Effects of early resource-limiting conditions on patterns of growth, growth efficiency, and immune function at emergence in a damselfly (Odonata: Coenagrionidae)

C. Dmitriew, M. Cooray, and L. Rowe

Abstract: Periods of restricted growth during early development are expected to have detrimental effects on subsequent metrics of fitness, most prominently increases in age and decreases in size at maturity. However, in some cases, animals may compensate by altering foraging effort, growth efficiency, or patterns of resource allocation between critical traits prior to maturation. Yet, even when compensation for age and size is complete, brief periods of restricted growth may carry costs persisting in the long term, and compensatory tactics may themselves be costly. We investigated the long-term costs of early growth restriction and mechanisms of compensatory growth in the damselfly *Ischnura verticalis* (Say, 1839). Larvae were temporarily exposed to one of three feeding regimes in the early stages of development, after which food levels were restored. In the period of unrestricted growth prior to emergence, partial compensation for structural size in the lowest food treatment was observed, while both resource-limited groups accelerated mass gain relative to controls. Changes in food consumption and food conversion efficiency were ruled out as mechanisms for accelerating growth following diet restriction. We tested for changes in resource allocation patterns that could explain the observed compensatory growth and found that adult body shape may depend on early growth conditions in females. There was no evidence of detrimental effects on immune function at emergence, although males tended to have higher phenoloxidase activity (a measure of immunocompetence) than females.

Résumé : On s'attend à ce que des périodes de croissance réduite au début du développement puissent avoir des effets négatifs sur les métriques subséquentes de fitness, en particulier sur l'accroissement de l'âge et la diminution de la taille à la maturité. Cependant, en certains cas, les animaux peuvent compenser en changeant leur effort de recherche de nourriture, leur efficacité de croissance ou leurs patrons d'allocation des ressources entre divers caractères essentiels avant la maturation. Néanmoins, même si la compensation pour l'âge et la taille est complète, de brèves périodes de croissance restreinte peuvent entraîner des coûts qui persistent sur de longues périodes et les stratégies de compensation peuvent elles-mêmes être coûteuses. Nous avons étudié les coûts à long terme d'une restriction de croissance en début de cycle, ainsi que les mécanismes de croissance compensatoire, chez la demoiselle *Ischnura verticalis* (Say, 1839). Nous avons soumis temporairement des larves à l'un de trois régimes alimentaires durant les premières périodes du développement; le niveau d'alimentation a ensuite été rétabli. Durant la période de croissance sans contrainte avant l'émergence, il s'est fait une compensation partielle pour la taille du corps au niveau d'alimentation expérimentale le plus bas; en revanche, les deux groupes exposés à des ressources restreintes ont accéléré leur gain de masse par rapport aux témoins. Les changements de consommation de nourriture et d'efficacité de conversion de nourriture ne s'appliquent pas dans ce cas comme mécanismes explicatifs de la croissance accélérée après la restriction alimentaire. Nous avons vérifié l'existence de changements dans les patrons d'allocation des ressources qui pourraient expliquer la croissance compensatoire observée et nous trouvons que, chez les femelles, il est possible que la forme du corps de l'adulte dépende des conditions de croissances des jeunes larves. Il n'y a pas d'indication d'effets négatifs sur la fonction immunitaire à l'émergence, bien que les mâles aient tendance à avoir une plus forte activité de la phénoloxydase (une mesure de l'immunocompétence) que les femelles.

[Traduit par la Rédaction]

Introduction

There has been growing interest in how periods of poor growth associated with resource restriction impact fitness in the long term (e.g., Metcalfe and Monaghan 2001; Ali et al. 2003). While delays in development and reduced size at maturity are frequently observed in response to periods of resource limitation, many organisms are capable of accelerating growth rates when conditions improve, with consequent positive effects on development rate and size-dependent fitness traits such as fecundity (Honek 1993; Merrett 1994; Sokolovska et al. 2000) and male mating success and longevity (Trivers 1972; Nylin and Gotthard 1998; Jennions et al. 2001). However,

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growth is energetically expensive, particularly in terms of protein deposition (Jorgensen 1988), and therefore utilizes resources that might otherwise be allocated to different structures and functions. In general, reduced adult body size is expected to occur in organisms experiencing strong directional selection on development time, such as those experiencing seasonal time constraints (Rowe and Ludwig 1991; Abrams et al. 1996; Nylin and Gotthard 1998). However, organisms undergoing compensatory growth may also suffer consequences of initial poor growth independent of body size at maturity, including an increased risk of mortality (e.g., Gotthard et al. 1994; Blanckenhorn 1998; Ozanne and Hales 2005). Other possible costs include negative effects on immune function (Birkhead et al. 1999), subsequent growth (Morgan and Metcalfe 2001), male dominance status (Royle et al. 2005), swimming efficiency (Billerbeck et al. 2001), and scale strength in fish (Arendt et al. 2001).

