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LETTER

Alternative growth and energy storage responses to mortality threats in damselflies

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Abstract

The role of physiology in mediating the growth/predation risk trade-off has been largely ignored. We examined effects of predation risk on relationships between growth and storage molecules in *Enallagma aspersum* and *Ischnura verticalis* damselfly larvae that differ in this trade-off. In laboratory and field experiments, both species had similar growth and mortality rates and similar concentrations of storage molecules in the absence of mortality threats. However, in the presence of dragonfly predators *Ischnura* larvae had higher mortality rates and grew faster than *Enallagma* larvae. Consistent with the difference in growth rate, *Enallagma*'s total protein concentrations decreased under predation risk while those of *Ischnura* did not. Glucose and glycogen concentrations were not affected, while triglyceride concentrations were lower under predation risk in *Enallagma* but not in *Ischnura*. Species differences at the physiological level to the presence of mortality threats may be crucial to understanding patterns in metamorphic and post-metamorphic traits.

Keywords

Carry-over effects, energy storage, growth/predation risk trade-off, life history plasticity, physiological stress, predation risk.

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INTRODUCTION

Animals that grow faster often suffer higher rates of mortality from predators. This so-called growth/predation risk trade-off plays a fundamental role in shaping life histories, species interactions and ultimately community assembly (Houston *et al.* 1993; Werner & Anholt 1993; Leibold 1996, 1998; McPeek 1996; Wellborn *et al.* 1996). Despite its importance, surprisingly little attention has been given to the mechanism underlying the growth/predation risk trade-off (Noonburg & Nisbet 2005). The typical assumption is that the trade-off is primarily behaviourally mediated (Lima 1998). Consequently, many studies have shown that prey organisms reduce their activity in the presence of predators to reduce detection, and these responses are correlated with decreases in growth as well, presumably because decreased activity resulted in decreased feeding (reviewed in Lima & Dill 1990; Lima 1998). However, growth rate is determined by much more than simply activity. For example, several studies showed that animals' growth rates decrease under predation risk, and that these reductions could not be explained by a decrease in foraging rate (Duvall & Williams 1995; Hechtel & Juliano 1997; Boonstra *et al.* 1998; McPeek *et al.* 2001; Stoks 2001; Stoks & McPeek 2003b; McPeek 2004). In particular, physiological mechanisms influencing the processing of ingested food and the metabolic allocation of assimilated food may also play a key role in mediating the growth/predation risk trade-off (McPeek 2004; Noonburg & Nisbet 2005; Stoks *et al.* 2005).

Physiological mechanisms that may be involved in the trade-off are the allocation of assimilated food to build new tissue, to storage pools and the depletion of these storage pools under predation risk (Perrin & Sibly 1993). In arthropods, two types of storage pools can be distinguished. Arthropods can only increase in size at ecdysis when they shed their exoskeleton; at this time they can build new tissues to increase the sizes of body parts. During an instar, they must store accumulated amino acids to be used to build these new tissues in large storage proteins that are dissolved and circulate in the haemolymph (Telfer & Kunkel 1991). At a moult, these storage proteins are hydrolysed, and the liberated amino acids used to build the necessary structures to increase in size. A second type consists of molecules involved in the short-term (glucose and glycogen) or long-term (triglycerides) storage of energy to fuel behavioural

activity (Nation 2002). Under predation risk, reductions in these storage pools are likely for two reasons. First, behavioural responses to mortality threats may lower food intake, which will reduce the rates at which molecules are added to these pools. Second, prey animals may have physiological mechanisms that alter how assimilated food is allocated to various metabolic needs. For example, two recent studies demonstrated that fish invested less in energy storage when threatened by predators (Pratt & Fox 2002; Garvey *et al.* 2004). However, these studies could not discriminate whether this pattern was caused by behaviour or physiology. Identifying whether these storage pools are affected by predation risk will provide new insights into the possible consequences of non-consumptive predator effects. Such predator effects can be more important for population dynamics than the direct mortality imposed by predators in many species (McPeek & Peckarsky 1998; Preisser *et al.* 2005). Although most studies focused on predator-induced effects on life history traits like growth, age and size at emergence (Lima 1998), predator-induced reductions in energy storage pools may also have consequences on many other fitness components, for example linked to winter survival and mating success (Lee & Denlinger 1991; Plaistow & Siva-Jothy 1996).

