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Stronger compensatory growth in a permanent-pond *Lestes* damselfly relative to temporary-pond *Lestes*

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Compensatory growth where animals compensate for time stress or transient nutritional or thermal stress by accelerating their growth rate is widespread. We know, however, relatively little about the evolution and ecological correlates of compensatory growth. For this we need studies on congeneric species with known phylogenetic relationships that also focus on the associated largely understudied costs. Here we tested for compensatory growth and associated costs in response to time stress (manipulated by photoperiod) and a transient period of starvation or cooling in larvae of the permanent-pond damselfly *Lestes eurinus*, and compare the results with former studies on temporary-pond *Lestes*. Larvae showed full compensation in body mass at emergence for all combinations of time stress and starvation or cooling. Unexpectedly, compensatory growth to starvation or cooling was not stronger under time stress. Instead, males under time stress delayed emergence after these transient stressors. In line with a stronger compensatory growth response to time stress than to the other stressors, physiological costs in terms of a reduced investment in immune response (measured as phenoloxidase activity) and energy storage (measured as fat content) were detected only under time stress. Compared to temporary-pond *Lestes*, *L. eurinus* showed stronger compensatory growth to time stress. We hypothesize that the stronger compensatory (growth) response in permanent-pond *Lestes* co-evolved with their derived slower lifestyle when they invaded permanent ponds.

Given the strong link between adult mass and fitness (Roff 2002), animals are expected to compensate for any stress that would negatively affect final mass. Individuals can either compensate by taking longer to reach a given developmental stage or by accelerating the rate of mass accumulation (i.e. growth rate) to catch up (Metcalfe and Monaghan 2001). Although compensatory growth has received much attention, our insight in its evolution and ecological correlates is limited because only a small set of wild species has been studied (Ali et al. 2003, Margraf et al. 2003, Alvarez and Nicieza 2005), and few comparisons among species or populations with marked differences in life cycle strategies and environmental stressors have been made (Schultz et al. 2002, Sogard and Olla 2002, Alvarez and Metcalfe 2007). Patterns in the occurrence, strength and costs of compensatory growth may explain why animals do not always grow at their maximum rates (Arendt 1997). This is central to the study of growth trade-offs and will give insight when mass and age at maturity, key fitness components, are negatively related (Nylin and Gotthard 1998). Also, studies of multiple species in a phylogenetic context will allow inferences about the directionality of evolutionary responses (Pigliucci 2001), but are missing so far. Including costs of compensatory growth in such research programme will also elucidate potential constraints for the evolution of compensatory growth. Costs of rapid growth in general are understudied which holds especially for physiological costs (Arendt 1997, Gotthard 2001). The little evidence so far suggests that compensatory growth may result in a long-term lower investment in immune response and energy storage (Morgan and Metcalfe 2001, Rolff et al. 2004, Stoks et al. 2006a, 2006b).

The damselfly genus *Lestes* is an elegant system to study compensatory growth responses in an ecological and evolutionary context, because there is a striking dichotomy in life history strategies linked to species occupying temporary or permanent water bodies (Stoks and McPeek 2006). The large majority of *Lestes* species occupy temporary water bodies that intermittently dry in summer. These species all lay eggs in summer and the eggs remain in a diapause state, which is drought resistant, throughout autumn and winter. In the following spring, eggs hatch, and individuals spend 2–3 months in a rapidly developing aquatic larval stage. Adults emerge in summer (Jödicke 1997). Larvae of these species face strong time constraints in many generations because they must emerge before their pond dries. A second, permanent-pond group contains species with direct egg development. Eggs of these species hatch into aquatic larvae in late summer, and the larval phase lasts ca 10 months through winter and to the following spring, when individuals emerge as aerial adults in early summer. This life history strategy limits these

species to permanent ponds and lakes because of the overwintering aquatic larval stage (Stoks and McPeek 2003a, 2006). Phylogenetic analyses suggest that the temporary-pond life history with its diapausing eggs and short larval period is the ancestral state and that one habitat shift into permanent lakes gave rise to the permanent-lake life history with its direct egg development and a long larval period (Stoks and McPeek 2006). In line with these life history differences, routine growth rates, i.e. growth rates in non-stressed larvae, are higher in temporary-pond *Lestes* compared to permanent-pond *Lestes* (Stoks and McPeek 2003b).

Based on the change in routine growth rates in association with the habitat shift two opposite evolutionary outcomes with regard to the strength of compensatory growth can be expected depending on the relative strength of constraints and selective factors shaping compensatory growth (see Schultz et al. 2002 for a similar reasoning at the population level along a latitude gradient). First, temporary-pond *Lestes* with their rapid routine growth rates may already be growing near to their physiological maximum, and further increases to compensate for a growth delay may consequently be small. If true, permanent-pond *Lestes* with their lower routine growth rates should be able to show a larger compensatory growth response. Alternatively, the same selective factor (i.e. time stress imposed by hydroperiod) that likely shaped the higher routine growth rates in temporary-pond *Lestes* may also have selected for a larger compensatory growth response because the perceived growth delay is relatively higher and more costly to overall fitness under a time stress (Metcalfe et al. 2002). Under this scenario, permanent-pond *Lestes* that do not experience hydroperiod-imposed selection for a fast life history, likely will show a smaller compensatory growth response. Several studies have shown compensatory growth in *Lestes* (Discussion), but so far these were only performed on species of the temporary-pond group. To get insight in the evolution and ecological correlates of compensatory growth and to disentangle both evolutionary scenarios we here investigated the intensity of compensatory growth and its associated costs in a permanent-pond *Lestes*.