Increased food intake is one of the most common means of accelerating growth (Ali et al. 2003), yet may increase the risk of predation if foraging behaviour is conspicuous (e.g., Sih 1987; Lima and Dill 1990). Acceleration of growth rate might also occur by physiological mechanisms such as altered growth efficiencies (Boujard et al. 2000). However, these changes may in turn affect investment in stored resources (Bayne 2000, Stoks et al. 2006) or rate of protein turnover (Morgan et al. 2000), which is a measure of the rate of protein repair and maintenance. Reduced rate of protein turnover is associated with aging and cellular senescence (e.g., Ramsey et al. 2000) and may translate into reduced longevity among individuals experiencing poor early growth. Changes in adult allometries following periods of poor growth are not uncommon among insects and fish (Stevens et al. 1999, 2000; Alvarez and Nicieza 2005; Boggs and Freeman 2005; Dmitriew and Rowe 2005) and may have a number of undetermined consequences for fitness.

Damselfly growth rates are highly flexible and may increase in response to perceived time constraints (Strobbe and Stoks 2004) or following a period of restricted growth (Dmitriew and Rowe 2005). This is expected, given that female fecundity is often strongly associated with body size in this group (Sokolovska et al. 2000). However, in a study of lestid damselflies, De Block and Stoks (2005) found that adult fitness was also affected by the conditions experienced by juveniles independently of body size at maturity. In this paper, we extend the study of long-term costs of growth restriction during early development in damselflies by attempting to identify specific long-term costs as well as the mechanisms by which compensatory growth in body size is achieved.

Three main questions were addressed in this experiment. First, we evaluated the limits of compensatory ability in the larval damselfly *Ischnura verticalis* (Say, 1839) (Odonata: Coenagrionidae) by exposing them to three levels of resource restriction for a brief period followed by ad libitum feeding until emergence. Given the seasonal constraints in this temperate-zone species, as well as risk of predation during the larval stage, it was predicted that damselflies unable to compensate for growth lost during a period of extreme resource limitation would emerge at smaller body size rather than delaying emergence to increase in size. We also evaluated the allometric relationships between wing length and a composite measure of body size to determine whether adult body shape was affected by early larval diet. We chose to investigate the effects of early resource limitation on wing length because previous studies in butterflies have shown that diet affects the size of wings relative to the body (Boggs and Freeman 2005). Wing length or area is also likely to affect fitness because flight performance in insects may depend on the shape and size of the wing relative to the load (e.g., Kingsolver 1999; Frankino et al. 2005). Accelerated growth of certain body structures may be achieved by altering patterns of resource allocation within food-stressed individuals and may explain the increase in growth rate of structural body size observed in an earlier experiment (Dmitriew and Rowe 2005). Body size and mass often have positive effects on fecundity in insects including damselflies (e.g., Honek 1993; Sokolovska et al. 2000), and increases in structural size may reflect selection for greater storage capacity in adults, since damselflies continue to feed and store resources as adults prior to sexual maturation.

Previously, we found no evidence that food-restricted larvae increased their foraging effort during accelerated growth (Dmitriew and Rowe 2005). Therefore, we hypothesized that damselflies may achieve accelerated growth by increasing the efficiency at which food is converted into mass. Recently, Stoks et al. (2005) found that *Lestes sponsa* (Hansemann, 1823) reared under a photoperiod simulating lateness of season grew more quickly than control larvae experiencing an "early" photoperiod, although the opposite was true in the congeneric species *Lestes congener* Hagen, 1861 (Johansson and Rowe 1999). In the presence of a fish predator, time-stressed *L. sponsa* larvae ate more food and had higher growth efficiency than did controls (Stoks et al. 2005).

Finally, we then tested for reduced immune function at emergence as a potential long-term cost of restricted growth despite compensation for body size. While maintenance of immunity is often costly, and may trade off with growth rate (Schmid-Hempel 2003; Brommer 2004), it may have positive effects on survivorship, female preferences, or competitive ability (Yourth et al. 2002*a*, 2002*b*; Rantala and Kortet 2003; Koskimäki et al. 2004; Tsubaki and Hooper 2004).

Methods

General

Larval *I. verticalis* were collected from the Burns Conservation Area pond (Halton County, Ontario, Canada) on 17 September 2004. Over the next 3 days, individuals were weighed and head width measured and then placed individually into small plastic cups containing 100 mL of dechlorinated water with a wooden dowel functioning as a perch. Prior to the start of the experiment, larvae were held in an environmental chamber at 18 °C with a photoperiod of 14 h light : 10 h dark and fed daily with 6–8 *Daphnia magna* Straus, 1820. Starting on 7 October, damselflies were randomly assigned to one of three food treatments: fed ad libitum every day (high food, $N = 101$), every 2nd day (moderate food, $N = 101$), or every 4th day (low food, $N = 41$) for a period of 4 weeks, which we refer to as the "food manipulation period". A large number of larvae were required for the immune function assays; therefore, to keep numbers manageable, we only reared larvae from the high and moderate-food treatment groups for that experiment. Surplus *D. magna* remaining in the medium- and low-food cups were removed on nonfeeding days. A one-way ANOVA was performed on head width and mass prior to the food treatments to confirm that the groups did not differ significantly at the outset of the experiment ($F_{[2,240]} = 1.00$, $p = 0.40$ and $F_{[2,240]} = 0.92$, $p = 0.40$ for mass and head width, respectively).