In this study, we compare how mortality threats influence growth and pools of various molecules for species in two genera of damselflies. As theory predicts, *Enallagma* and *Ischnura* damselflies (Zygoptera: Coenagrionidae) appear to coexist as larvae because they settle the growth/predation risk trade-off in different ways (McPeek 1996, 1998). *Ischnura* larvae suffer higher mortality from the top predator (fish or dragonfly larvae) but grow substantially faster than *Enallagma* larvae (McPeek 1998). These growth differences in the presence of predators do not result from *Ischnura* larvae ingesting more food; rather, *Ischnura* larvae grow faster than *Enallagma*, because they convert more ingested food into their own biomass than do *Enallagma* larvae (McPeek *et al.* 2001; McPeek 2004). These growth differences are not apparent when larvae are held in complete isolation of all mortality threats. These results indicate that in these damselflies behaviour does not mediate the growth/predation risk trade-off (McPeek 2004). Instead, *Ischnura* and *Enallagma* species have different levels of physiological responses to the presence of mortality threats that decrease growth to different degrees (McPeek *et al.* 2001; McPeek 2004). One way to test the efficacy of the physiological mechanisms mediating growth differences between these taxa is to examine the levels of storage molecules possessed by larvae of these two genera when held under different mortality threats. We predicted that species showing lower growth rates under mortality threats should have (1) lower total protein levels, which should reflect differences in the concentrations of protein storage molecules; and (2) lower energy storage pools, because their physiological responses to the mortality threats in some way prevent them from accumulating these molecules.

To test these predictions, we performed a series of laboratory and field experiments that placed *Ischnura* and *Enallagma* larvae under various levels of mortality threat, and compared the concentrations of these molecules in larvae. Because, previous work showed the generality of the growth and physiological responses across *Ischnura* and *Enallagma* species living in both fish lakes and fishless lakes (McPeek 2004), we have performed this more detailed study on *Ischnura verticalis* (Say) and *Enallagma aspersum* (Hagen) as representatives of the two genera. These two species are found together at ponds and lakes that are dominated by large dragonfly predators across eastern North America.

METHODS

Laboratory experiment

We quantified the changes in growth rate and storage pools under predation risk in a short-term (5 days) experiment with two treatments: one in which larvae were held in complete isolation of all mortality threats, and another in which larvae were held in the presence of other damselflies and predatory *Anax* dragonflies. *Anax* is the dominant predator in fishless ponds in eastern North America (McPeek 1990; Stoks & McPeek 2003a). Coenagrionid damselfly larvae are cannibalistic and perceive each other as predators (McPeek & Crowley 1987). We only included the combined exposure treatment as previous experiments showed that digestive physiological responses of *Enallagma* and *Ischnura* larvae to the presence of other damselflies and to the combined presence of other damselflies and other predators are not different (McPeek *et al.* 2001). Fifteen larvae of each species were included in each of these two treatments. We used similar rearing conditions as described in McPeek *et al.* (2001) and McPeek (2004). Under these conditions, *Enallagma* and *Ischnura* growth rates decrease in the presence of a predator without associated decreases in food uptake due to decreases in the amount of assimilated food that is converted into body mass (McPeek 2004).

Larvae were collected on 22 September 2004 in Sylvester's pond, a small fishless farm pond in Norwich, VT, USA and housed in 20 mL glass vials containing pond water and a wooden dowel to serve as perches and were fed *Daphnia pulex* ad libitum every day. Vials were maintained in large trays with cardboard dividers to isolate larvae from each other, and held in an incubator at 21 °C on a 14L : 10D photoperiod.

We installed the experimental conditions, 1 day after a larva moulted in the laboratory. Larvae assigned to the treatment without predators were placed individually in a

new 20 mL glass vial in another tray with cardboard dividers. Larvae assigned to the treatment with predators were placed individually in a new 20 mL glass vial that was floated in a 2 L aquarium containing four penultimate instar *A. junius* dragonfly larvae. To the top of each vial, a 45-mm-tall cylinder of 0.5-mm-mesh Nitex cloth was attached with a cable tie, and this cloth was attached to a small Styrofoam float with a metal insect pin. As water could freely pass through the cloth, damselfly larvae could receive both visual and chemical stimuli from the dragonfly predators in the aquarium and from other damselflies in similar floating vials. Four vials, two with *Enallagma* larvae and two with *Ischnura* larvae were present in each aquarium. Larvae were randomly reassigned to aquaria each day. Dragonfly larvae in the aquaria were daily fed two *E. aspersum* and two *I. verticalis* larvae. Cardboard trays and aquaria with vials were placed in the same incubator as before. Larvae were fed 20–30 *D. pulex* daily. This ration was adjusted for each larva so that at least one or two uneaten *Daphnia* were recovered the next day. *Daphnia* were size-sorted before being fed to larvae: only those passing through a 1.00-mm and retained by a 0.71-mm mesh sieve were used.