The same factors that play at the species level may also shape the occurrence and strength of compensatory growth within species. Stressors typically co-occur in nature (Sih et al. 2004) and compensatory growth may occur in response to several stressors, including time stress, transient nutritional and thermal stress (Metcalfe and Monaghan 2001). It has been hypothesized that under time stress larvae should show a stronger compensatory growth response to starvation or cooling because of the reduced time available to restore body mass (Metcalfe et al. 2002). Animals often face time stress because they have to reach a given stage before a certain time (e.g. the onset of winter or pond drying, Rowe and Ludwig 1991, Abrams et al. 1996). This hypothesis of a synergistic compensatory response between time stress and other transient stressors has rarely been tested. In the damselfly *L. viridis* no interaction between the compensatory growth response to time stress and starvation was observed (Stoks et al. 2006b). However, *L. viridis* shows the typically high routine growth rates of species living in temporary ponds (Stoks and McPeek 2003b), which therefore may not be able to synergistically increase growth rate. Under this scenario, a synergistic response between time stress and transient food stress may be more likely in permanent-pond *Lestes* than in temporary-pond *Lestes*.

We studied compensatory growth and its associated costs to a combination of time stress and a transient period of nutritional or thermal stress in a larval rearing experiment from the egg stage until adult emergence of the permanent-pond *Lestes L. eurinus*. We imposed time stress by manipulating the perceived hatching date of the larvae. Larvae that experience a photoperiod indicating a late hatching date in summer are time-stressed in the sense they have a shorter larval growing period available compared to larvae that experience an early-hatching date. Adults that emerge late may have a lower lifetime mating success (Anholt 1991) as weather conditions deteriorate throughout the reproductive season. Furthermore, their offspring will hatch later and therefore will be smaller and more vulnerable to cannibalism by offspring from early-emerged adults (Anholt 1994). Besides time stress and transient nutritional stress, we also imposed transient thermal stress (i.e. a period with depressed temperatures as experienced during winter) to induce a growth delay hence a compensatory growth response. *L. eurinus* winters in the last larval instars and regains normal growth in early spring. However, spring conditions in New England may be variable and subsequent periods of cold temperature are common (De Block unpubl.). Besides comparing our results with those obtained in temporary-pond *Lestes* we tested following a priori predictions. (1) Under time stress larvae will reduce age at emergence, and show compensatory growth to keep mass at emergence as constant as possible (Rowe and Ludwig 1991, Abrams et al. 1996). (2) After starvation or cooling larvae will show compensatory growth and delay emergence to keep mass at emergence as constant as possible (Metcalfe and Monaghan 2001). (3) Under time stress larvae should show a stronger compensatory growth response to starvation or cooling because of the reduced time available to restore body mass (Metcalfe et al. 2002). (4) Compensatory growth will result in a lower investment in immune response and energy storage (Arendt 1997, Gotthard 2001). For this we scored the activity of phenoloxidase (PO), a key variable in insect immune function (Siva-Jothy et al. 2005), and total fat content, the most important long-term energy storage pool in insects (Klowden 2002). Both PO and fat content are important physiological traits as they are linked to adult fitness in damselflies (Plaistow and Siva-Jothy 1996, Rolff et al. 2004).

Methods

We set up a randomized full factorial 2 (photoperiod treatment) $\times 3$ (starvation/cooling treatment) rearing experiment. The photoperiod treatment started in the egg stage and ran until adult emergence, and exposed animals to the light regime that would be experienced by larvae that hatched early (low time stress) and late (high time stress) in the season. The starvation/cooling treatment was imposed for one week, two weeks after the end of the artificial winter period. We chose not to impose the starvation/cooling

treatment before winter as at that moment many larvae were too small to weigh without damage.

Vegetation with eggs of *L. eurinus* was collected on 5 August 2004 at Sylvester Pond (Norwich, VT, USA). The vegetation was randomly divided in two groups and placed in plastic containers with water in a temperature controlled room at 20°C. One group was kept at the photoperiod of the actual date minus three weeks (early photoperiod; starting at the photoperiod of 15 July) and the other group at the photoperiod of the actual date plus three weeks (late photoperiod; starting at the photoperiod of 1 September). Containers were checked daily for freshly-hatched larvae. Once hatched, larvae were reared separately in 100-ml plastic cups filled with spring water until metamorphosis. Early and late photoperiods were adjusted every week to simulate the natural progress of the light cycle. The same temperature controlled room was divided in two compartments using black opaque plastic. All larvae of the same photoperiod treatment shared the same compartment at any given time. To minimize potential confounding differences between the compartments, larvae and their respective photoperiod were rotated between compartments biweekly (Johansson and Rowe 1999). Each larva was fed one ration of *Artemia* nauplii six times a week (one ration = 224 ± 19 nauplii [mean ± 1 SE]; n = 6 random samples). This corresponds with high food levels (Johansson et al. 2001).

New England ponds are typically covered with ice from late November onwards, and water temperatures under the ice are 3–4°C (De Block et al. 2007). So when light regimes of the two photoperiod treatments matched the photoperiod of 22 November (actual dates being 15 December and 1 November for the early and late photoperiod treatments, respectively) winter conditions were initiated. Therefore, cups with larvae were transferred randomly to one of two incubators. The temperature in the incubators was lowered to 4°C over 24 h. The photoperiod was kept constant at L:D 9:15, indicating winter, and larvae were fed *Artemia* nauplii ad libitum twice a week. Field experiments have shown that *L. eurinus* larvae feed and grow, albeit very slowly, throughout the winter (De Block et al. 2007). Larvae were rotated between incubators biweekly.

