slightly longer than the caddisfly larva (1.2–1.3 × body length), but is proportionately wide compared to the cases of the other species (Fig. 2). *L. externus* case size, shape, and composition vary considerably among populations; at our main study site, the cases are composed of haphazardly placed fragments of sedge stems (*Carex aquatilus*), and the width is typically at least 2/3 × length (final instar case width 12–14 mm, length 18–22 mm). The three other species have relatively narrow, tubular cases with width/length < 1/3. *Agrypnia* is the largest bodied species (final instar length, 17–22 mm) with large

Fig. 2 Case structure and relative sizes of **a** *Asynarchus nigriculus*, **b** *Limnephilus picturatus*, **c** *Limnephilus externus*, and **d** *Agrypnia deflata*

a **A. nigriculus**

1 cm

b **L. picturatus**

c **L. externus**

d **A. deflata**

cases composed of whorls of stems from the submergent plant, *Isoetes bolanderi*. The cases are disproportionately long (1.7–1.8 × body length) compared to the other species (Fig. 2). The two smallest species, *L. picturatus* and *A. nigriculus*, are similar in body size (final instar length 12–16 mm), but differ slightly in proportional case length (1.4–1.5 vs. 1.1–1.2 × body length) and composition (longitudinal sedge stems vs. bark, wood, and spruce needles, respectively).

Methods

Salamander predation on case and caseless caddisflies

To determine the role of cases in reducing salamander predation, we conducted experiments with cased and caseless caddisfly larvae in microcosms [plastic storage containers (0.25 m² bottom area) filled with filtered pond water (15 cm depth), 2.5 cm of detritus, one clump of emergent vegetation (*Carex aquatilis*), three rocks, and three pieces of woody debris to create semi-natural levels of habitat heterogeneity and encounter rates; after Wissinger et al. (1996, 1999a). Larvae of each caddisfly species were obtained from shallow ponds in a subalpine (3,400 m elevation) wetland complex at the Mexican Cut Nature Reserve near the Rocky Mountain Biological Laboratory in central Colorado, USA (Wissinger et al. 1999b). The microcosms were housed in a portable field laboratory adjacent to one of the ponds.

One paedomorphic salamander larva (SVL 92 ± 3 mm) was added to each microcosm for 24 h before the trials to standardize hunger levels. We introduced ten final (fifth) instar larvae, either with or without their cases to each microcosm 10 min before the behavioral trials. Final instars of each species (body metrics and mass given in Wissinger et al. 2003) were chosen randomly from a common pool and assigned to treatments and replicates. Each larva was removed from its case by probing posteriorly with a blunt probe. Larvae used in the cased treatments were allowed to re-enter their cases whereas those used in caseless treatments were not. Four observers conducted trials (two cased and two caseless) for 1 h between 09:00–10:00, 10:30–11:30, and 12:00–13:00 on two consecutive days (12 replicates of cased and caseless trials for each of four species). Observers were randomly assigned to the different treatments, and we found no observer bias during initial statistical analysis. The observation times fell within the peak period (mid-morning to early afternoon) of foraging activity observed for paedomorphic salamanders in natural populations at our study site (D. Weigel, S. Wissinger and H. Whiteman, unpublished data). The trials for *A. deflata, L. picturatus, A. nigriculus*, and *L. externus* were conducted in four consecutive weeks from mid-July to mid-August 1997 corresponding to the natural phenological sequence of the availability of final instars (see Wissinger et al. 2003). Because of seasonal differences in the availability of the

different species, species comparisons of vulnerability to salamander and beetle are necessarily confounded by date.

During each trial we recorded the number of times a salamander detected (movement towards a caddisfly larva), pursued (assumed a characteristic poised-for-attack posture), attacked (attempted ingestion by buccal suctioning), and captured (larva ingested with or without case) caddisflies (after Holomuzki 1989; Wissinger et al. 1999a). After the trials, caddisflies (including replacements of animals captured during the behavioral observations) were left in the arenas for 48 h after which we censused the number surviving. Initially, we explored the effects of blocks (time of day) on the analyses and found no main- or interaction-effect differences among treatments. We used two-way ANOVA to compare the survival of the four species with and without cases. Predation on caseless larvae was extremely fast (i.e., all or most were detected, pursued, attacked, and captured immediately); thus, we could only document the details of interactions between salamander foraging behaviors and caddisfly defense strategies in cased treatments. In cased treatments, we used one-way ANOVA to compare the number of detections, pursuits, and attacks for each species after testing for assumptions of normality and homogeneity of variances. Individual means were compared using Scheffé's multiple contrasts when one-way ANOVAs were significant. Because of departures from parametric assumptions, we used a Kruskal–Wallis test to compare captures, pursuits/detection, attacks/pursuit, and captures/attack among species.