We quantified growth rate based on the increase in wet mass from the start until the end of the 5 day period as [ln(final wet mass) – ln(initial wet mass)]/5 days. Wet masses were measured to the nearest 0.01 mg using an Ohaus Analytical Plus electrobalance (Ohaus, Florham Park, NJ, USA) after gently blotting larvae dry with absorbent tissue. At the end of each experiment, larvae were stored individually in microcentrifuge tubes in a −80 °C freezer for later quantification of storage pools. All larvae were processed within 6 months of storage in the freezer.

We analysed the effects of species and predator treatment on growth rate and mass-corrected levels of molecules with ANCOVAs. Mass corrections were done by dividing the total quantity of a molecule measured for an individual by the dry mass of the individual. This enables comparison among studies as opposed to presenting least squares means. Dry masses were obtained from wet masses, assuming that dry mass is 15.0% of wet mass in coenagrionid larvae (McPeek *et al.* 2001).

Field enclosure experiment

To quantify the effects of predators on growth rate and molecule concentrations in the field, we also performed a field enclosure experiment with three treatments: a low-density treatment without predator, and two high-density treatments, one with and the other without predator. The low-density treatment (16 larvae in an enclosure with a bottom area of 0.073 m^2 giving a density of 220 larvae m^{-2}) without predators paralleled the 'complete isolation' laboratory treatment which was logistically not feasible to install. This is a reduced mortality risk treatment as previous work has shown that growth rates in field enclosures only decrease at total densities of *c.* 400 larvae m^{-2} (McPeek 1998). The high-density treatment with predator mimicked the natural conditions in the pond by having larvae at roughly their natural density (60 larvae per enclosure; *c.* 820 larvae m^{-2}) and having one *A. junius* larva to feed on the damselflies; this treatment was meant to parallel the 'mortality risk' treatment in the laboratory. In addition, we also installed a high-density treatment without *Anax* predator. Based on previous field experiments (McPeek 1990, 1998), we expected that *Enallagma* larvae in this treatment would have high survival as in the low-density treatment and slow growth rate as in the high-density treatment with the *Anax* predator. The low-density treatment was replicated five times; both treatments at high density were replicated three times.

To establish the low and high-density treatments, we added eight or 30 damselfly larvae of each species to the enclosures, respectively. The resulting densities are within the range of observed damselfly densities in fishless lakes (McPeek 1998). Larvae were collected by dip net in Sylvester's Pond on the same day. One penultimate-instar *Anax* dragonfly larva was added to three cages 4 days after the damselfly larvae were added. The experiment was started on 21 September, and terminated on 6 October. All larvae recovered from enclosures were sorted to species, gently blotted dry, and submerged in liquid nitrogen in the field.

The methods used in the enclosure experiment closely followed those of previous studies (McPeek 1990, 1998; Stoks & McPeek 2003a). Experiments were carried out in semi-permeable enclosures in which small prey items could pass freely in and out of the enclosures. Enclosures were cylindrical chicken-wire frame cages (30 cm diameter, 1.2 m high) covered with a bag made of nylon mosquito netting (0.6 × 1.2 mm mesh size). The nylon bag was filled with *Chara* vegetation in natural densities. Eleven enclosures were placed in water at a depth of 80 cm with their open top extending 40 cm above the water level. No immigration of aquatic insects was detected. Enclosures were placed 1 week before adding the damselfly larvae to allow colonization by prey items. Coenagrionid damselfly larvae show natural mortality and growth rates under appropriate treatments in these enclosures (McPeek 1990, 1998).

