Time constraints and flexibility of growth strategies: geographic variation in catch-up growth responses in amphibian larvae

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Summary

1. As size is tightly associated with fitness, compensatory strategies for growth loss can be vital for restoring individual fitness. However, immediate and delayed costs of compensatory responses may prevent their generalization, and the optimal strategy may depend on environmental conditions. Compensatory responses may be particularly important in high-latitude habitats with short growing seasons, and thus, high-latitude organisms might be more efficient at compensating after periods of unfavourable growth conditions than low-latitude organisms.

2. We investigated geographical differences in catch-up growth strategies of populations of the common frog (*Rana temporaria*) from southern and northern Sweden in two factorial common garden experiments involving predation risk and two different causes of growth arrest (nutritional stress and low temperatures) to evaluate how the compensatory strategies can be affected by context-dependent costs of compensation. Larval and metamorphic traits, and post-metamorphic performance were used as response variables.

3. Only northern tadpoles exposed to low food completely caught up in terms of metamorphic size, mainly by extending the larval period. Low food decreased survival and post-metamorphic jumping performance in southern, but not in northern tadpoles, suggesting that northern tadpoles have a better ability to compensate after periods of restricted food.

4. Both northern and southern tadpoles were able to metamorphose at the same size as control tadpoles after being exposed to low temperatures, indicating that consequences of variation in temperature and food availability differed for tadpoles. However, the combination of low temperatures and predation risk reduced survival in both southern and northern tadpoles. Also, predation risk decreased energy storage in both experiments.

5. Our results highlight the influence of climatic variation and the type of stressor as selective factors shaping compensatory strategies.

Key-words: catch-up growth, life history strategies, metamorphosis, *Rana temporaria*, time-stressed populations

Introduction

In most animals, there is a positive association between size and fitness (Roff 1992). Larger individuals may have higher social status, lower risk of starving or being preyed upon, and greater reproductive success (Roff 1992; Arendt 1997). Environmental factors like low resource availability and temperature fluctuations can decrease growth and exert a negative influence on fitness (e.g. Kingsolver & Huey 2008; Amarasekare & Savage 2012). Consequently, to minimize fitness loss, selection may have favoured potential for growth recovery after periods of slow growth. There are two ways by which organisms can compensate for transitory growth deceleration (reviewed in Hector & Nakagawa 2012). The first involves changes in the timing of life cycle events (e.g. metamorphosis,
Compensatory strategies may be especially important in animals with complex life cycles, where size at and timing of crucial life-history switch points can strongly influence fitness (Stearns & Koella 1986). In amphibians, size differences at metamorphosis tend to persist through later life and individuals metamorphosing early and at large size often enjoy higher fitness through the positive effects on survival and reproductive success (e.g. Smith 1987; Semlitsch, Scott & Pechmann 1988; Alteweg & Reyer 2003). Both size at metamorphosis and developmental time are influenced by environmental conditions, and the latent costs of late metamorphosis and small metamorphic size can have selected for compensatory strategies during early ontogeny (Stoks, De Block & McPeek 2006; Capellán & Nicieza 2007).

The common frog, Rana temporaria L., is a widespread anuran in Europe (Gasc et al. 1997) and an excellent study species for investigating geographic variation in compensatory strategies. Along the latitudinal gradient across Scandinavia, tadpoles of R. temporaria exhibit increasing routine growth and development rates towards higher latitudes (e.g. Laugen et al. 2003; Lindgren & Laurila 2009). Because of the more stringent time constraint in the north, the costs of delaying growth and development should be larger for high-latitude tadpoles. For example, as body size is positively correlated with overwinter survival in many ectotherms (e.g. Alteweg & Reyer 2003; Munch, Mangel & Conover 2003; Sears 2005), size at metamorphosis may have especially high fitness value in high-latitude populations, where post-metamorphic juveniles have very little time available for foraging before the onset of winter (Laugen et al. 2003). Also, predator densities are lower in high-latitude ponds (Laurila, Lindgren & Laugen 2008), possibly reducing the cost of increased predation risk for northern tadpoles. Consequently, selection may have favoured stronger compensatory responses in northern populations.