Beetle predation on case and caseless caddisflies

To determine the relative vulnerability of different caddisfly species to beetle predation, we conducted 48 h survival trials comparable to those described above for the salamanders; i.e., we isolated one final instar *Dytiscus* larvae in each microcosm for 24 h before adding either ten cased or ten caseless final instar larvae of each species ($n = 12$ for each treatment). The trials for *A. deflata, A. nigriculus, L. picturatus* and *L. externus* were conducted in four consecutive weeks from mid-July to mid-August 1998, corresponding to the phenological sequence in the appearance of final instars at our study site (Wissinger et al. 2003). Each caddisfly larva used in the trials was removed from its case by probing posteriorly with a blunt probe. Larvae in the cased treatments were allowed to re-enter their cases before addition to the microcosm, whereas those in caseless treatments were not. During these trials, we observed that beetles were often distracted by secondary prey during the attack sequence and abandoned one prey to attack other individuals. Thus, in order to quantify the effectiveness of larval cases in deterring predation after attack (captures/attacks), and the effects of cases on handling time, we conducted a separate set of behavioral experiments in small observation arenas (100 cm²; 7 cm depth mesh
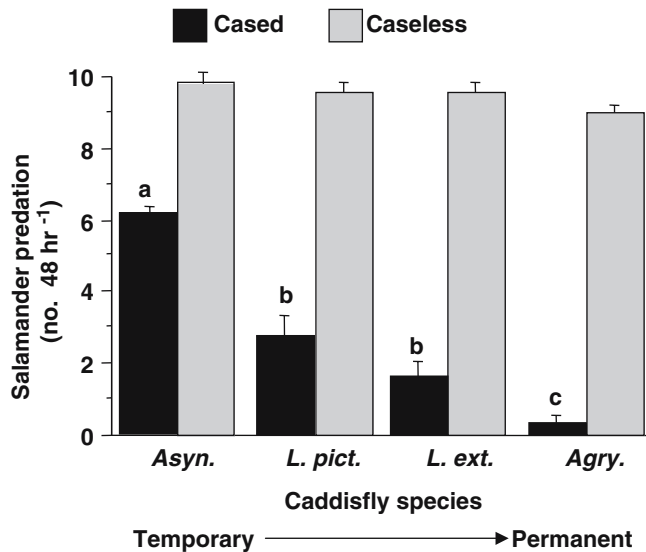
Fig. 3 Salamander predation on cased (*filled box*) and caseless (*shaded box*) caddisflies (mean ±1 SE) in a 48 h microcosm experiment. Lower case letters group means that did not differ based on Scheffé's test after a one-way ANOVA on cased larvae

substrate) with a single final instar beetle larva (40–50 mm total length) and a single caddisfly larva. *Dytiscus* larvae were isolated for 24 h before the trials to standardize hunger levels. Each beetle larva was used in only one trial before returning it to the source pond. Four observers conducted 1 h trials (two cased and two caseless) between 09:00–10:00, 10:30–11:30, and 12:00–13:00 on two consecutive days (12 replicates of cased and caseless trials for each of four species). Preliminary trials indicated no effects of time of day (i.e., 9:00–15:00 h) on beetle predatory behavior (S. Wissinger and W. Brown, unpublished data).

Dytiscus larvae feed by sucking the body fluids of their prey through enlarged, hollow mandibles (Formanowicz 1982). In each trial, we recorded whether or not an attacked caddisfly was eventually consumed (injected with proteolytic enzymes that resulted in the rapid discoloration and eventually death) or escaped, and measured handling time as the total time spent between first attack and capture or departure. We compared the proportion of cased and caseless larvae of each species that were captured using a χ^2 test for deviations from the two-way contingency null hypothesis that case status had no effect on the success of attacks. We used one-way ANOVA to compare the average beetle handling times for cased larvae of different species.

Beetle predation on pupae

Three of the caddisfly species (*A. deflata, L. externus, L. picturatus*) pupate by burrowing into soft substrates, and both field observations and preliminary experimental trials indicated that *Dytiscus* larvae do not prey on buried pupae (S. Wissinger, unpublished data). In con-

trast, late fifth instar *A. nigriculus* add stones to their cases and then pupate on exposed hard substrates (wood, rock, or other conspecific cases). Late instar *Dytiscus* larvae become abundant during *Asynarchus* pupation, and we have frequently observed them attacking *Asynarchus* pupae. To test the efficacy of stone cases in reducing beetle predation, we conducted a field experiment in late July in shallow mesocosms (plastic wading pools -50 cm water depth, 1.5 m² area) placed near the ponds. We added 5 cm of natural vernal wetland substrate from the adjacent pond to each of the mesocosms. Five received unaltered substrate (bits of wood, pine needles and other vegetation and mineral fragments), and five received substrate from which the mineral fragments had been removed by repeatedly elutriating the organic fraction. Several large rocks were added to the microcosms to provide pupation sites. On July 23, we placed 50 final (fifth) instar *Asynarchus* larvae with no mineral fragments on their cases into each mesocosm. Larvae in both treatments pupated during the last week of July when it became obvious that our manipulation had been effective; i.e., five replicates had pupae with stony cases and five had cases composed mainly or entirely of spruce needles, bark, and other bits of vegetation. We added three final instar *Dytiscus* larvae to the mesocosms on August 5 for 5 days, and then removed the beetles and counted the surviving pupae. The short duration of the experiment was necessary to minimize the confounding effects of the loss of pupae to emergence.