Mortality and growth rates were calculated separately for each species in each enclosure. Mortality rate for each cage was calculated as –[ln(number recovered) – ln(number added)]/15 days. Growth rate for each species in each enclosure was based on the mean wet mass of recovered larvae, and the mean wet mass of a random sample of 30 larvae of each species collected when the experiment was initially stocked with larvae. To quantify molecules, we analysed 15 larvae per species from the initial sample and

15 larvae per species for each of the three treatments at the end of the experiment (total of 120 larvae). The 15 larvae per species by treatment combination were evenly sampled from the replicate enclosures: for the low-density treatment, we randomly analysed three larvae per enclosure, for both high-density treatments we randomly analysed five larvae per enclosure. Three measurements of triglyceride content were considered as outliers (> 3 SD larger than the mean) and not included in the analyses. No transformations were done as assumptions of ANOVA were not violated. We used enclosures as our experimental units and therefore calculated enclosure means for each molecule concentration. Because each enclosure contained the two focal species, we performed repeated measures ANOVAs (rmANOVAs) with predation risk treatment as independent variable and the response variables for each species as sets of 'repeated' measures on each experimental unit. In this analysis, significance of within subjects effects would identify a difference in the means of the two species between the treatments, and an interaction between the predator treatment and the within-subjects term would indicate that the species responded differently to the treatments.

Based on previous work (McPeek 1998; McPeek *et al.* 2001), we had *a priori* expectations that treatment effects would differ between the species and different variables would show different patterns of response for each species. Therefore, we constructed two sets of linear, orthogonal contrasts, and tested the interaction between species and each of these contrasts. For mortality rate, we expected strong predator effects for *Ischnura* and weaker effects for *Enallagma*, and no species differences in mortality changes at low and high density in the absence of the *Anax* predator. Therefore, a first contrast tested for differences between the combined response in the treatments without *Anax* (the low-density treatment and the high-density treatment without *Anax*), and the high-density treatment with *Anax*. A second contrast tested for differences between the high-density treatment without *Anax* and the high-density treatment with *Anax*. For growth rate and molecule concentrations, we expected strong negative effects of density for *Enallagma* and weaker effects for *Ischnura* and no additional effects of the *Anax* predator at high larval density where larvae already suffered considerable predation risk by conspecifics. Therefore, a third contrast tested for differences between the low-density treatment and the combined response at the high-density treatments (without and with *Anax*). A fourth contrast tested for differences between the high-density treatments without *Anax* and with *Anax*.

Quantification of storage molecules

To quantify molecular pools, we measured the amounts of free glucose, glycogen, triglycerides and total proteins in each larva. We were unable to measure the amounts of storage proteins directly, and so we use total protein as a surrogate measure. Insects also store glucose in the disaccharide trehalose, but preliminary measures indicate that trehalose concentrations in damselfly larvae are < 10% of free glucose concentrations (M.A. McPeek, unpublished data). Because of the expense of measuring trehalose, we have not measured this small fraction of the total glucose pool.

For quantification of the molecular pools, each larva was homogenized in a microcentrifuge tube in 150–300 μL of 50 mM imidazole buffer, depending on its wet mass, while on ice. To prevent protein degradation, the imidazole buffer contained a broad spectrum protease inhibitor cocktail at the manufacturer's recommended concentration (Complete Protease Inhibitor Cocktail, Roche Diagnostics, Penzberg, Germany). This sample was then centrifuged at 1310 ***g*** for 10 min at 4 °C. The supernatant was then assayed for the concentrations of the various molecules. Throughout this procedure samples were maintained on ice, and assays were performed within 2 h of homogenization. Quantification of each molecule was done colorometrically using a Molecular Devices (Sunnyvale, CA, USA) SpectraMax 190 96-well plate spectrophotometer at a temperature of 25 °C. Each molecule was assayed in duplicate, and the mean of the duplicate readings was taken as the measurement for that individual.

To quantify the total amount of free glucose plus glycogen in each sample, we used the Glucose oxidase method (Trinder 1969) closely following the protocol provided by Thermo Electron (Louisville, CO, USA). We added 75 μL of H_2O containing 1 U of amyloglucosidase to 25 μL of the supernatant. This mixture was incubated for 1 h at 37 °C to hydrolyse the glycogen into glucose. Total glucose in the sample was then determined using the Glucose Oxidase Method Kit (Thermo Electron) by adding 25 μL of the resulting sample to 200 μL of the enzymatic glucose reagent, and reading the absorbance at 500 nm after 20 min of incubation at room temperature in the dark. To quantify the amount of only free glucose, we followed the same procedure except that the initial 25 μL supernatant sample was added to 75 μL of H_2O without amyloglucosidase. The difference in glucose content between these two samples equalled the amount of glucose stored in glycogen.