Following the food manipulation period (4 November), larvae were again weighed and measured. From this point onward, larvae in all treatments were fed ad libitum until emergence. Temperature was lowered to 9 °C for 2 weeks and then to 4 °C (the temperature of water under ice) to simulate winter conditions. Photoperiod was set to 9 h light : 15 h dark, approximating local winter conditions. On 17 January, photoperiod was set to 12 h light : 12 h dark and temperature to 18 °C (simulating spring conditions) until emergence. This period is referred to as the "spring growth period". Throughout the larval stage, containers were frequently cleaned to remove fecal pellets and dead *D. magna*. Water was changed approximately once per month. Prior to emergence, a piece of nylon netting was placed over each cup to contain emerging adults, which were collected within 24 h. Throughout the experiment, cups were checked daily for exuviae (to estimate development rate) and dead individuals. All animals alive for a given measurement were included in analyses even if they subsequently died prior to emergence; their removal from the analyses had no significant effect on the outcomes.

Growth and development

Head width and mass were measured monthly during the larval stage and at emergence. Before each weighing, larvae were starved for 24 h to allow evacuation of the gut. All larval measures are of wet mass (larvae were blotted on a tissue to remove excess water prior to weighing). At emergence, larvae were sexed and wet mass, head width, pronotum width, and wing length were recorded for all individuals. Because damselflies eject water during emergence, adult wet mass could not be directly compared with larval mass. Therefore, larval dry mass was estimated using the conversion factor dry mass = 0.1497 × wet mass as calculated by McPeek et al. (2001) for several damselfly species, including *I. verticalis*. To estimate adult dry mass, a subset of emerging adults was dried for 24 h at 60 °C and weighed (low food, $N = 31$; moderate food, $N = 40$; high food = 34). The balance of the adults were set aside for the phenoloxidase assay, since this experiment required removal of the hemolymph from live specimens. Growth rate was calculated as the change in head width or mass over time (number of days between measurements) and compared among treatments using ANOVA.

A composite measure of body size was calculated using a principal component analysis (PCA) on head width, pronotum width, and body mass. Because there were significant differences between the sexes for mass, separate PCAs were performed for males and females. For females, these traits accounted for 59% (eigenvalue = 1.76) of the variation in PC1, with loadings of 0.535, 0.561, and 0.631, respectively. For males, loadings were 0.547, 0.581, and 0.603 and accounted for 70% of the variation in PC1 (eigenvalue = 2.09). The effect of larval food treatment on body shape was determined using an ANCOVA for treatment and sex with PC1 as the covariate.

Food consumption and food conversion efficiency

Food conversion efficiency during the food manipulation period was determined using a modification of McPeek's (2004) protocol with a randomly selected subset of larvae from the high- ($N = 39$) and moderate-food ($N = 35$) treatments. Over a 4 day period (27–30 October), damselflies were fed 10 *D. magna* per day (mean dry mass of daily ration = 1.16 (±18.7) mg, $N = 10$). Larvae in the low-food treatment were fed this ration on the first and third day only. Uneaten *D. magna* were collected daily and placed in tinfoil containers. At the end of the observation period, *D. magna* were dried at 60 °C for 24 h and weighed. As per Stoks et al. (2005), assimilated food was calculated as mean dry mass of ration – dry mass of uneaten food – dry mass of fecal pellets and assimilation efficiency was calculated as assimilated food / food consumed. Food conversion efficiency was calculated as change in dry mass / assimilated food and growth efficiency as change in dry mass / food consumed. Larval dry mass was estimated using the conversion factor described above. This procedure was repeated during the spring growth period when compensatory growth was predicted to occur (26–29 January); however, at this time, larvae from all three treatments were analysed ($N = 39$, 38, and 36 for high, moderate, and low food, respectively).