Results

Salamander predation on caddisfly larvae

Salamanders consumed nearly all (10/10) caseless larvae of all four species of caddisflies (Fig. 3). Across all species, predation was significantly lower on cased than on caseless larvae (two-way ANOVA main effect of treatment $F_{3,40} = 1076$; $P < 0.001$), but there was a significant interaction between treatment and caddisfly species ($F_{3,40} = 10.8$; $P < 0.001$). The interaction reflected the relatively large number of cased *Asynarchus* that were eaten compared to the relatively small number for the other three species (Fig. 3). Salamander predation was significantly greater on cased *Asynarchus* than on cased *Limnephilus* species, which in turn was greater than on cased *Agrypnia* (Fig. 2; Scheffé's test $P < 0.05$ between groups; one-way ANOVA $F_{3,20} = 22.8$, $P < 0.01$). On average, predation was more than five times greater on cased *Asynarchus* larvae than on cased *Agrypnia* larvae.

During the 1 h behavioral trials, we observed a stereotypic sequence of steps that typify salamander predation on caddisflies. Salamanders exhibited a clear head-turning behavior when they perceived the movements of caddisflies (prey "detection"), and then moved towards the detected caddisfly and assumed a pre-attack

position in a head down poised-to-strike posture (prey "pursuit"). If the salamander detected continued movement, it snapped at the potential prey (attacks), and either consumed the case and larva, extracted the larva, or orally manipulated the case to dislodge the larva (prey "capture"). Salamander predation on larvae of each species of caddisfly differed at each of these steps in the predation sequence (Fig. 4). *Asynarchus* larvae were detected, pursued, and attacked significantly more times by salamanders than *Agrypnia*, which were detected, pursued, and attacked more times than the two *Limnephilus* species, which did not differ (Scheffé's test $P < 0.05$ between groups; one-way ANOVA $F_{3,20} = 30.6$ for detections; one-way ANOVA $F_{3,20} = 31.8$ for pursuits; one-way ANOVA $F_{3,20} = 29.8$ for attacks; $P < 0.001$; Fig. 4). Overall, more than three times the number of cased *Asynarchus* larvae were captured per trial than the other three species (one-way ANOVA $F_{3,20} = 24.4$; Scheffé's test $P < 0.05$), and the two species of *Limnephilus* were captured more often than those of *Agrypnia* (Scheffes' post-hoc $P < 0.05$).

Interspecific differences in captures rates depended both on larval behaviors and on case construction. Salamanders always pursued larvae after detection (i.e., pursuits/detection ~ 1 for all species; Fig. 5a), but attacks/pursuits were higher on *Asynarchus* than on the other species (Fig. 5b; Scheffé's test $P < 0.05$; one-way ANOVA $F_{3,20} = 6.83$; $P = 0.002$). This high attack rate was related mainly to the short time that *Asynarchus* spent withdrawn and immobile in the case after approach by salamander predators (i.e., after pursuit). In contrast, larvae of the other three species withdrew into their cases and remained motionless until salamanders moved to a new foraging location, effectively exceeding the salamander giving up time. Despite high attacks/pursuit, *Asynarchus* larvae were no more likely to be captured, once attacked than *L. picturatus* (Scheffé's test $P > 0.05$; one-way ANOVA $F_{3,20} = 3.61$; $P = 0.04$). All three limnephilid species were captured more often per attack than *Agrypnia* (Scheffé's test $P < 0.05$; Fig. 5c). Salamanders rarely swallowed the large cases of *Agrypnia*, but instead attempted to capture larvae by orally grasping the cases and attempting to dislodge them with forceful buccal pumping (analogous to smoking a cigar). The only *Agrypnia* larva that was captured was extracted from its case after extended handling (18 min). In contrast, salamanders captured the relatively small *A. nigriculus* and *L. picturatus* larvae quickly by swallowing both case and larva. *L. externus* larvae were captured mainly by extraction from their large cases.
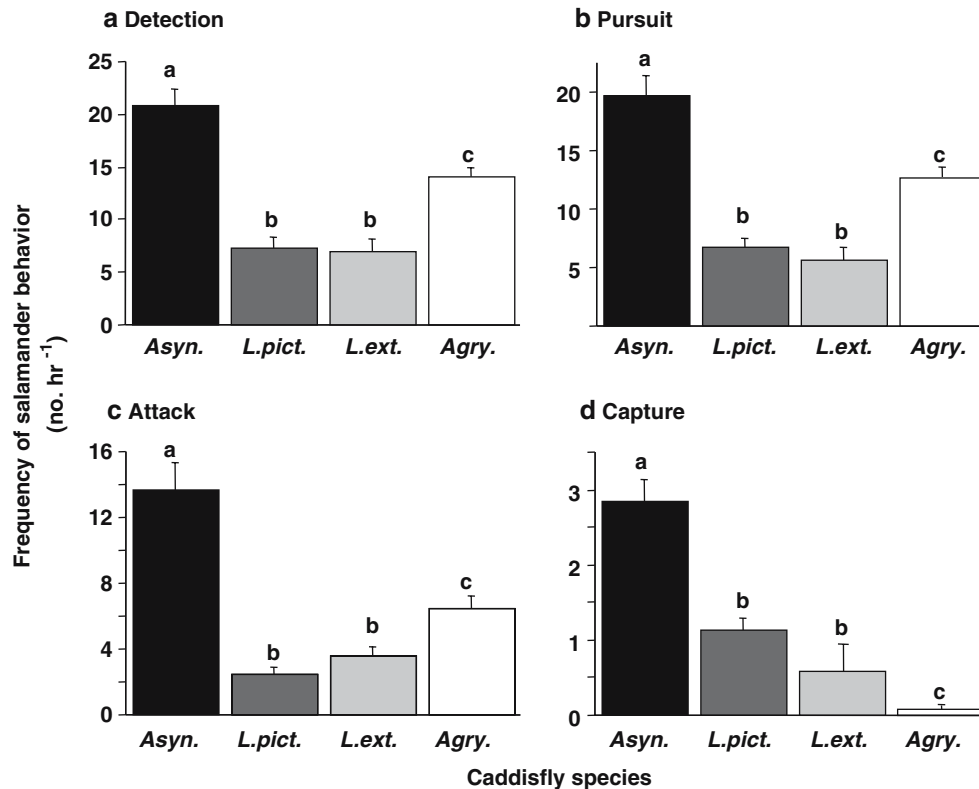