After three months on these winter conditions, spring conditions were established. The temperature in the incubators was raised to 15°C over 24 h, the photoperiod was set to the photoperiod of 1 April, and larvae were fed one ration of *Artemia* nauplii six times a week. Photoperiods were again adjusted every week to simulate the natural progress of the light cycle. Both the early and the late photoperiod larvae were reared at the same photoperiod after their artificial winter because early- and late-hatched larvae begin further development at the same time after winter (when temperature levels increase above 7°C) (Corbet 1999). Larvae that perceived a late hatching date (i.e. that experienced the late photoregime before winter) were, however, younger and smaller when they entered spring conditions (Results). As they experienced the same photoperiod regime in spring at smaller ages/sizes, they still perceived a higher time stress during this period compared to larvae that perceived an early hatching date (Strobbe and Stoks 2004).

Two weeks later, all larvae were weighed. Larvae were then assigned to one of three starvation/cooling treatments (hereafter called the SC treatment) for the next seven days: (1) control treatment: fed daily and 15°C, (2) starvation treatment: fed only on day 4 and 15°C, and (3) cooling treatment: fed daily and 4°C. After this 7-day SC-period, all larvae were weighed again. Larval wet mass was measured by gently blotting larvae dry with absorbent tissue and weighing them to the nearest 0.01 mg on an electronic microbalance. To ensure empty guts when weighing, the larvae were not fed during the preceding 24 h.

One month after switching to spring conditions, larvae were taken out of the incubators and transferred to the same compartmentalized room as used before winter at 20°C. When larvae molted into the final instar, the number of *Artemia* nauplii per food ration was increased (one ration = 952 ± 11 nauplii [mean ± 1 SE]; n = 6 random samples). Throughout the experiment, cups were checked twice a day for dead and emerged individuals. Adults were weighed to the nearest 0.01 mg the morning following emergence. Relative growth rates were calculated as $[\ln(mass_{end}) - \ln(mass_{ini})]/(t_{end} - t_{ini})$ with $mass_{end}$ and $mass_{ini}$ being masses at the end (t_{end}) and start (t_{ini}) of a growth period. Three growth periods were distinguished: the pre-SC period (from hatching till the start of the SC period), the SC-period, and the post-SC period (from the end of the SC period till emergence). For the pre-SC period $\ln(mass_{ini})$ was set to zero (Johansson and Rowe 1999).

Phenoloxidase (PO) activity was measured at emergence following the protocol of Rolff et al. (2004). We first cut off head and abdomen from the thorax. Haemolymph extracts were obtained by perfusing the thorax of an individual with 0.3 ml of cacodylate buffer (0.01 M Na-Coc, 0.005 M $CaCl_2$). The haemolymph extract was collected in a 1.5-ml microcentrifuge tube and immediately frozen at −80°C. For the spectrophotometric assay, the samples were thawed on ice. The cell walls were removed via centrifugation (4°C, 4000 rpm, 15 min). Twenty µl of the supernatant was added to 60 µl L-dihydrophenylalanine (10 mM in cacodylate buffer) and 120 µl phosphate buffered saline. The reaction was allowed to proceed for 30 min at 30°C. During the reaction PO catalyses the transition from L-Dopa to dopachrome. Dopachrome can then be measured photometrically. Readings were taken every 10 s on a plate reader. Enzyme activity was measured as V_{max} (the slope of the reaction curve during the linear phase of the reaction). Samples were run in duplicate and the mean of both readings of each sample was used for further analysis.

Afterwards, fat at emergence was measured. Therefore, all adult body parts (head, thorax and abdomen) were placed in 1.5-ml microcentrifuge tubes and dried for >48 h at 60°C before their dry mass was taken to the nearest 0.01 mg. To extract fat 1 ml of dichloromethane was added to each microcentrifuge tube, which were shaken for 24 h. The dichloromethane was removed from each microcentrifuge tube and larvae were dried and weighed again. The difference in mass equals the amount of fat of a larva.

We tested for effects of photoperiod and SC treatment on survival with a loglinear model. Note that we did not include sex as we could not sex larvae initially. Because we had repeated measurements on the same larva, repeated-measures analyses of variance (RM-ANOVAs) were run for mass and relative growth rate. In these analyses, photoperiod, SC treatment and sex were included as independent

variables. To specifically test whether differences in relative growth rate between photoperiod treatments were not due to differences in mass we also evaluated the effect of photoperiod on relative growth rates during a given period with mean larval mass during that period as a covariate. We tested for the effects of photoperiod, SC treatment and sex on PO activity and fat content at emergence with mass at emergence as a covariate in a MANCOVA. Age at emergence and PO activity were ln-transformed to meet assumptions of AN(C)OVA. As we were specifically interested whether the starvation and cooling treatment had a differential effect, we set two a priori orthogonal contrasts. A first contrast compared the control treatment with the combined starvation and cooling treatments, a second one compared the starvation with the cooling treatment. Since multiple contrasts were made, we compared their p-values against a corrected α-level. For most response variables only these two contrasts were tested, hence α-adjusted was set at 0.025 (0.05/2). In the case of age at emergence the two contrasts were made separately by sex, hence α-adjusted was set at 0.0125 (0.05/4). Sample sizes slightly differed between end points. Analyses on all available data or analyses only including data for larvae of which all end points were available gave similar significance patterns and only the latter analyses will be presented.

Results

Larval survival did not vary among levels of the photoperiod (loglinear analysis, $\chi^2_1 = 1.97$, $p > 0.15$) or SC treatments ($\chi^2_1 = 5.14$, $p = 0.08$). Overall survival was 81.9%.

