BEHAVIOURAL ECOLOGY

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Resource limitation, predation risk and compensatory growth in a damselfly

Abstract Periods of poor nutrition during early development may have negative fitness consequences in subsequent periods of ontogeny. In insects, suppression of growth and developmental rate during the larval stage are likely to affect size and timing of maturity, which in turn may lead to reduced reproductive success or survivorship. In light of these costs, individuals may achieve compensatory growth via behavioural or physiological mechanisms following food limitation. In this study, we examined the effects of a temporary period of food restriction on subsequent growth and age and size at maturity in the larval damselfly *Ischnura verticalis* (Odonata: Coenagrionidae). We also asked whether this temporary period of reduced nutrition affected subsequent foraging behaviour under predation risk. *I. verticalis* larvae exposed to a temporary food shortage suffered from a reduced growth rate during this period relative to a control group that was fed ad libitum. However, increased growth rates later in development ensured that adult body size measurements (head and pronotum widths) did not differ between the treatments upon emergence. In contrast, adult dry mass did not catch up to that of the controls, indicating that the increased growth rates for size dimensions occur at the cost of similar gains in mass. Predators reduced foraging effort of larvae, but this reduction did not differ between control larvae and those previously exposed to poor nutrition.

Keywords Foraging behaviour · *Ischnura verticalis* · Life-history plasticity · Odonata · Trade-offs

Introduction

Life in variable environments favours adaptive plasticity in metric and life history traits. According to theory, timing of and size at maturity will vary in predictable ways depending on ecological factors such as resource availability and predation risk (Rowe and Ludwig 1991; Abrams and Rowe 1996; Nylin and Gotthard 1998). These two life history traits tend to trade off with one another; while increasing size at emergence may elevate fecundity, accumulating size may increase instantaneous mortality or increase the probability of mortality prior to reproduction through a delay in maturation. These effects may further depend on the environmental conditions experienced during development, since levels of nutrition and predation risk are likely to have strong effects on growth, development, and instantaneous rates of mortality (reviews in Nylin and Gotthard 1998; Metcalfe and Monaghan 2001).

Somatic tissue growth is restricted to the larval stage, and fecundity tends to be an increasing function of body size in insects including odonates (Honek 1993; Sokolovska et al. 2000). Therefore, future reproductive success depends in large part on foraging and growth occurring prior to emergence. While insects may benefit by prolonging the larval stage to accumulate further mass, there are a number of potential benefits to early maturation. These include a reduction in cumulative predation risk, lengthening of the reproductive season and, in temperate-zone species, completion of maturation in time to maximise reproductive success prior to the onset of winter (e.g. Fincke 1986; Johansson and Rowe 1999; Johansson et al. 2001). Species may likewise have plastic development schedules in response to seasonal or other absolute time constraints. In *Lestes* damselflies, when photoperiod was adjusted to mimic late season, larvae responded with increased foraging effort and growth rate at the cost of increased predation risk (Johansson et al. 2001). Acceleration of pond drying had a similar effect in pool frogs, as tadpoles increased their development rate in

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order to reach the adult stage before the pond disappeared (Altwegg 2002).

Given the trade-off between accumulating mass and the delays to maturity required to do so, periods of resource limitation may favour compensatory growth later in life, especially when the growth period is constrained by seasonality (Metcalfe and Monaghan 2001). However, while compensatory growth may offset some costs of periods of resource limitation, it may itself be costly. For example, butterflies and wood frog tadpoles are more likely to increase foraging rates when resource levels are low, and suffer from proportional increases in predation (Gotthard 2000; Anholt and Werner 1998). Intrinsic costs of accelerated growth effort may include reduced longevity and depressed immune function (reviewed in Metcalfe and Monaghan 2001). Alternatively, limited resources may be preferentially allocated to the most critical requirements (i.e. Stevens et al. 1999; Morgan and Metcalfe 2001).