Fig. 4 Components of salamander foraging behavior of cased caddisfly larvae during 1 h trials in microcosms: **a** Detection = salamander detects presence of moving caddisfly larva, **b** pursuit = salamander approaches caddisfly larva and assumes attack position, **c** attack = salamander lunges at caddisfly, and **d** capture = salamander either extracts caddisfly larva from case or ingests larva and case. Values indicate the mean number (± 1 SE) of each of the behaviors. Lower case letters group means that did not differ based on Scheffé's test after a one-way ANOVA on cased larvae

a Pursuits per detection

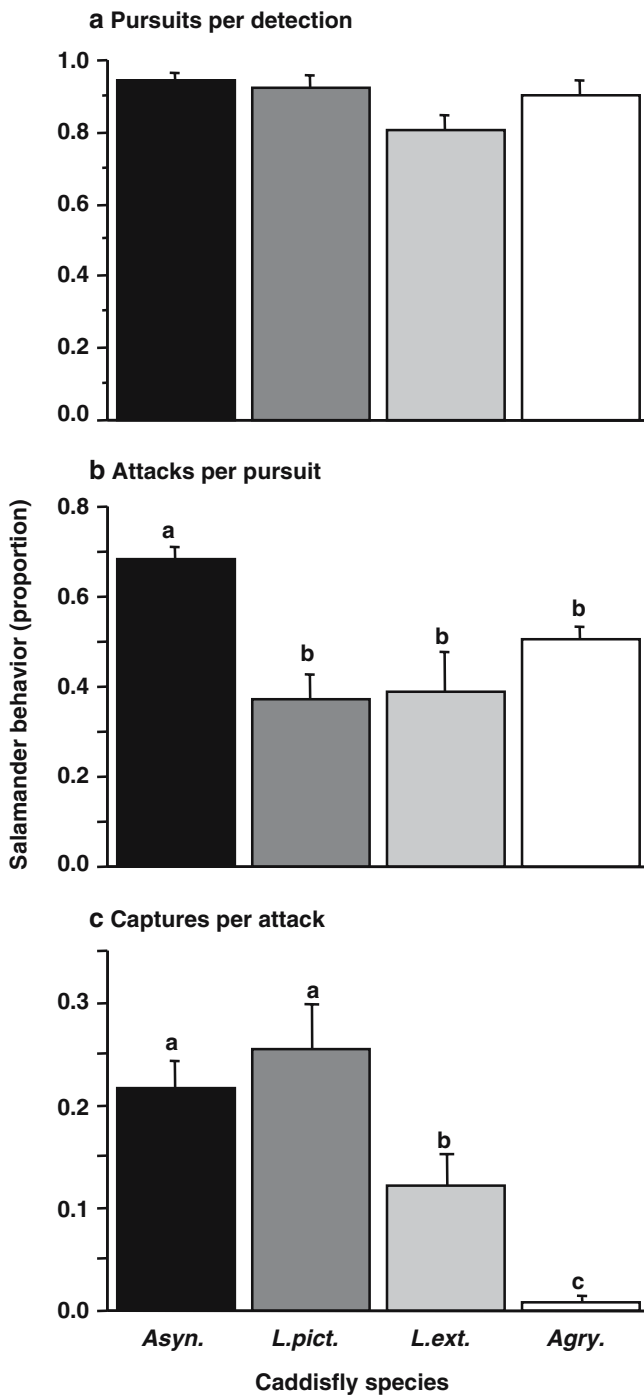## b Attacks per pursuit

c Captures per attack

Fig. 5 Salamander foraging behavior of cased caddisflies in microcosms: **a** pursuit per detection, **b** attack per pursuit, **c** capture per attack (proportion ±1 SE) based on the values given in Fig. 4. Lower case letters group species that did not differ based on Scheffé's test after a one-way ANOVA on cased larvae