Animals reared in the late photoperiod emerged as adults ca 35 days earlier in age than those in the early photoperiod, a reduction of 13% (Fig. 1: $F_{1,305} = 2271.36$, $p < 0.0001$). Note that taking into account the imposed time shift of 42 days, the perceived age of emergence was still ca seven days higher in animals reared in the late photoperiod (ANOVA on perceived age, $F_{1,305} = 99.16$, $p < 0.0001$). The three-way interaction among photoperiod, SC treatment and sex for age at emergence was nearly significant ($F_{2,305} = 2.93$, $p = 0.088$), because responses were different between early and late photoperiods. In the early photoperiod, adults emerged at similar ages across all treatments (all main effects and interactions with $p > 0.34$ within the early photoperiod). However, in the late photoperiod, animals responded to the SC treatments in age at emergence in a sex-specific way (SC treatment $\times$ sex, $F_{2,145} = 4.02$, $p < 0.05$). Males in the late photoperiod emerged earlier in the control than in the starved or cooled treatment levels (contrast, $F_{1,71} = 14.80$ $p < 0.001$), which did not differ (contrast, $F_{1,71} = 3.58$, $p = 0.063$, α-adjusted $= 0.0125$). Control females in the late photoperiod did not differ in age at emergence from starved or cooled larvae (contrast, $F_{1,74} = 0.19$, $p > 0.66$), neither differed starved larvae from cooled larvae (contrast, $F_{1,74} = 5.12$, $p = 0.027$, α-adjusted $= 0.0125$).

The effect of photoperiod on body mass changed through time (Fig. 2: RM-ANOVA, photoperiod $\times$ time, $F_{2,610} = 243.67$, $p < 0.0001$). At the start and at the end of the SC period, larval mass was lower in the late photoperiod than in the early photoperiod treatments (both $p < 0.0001$), which is consistent with developmental age differences at that moment. In contrast, by the time adults emerged, masses did not differ among photoperiod treatments ($F_{1,305} = 0.16$, $p > 0.69$), although their ages were quite different (Fig. 1). Also patterns in mass changed through time among SC treatments (Fig. 2: RM-ANOVA, SC treatment $\times$ time, $F_{4,610} = 11.93$, $p < 0.0001$). Prior to their allocation, larval masses in the various SC treatments, were not different ($F_{2,305} = 0.60$, $p > 0.54$). At the end of the SC period, mass was lower in starved and cooled larvae than in control larvae (contrast, $F_{1,305} = 11.99$, $p < 0.001$), and cooled larvae had a lower mass than starved larvae (contrast, $F_{1,305} = 5.53$, $p = 0.019$, α-adjusted $= 0.025$). However, at emergence, masses did again not differ among the SC treatments ($F_{2,305} = 1.54$, $p > 0.21$). At any point in time females were heavier than males (Fig. 2: sex, $F_{1,305} = 23.73$, $p < 0.0001$).

We also analyze relative growth rate, because it is an integrative measure of changes in age and body mass. Photoperiod and SC treatments interacted in a time and sex specific way in shaping relative growth rate (Fig. 3: RM-ANOVA, photoperiod $\times$ SC treatment $\times$ sex $\times$ time, $F_{4,416} = 3.16$, $p < 0.05$). To dissect this four-way interaction we performed separate ANOVAs for the three periods. During the pre-SC period, larvae in the late photoperiod had higher relative growth rates than larvae in the early photoperiod ($F_{1,305} = 24.15$, $p < 0.0001$), but relative

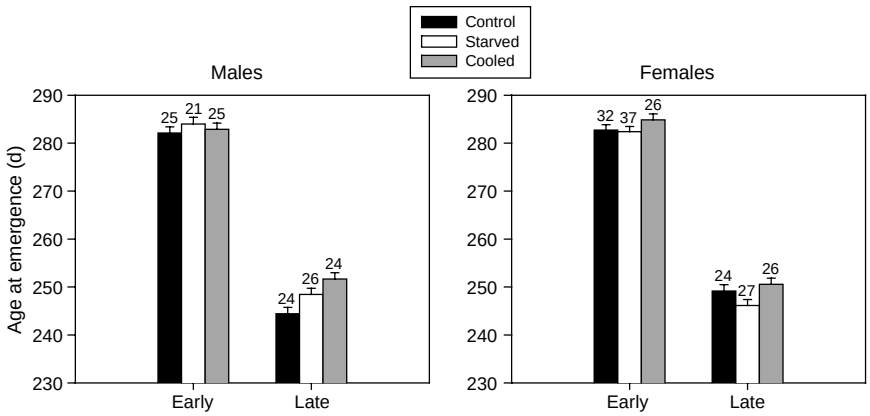

Fig. 1. Mean (+1 SE) age at emergence of *Lestes eurinus* larvae as a function of photoperiod, SC (starvation or cooling) treatment and sex. Numbers above bars represent sample sizes.