Triglyceride content of a sample was determined using the triglycerides lipase/peroxidase method (Fossati & Prencipe 1982; McGowan *et al.* 1983) closely following the protocol provided by the Infinity Triglyceride kit of Thermo Electron. Two point five microlitre of supernatant was added to 125 μL of triglyceride reagent, and the absorbance measured at 520 nm after 10 min of incubation at room temperature in the dark. Several of the triglyceride values were estimated to be slightly negative based on the standard

lines converting absorbances to concentrations. We included these negative values in analyses, even though negative amounts are not possible; remember that these values are all measured relative to a standard curve. Analyses of data replacing the negative values with zeroes gave identical conclusions.

Total protein content of a sample was quantified using the Bio-Rad (Hercules, CA, USA) protein assay based on the Bradford method (Bradford 1976). The supernatant was first diluted 200 times with water, and 160 μL of this diluted supernatant was combined with 40 μL of dye reagent. The absorbance was read at 595 nm after 15 min of incubation at room temperature. A bovine gamma globulin standard (Bio-Rad catalogue number 500-0005) was used to construct standard curves.

RESULTS

Laboratory experiment

Both species grew more slowly in the presence of the *Anax* predators (ANOVA, predator effect: $F_{1,56} = 36.77$, $P < 0.0001$; Fig. 1). *Enallagma* growth was more greatly depressed (62.9%) than *Ischnura* (25.8%) by the dragonfly's presence (predator × species: $F_{1,56} = 5.15$, $P = 0.027$).

The response of molecule concentrations to predators differed between the two species (MANOVA, predator × species: $F_{4,53} = 8.18$, $P < 0.0001$). Triglyceride and total protein concentrations in *Ischnura* larvae were similar in the two treatments, but *Enallagma* larvae had less triglyceride and total protein per unit mass in the presence of dragonflies as compared with when they were held in isolation (ANOVAs, predator × species terms for triglycerides, $F_{1,56} = 22.29$, $P < 0.001$; for proteins, $F_{1,56} = 9.50$, $P = 0.0032$; Fig. 2c–d). Glucose and glycogen levels were not affected by exposure to predators in either species (all $P > 0.20$) (Fig. 2a,b).

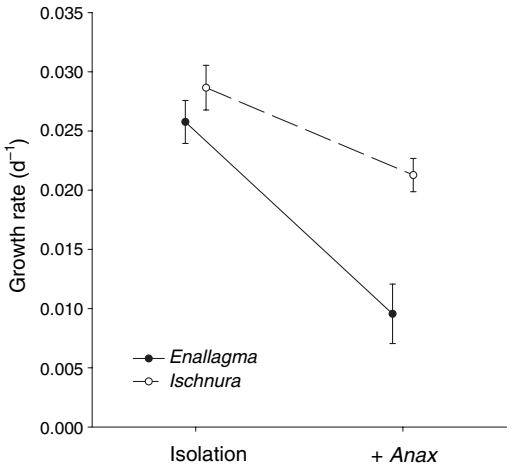

Figure 1 Growth rate of *Enallagma* and *Ischnura* larvae in isolation and in the combined presence of conspecific larvae and *Anax* dragonfly larvae in the laboratory experiment. Means ± 1 SE are presented.

Field enclosure experiment

Both species had a higher mortality rate in the presence of the dragonfly, with *Ischnura*'s mortality rate being significantly higher than that of *Enallagma* (rmANOVA, species × contrast 1; Table 1, Fig. 3a). Mortality rates in the absence of *Anax* did not differ between high density and low density (contrast 2; Table 1). While *Enallagma* grew slower at high density than at low density, this was not the case for *Ischnura* (species × contrast 3; Table 1, Fig. 3b). As a result, the two species' growth rates were similar at the low density, but *Ischnura* growth rates were higher than those of *Enallagma* at high density. Growth rates at high density were not affected by the presence of the *Anax* predator (contrast 4; Table 1).

At the start of the experiment, molecule concentrations were not different between the species (MANOVA, species main effect: $F_{4,25} = 1.12$, $P = 0.37$; Fig. 2a–d), but molecule concentrations responded differently to mortality threats in the two species (species × contrast 3; Table 1, Fig. 4). Triglyceride and total protein concentrations in *Ischnura* larvae were similar at the low-density and both high-density treatments, but *Enallagma* larvae had less triglyceride and total protein per unit mass at high density in both the absence and presence of dragonflies as compared with when they were held at low density (contrast 4; Table 1, Fig. 4c,d). Glucose and glycogen levels were not different among the treatments (Table 1, Fig. 4a,b).