Ischnura verticalis (Odonata: Coenagrionidae) is a univoltine damselfly common to ponds across southern Ontario. This is an appropriate system for studying the effects of a trade-off between size and timing of maturity under conditions of resource limitation, since natural populations of *I. verticalis* larvae are subject to strong fluctuations in food supply due to seasonality and other factors (Baker 1989). While they appear to be fairly resilient to temporary food shortages (Richardson and Baker 1997; Baker 1989), growth and development rates, and in extreme cases survivorship, may be affected over the long term (Dixon and Baker 1988). Damselflies show plasticity of behaviour in response to predation risk, and growth and development rates respond to seasonal time constraints (Johansson and Rowe 1999; Johansson et al. 2001; Stoks et al. 2001). Larval damselflies, including *I. verticalis*, reduce foraging rates and suffer from reduced efficiency in converting food to mass in the presence of predators (Dixon and Baker 1988; McPeek et al. 2001). Resource limitation is likely to have important consequences during early development in particular, leading to the suppression of growth and development rates which, in turn, may affect survivorship and reproductive success later in life.

In this study, we examined the effects of a temporary period of reduced nutrition early in development on subsequent growth and age and size at maturity. We also asked whether this also affected subsequent foraging behaviour under predation risk. Larval growth rates were compared following exposure to the two food treatments (temporary food shortage or fed ad libitum), and the presence or absence of a predator was used in order to test for a mediating effect of delayed growth and development on subsequent foraging behaviour. Increased growth rates are predicted in the later instars for larvae that suffered food shortage earlier in life. These larvae are also expected to forage at higher rates in the presence of predators than will control larvae. Development time is expected to be less sensitive to food limitation than growth rate because of seasonal constraints on this temperate-zone species.

Materials and methods

Larval *I. verticalis* were collected using a dip net from the Burns Conservation Area pond in Halton County, Ontario, Canada, on 20 September, 2002. Individuals were placed separately in labelled 100-ml plastic cups filled with water and containing a wooden dowel 100×2 mm. Larvae were kept at room temperature (water temperature approximately 18°C) for 4 weeks, after which they were transferred to an environment chamber with a 12:12 light/dark cycle and a temperature of 12°C, replicating natural autumn conditions in southern Ontario. Throughout this period, damselflies were fed ad libitum with *Daphnia magna* from laboratory stock. The damselflies were randomly divided into two equal groups for the pre-observation food ration treatment on 19 October, and the temperature was raised to 18°C to stimulate foraging. Over a 4-week period, the first group (high food, n=88) was fed daily ad libitum and the second group (low food, n=89) was fed ad libitum every fourth day. In the latter treatment, excess daphnia were removed 24 h after they were added.

On 16 November, the temperature was lowered in two 7°C increments over 24 h to 4°C, the approximate temperature of water under ice in the winter. On 1 March, water temperature was increased to 16°C over a 24-h period (simulating natural spring conditions). Henceforth, both groups were fed ad libitum daily until the end of the experiment. Cups were checked daily for exuviae and dead individuals throughout the entire experiment. Prior to maturation, a piece of netting was placed over the cup to contain any emerging adult damselflies.

Growth and development

Various size metrics were measured on three dates: 2–6 January, 16 and 17 March and following emergence. Head width (measured as the widest point from the outside margin of the eyes), maximum pronotum width and wet mass were measured for all larvae. Prior to measurement, larvae were starved for 24 h to control for weight of undigested food in the gut and were placed on a tissue to absorb excess moisture. Adult mass was measured as the dry weight following drying at 60°C for 24 h.