In this study, we analysed the patterns and costs of catch-up growth responses in latitudinally separated amphibian populations. We used individuals from four R. temporaria populations originating from the two latitudinal extremes across Sweden, compared their ability to compensate in terms of age and size at metamorphosis and explored differences in potential costs following the growth delay in terms of survival, decreased lipid levels, impaired locomotor performance and increased activity rates in the presence of predators. We conducted two experiments: in the first, we arrested growth by using low food levels, whereas in the second experiment, we reared the tadpoles at low temperature. In both experiments, the main stress factor was combined with the presence of predator, which allowed us to investigate whether
predator presence affected compensatory abilities, as well as synergistic effects between temperature or food stress and predator stress. Although predation is considered a major cost of compensatory growth responses (Mangel & Munch 2005; Dmitriew 2011), few studies have explored whether predator stress can have an effect on compensatory strategies (but see Dmitriew & Rowe 2005; Stoks et al. 2005).

We made the following predictions: (i) northern tadpoles will show stronger compensation than southern populations after a period of low temperature or low food, with metamorphic timing and size being more similar to that of tadpoles growing under continuous favourable conditions. Alternatively (ii), southern tadpoles will show stronger compensatory responses because the northern tadpoles are already growing at their physiological maximum levels. (ii) Low temperature and low food levels will induce extended larval periods. (iv) Compensation will be partially attained by increasing risk-taking behaviour, and it will affect negatively juvenile lipid levels and locomotor performance. (v) The costs of compensation will be greater in the populations showing stronger compensatory responses. (vi) As catch-up growth responses should increase predation risk, the compensatory responses will be weaker in predator-exposed tadpoles.

Materials and methods

We conducted two experiments during two consecutive years. In the first experiment, we compared the growth patterns of four R. temporaria populations after a 12-day period of food restriction (c. 35% of the total larval period; food manipulation experiment, henceforth FME). In the second experiment, we compared the same populations in their response and recovery from a 7-day low-temperature period (c. 20% of the total larval period; temperature manipulation experiment, henceforth TME).

Freshly laid eggs of R. temporaria were collected from four populations located at two extremes of the latitudinal gradient across Sweden (Fig. 1). In both experiments, we sampled c. 500 eggs per clutch from each of ten clutches in each locality. The eggs were transported to the laboratory in Uppsala, and each clutch was distributed evenly in two 3-L buckets. Throughout the study, the eggs and tadpoles were kept in a 19 °C temperature-controlled room (except during the low-temperature treatment in TME) under 16L:8D photoperiod. After hatching, tadpoles were fed finely chopped and lightly boiled spinach ad libitum (except during the low-food treatment in FME). To ensure homogenous water quality, we used reconstituted soft water (RWS: APHA 1985). Water was completely changed every three days before the start of the experiments and every seven days during the experiments. When tadpoles had reached Gosner stage 25 (complete gill absorption; Gosner 1960), 100 tadpoles from each clutch were pooled into a single bucket. Tadpoles from each population were then haphazardly divided into groups of ten individuals, and each group allocated to an experimental container (day 0 of the experiment). Note that because of the differences in phenology (Fig. 1), the date of commencement of these experiments varied among populations.

In both experiments, half of the tadpoles were reared with a caged predator, and the other half in a predator-free environment. Late-instar Aeshna dragonfly larvae, collected from ponds near Uppsala, were used as predators. These dragonflies occur throughout Scandinavia and are voracious predators of tadpoles. The two experiments were designed according to a 2 × 2 factorial randomized block design, with geographic area (low or high latitude), predation risk (predator present or absent), and either feeding regime (continuous or restricted) or temperature (constantly high or temporarily low; see below) as factors. All these factors were treated as fixed effects. Population origin (Fig. 1) was included as a random effect nested under area. There were six replicates per treatment combination for each population (96 experimental units).