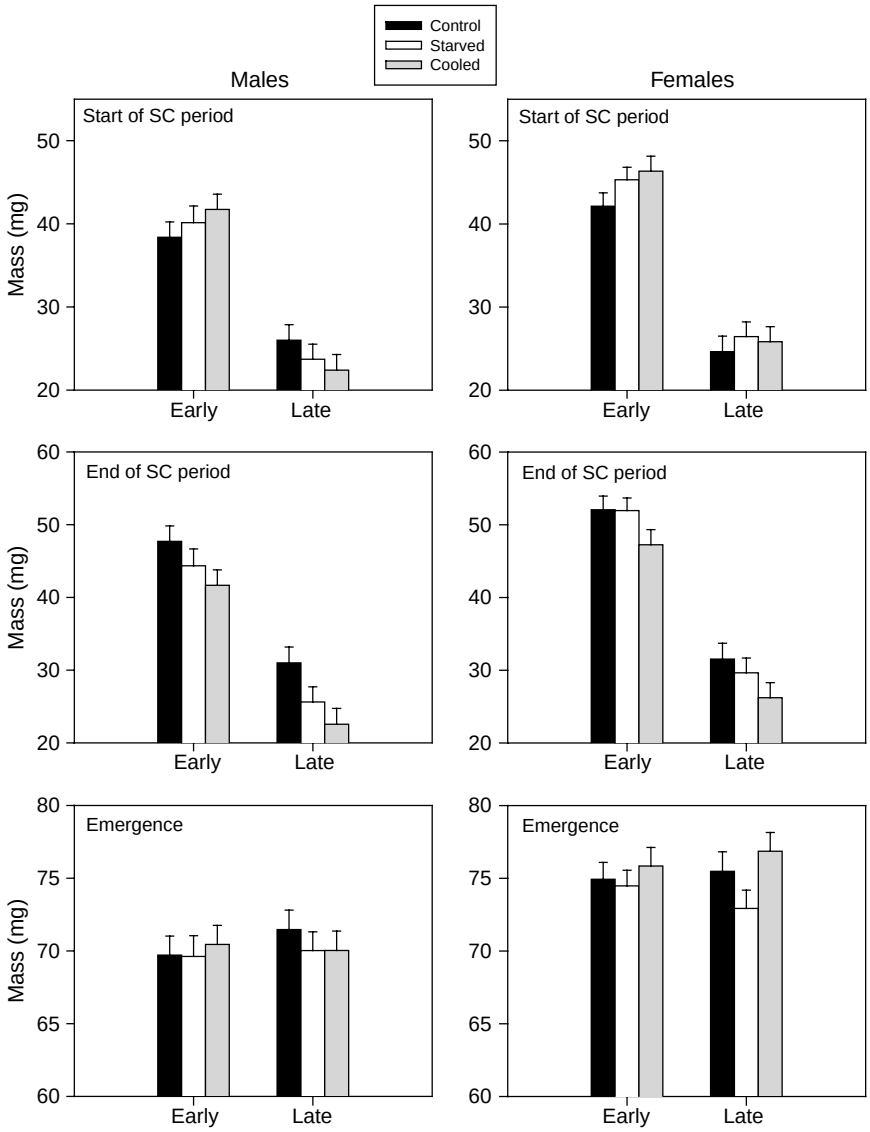

Fig. 2. Mean (+1 SE) body mass of *Lestes eurinus* larvae as a function of photoperiod, SC (starvation or cooling) treatment and sex at three successive moments: start and end of the SC period, and at emergence. Sample sizes as in Fig. 1.

growth rates among the SC treatments did not differ ($F_{2,305} = 0.20$, $p > 0.82$). These differences in relative growth rate between photoperiod treatments remained when correcting for the mean mass during the pre-SC period ($F_{1,304} = 911.89$, $p < 0.0001$). Overall, females had higher relative growth rates than males ($F_{1,305} = 5.14$, $p < 0.05$).

During the SC period, relative growth rate showed a 3-way interaction among photoperiod, SC treatment and sex (Fig. 3: $F_{2,208} = 4.74$, $p < 0.01$). In the control treatment, larvae in the late photoperiod gained mass faster than larvae in the early photoperiod ($F_{1,51} = 10.77$, $p < 0.01$), and this effect tended to be more pronounced in females ($F_{1,51} = 3.94$, $p = 0.053$). These effects remained when correcting for the mean mass during the SC period (photoperiod: $F_{1,50} = 4.83$, $p < 0.05$, photoperiod × sex: $F_{1,50} = 3.70$, $p = 0.060$). Not surprisingly, relative growth rates in starved and cooled larvae were very low and were significantly lower than in control larvae (contrast, $F_{1,208} = 287.51$, $p < 0.0001$, α-adjusted $= 0.025$), and relative growth rates did not differ between starved and cooled larvae (contrast, $F_{1,208} = 2.06$, $p > 0.15$).

During the post-SC period, relative growth rates were independently affected by photoperiod and SC treatments (Fig. 3: photoperiod: $F_{1,305} = 339.22$, $p < 0.0001$; SC treatment, $F_{2,305} = 10.16$, $p < 0.0001$; photoperiod × SC treatment, $F_{1,305} = 0.39$, $p > 0.67$). Larvae reared in the late photoperiod grew substantially faster than larvae in the early photoperiod, also when correcting for the mean mass during the post-SC period ($F_{1,304} = 130.70$, $p < 0.0001$). In general, relative growth rates increased from control to starved to cooled SC treatment levels (contrast of control vs starved and cooled larvae, $F_{1,305} = 13.93$, $p < 0.001$; contrast of starved vs cooled larvae, $F_{1,305} = 6.68$, $p = 0.010$, α-adjusted $= 0.025$).