Beetle predation on caddisfly larvae

In the 24 h microcosm experiment, beetle larvae consumed all caseless larvae of all species of caddisflies (Fig. 6). In some instances we observed ''wasteful killing'', i.e., caseless larvae were killed (injected
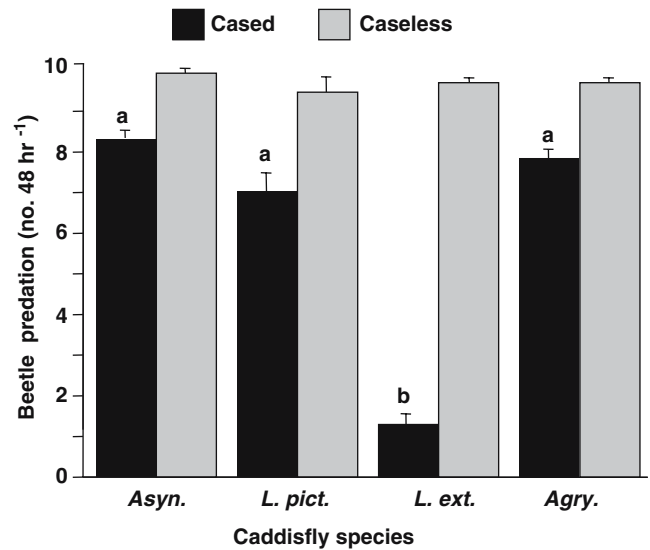

Fig. 6 Beetle larva predation (mean ±1 SE) of cased (*filled box*) and caseless (*shaded box*) caddisflies in 48 h microcosm experiment. Beetle predation differed significantly between cased and caseless larvae of all species. Lower case letters group species of cased larvae that did not differ based on Scheffé's test after a one-way ANOVA

with proteolytic enzymes), but not consumed (i.e., predigested body fluids of killed caddisflies were not removed by the beetles). Beetle larvae captured fewer cased than caseless caddisfly larvae, and the effect of cases on survival (two-way ANOVA main effect of cases $F_{1,40} = 188$; $P < 0.001$), varied among species (Fig. 6; two-way ANOVA main effect of species $F_{3,40} = 44.7$; $P < 0.001$; case by species interaction $F_{3,40} = 43.62$; $P < 0.001$). Beetles captured fewer cased *L. externus* than *L. picturatus*, and fewer of both than either *Asynarchus* or *Agrypnia* (Scheffé's test $P < 0.05$ between groups based on one-way ANOVA on cased larvae $F = 57.8$; $P > 0.001$).

We observed nearly identical results in the small observation arenas where we could isolate the predation sequence on one caddisfly (Fig. 7). When beetle larvae attacked caseless caddisflies, they grabbed larvae with their mandibles and injected a proteolytic enzyme that discolored and killed the larvae. For cased caddisflies, the approach of a beetle larva caused larvae to withdraw into their cases, which the beetles then grasped and began to probe with their mandibles. All cases increased handling time (two-way ANOVA main effect of cases $F_{1,40} = 627$; $P < 0.001$), but to differing degrees for each species (main effect of species $F_{3,40} = 31$; $P < 0.001$; cases by species interaction $F_{2,40} = 36$; $P < 0.00$; Fig. 8). Beetle handling times were longer for *L. externus* than for *L. picturatus* and *Agrypnia*, which were greater than for *Asynarchus* (Scheffé's test $P < 0.05$ between groups based on one-way ANOVA on cased larvae $F = 40.9$; $P > 0.001$). Beetles easily penetrated the relatively thin, tubular cases and located the larvae of *Asynarchus* and *Agrypnia* with their mandibles. Once attacked, caddisfly larvae often emerged partly or completely (especially