We used opaque plastic containers (38 × 28 × 13 cm) as experimental units. Each container was filled with 10 L of RSW and provided with cylindrical transparent mesh-bottom predator cage (diameter 11 cm, height 21) hung 2 cm above the container bottom. One Aeshna larva was placed in each predator cage in the containers assigned to the predator treatment. In the predator-absent treatment, the cages remained empty. The predators were fed two R. temporaria tadpoles daily so that tadpoles received both visual and chemical cues from predation. The containers were arranged into four vertical blocks to account for a known temperature gradient within the laboratory room.

In FME, all tadpoles were fed ad libitum on days 1–7. On days 8–19, those assigned to the low-food treatment were fed 1/6 of the ad libitum ration, whereas the constant-food tadpoles were kept at ad libitum level. After day 19, all tadpoles were fed ad libitum until metamorphosis. Activity was measured on days 10, 16, 19 (i.e. during the period of food restriction in the treatment tanks), 20 and 23. The number of tadpoles active (i.e. swimming or actively feeding) in each tank during a 10-s observation period

Fig. 1. Map showing the locations of the studied Rana temporaria populations and their coordinates. The southern populations (Ålskog and Måryds) were collected on 5–6 April 2008 and 4 April 2009 and the northern populations (Jukkasjärvi and Björkliden) on 6 June 2008 and 17 May 2009.
was scored six times a day (between 10 a.m. and 4 p.m.), and the mean of the six observations for each tank was used as the response variable.

In TME, all tadpoles were kept at 19 °C for the first 7 days. On days 8–14, tadpoles assigned to the low-temperature treatment were maintained at 10 °C. The treatment period was shorter than in FME to produce similar relative differences in body mass between control and treatment animals in both experiments. Behavioural measurements were carried out as described above on days 10, 14 (period of low temperature in the treatment tanks), 15, 17 and 22.

In both experiments, containers were checked once a day when the tadpoles approached metamorphosis. Metamorphosed individuals (Gosner stage 42) were removed, and mass at metamorphosis was measured to the nearest 0·1 mg with a digital balance after gently blotting the metamorphs on a paper towel to remove excess water. To minimize imposing additional stress on experimental tadpoles, we measured body mass only at two time points, one day following the low growth period and once tadpoles had reached metamorphosis. Therefore, and because of the nonlinearity of tadpole growth (see examples in Wells 2007), we did not take direct estimations of growth rates. However, even with more frequent size measurements, demonstrating compensatory growth sensu stricto is a difficult task in a rapidly developing species like *R. temporaria*, because growth rate is intimately entwined with body size and, importantly, with developmental stage. The duration of the larval period was defined as the number of days elapsed between the start of the experiment and metamorphosis. Once weighed, the metamorphs were placed in individual 0·8-L opaque plastic vials, filled with c. 0·5 cm of RSW and with a small stone for resting, until complete tail absorption (Gosner stage 46).

Locomotor performance was examined at the day juveniles reached Gosner stage 46. Jumping capacity was tested in a linear test track by gently prodding the froglet on the urostyle to induce jumping, and scoring the maximum jump distance from two jump series recorded with 1-hour interval (see Orizaola & Laurila 2009 for methodological details). After jumping tests, a photograph was taken to each juvenile to examine tibiofibula length after which the froglets were euthanized with MS222 and preserved at −80 °C.

To estimate energy reserves, the preserved tadpoles were lyophilized, then oven-dried at 37 °C overnight and weighed to the nearest 0·1 mg with a digital balance (total dry mass). We extracted the total content of non-polar lipids by Soxhlet method using petroleum ether and seven cycles of lipid extraction (c. 20 min per cycle). Petroleum ether is highly efficient for the extraction of non-polar (storage) lipids, with little removal of polar (structural) lipids (Dobush, Ankney & Kremenetz 1985). After the extraction, we oven-dried the samples for 24 h and weighed them again (lean dry mass). Lipid content was calculated as the difference between total and lean dry mass (see Nicieza, Álvarez & Atienza 2006).