Photoperiod and sex independently affected PO activity and fat content at emergence (Fig. 4a–d: MANCOVA, photoperiod, $F_{2,303} = 14.52$, $p < 0.0001$; sex, $F_{2,303} = 5.51$, $p < 0.01$). Neither the SC treatment, nor any interactions affected PO activity at emergence (all $p > 0.22$). Univariate

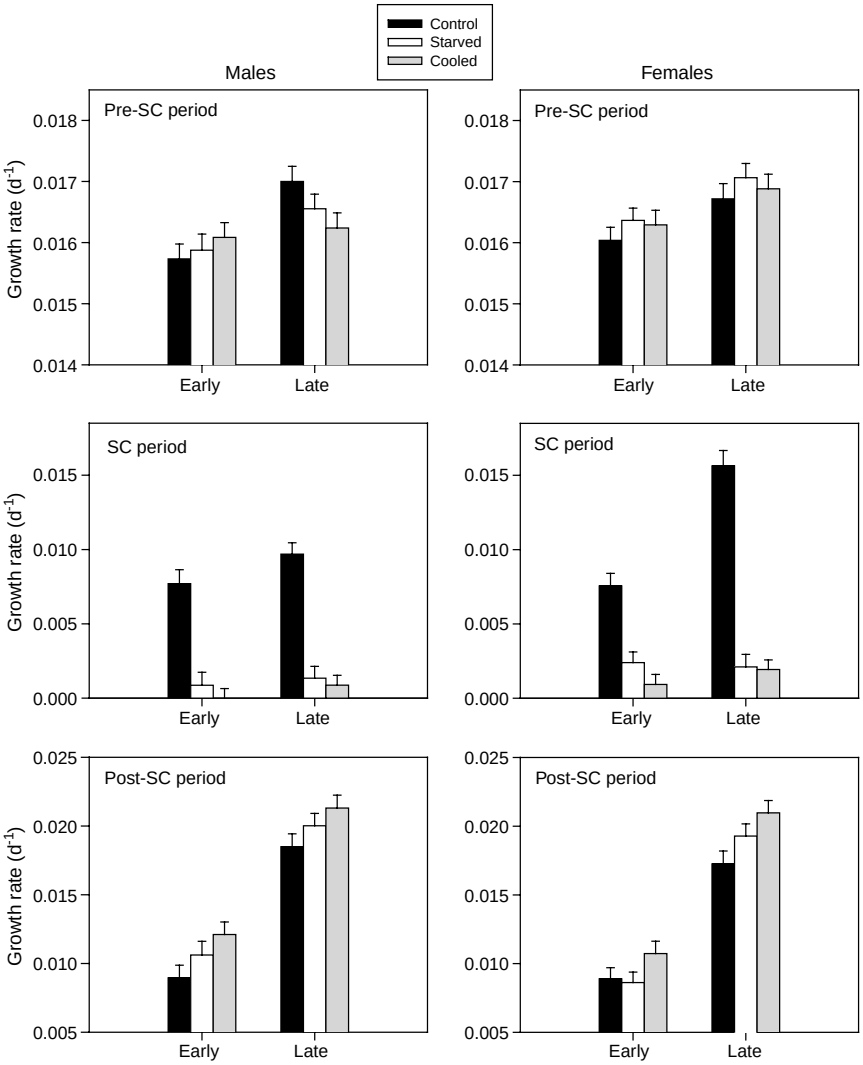

Fig. 3. Mean (+1 SE) growth rate of *Lestes eurinus* larvae as a function of photoperiod, SC (starvation or cooling) treatment and sex during three successive periods: pre-SC period, SC period and post-SC period. Sample sizes as in Fig. 1.

ANCOVAs showed that both PO activity and fat content were lower in the late photoperiod than in the early photoperiod (PO activity: $F_{1,304} = 14.59$, $p < 0.001$; fat content: $F_{1,304} = 14.52$, $p < 0.001$). Overall, females had a higher PO activity than males (Fig. 4a-b: $F_{1,304} = 7.63$, $p < 0.01$). Sexes did not differ in fat content (Fig. 4c–d: $F_{1,304} = 3.43$, $p = 0.07$).

Discussion

Lestes eurinus larvae showed strong compensatory responses to the imposed combinations of time stress and transient nutritional or thermal stress, which resulted in full compensation in body mass at emergence irrespective of the combination of stressors involved. In this experiment, we simulated larvae that hatched at different times (i.e. photoperiod treatment) and that experienced a seven-day period of either starvation or return to winter temperatures (i.e. SC treatment). These treatments generated great differences in age at emergence, and differences in larval masses and rates of mass accumulation throughout the larval period (Fig. 1–3). However, by the time they emerged as adults, no differences in mass were apparent between any of the treatments, indicating that all animals had fully compensated for various growth deficiencies. As predicted, these responses demonstrate that *L. eurinus* larvae showed compensatory growth responses to time stress (Rowe and Ludwig 1991, Abrams et al. 1996) and after a period of suboptimal growth conditions caused by starvation or cooling (Morgan and Metcalfe 2001). Compensatory responses were also accomplished by emerging earlier in response to time stress and by emerging later in response to the growth delay due to starvation and cooling. The latter mass-compensatory mechanism, however, only occurred in males under time stress. The compensatory responses were much stronger to time stress than after starvation or cooling. This is in line with the fact that the imposed photoperiod treatment simulated a growth delay of six weeks, while the SC treatment only lasted for one week. Moreover, compensatory growth was more pronounced in previously-cooled larvae than in previously-starved larvae. This may be due to the fact that at the end of the SC period mass was lower after the cooling than after the starvation