Immune function: phenoloxidase activity

Phenoloxidase, an enzyme associated with the melanotic encapsulation response in insects (Söderhäll and Cerenius 1998), was assayed to estimate immunocompetence of damselflies in the high- ($N = 32$) and moderate-food ($N = 29$) treatments. After weighing, hemolymph was collected from adults within 24 h of emergence (and after complete expansion of the wings and abdomen) by perfusing the abdomen with 0.5 mL of Dulbecco's phosphate-buffered saline (Sigma-Alpha). The hemolymph and buffer were collected and placed immediately in a microcentrifuge tube in dry ice. Samples were then stored at –80 °C and phenoloxidase analysis was performed within a week of extraction. Samples were thawed and cell walls removed by centrifugation (at 4 °C and 2800 r/min, 730*g*, for 15 min). A 100 µL aliquot of the supernatant and 100 µL of 10 mmol/L L-dopa was added to a multiwell microassay plate, and each sample was run in triplicate. Readings were taken every 10 s at 492 nm (Spectramax Plus 384 spectrophotometer, Softmax Pro version 3.1.2; Molecular Devices Corporation 2000) and 30 °C for 45 min. Phenoloxidase activity was measured as the slope of the reaction curve during the linear phase (between 1600 and 2600 s). For each individual, the strength of the immune response was calculated as the mean slope of the replicates for which the R^2 value for the linear regression over the specified time period was at least 0.98. Lower values are likely to indicate a poor reaction; 15 samples were rejected, as no single replicate met this criterion. Phenoloxidase activity was analysed in an ANOVA (treatment × sex), with phenoloxidase activity size-corrected to account for potential increases in phenoloxidase for larger volumes of hemoymph.

Results

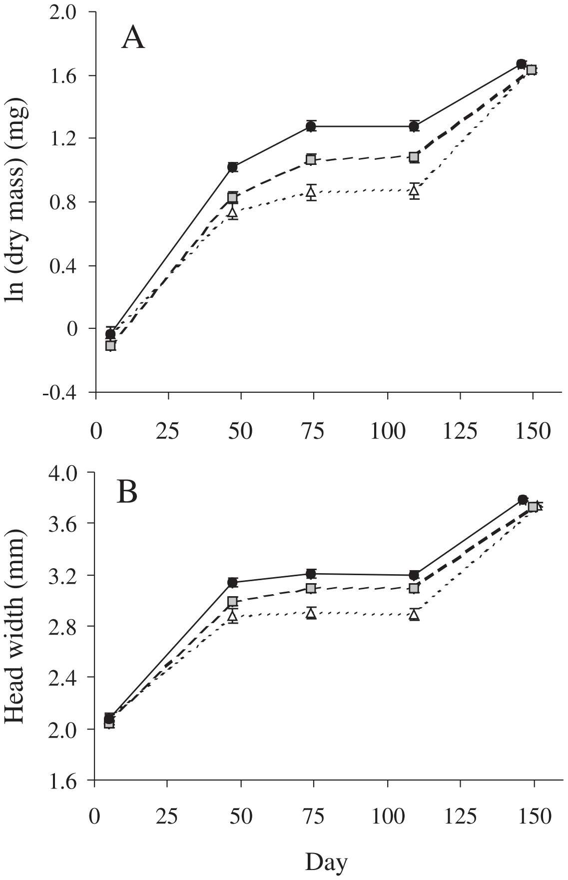
Growth compensation

Of 243 damselflies used in the experiment, 184 survived to emergence Mortality did not differ among the three treatment groups (low food: 15.8%; moderate food: 21.2%; high food 25.3%; $\chi^2_{[2]} = 2.2$, $p = 0.33$). Seven damselflies failed to emerge within 1 month of the others and were excluded from the analysis as outliers: two each from the high- and moderate-food treatment and three from the low-food treatment.

As expected, growth rate during the food manipulation period was an increasing function of food ration (ANOVA — $F_{[2,240]} = 9.83$, $p < 0.0001$ and $F_{[2,236]} = 13.3$, $p < 0.0001$ for head width and ln(wet mass), respectively), resulting in significantly larger and heavier larvae in the moderate- and high-food treatments (ANOVA head width post treatment: $F_{[2,240]} = 11.0$, $p < 0.0001$; ln(wet mass) post treatment: $F_{[2,236]} = 18.7$, $p < 0.0001$). Over the winter period, little growth occurred and there were no significant differences in head width growth (change in head width / head width prior to winter: $F_{[2,222]} = 1.77$, $p = 0.17$). However, the amount of mass gained was less for larvae in the low-food treatment, despite correcting for mass at the start of winter (change in ln(mass) over winter / ln(prewinter mass): $F_{[2,219]} = 4.22$, $p = 0.016$, post hoc Tukey's HSD: $L < M = H$). During the spring growth period, head width of larvae from the low-food treatment increased significantly faster than in the control and moderate-food treatments ($F_{[2,177]} = 5.03$, $p = 0.0075$; post hoc Tukey's HSD: $L > H = M$) (Fig. 1). If growth compensation in head width occurred in the moderate-food treatment, it was not detectable, and control larvae emerged at a larger size than the moderate-food treatment larvae (sex: $F_{[2,174]} = 0.15$, $p = 0.70$; treatment: $F_{[2,174]} = 3.47$, $p = 0.033$; sex × treatment: $F_{[2,174]} = 0.34$, $p = 0.71$; Tukey's HSD for treatment: $H > M$) (Fig. 1). The rate of dry mass gain during this period was highest in the low-food treatment and lowest in the high-food larvae (change in ln(dry mass): $F_{[2,102]} = 7.97$, $p = 0.0006$), which resulted in complete compensation of dry mass by emergence such that emerging adults from all three groups had dry masses that did not differ significantly either by sex or by treatment (ln(dry mass) — sex: $F_{[1,98]} = 2.97$, $p = 0.09$; treatment: $F_{[2,98]} = 1.07$, $p = 0.34$; sex × treatment: $F_{[2,98]} = 0.54$, $p = 0.58$). Wet mass at emergence was significantly higher for females but there was no significant difference among treatments (ln(wet mass): sex: $F_{[1,174]} = 29.3$, $p < 0.0001$; treatment: $F_{[2,174]} = 2.86$, $p = 0.06$; sex × treatment: $F_{[2,174]} = 0.21$, $p = 0.80$).