DISCUSSION

The data from our field experiment are consistent with the growth/predation risk trade-off (Werner & Anholt 1993) and previous field experiments comparing survival and growth of these two genera (McPeek 1998). In the presence of a dragonfly predator both growth rate and mortality rate were higher in *Ischnura* than in *Enallagma*. Growth differences between genera were absent at low density, but at high-density *Enallagma* had a much lower growth rate than at low density, while growth rate of *Ischnura* was not affected by density (Fig. 3b). Growth differences between the genera were also observed in the presence of a dragonfly predator in the laboratory experiment where larvae were fed *ad libitum* food amounts (see also McPeek *et al.* 2001; McPeek 2004). This indicates that food limitation at high density alone cannot explain our results (see also McPeek 1998). Damselfly larvae are

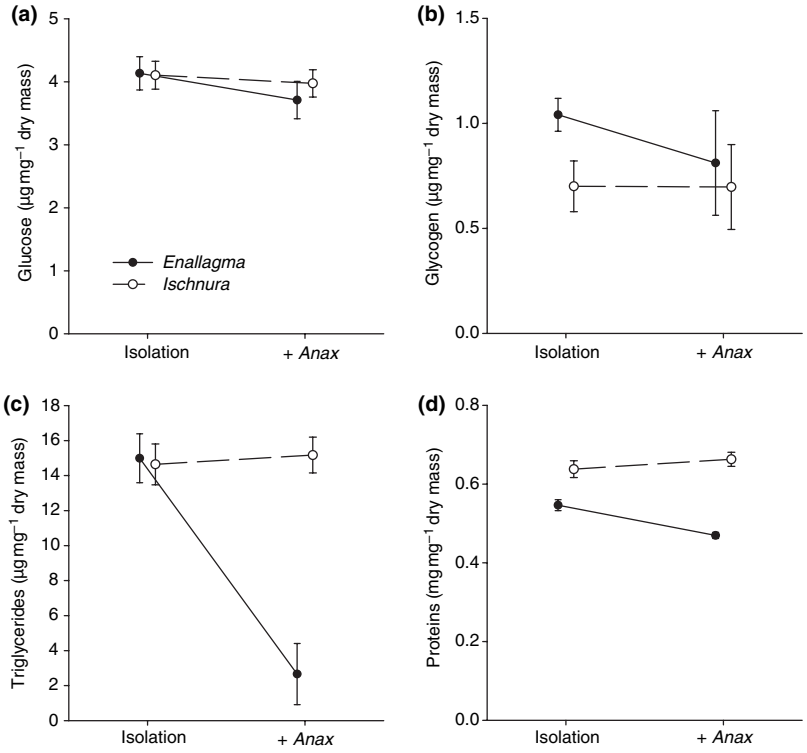

Figure 2 Mass-corrected levels of glucose (a), glycogen (b), triglyceride (c) and protein (d) of *Enallagma* and *Ischnura* larvae in isolation and in the combined presence of conspecific larvae and *Anax* dragonfly larvae in the laboratory experiment. Means ± 1 SE are presented.

Table 1 Contrast analyses testing for differences in mortality rate, growth rate and mass-corrected levels of storage molecules among treatment levels for *Enallagma* and *Ischnura*

Response variable	Species × Contrast	*Enallagma*	*Ischnura*	Species × Contrast	Both species
	Contrast 1 (low density no *Anax* and high density no *Anax* vs. high density with *Anax*)			Contrast 2 (low density no *Anax* vs. high density no *Anax*)	
Mortality rate	10.85*	241.62***	56.65***	0.21	4.72
	Contrast 3 (low density no *Anax* vs. high density no *Anax* and high density with *Anax*)			Contrast 4 (high density no *Anax* vs. high density with *Anax*)	
Growth rate	5.58*	30.14***	0.26	0.05	0.40
Glucose	2.42	2.61	0.57	0.19	1.06
Glycogen	3.27	2.87	1.17	3.09	4.10
Triglycerides	5.26*	16.83**	0.04	0.30	0.45
Proteins	9.41*	28.75***	0.56	1.98	2.47

When the interaction between species and the contrast was significant, contrasts are given separately per species, otherwise the joint contrast for both species is given. Given are *F*-values from rmANOVAs with d.f. = 1, 8.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

cannibalistic (McPeek & Crowley 1987), and therefore represent mortality threats to one another in addition to the threats posed by other predators. Growth rates in the presence of predators in these laboratory experiments are very similar to growth rates observed in the field (McPeek *et al.* 2001; McPeek 2004; this study), indicating that the differential responses of *Ischnura* and *Enallagma* species to mortality threats is the primary cause of their growth differences in the field.