Foraging behaviour under predation risk

The behavioural assay was initiated on 3 March, following the spring temperature increase. Twelve plastic tanks 60×45×15 cm were filled with water and permanently aerated with a small corner filter. Plastic grille dividers placed one-third into the length of the tank permitted visual and chemical cues of an insectivorous fish (the golden shiner, *Notemigonus crysoleucas*) to reach damselflies in the predator treatment. One fish was placed in each of the six tanks and fed daily with commercial fish pellets. The section of the tank containing fish was covered with a piece of netting to prevent them from

jumping out. All surviving damselfly larvae were transferred to plastic cups floated with polystyrene, which had part of the side of the cup replaced with mesh screen to permit water flow. Individuals from both food treatments were randomly placed in the fish and fishless tanks, resulting in six low food and five high food individuals per tank. Larvae were maintained under these conditions until emergence. Dead individuals were replaced with reserves (collected at the same time as the individuals used in the experiment) to maintain equal densities, although the replacement larvae were not used in the subsequent observations of foraging behaviour.

Foraging behaviour for a subset of the larvae was observed over a 1-week period (10–16 March) by a single observer. The experimental design consisted of a 2×2 factorial design (previous food level by predation): (1) high food, predator (n=19); (2) high food, no predator (n=22); (3) low food, predator (n=18); and (4) low food, no predator (n=20). Larvae were observed for a 15-min period, and observations were alternated between larvae from each of the four treatments to eliminate any confounding time-of-day effects. As per Johansson and Rowe (1999), the behaviours noted included "orient" (orientation towards a prey item with no leg movement), "advance" (movement of one or more legs in the direction of a prey item), "strike" (rapid extension of the labium in the direction of a prey item) and "capture" (successful capture of a prey item). Other conspicuous activity included "swimming" and "walking" (movements not directed towards a prey item). These behaviours are not strictly foraging behaviours and were therefore not included in the principle component analysis, although they may be indirectly associated with moving to a more profitable foraging patch (see Johansson et al. 2001).

Analysis

Initial differences in body size following food treatments were measured in January and analysed with one-way ANOVA. The changes in mass, head width and pronotum width that occurred between March and emergence in April were analysed separately with two-way ANOVAs. Because individuals were grouped into bins after 3 March, and each bin occurred in only one fish treatment, we removed potential bin effects by including the term "bin nested within fish" in a nested ANOVA that included the categorical treatment variables (fish, food, and fish × food). For all tests, bin was not significant. Nonetheless, we kept the factor bin in the analyses as reported below. For the behaviour experiment, the number of behavioural variables was reduced using principal component analysis (PCA) on correlations. PCA scores were then analysed with the nested ANOVA as described above. In this case, there was a significant effect of bin ($F_{1,10}$=2.49, p=0.014). All analyses were implemented with the statistical software package Systat 10.0.

Results

Out of 129 individuals that were weighed at least once, 111 survived to emergence. Eight individuals escaped prior to measurement of adult body size, but at least two measurements (head width, pronotum width or mass) were available for the remainder of the adults. All data available was used (for example, larval size and behavioural measurements for individuals that failed to survive to emergence were included in the analysis as their omission had no detectable effect on the outcome of the statistical analysis).

Growth and development

The initial food level manipulation was successful in reducing mass, pronotum and head width growth in the low food treatment. By January, mean head width was 2.62 and 2.34 mm in the high and low food treatments, respectively ($F_{1,128}$=16.2, p<0.0001); pronotum width 1.30 and 1.16 mm ($F_{1,128}$=17.1) and mass 14.1 and 8.92 mg ($F_{1,128}$=33.5, p<0.0001). In order to determine whether these size differences reflected a relative increase in condition for those individuals fed ad libitum rather than an accelerated development rate, we performed an ANCOVA on mass with head width as the covariate. Mass was significantly lower in the low food treatment than in the control ($F_{1,122}$=29.7, p<0.0001); therefore, it is concluded that the larvae exposed to low food levels were in poorer condition than those in the high food treatment.