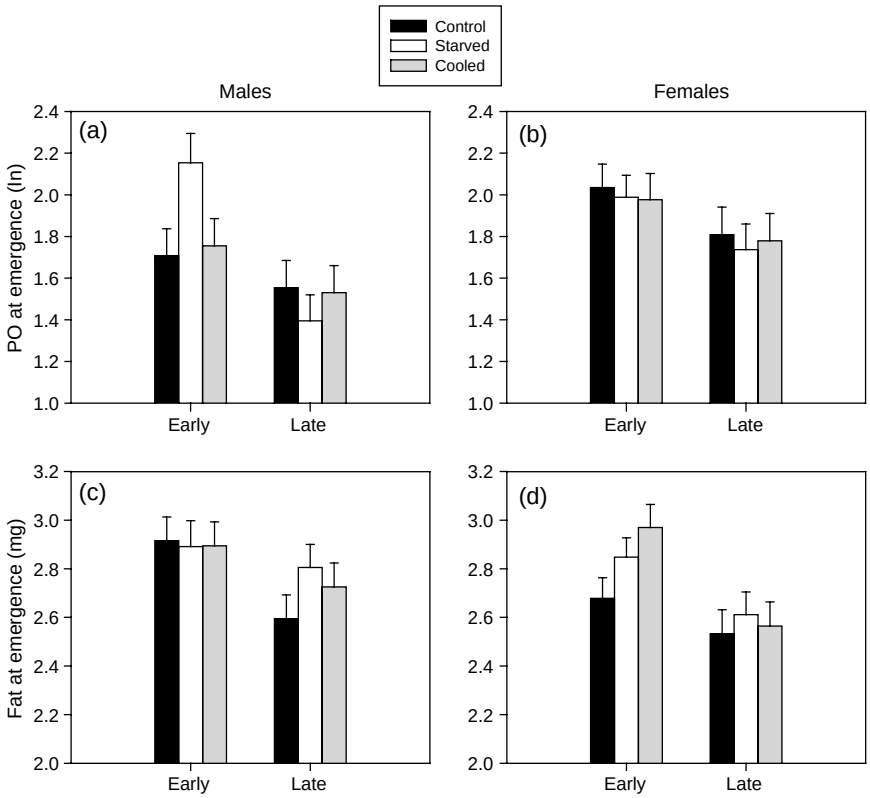

Fig. 4. Mean PO (phenoloxidase) activity and fat content of *Lestes eurinus* larvae as a function of photoperiod, SC (starvation or cooling) treatment and sex. Values are least-squares means corrected for mass at emergence. Enzyme activity was measured as V_{max} (maximum velocity; the slope of the reaction curve during the linear phase). Sample sizes as in Fig. 1.

treatment. Alternatively, starvation may cause greater physiological adjustments than cooling, so causing a longer recovery time.

Metcalfe et al. (2002) hypothesized that within species animals under time stress should show a stronger compensatory growth response to a period of reduced growth because of the reduced time available to restore body mass. Our data do not support this hypothesis as the compensatory growth response to starvation and cooling was the same in both photoperiods. Instead, males in the late photoperiod but not in the early photoperiod delayed emergence in response to starvation or cooling. Studies on compensatory growth typically do not consider the latter mass-compensatory mechanism. However, our results suggest that these mechanisms may complement one another. Note that despite this delay in emergence date, within the same SC treatment males under time stress still emerged more than 30 days before males in the early photoperiod.

Both observed mass-compensatory mechanisms, a delay in emergence and compensatory growth, likely carry costs. The delay in emergence after starvation or cooling may negatively affect fitness. For example, cannibalism occurs frequently in *Lestes* (De Block and Stoks 2004a), and the offspring from animals that emerge and reproduce later will hatch later and therefore will be smaller and more vulnerable to cannibalism by offspring from early-emerged animals (Anholt 1994). Also, later emerged adults may have a lower lifetime mating success (Anholt 1991) as weather conditions deteriorate later in the flight season. Note that the same reasons induce time stress in the larval stage. Compensatory growth to time stress was associated with a lower PO activity and a lower fat content at emergence, both of which negatively covary with adult fitness in damselflies (Plaistow and Siva-Jothy 1996, Rolff and Siva-Jothy 2004). Physiological costs may therefore play an important role in shaping carry-over costs from the larval stage where compensatory growth occurs into adult fitness. Physiological costs of compensatory growth have been largely ignored (Gotthard 2001). Only recently have studies begun to examine these compensatory growth costs (Morgan and Metcalfe 2001, Rolff et al. 2004, Stoks et al. 2006a, 2006b). These costs may result from a resource-based trade-off where fewer resources are allocated to energy storage and immune response at the expense of overall mass gain. In support of this, *Lestes* partly generate compensatory growth to time stress by allocating more assimilated food into growth, hence away from other processes (Stoks et al. 2005). However, such costs were not apparent in response to our SC treatment. Perhaps, the compensatory growth responses to the SC treatment were too weak to measure an appreciable cost in PO activity or fat storage. A reduction in fat content after compensatory growth to starvation was demonstrated in another *Lestes* where a longer starvation period was imposed and a greater compensatory growth response was observed to this stress (Stoks et al. 2006b).

The compensatory responses to time stress (i.e. between the photoperiod treatments) by the permanent-pond *Lestes L. eurinus* in this study were much stronger than we or others have observed in previous studies of temporary-pond

Lestes. Under time stress, *L. eurinus* showed a 13% lower age at emergence and a 18% higher growth rate (calculated over the entire larval period; note there is a ca 100% higher growth rate under time stress during the post-SC period). Under similar levels of time stress (at least six weeks difference in photoperiod) and with no threat of predation or food stress, temporary-pond *Lestes* show a much smaller change in age at emergence (one-sample t-test, $t_{12} = 6.76$, $p < 0.0001$) and growth rate ($t_{12} = -9.49$, $p < 0.0001$) (averaged across a number of studies: −6% and +2%, respectively, Table 1). In most of these studies on temporary-pond *Lestes* the diet of *Artemia* nauplii was supplemented in the final instar with chironomids, enchytritraeid worms or daphnids (Table 1). This was not the case in the present study, making our comparison conservative. One may argue that we based our conclusion on only one population of *L. eurinus* which therefore may not apply to other populations of this species. However, strong population differentiation in larval life history is unlikely in *L. eurinus* as it always occupies fishless, permanent ponds and shows a very similar life history: being always univoltine with a direct egg development and an overwintering larval stage of about 10 months (Stoks and McPeek 2006). This large difference in development time compared to temporary-pond *Lestes*, which have a larval stage of about 2–3 months, likely swamps any interpopulation variation in response to time stress within *L. eurinus*.