Larvae in the control treatment emerged about 3 days earlier, on average, than those in the moderate- and low-food treatments ($F_{[2,182]} = 7.63$, $p = 0.0007$; Tukey's HSD: $H < M = L$) (Fig. 1). Initial analysis showed no effect of sex on larvae growth rates; therefore, sex was not included in the analyses.

Fig. 1. Growth trajectories of (A) ln(dry mass) and (B) head width of damselflies *Ischnura verticalis* reared at three food treatments from day of collection to emergence. The slope of the line indicates growth rate for each treatment group (solid line, control treatment; dashed line, moderate-food treatment; dotted line, low-food treatment). Error bars indicate 1 SE.

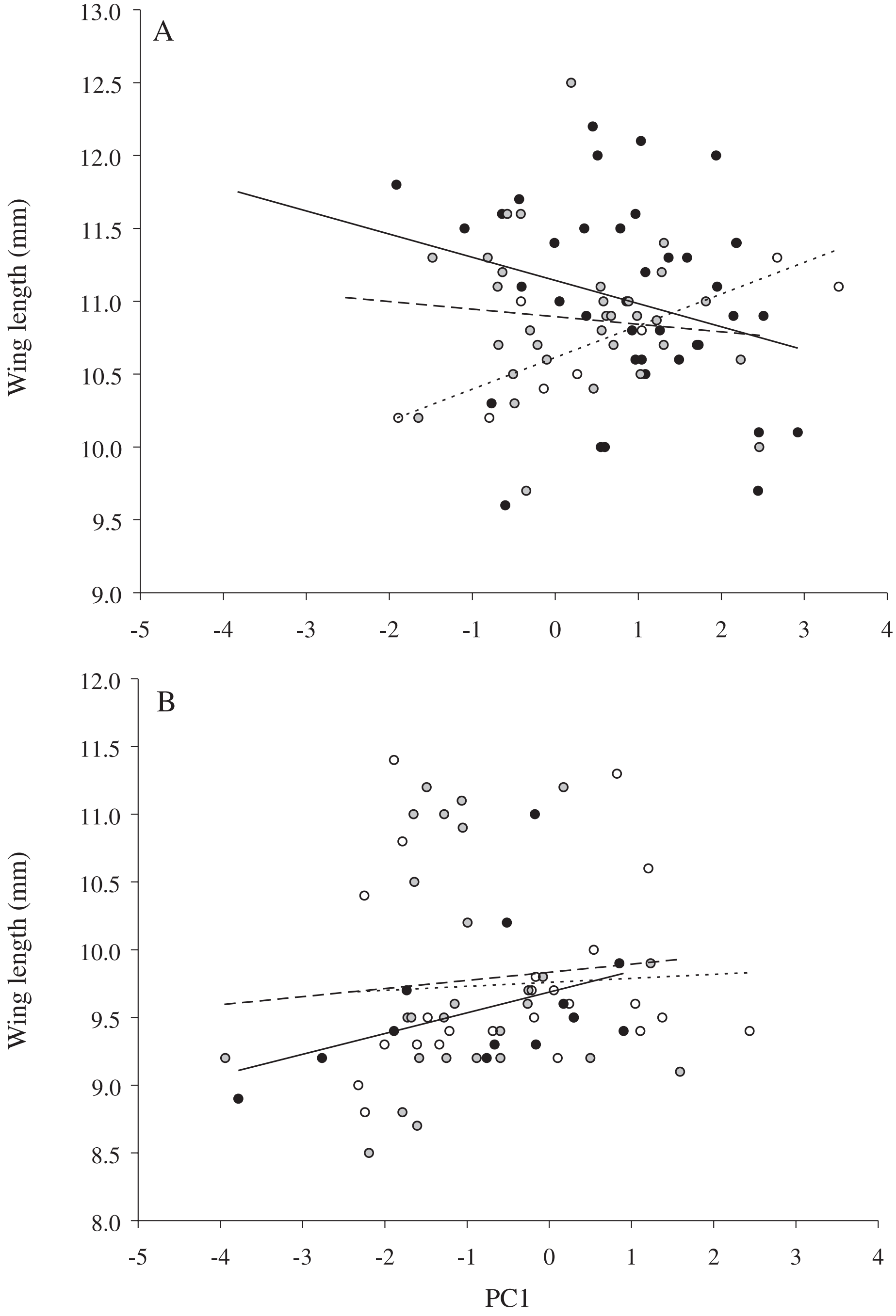


The allometric relationship between wing length and body size (PC1) was compared among food treatments for each sex using sex-specific PCAs. Although mean wing length relative to body size did not depend on treatment, there was a significant interaction between treatment and adult body size in females, with low-food females showing a positive relationship between wing length and size (Fig. 2A, Table 1). There was no relationship between wing length and body size for females in the moderate- and high-food treatment groups or for any of the male treatment groups (Fig. 2B, Table 1). Wet mass at emergence was used to increase the sample size available for analysis, since dry mass was not available for all individuals; however, the results of the analysis using dry mass are not qualitatively different (not shown).

Growth and food conversion efficiency

During the food manipulation period, larvae in the high-food treatment consumed a greater proportion of the food mass provided to them than did larvae in the moderate-food treatment (ANCOVA for food treatment with initial mass as the covariate: $F_{[3,66]} = 4.75$, $p = 0.03$) (Fig. 3). The amount of food assimilated was significantly higher in the high-food treatment because they had the opportunity to feed daily rather than every 2nd day ($F_{[3,66]} = 5.62$, $p = 0.02$). However, there were no treatment effects in any of the conversion variables measured (assimilation efficiency: $F_{[5,66]} =$