Fig. 7 Number of behavioral trials (1 h; $n = 12$) in which individual cased and caseless caddisflies were eaten by beetle larvae for **a** *Asynarchus nigriculus*, **b** *Limnephilus picturatus*, **c** *Limnephilus externus*, and **d** *Agrypnia deflata*. *Asterisks* indicate a significant difference in the number of successful captures in trials with and without cases based on χ^2 tests ($P < 0.05$)
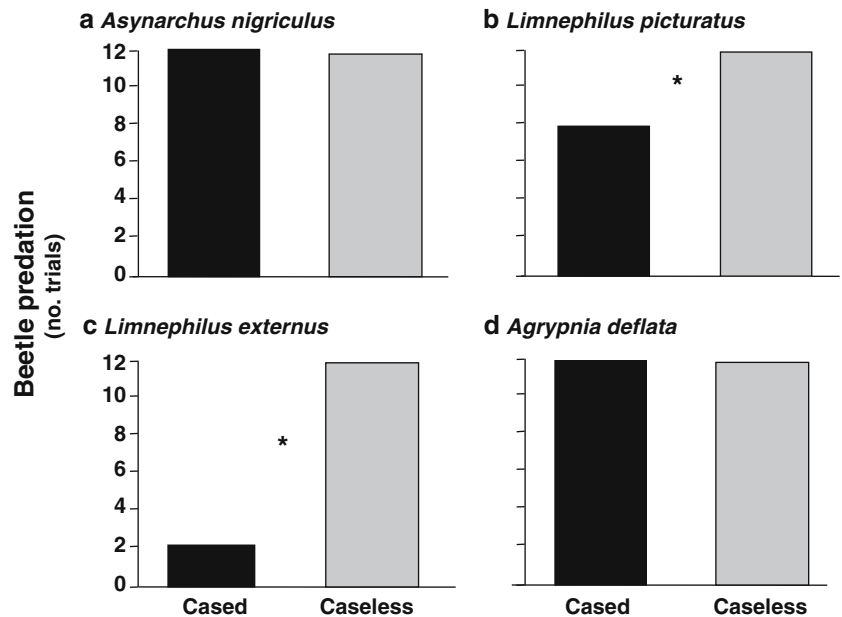
Agrypnia) from their cases where they were consumed by the attacking beetle larva. In contrast, the relatively thick-walled cases of *L. externus*, and to a lesser extent, *L. picturatus*, often prevented beetles from locating the caddisfly larva inside, and beetles often had to surface to breathe before returning for successive attempts at capture. In most trials (10/12), *L. externus* larvae retreated and remained in their cases where attacking beetles could not locate them despite repeated case probing with their mandibles. *L. externus* larvae did not move during the remainder of the trials, even when beetle larvae stopped attacking after multiple failed attempts (7/12 trials).

In the field experiment in which we manipulated *Asynarchus* pupal case material, more than twice the number of pupae with stone cases survived in the presence of *Dytiscus* beetle larvae than those with organic cases ($T = 4.91$; $P = 0.0006$; Fig. 9).

Discussion

Cased caddisflies exhibit species replacements along permanence gradients in several types of lentic habitats (Otto 1976; Zamora-Munoz and Svensson 1996; Wissinger and Gallagher 1999; Wissinger et al. 2003), but, as for most taxa of aquatic invertebrates, relatively little is known about the underlying tradeoffs. The portable cases of some species are known to deter predators (Otto 1976, 1982; Otto and Svensson 1980; Johansson 1991; Johansson and Johansson 1992; Nislow and Molles 1993; Johansson and Englund 1995; Otto and Johansson 1995), and we hypothesized that differences in case structure (Fig. 2) should reflect differences in the protective role of cases against the different types of predators in permanent and temporary habitats. We found

that caddisfly larvae without cases are more vulnerable to both salamander and beetle predators than those with cases (Figs. 3, 6), and that cased larvae of the different species are least vulnerable to the type of predator with which they coexist. However, the degree to which differences in vulnerability are a result of the protective role of cases per se versus other traits (defense behaviors, developmental phenology) varied considerably among species.

Caddisfly defense against salamander predators

The relative vulnerability of caddisflies to salamander predation was consistent with our prediction; i.e., the temporary-habitat species that rarely co-occurs with salamanders (*A. nigriculus*) was much more vulnerable than the species that are common in permanent habitats. Frequent detections, frequent attacks, and small, easily ingested cases (Figs. 2, 4) all contributed to the high capture rates of *A. nigriculus* by salamanders. In contrast, the least vulnerable species (*A. deflata*) was rarely captured despite high detection and attack rates by salamanders. When attacked by salamanders, *A. deflata* retreat to the rear of their long cases (1.7–1.8 × larval body length Fig. 2), where they frequently avoid capture. This differs from the other two relatively invulnerable species (*L. externus* and *L. picturatus*), which, once attacked by salamanders, are captured more frequently than *A. deflata*. The primary defense of the two *Limnephilus* species is to avoid detection by retreating into their cases and remaining motionless in the presence of foraging salamanders. A freeze response inside the case by both species frequently exceeds predator "giving up time"; i.e., in the absence of any detectable movement, salamanders move to a new location in search of
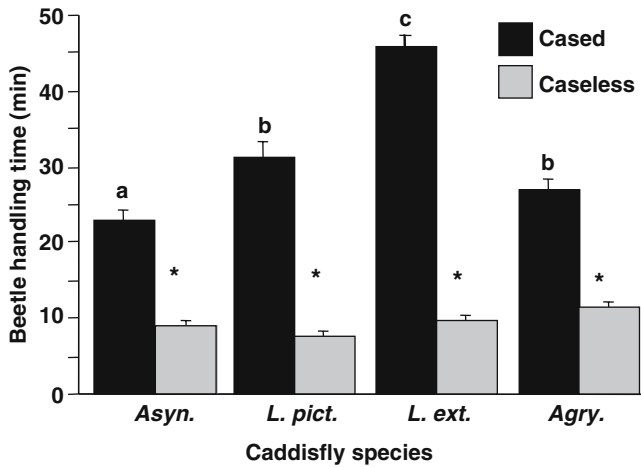
Fig. 8 Beetle handling time (mean ±1 SE) of cased (*filled box*) and caseless (*shaded box*) caddisflies during 1 h behavioral trials in microcosms. *Asterisks* indicate means differed ($P < 0.05$) between cased and caseless larvae based on one-way ANOVA for each species. Lower case letters group species that did not differ based on Scheffé's test after a one-way ANOVA on cased larvae