**Statistical Analyses**

Analyses on mass and larval period were conducted on container means using mixed model ANOVAs where area, food/temperature and predator were treated as fixed effects and population was a random factor nested within area (north or south). We used mixed model ANCOVAs to analyse variation in jumping ability (using tibiofibula length as a covariate) and fat reserves (with dry body mass as a covariate). The random effect was estimated with REML and fixed effects with Type III mean squares in Proc Mixed in SAS (SAS Institute Inc., Cary, North Carolina, USA). Mortality had a significant effect on mass at metamorphosis in the TME and was included as a covariate in this analysis (mortality had no significant effects in other analyses). The original models included all interactions, but all non-significant higher level interactions were removed from the final models. Block did not affect any of the response variables and therefore was not included in the analyses.

Analyses of activity were performed on arcsin-transformed means of container-specific proportions using observation day as a repeated measure in a repeated-measures ANOVA (Proc Repeated in SAS). Each day was then analysed separately using ANOVA. As there was no detectable variation between the populations within area in any of the activity analysis, the population term was removed from the final model. Mortality was analysed as a binary variable with a mixed model GLMM in Proc GLIMMIX in SAS.

**Results**

**Food Manipulation Experiment**

**Tadpole body mass (day 19)**

The 12-day food restriction treatment decreased mass significantly (*F*1, 86 = 781·4, *P* < 0·001, see S1 for complete analyses). The effects of food treatment varied with geographic area and predator treatment, and southern populations experienced a greater decrease in mass than northern populations in the presence of the predator (53% mass loss on average in the North vs. 57% in the South), but were less affected in the non-predator treatments (53% mass loss on average in the North vs. 45% in the South), bringing about a significant area × food × predator interaction (*F*1, 86 = 11·39, *P* = 0·001; Fig. 2a,b).

**Behaviour**

Northern tadpoles were generally more active than southern tadpoles (59% more on average, *F*1, 88 = 184·85, *P* < 0·001, see S2 and S3 for complete analysis), and predator-exposed tadpoles were less active than controls (58% less on average, *F*1, 89 = 1081·28, *P* < 0·001; Fig. 3). Food-restricted tadpoles were more active than controls during the low-food period (49% more on average), but not during realimentation resulting in a significant day × food interaction (*F*3, 352 = 32·25, *P* < 0·001; Fig. 3a,b). In the absence of predators, low food level increased activity similarly in northern and southern tadpoles. However, in the presence of predators, the patterns showed by northern and southern tadpoles differed; northern tadpoles showed a stronger response (i.e. increase in activity) to food shortage at the beginning of the manipulation period (88% more active on average, area × food × predator, day 10; *F*1, 88 = 33·22, *P* < 0·001) and a lower response at the end (42% less active on average, day 19:...
Once the low-food tadpoles were again allowed to feed at *ad libitum* levels, their activity was similar to that of the control tadpoles (day 20: $P = 0.665$, day 22: $P = 0.246$). However, a significant predator *×* food interaction on day 20 ($F_{1, 91} = 6.26$, $P = 0.014$) indicated that previously food-restricted ‘non-predator’ tadpoles had higher activity as compared to ‘non-predator’ controls (16% higher on average), whereas previously food-restricted predator-exposed tadpoles were less active than predator-exposed control tadpoles (41% higher on average).

**Mortality**

Mortality was 10.6% in FME, with the number of metamorphs from each experimental container varying between five and ten (average 8.95). Food restriction and predator presence increased mortality in the southern (c. 16% on average, $F_{1, 88} = 12.85$, $P = 0.001$; Fig. 3a,b). Once the low-food tadpoles were again allowed to feed at *ad libitum* levels, their activity was similar to that of the control tadpoles (day 20: $P = 0.665$, day 22: $P = 0.246$). However, a significant predator *×* food interaction on day 20 ($F_{1, 91} = 6.26$, $P = 0.014$) indicated that previously food-restricted ‘non-predator’ tadpoles had higher activity as compared to ‘non-predator’ controls (16% higher on average), whereas previously food-restricted predator-exposed tadpoles were less active than predator-exposed control tadpoles (41% higher on average).