Fig. 2. Change in wing length of (A) female and (B) male *I. verticalis* with respect to total body size (PC1). Solid circles and solid line, high-food treatment; shaded circles and broken line, moderate-food treatment; open circles and dotted line, low-food treatment.



0.82 $p = 0.37$; growth efficiency: $F_{[5,66]} = 1.42$, $p = 0.24$; and conversion efficiency: $F_{[5,66]} = 0.19$, $p = 0.66$).

During the spring period, there was no difference in the proportion of food consumed among the three treatment groups (ANCOVA with initial mass as covariate: $F_{[5,99]} = 0.46$, $p = 0.63$) or in any of the four digestive variables measured (food assimilated: $F_{[5,99]} = 0.39$, $p = 0.68$; assimilation efficiency: $F_{[5,99]} = 0.04$ $p = 0.96$; growth efficiency: $F_{[5,99]} = 1.98$, $p = 0.14$; and conversion efficiency: $F_{[5,99]} = 1.05$, $p = 0.35$) (Fig. 3).

Phenoloxidase assay

There was no effect of food treatment on size-corrected phenoloxidase activity ($V_{\max}$/ln[mass]) in newly emerged adults; however, male damselflies showed a stronger immune response (two-way ANOVA — sex: $F_{[1,56]} = 6.48$, $p = 0.014$; treatment: $F_{[1,56]} = 0.61$, $p = 0.43$; sex × treatment: $F_{[1,56]} = 0.0019$, $p = 0.97$) (Fig. 4).

Discussion

In this study, we identified a number of long-term effects of growth restriction in damselfly larvae. A period of poor resource availability early in development resulted in reduced structural size at emergence, compensatory increases in the rate of structural growth and mass gain late in life,

Fig. 3. Proportion of food consumed by food level treatment during the food manipulation and the spring growth period of the damselfly *I. verticalis*. Solid bars, high-food treatment; shaded bar, moderate-food treatment; open bars, low-food treatment. Error bars indicate 1 SE.

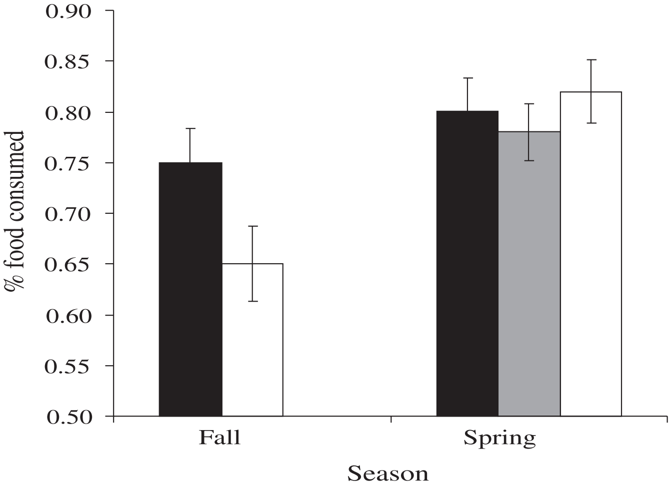
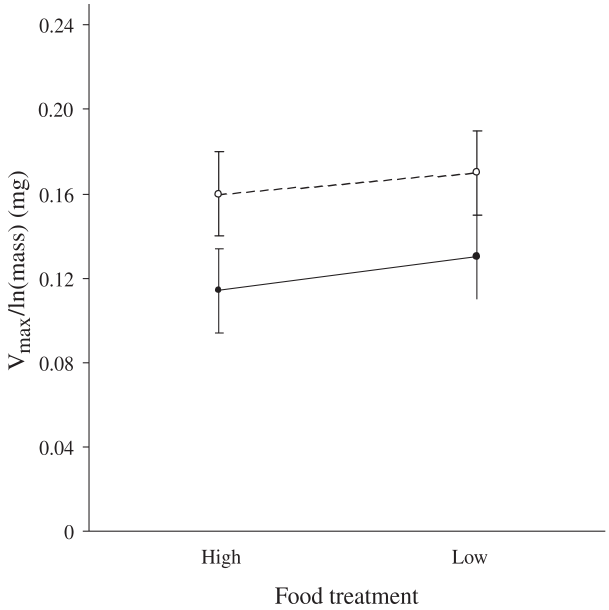