other prey (also see Holomuzki 1989; Johansson 1991; Johansson and Englund 1995; Wissinger et al. 1999a). Thus, effective pre-attack behaviors that reduce the probability of detection by salamanders are the primary defense of these *Limnephilus* spp., and cases act mainly as a secondary defense when that primary defense fails (as in Galatowitsch and Mumme 2004). The large cases of *L. externus* larvae are more effective than those of *L. picturatus*, which, once detected and attacked, are as likely to be captured as *A. nigriculus* larvae.

Cases and beetle predation

Foraging behaviors of *Dytiscus* beetle larvae vary considerably among species and even within a species across prey types (Formaniwicz 1982; Johansson and Nilsson 1992; Kriska and Schmera 2002). Larvae of the *Dytiscus* species at our study site (*D. dauricus*) attack caddisflies by grasping the case and probing the sides with hollow mandibles (as observed in *D. circumcinctus* in Johansson and Nilsson 1992). When a caddisfly is pierced by a beetle's mandibles and injected with proteolytic enzymes, it often crawls out of the case at which point it is directly grasped by the beetle. Given this attack mode, it is not surprising that *L. externus*, the species with the stout case was much less vulnerable to beetle predators than the three species with narrow tubular cases (Figs. 6, 7). The efficacy of the bulky cases in protecting *L. externus* from attacking beetles is emphasized by the long handling time compared to the other species (Fig. 8). Of the other species, the high vulnerability of *A. deflata* also fit our prediction, given that beetles are rarely abundant in permanent ponds because of salamander predation (Wissinger et al. 1999b).

The vulnerability of the other two species (*A. nigriculus* and *L. picturatus*) to beetle predation is not con-

sistent with the prediction that cases should be most effective against the predators with which species typically coexist. One explanation is that these caddisflies develop rapidly and pupate in midsummer before late-instar beetle larvae become abundant (Wissinger et al. 2003). *L. picturatus* burrow into soft substrates to pupate where they are undetectable by foraging beetle larvae. *A. nigriculus* pupate on exposed hard substrates where they are detected and often attacked by foraging beetle larvae (S. Wissinger, personal observation). The results of the experiment with *A. nigriculus* pupae demonstrate that switching to mineral cases during pupation more than doubles their survival rate compared to pupating with organic cases (Fig. 9). This shift in case composition occurs even in predator-free habitats, suggesting it is less plastic than that observed in caddisflies that only build mineral larval cases in the presence of predators (e.g., Otto and Svensson 1980; Nislow and Molles 1993). The switch to mineral cases in *A. nigriculus* occurs abruptly before pupation, perhaps to minimize the energetic costs of carrying stone cases (Otto and Svensson 1980; Otto 2000).

Caddisfly defense along permanence gradients

Although many studies have shown that cases decrease the vulnerability of caddisflies to predators, only a few have assessed the degree to which the effectiveness of cases is predator specific (Johansson 1991; Johansson
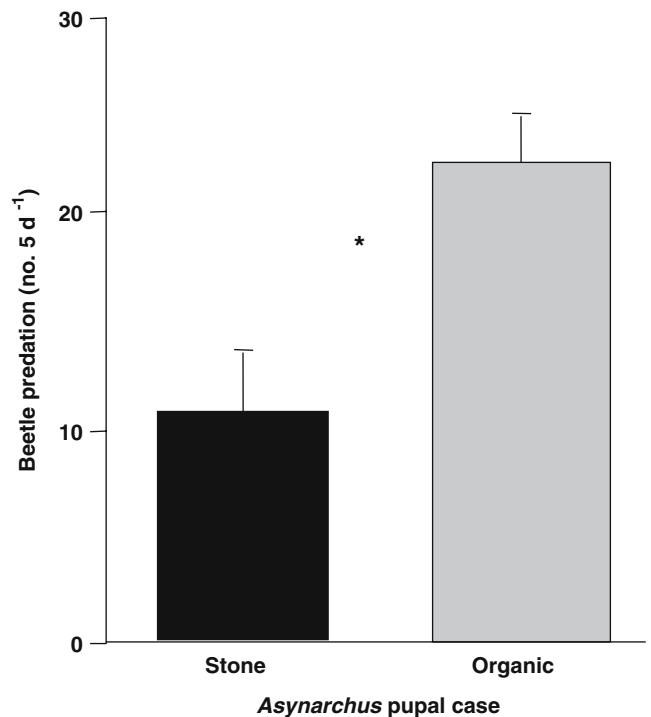