Previous laboratory experiments showed that *Ischnura* and *Enallagma* species ingest the same amounts of food in both the presence and absence of predators over the course of days (McPeek *et al.* 2001; McPeek 2004). In addition, the presence of mortality threats does not

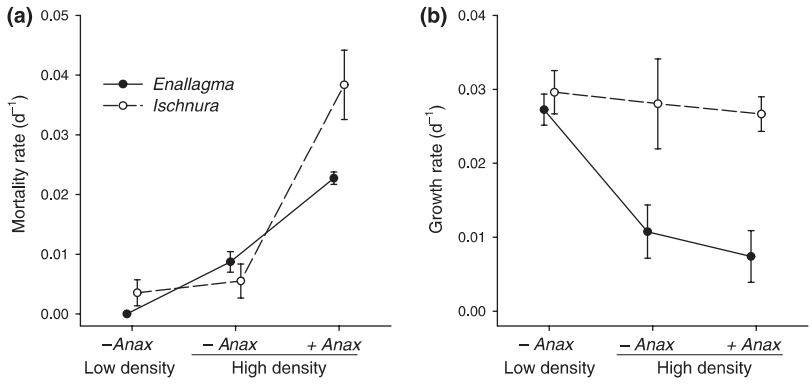

Figure 3 Mortality rate (a) and growth rate (b) of *Enallagma* and *Ischnura* larvae in the different predation risk treatments in the field enclosure experiment. Means ± 1 SE are presented. There was no variation for the mortality rates of *Enallagma* at low density in the absence of *Anax*.

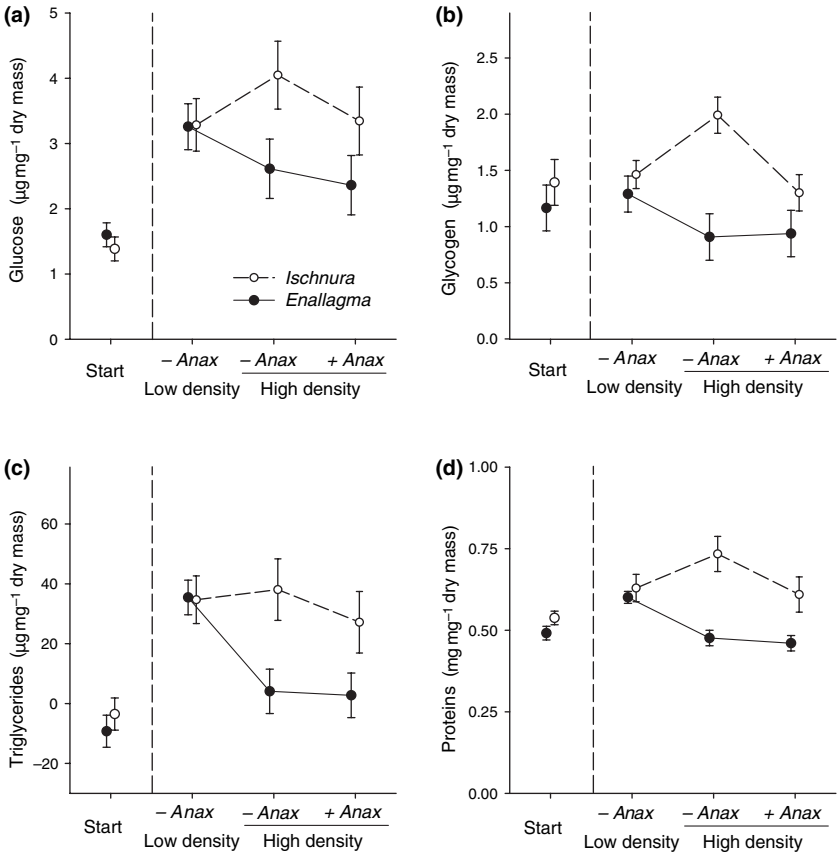

Figure 4 Mass-corrected levels of glucose (a), glycogen (b), triglyceride (c) and protein (d) content of *Enallagma* and *Ischnura* larvae at the start and in the different predation risk treatments at the end of the field enclosure experiment. Means ± 1 SE are presented.

negatively affect the amount of ingested food that is assimilated (McPeek 2004). The primary difference between the genera appears to result from differences in what is done with this assimilated food. *Enallagma* species, including the study species, show a larger reduction in the efficiency of converting assimilated food into their own biomass in the presence of mortality threats than *Ischnura* (McPeek *et al.* 2001; McPeek 2004). Thus, the difference in growth between the genera must lie in the molecular machinery they possess to process assimilated food into the various metabolic needs.