Table 1. ANCOVA testing the effect of early resource treatment on the relationship between wing length and a composite measure of adult body size (PC1 = head width, pronotum width, and ln(wet mass)) of the damselfly *Ischnura verticalis*.

	df	*F*	*p*
Females			
Treatment	2	1.14	0.33
PC1	1	1.49	0.23
Treatment × PC1	2	3.33	0.041*
Error		73	
Males			
Treatment	2	0.66	0.42
PC1	1	0.39	0.68
Treatment × PC1	2	0.28	0.76
Error		59	

Fig. 4. Effect of food treatment and sex on mass-corrected immune function (V_{max}/ln(mass)) of the damselfly *I. verticalis* at emergence. Solid circles, males; open circles, females. Error bars indicate 1 SE.



and changes in the scaling relationship between wing length and body size in females. There was no evidence that larvae increased food intake or growth and conversion efficiencies to achieve accelerated growth as was predicted; we also did not observe effects of early growth restriction on immune function at emergence.

Compensatory growth

Organisms show a variety of responses to periods of growth restriction. Poor growth may cause individuals to delay life-history transitions (e.g., maturity) and undergo these transitions at a smaller size; both factors may reduce subsequent fitness. However, many species demonstrate the ability to compensate behaviorally or physiologically for delayed growth, thereby reducing some of these costs (Rowe and Ludwig 1991; Abrams et al. 1996). In the current experiment, as has been demonstrated previously (Dmitriew and Rowe 2005), damselflies underwent compensatory growth following diet restriction early in development. In contrast with our previous study, only larvae reared at very low food, and not those at a moderate level of food restriction, showed evidence of compensatory growth in structural size. Moreover, in the previous study, accelerated growth resulted in complete compensation for structural size by emergence (Dmitriew and Rowe 2005), while only partial compensation was observed in the experiment reported here. The timing of the period of diet restriction in the current study was later in development than in the previous study, with mean head width being greater (3.10 ± 0.2 and 2.48 ± 0.3 mm, respectively) in the current study following the food manipulation. This suggests that the timing of environmental stress may limit the ability to catch up, which fits with theoretical predictions stating that compensation is most likely to occur when the period of resource restriction is early in life (Mangel and Munch 2005). The hypothesis that timing of resource limitation determines the ability to compensate in *I. verticalis* must be confirmed by directly comparing the compensatory ability of individuals exposed to resource limitation in different instars in a single experiment.

Larvae from both the low- and moderate-food treatments compensated by increasing the rate of mass gain relative to controls and emerged at similar mass. Interestingly, both sexes had a similar dry mass at emergence, but adult wet mass was greater for females; there was also a marginally significant difference in adult wet mass (but not dry mass) among treatments. These differences may reflect sex and treatment effects on body composition (see Bayne 2000), since animals with varying levels of fat and protein contain different proportions of water. This may introduce some error into our calculation of dry mass growth rate, since the conversion factor used is not specific to condition, which would affect our estimate of the change in dry mass for larvae in the low-food treatment. However, since the sexes did not differ in wet mass as larvae, the conversion factor should be accurate for both males and females.

Food intake and conversion and growth efficiency

Larvae in the high-food treatment consumed more food relative to body mass than did larvae in the moderate-food treatment. This was surprising, as we predicted that given the reduced rations, larvae in the moderate-food treatment should have consumed as many of the available *D. magna* as possible. In previous studies of resource limitation in this species, larvae fed low daily rations tended to consume all food provided (Baker 1988). In the present study, larvae were fed ad libitum on each day of feeding, varying the frequency of feeding days by treatment. Damselflies are known to "waste" prey items, possibly because signals from an empty midgut cause larvae to continue foraging even when the foregut is full. Captured prey are thus killed but not consumed, even when space later becomes available in the foregut, as damselflies feed exclusively on live prey (Johnson et al. 1975). Larvae fed every 2nd day may have slower passage of material through the gut if metabolic rate is generally reduced during periods of low food.