By emergence, the means for both head and pronotum width for low food larvae were no longer significantly different from the high food treatment ($F_{1,95}$=0.0029, p=0.96 and $F_{1,98}$=0.57, p=0.45, respectively). Larvae in the low food treatment showed a tendency towards delayed emergence, although this was not statistically significant (mean delay 4.5 days, range 196–253 days; $F_{1,107}$ = 3.8, p=0.055, Fig. 1). Moreover, the mean slope for both head and pronotum width growth over time (between March and adult emergence size) was significantly greater in the low food treatment (head width: $F_{1,95}$=10.9, p=0.0014; pronotum width: $F_{1,98}$=11.6, p=0.0010), indicating that there was a compensatory increase in growth rate of the body (Fig.1). There was no significant interaction between fish and food treatments ($F_{1,93}$=0.12, p=0.73).

No direct comparison between rates of increase in mass could be made between the food level treatments, because mass of adults was measured as dry weight and live larvae as wet weight. By emergence, mass was still reduced in the low food treatment (5.6 and 5.1 mg for high and low food treatments, respectively. $F_{1,94}$=7.00, p=0.0098). Thus, although there may have been some level of compensatory growth in this metric, it was not detectable in our analysis.

Predation risk had no detectable significant effect on adult head or pronotum width ($F_{1,95}$=0.0025, p=0.96 and
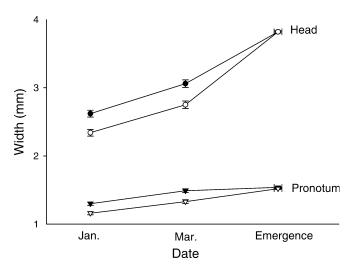
Fig. 1 Effect of food treatment on head and pronotum growth in the larval damselfly *Ischnura verticalis*. *Circles* represent mean head width, *triangles* mean pronotum width. *Open markers* refer to individuals raised at low food levels and *closed markers* to larvae raised at high food levels. *Error bars* represent one standard error from the mean
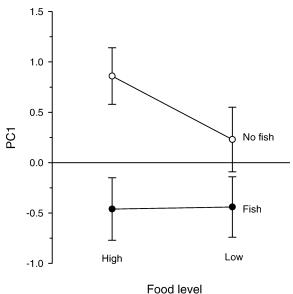

Fig. 2 Effect of food level and predatory fish (golden shiner, *N. crysoleucas*) on foraging behavior (PC1) of larval *I. verticalis*. *Open circles* represent predator-free treatment, *solid circles* predator treatment. Error bars represent one standard error from the mean

$F_{1,98}$=0.24, p=0.62, respectively), nor was emergence date delayed in the presence of fish ($F_{1,107}$=0.073, p=0.79). Larvae that were exposed to a predator emerged at a significantly reduced body mass: dry mass at emergence was 5.1 mg compared to 5.6 mg in the controls ($F_{1,93}$=5.33, p=0.024).

Behaviour

The four foraging behaviours, orient, strike and advance, loaded heavily onto the first principle component axis (Table 1). Capture loaded more heavily onto PC2, perhaps because while feeding on a captured prey item, a larva could not be engaged in the foraging behaviours. Presence of fish significantly reduced the foraging behaviours associated with PC1 ($F_{1,76}$=10.8, p=0.0016), but there was no effect of food level or interaction between food level and predator ($F_{1,76}$=1.06, p=0.32 and $F_{1,76}$=1.15, p=0.29, respectively) (Fig. 2). The reduction in foraging effort in response to predation did not lead to a significant difference in head or pronotum width; however, the decline in foraging rate likely contributed the observed reduction in adult mass (see previous section).