The stronger compensatory response in permanent-pond *Lestes* is probably due to the evolved slower life style and the lack of a threat of pond drying. Phylogenetic reconstructions indicate that the *Lestes* lineage that shifted from temporary ponds to permanent water bodies evolved from a life history strategy with a diapausing egg stage and a short larval period to one with immediately developing eggs and a long larval period (Stoks and McPeek 2006). The longer larval growth period probably relaxed selection for rapid routine growth as rapid growth may carry costs, here identified as a reduced investment in immune response (PO) and in energy storage (fat content). This presumably caused the lower routine growth rates observed in the permanent-pond *Lestes* (Stoks and McPeek 2003b, Table 1). The much shorter larval growth period and higher routine growth rates in temporary-pond *Lestes* (Stoks and McPeek 2003b, Table 1) may give them less opportunity to further accelerate development and growth as they may be near their physiological limits (see also Sogard and Olla 2002 for a similar reasoning for two unrelated fish species). The higher growth rates in temporary-pond *Lestes* are due to higher conversion efficiencies of assimilated food into body mass (Stoks and McPeek 2003b). Also, because the pond may dry – which would result in larval mortality – temporary-pond *Lestes* do not have the luxury of delaying emergence to accumulate greater mass, as *L. eurinus* does. Taken together, we therefore hypothesize that the stronger compensatory response in permanent-pond *Lestes* co-evolved with their derived slower lifestyle when they invaded permanent ponds.

We know relatively little on the macro-evolution and ecological correlates of compensatory growth (Ali et al. 2003, Alvarez and Nicieza 2005). Studies that compared

Table 1. Overview of the strength of compensatory responses to time stress in *Lestes* species. Early and late denote the photoperiod treatments that when started refer to hatching dates early (low time stress) and late (high time stress) in the larval growth season.

Species	Sample size		Age at emergence (days)¤			Growth rate (across entire larval period)¤			Reference£
	Early	Late	Early	Late	Difference (%)$	Early	Late	Difference (%)$	
Temporary-pond *Lestes*									
L. congener	78	89	64.6	62.3	−4***	0.0331	0.0341	+3*	1
L. congener†	17	24	112	100	−11***	0.053	0.048	−9*	2
L. dryas	76	72	46.5	44.2	−5***	0.0450	0.0453	$+1^{NS}$	1
L. forcipatus	58	69	66.3	63.8	−4**	0.0347	0.0356	$+3^{NS}$	1
L. sponsa†	19	20	110.3	100.8	−9***	0.0170	0.0156	−8**	3‡
L. sponsa†	19	20	119.5	115.8	−3*	0.0176	0.0175	-1^{NS}	3‡
L. sponsa†	30	30	112.2	101.1	−10***	0.0431	0.0480	+11***	4
L. viridis†	48	48	67.5	64.3	−5***	0.0328	0.0322	−2**	5
L. viridis	72	76	86.0	82.8	−4***	0.0246	0.0245	0^{NS}	6
L. viridis†	294	244	79.9	74.1	−7***	0.0313	0.0331	+6***	7
L. viridis	88	74	60.8	61.4	$+1^{NS}$	0.0321	0.0315	-2^{NS}	8
L. viridis†	156	124	78.3	71.5	−9***	0.0338	0.0362	+7***	9
L. viridis†	52	55	71.6	64.2	−11***	0.0355	0.0395	+11***	10
Permanent-pond *Lestes*									
L. eurinus	57	48	282.5	246.9	−13%***	0.0098	0.0116	+18***	11

¤Growth rates calculated for the entire larval period as ln(dry mass at emergence)/age at emergence. For some studies of *L. viridis* only wet mass data were available; here dry mass was calculated as wet mass/3.79 based on studies that measured both wet mass and dry mass. When different treatments were present, the treatment with optimal growth conditions (i.e. high food, no predator) was chosen. If larvae from several hatching dates were used, an overall mean was calculated.

†Increased food ration in final instar by adding chironomids, enchytritraeid worms or daphnids to the diet.

‡Results from the ''Predation experiment'' and the ''Resource experiment'', respectively.

$p-values: NS: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

£1: De Block et al. (in press), 2: Johansson and Rowe (1999), 3:Johansson et al. (2001), 4: Stoks et al. (2005), 5: De Block and Stoks (2003), 6: De Block and Stoks (2004b), 7: De Block and Stoks (2005), 8: Rolff et al. (2004), 9: Stoks et al. (2006a), 10: Stoks et al. (2006b), and 11: present study.

compensatory growth among species to now have focused on species from different genera (Sogard and Olla 2002, Tammaru et al. 2004) or have not considered phylogenetic relationships and directionality of evolutionary change (Nylin et al. 1996, Alvarez and Nicieza 2005). Although such studies are highly informative, they cannot make inferences about the direction of evolution and therefore about what change in selective agents have caused the evolutionary responses. Interpretations of our results are based on our phylogenetic hypotheses about the polarity of the habitat shift that produced *Lestes* species in temporary and permanent ponds. More studies on various taxa conducted in a phylogenetic context are needed to unravel the evolution of compensatory growth and key selective agents. Such studies will be insightful because reciprocal shifts of lineages between two selective regimes do not necessarily result in reciprocal responses to these selective changes (Stoks and McPeek 2006), and thus whether a selection pressure has been added or removed from a lineage can be critical to interpreting the resulting evolutionary responses.

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