Although growth rate of larvae prior to emergence was accelerated in the low-food treatment, we found no difference in either the number of prey items consumed or in food conversion efficiency over a 4-day period during this stage of development. Therefore, the mechanism by which larvae increase the rate of growth of structural body size and mass remains unclear. It is possible that our measurements were not sufficiently accurate to reveal changes in growth efficiencies or that measurements of the digestive variables, which took place within a few days of establishing the "spring" growth conditions, occurred before compensatory growth was initiated. Although the increases in structural size may result from changes in allocation between components of body size (see below), the observed increase in rate of mass gain is not explained by these trade-offs in allocation.

Allometry

Effects of early larval diet on allometry may have contributed to the observed increase in growth rate of certain components of structural size, as allocation of resources may change under stress (e.g., Stevens et al. 1999, 2000; Boggs and Freeman 2005). We found no effect of early diet on the relationship between wing length and body size emergence in either males or females, in contrast with our prediction that females should preferentially allocate limited resources towards structural size over wing length. However, we did observe a tight positive relationship between body size and wing length in the females reared at low food, which was absent in males and among females reared at the moderate- and high-food levels, although the implications of this result are unclear.

Immune function

We found that immune function was conserved for larval damselflies exposed to a moderate level of resource limitation, as they showed no difference in phenoloxidase activity relative to controls fed ad libitum throughout the experiment. This indicates that *I. verticalis* maintains immune function even when resource limitation results in emergence at smaller adult size. By contrast, in other insects, and including other species of damselfly, resource restriction has been shown to lead to a reduction in immune variables such as phenoloxidase activity and hemocyte number (e.g., Feder et al. 1997; Siva-Jothy and Thompson 2002; Rolff et al. 2004). Larvae reared under time constraints also had lower phenoloxidase activity in the damselfly *Lestes viridis* (Vander Linden, 1825) (Stoks et al. 2006). Given that severe infections may have a strong impact on condition and overall fitness, the advantages of conserving immune function despite resource limitation, or having the ability to quickly restore immune function once conditions improve, may outweigh the maintenance costs (Lochmiller and Deerenberg 2000). Emerging damselflies may be particularly vulnerable to parasites. For example, larvae are frequently parasitized by mites that commence feeding following emergence (Yourth et al. 2002*a*, 2002*b*; Tsubaki and Hooper 2004) and there was positive selection on immune variables in a natural population of damselflies (*Calopteryx splendens* (Harris, 1782) (Rolff and Siva-Jothy 2004). It has been suggested that while phenoloxidase levels may be rapidly upregulated following restoration of resource levels, substrates downstream of phenoloxidase in the immune response may be limiting (e.g., Feder et al. 1997; Siva-Jothy and Thompson 2002). Further, as a precursor to melanin, there may be other selective pressures maintaining phenoloxidase levels, such as natural selection on thermoregulation (e.g., Ellers and Boggs 2004).

Sexual dimorphism in immune function

Sex differences in immunity is common among insects (Sheridan et al. 2000; Rolff 2002; McKean and Nunney 2005), but there is no general pattern of greater female immunity as is observed in vertebrates (Zuk and McKean 1996). Sexual dimorphism in immunocompetence has been investigated quite extensively in odonates and appears to be highly variable and dependent on the species, conditions, or assay used (e.g., Braune and Rolff 2001; Rolff 2001; Siva-Jothy et al. 2001; Siva-Jothy and Thompson 2002). Recent theoretical work has focused on how life history differences between the sexes or differential exposure to immune challenges may predict patterns of sexual dimorphism in immune function (Rolff 2002; McKean and Nunney 2005). Increased phenoloxidase levels in male *I. verticalis* may reflect selection for greater investment in immunity; however, in addition to its role in the encapsulation of foreign material, phenoloxidase causes the hardening and dark coloration of the cuticle. Therefore, increased levels of phenoloxidase in males may also be the result of selection on other traits such as sexual selection for color patterns (Córdoba-Aguilar 2002; Svensson et al. 2004) or thermoregulation (reviewed in Caro 2005). However, this is unlikely to be the case in *I. verticalis*, which is not a strongly dimorphic species and lacks the wing spots present in other damselfly species.

Summary

We have shown that temporary resource restriction early in development has effects lasting to emergence in the damselfly *I. verticalis*. We observed acceleration of the growth rate of structural body size, yet compensation was incomplete. This result was in contrast with previous studies and may be due to differences in the timing of resource limitation. Damselflies did not compensate for poor growth by increasing food intake or growth efficiency following restoration of food levels. Finally, we found no evidence that stressed larvae allocated resources differently between mass and structural size or between body size and wing length or that investment in immune function was affected. Therefore, any costs of accelerating growth under these conditions remain to be determined. Future research efforts should focus on the role of timing of periods of growth restriction on compensatory growth and trade-offs as well as closer examination of changes in body composition.

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