Fig. 9 Beetle predation (mean ±1 SE) of stone case (*filled box*) and organic case (*shaded box*) *Asynarchus* pupae in a 5-day mesocosm experiment. *Asterisk* indicates a significant difference ($P < 0.05$) based on *t*-test

and Johansson 1992; Nislow and Molles 1993). In our study, there is only one species (*A. deflata*) for which the design of the case appears to have tradeoffs in effectiveness against different types of predators. The disproportionately long tubular cases (1.7–1.9 × body length) of *A. deflata* provide an effective refuge from salamander predators in permanent pond, but are ineffective against beetles, a predator with which this species rarely co-occurs. This tradeoff resembles that observed for different species of damselflies and *Rana* tadpoles that exhibit behaviors that are either effective against dragonfly or fish predators (e.g., Werner and McPeek 1994; McPeek 1990b; Stoks and McPeek 2003). Salamanders should indirectly benefit *A. deflata* by excluding *Dytiscus* beetles from permanent ponds, much in the way centrarchid fish indirectly benefit permanent pond amphibians and damselflies by excluding large dragonflies (Werner and McPeek 1994).

The larval cases of *A. nigriculus* and *L. picturatus* play a relatively minor role in defense against both types of predators. The tradeoffs that underlie distributional patterns of these species are similar to those described for hylid frog tadpoles in which traits that facilitate rapid growth in temporary habitats render tadpoles vulnerable to permanent-habitat predators (Skelly 1995, 1997; Leips et al. 2000). The growth rate–vulnerability tradeoff for *A. nigriculus* involves several traits including (1) high activity (Wissinger et al. 1999a), (2) mob cannibalism, which provides a diet supplement but attracts predators (Wissinger et al. 2004a), and (3) minimal investment in case size (only 1.1–1.2 × body length). Given the energetic costs of making silk (Huryn and Wallace 2000; Stevens et al. 2000), temporary habitat caddisflies should invest minimally in cases, unless they play a role in delaying desiccation (Zamora-Munoz and Svensson 1996). Because *A. nigriculus* cases deter cannibalism (Wissinger et al. 2004b), investment in cases is probably a compromise between allocating energy to rapid development and the minimum case size that protects individuals from conspecifics.

The ineffectiveness of *A. nigriculus* and *L. picturatus* cases against beetle predators is probably mitigated by early pupation. Larval development is rapid in both species and elsewhere we argue that this facilitates the timely escape from early-drying *vernal* habitats (Wissinger et al. 2003). The results presented here suggest that rapid development also reduces temporal overlap with an *autumnal* habitat predator (beetle larvae) to which both species are extremely vulnerable. This might explain why these species emerge as early in autumnal (no time constraint) as in vernal habitats, thus forgoing the benefits of extending the larval growth period (increased adult body size and fecundity; Peckarsky et al. 2001).

Developmental phenology plays a different role in defense against predators for *L. externus*. Larvae quickly molt through the first four instars during early summer, but then persist (in the absence of drying) in the fifth (final) instar for 4–8 week in late summer during which time body size and the proportional size of cases increases dramatically. The large cases of final instars are effective against both beetle and salamander predators. The relative invulnerability of *L. externus* to both salamanders and beetles is consistent with the wide distribution of this species across permanent and autumnal temporary habitats. The traits that facilitate coexistence with predators (protracted larval development, investment in large cases, low activity rates; Wissinger et al. 1999a) probably prevent this species from exploiting the vernal wetlands inhabited by *A. nigriculus* (Fig. 1).

In summary, several types of tradeoffs that are similar to those previously described for damselflies and amphibians appear to underlie the realized distributions of subalpine caddisflies along a permanence gradient. The low vulnerability of caddisfly species to the predators with which each typically coexists depends on the interactive effects of several phenotypic traits that have probably evolved in concert (Richardson 2002). For two species, predator avoidance depends on a combination of a relatively large investment in cases and behaviors to effectively exploit the protective value of those cases (*L. externus* and *A. deflata*); whereas in the other two species with low investment in cases (*A. nigriculus* and *L. picturatus*), it depends on rapid development that facilitates the use of vernal habitats, and reduces temporal overlap with autumnal-habitat predators. Expanding comparative trait analyses to other species of phryganeid and limnephilid caddisflies will be required to determine whether the particular tradeoffs that we observed in this study are of general importance for explaining patterns of distribution and the diversification of lineages in these taxa (as in McPeek and Brown 2000; Richardson 2002).

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