Table 1 Loading of foraging behaviours onto first three PC axes. Bold face indicates heavy loadings

Behaviour	PC1	PC2	PC3
Orient	**0.519**	−0.459	−0.273
Strike	**0.532**	0.0260	**−0.636**
Capture	0.434	**0.733**	0.405
Advance	**0.509**	−0.430	**−0.776**

Discussion

Damselfly larvae decreased foraging activity in the presence of fish as predicted based on previous studies of this species and other damselfly species (Dixon and Baker 1988; Baker et al. 1999; McPeek et al. 2001; Stoks et al. 2001), but there was no interaction effect with our food treatment as we had expected. In other words, larvae in the low-food treatment, although delayed in growth and development, did not respond in subsequent periods by increasing foraging effort in the presence of fish. This may indicate that the potential benefits of reducing development time and increasing stored resources are more than counterbalanced by the risk of pre-reproductive mortality due to predation, or that we were simply unable to detect an effect that was there. Although adult mass was lower in the fish treatment, there was no significant relationship between foraging effort and mass at emergence. McPeek (2004) has previously demonstrated that depressed growth rates in the presence of fish are not attributable solely to behaviour, since larval *I. verticalis* exposed to predators consumed comparable quantities of food as those in a predator-free environment. However, the former group grew at a slower rate, possibly as a result of a physiological mechanism such as stress.

Head and pronotum widths were limited by food level early in development. Yet, by adulthood, the initial size differences between larvae raised on different food regimes had been resolved by a compensatory increase in growth rates. There was also a trend towards delay in emergence for low-food larvae, although this effect was not significant. Any delay in development would be contrary to our prediction that larvae would not delay emergence, given the time constraints imposed by the finite length of the reproductive season. The larvae in this

experiment may have received insufficient cues as to time in season, since photoperiod was kept at 12:12 throughout the experiment. Previous experiments with damselflies have shown that development rates were accelerated and length of the larval stage shortened in response to manipulation of photoperiod (Johansson and Rowe 1999). Alternatively, the effect of the temporary low food conditions may have been too much to correct through compensatory growth alone, which may have led to a delay in emergence time.

Compensatory growth is known in a number of taxa and is often achieved through increased food intake (review in Metcalfe and Monaghan 2001). In butterflies, increased foraging rates occurred following a period of resource deprivation, despite an accompanying increase in predation risk (Gotthard 2000). Other species are known to show compensatory growth in certain body size measures through the reallocation of limited resources to the most critical requirements. For example, salmon may show compensatory increases in lipid storage or skeletal growth depending on season (Morgan and Metcalfe 2001). Different caddisfly species maintain abdomen size at the cost of thorax size depending on certain life history traits (Stevens et al. 2000). For example, in a short-lived, high fecundity species with limited resources, abdominal size (and therefore lipid storage) was comparable to a control group, while thorax size was reduced (Stevens et al. 1999). In *I. verticalis*, head and pronotum width showed catch-up growth towards the end of the larval stage, while mass at emergence was significantly depressed by nutritional condition. When resources are limiting, damselflies may allocate resources preferentially to increasing body size, potentially at the cost of stored energy reserves such as fat. Because adult body size is fixed, resources accrued by adult feeding can be used for maintenance and reproduction alone, although adult body size may limit resource accrual and storage. Adult feeding in *I. verticalis* may have a stronger influence on egg production than larval feeding in the final instar (Richardson and Baker 1997), indicating that while body size is fixed at maturity, reserves can be increased during adulthood. Indeed, mass at emergence appeared to be more sensitive to nutrition, being reduced in response to reduction in foraging caused by fish predators, while other aspects of body size were not affected.

In summary, larvae exposed to a temporary period of low resource availability experienced reduced growth rates. By emergence, compensatory increased in head and pronotum width growth rates resulted in damselflies emerging at a mean body size comparable to that of controls. However, this increase in growth rate was apparently not attributable to increased foraging activity, and there was no tendency of previously food-limited larvae to increase risky foraging in the presence of predators. In fact, larvae exposed to fish predators decreased their foraging activity regardless of previous resource levels, which may have contributed to a reduction in adult body mass, though not head or pronotum width, compared to individuals that were not exposed to a predator. Limited larval resources may have been allocated to increasing body size, which is fixed at emergence, at the cost of reduced energy storage. The resulting reduction in body mass at emergence may have consequences for future fecundity (Sokolovska et al. 2000).

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