Our laboratory and field results confirm this conclusion, as *Enallagma* larvae had lower concentrations of total proteins and triglycerides in the presence of mortality threats than *Ischnura* larvae. Our total protein measures can account for 60–70% of the total dry mass of larvae,

meaning that differences in the accumulation of mass by larvae over the course of the experiments was primarily the result of differences in protein accumulation. Within an instar, damselfly larvae, like other arthropods, store amino acids in large storage molecules (Telfer & Kunkel 1991) in their haemolymph. We hypothesize that the presence of mortality threats induce molecular physiological changes in *Enallagma* larvae that either decrease the production rate of these storage proteins or increase their degradation rate. Parallel responses in other metabolic pathways may influence triglyceride concentrations. Interestingly, concentrations of glucose and glycogen, the molecules that fuel short-term behavioural and physiological demands were not affected. Various stressors have been shown to elevate haemolymph levels of neurotransmitters such as octopamine, dopamine and serotonine in insects (e.g. Woodring *et al.* 1988; Adamo *et al.* 1995; Hirashima *et al.* 2000; Gruntenko *et al.* 2004). These neurotransmitters are the mediating signalling system for such physiological responses. Increased levels of these neurotransmitters cause increased mobilization of carbohydrates and lipids into the haemolymph (Fields & Woodring 1991; Meyer-Fernandes *et al.* 2000) and are associated with decreased growth rates (Woodring *et al.* 1988). Whatever the exact mechanism, the fact that many studies have shown growth reductions in prey animals that cannot be explained by reduced foraging (see Introduction) suggests that predator-induced physiological responses may be widespread, and these changes may often manifest themselves as differences in the concentrations of critical molecules like triglycerides and proteins. Interestingly, our results also suggest that species may differ largely in these predator-induced physiological responses, and that this may be linked to how they settle the growth/predation risk trade-off.

The negative, non-consumptive effects of predation risk on the concentrations of critical molecules may cause long-term fitness costs in the larval and adult stage. Coenagrionid damselfly larvae overwinter in their pond and emerge the following spring. Winter survival is likely to be dependent upon long-term energy storage (e.g. triglycerides) (Lee & Denlinger 1991), which may lead to reduced winter survival in predator-stressed larvae. Further, lower energy pools are likely to persist into the adult stage and may affect survival to maturation and mating success (Plaistow & Siva-Jothy 1996). These reductions in energy pools are not captured by measuring typical life history variables like age and size at emergence and therefore may cause hidden carry over effects to the adult stage (De Block & Stoks 2005). Predator-induced changes in physiology that bridge the transition between the larval and adult stage may be widespread in animals with a complex life cycle. For example, Benard & Fordyce (2003) showed that in larval western toads, *Bufo boreas*, age and size at emergence were not affected by predation risk in the larval stage but that tadpoles reared under predation risk had reduced post-metamorphic chemical defences, suggesting a physiological carry-over effect from the larval into the adult stage. As suggested by our results, such carry-over effects may strongly differ between species that differ in how they settle the growth/predation risk trade-off.

A recent review showed that predator-induced plasticity in metamorphic traits in animals with a complex life cycle may be related to predator-induced changes in larval morphology and behaviour and urged that future work should incorporate more detailed studies of growth rate, morphology, and behaviour during the larval period (Benard 2004). Based on this study and our previous work on this problem, we also submit that the physiological consequences of stress from various agents of mortality may be at least as important as morphology and behaviour to understanding patterns in both metamorphic and post-metamorphic traits within and among species. Damselflies respond to predators and other mortality threats by decreasing activity, but these behavioural responses cannot account for the concomitant alterations of growth rates that are seen in the presence of these mortality threats (McPeek *et al.* 2001; Stoks & McPeek 2003b; McPeek 2004). Changes in growth are only accountable by understanding physiological changes in assimilation and utilization of ingested resources (McPeek 2004). Moreover, the results of the current study challenge many current formulations of physiological models that try to understand the population dynamic and ecosystem function consequences of energy allocation in individual organisms (cf. Gurney & Nisbet 2004; Kooijman *et al.* 2004).

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