**Metamorphic traits**

Control tadpoles metamorphosed earlier than food-restricted tadpoles (> 4 days earlier on average, $F_{1, 86} = 185.69$, $P < 0.001$; Fig. 2a,b; see S1 for complete analysis), and there was a marginally non-significant area effect with southern tadpoles having longer larval periods across all treatment combinations (>6 days on average, $F_{1, 2} = 16.1$, $P = 0.057$, Fig. 2). Predator exposure increased developmental time (c. 2.5 days on average, $F_{1, 86} = 57.77$, $P < 0.001$), and developmental time was less affected by food restriction in predator-exposed tadpoles (c. 3 days) than in tadpoles raised without predators (c. 5.5 days, food *×* predator: $F_{1, 86} = 12.17$, $P = 0.001$; Fig. 2a,b).

Southern tadpoles exposed to low food had lower mass at metamorphosis than control tadpoles (20% lower on average), whereas no such effect was found in the northern tadpoles, bringing about a significant area *×* food interaction ($F_{1, 86} = 19.53$, $P < 0.001$; Fig. 2a,b). Predation risk increased mass at metamorphosis in tadpoles in both areas (11% heavier on average, $F_{1, 86} = 31.45$, $P = 0.001$).

**Post-metamorphic traits**

Overall, size-corrected jump length was not affected by food ($P = 0.979$; Fig. 5a; see S5 for complete analysis). However, a marginally non-significant area *×* food interaction ($F_{1, 90} = 3.92$, $P = 0.051$; Fig. 5a) arose, because southern froglets made shorter jumps in the food-restricted than in the control treatment (4% shorter on average), whereas no such difference was found in the northern froglets. Predator exposure increased jump length in both areas (4% on average, $F_{1, 90} = 4.49$, $P = 0.037$).

We found significant effects of area, predation risk, and food treatment on size-adjusted lipid levels. Lipid levels were higher for southern than for northern froglets (6%
higher on average, \( F_{1, 91} = 4.36, P = 0.04 \); Fig. 5b), higher for low food than for control treatment (12% on average, \( F_{1, 91} = 17.91, P < 0.001 \); Fig. 5b), and higher for non-exposed than for predator-exposed individuals (17% on average, \( F_{1, 91} = 54.79, P < 0.001 \), Fig. 5b). None of the interaction terms had significant effects on lipid levels (\( P > 0.38 \) in all cases).

**TEMPERATURE MANIPULATION EXPERIMENT**

**Tadpole body mass (day 14)**

Overall, the 7-day low-temperature treatment decreased body mass (42% on average, \( F_{1, 88} = 382.3, P < 0.001 \), see S1 for complete analysis; Fig. 2a,b), and the effects varied with geographic area with northern populations experiencing a greater reduction in mass than southern populations (29% greater on average, area \( \times \) temperature: \( F_{1, 88} = 13.8, P < 0.001 \); Fig. 2). Predator presence had a negative effect on mass at day 14 for southern tadpoles (14% lower mass on average than controls), but not for northern tadpoles as indicated by the marginally non-significant area \( \times \) predator interaction (\( F_{1, 88} = 3.8, P = 0.055 \)).

**Behaviour**

Overall, predator presence reduced activity to very low levels independently of food levels both in southern and in northern populations (\( F_{1, 89} = 1081.28, P < 0.0001 \)). In the absence of predators, tadpoles were much less active when exposed to low temperature than those kept at
constant high temperature (64% less active on average, predator × temperature day 10: $F_{1,90} = 25.06, P < 0.0001$; day 14 $F_{1,90} = 6.12, P = 0.015$, Fig 3c,d; see S2 and S3 for complete analysis). After the cold treatment, the activity rates of control tadpoles (19 °C) remained basically unchanged in the absence of predators, whereas the activity of low-temperature tadpoles increased rapidly and exceeded that of controls (10% higher on average, predator × temperature day 15: $F_{1,90} = 3.72, P = 0.057$; day 17 $F_{1,91} = 4.63, P = 0.034$, Fig 3c,d; see S2 and S3 for complete analysis). This pattern was consistent in northern and southern populations, suggesting a behavioural compensatory response. At the beginning of the low-temperature treatment, northern tadpoles were more active than southern tadpoles only when predator was absent (41% more active on average, day 10: area × predator: $F_{1,90} = 9.34, P = 0.003$).

Mortality

Overall mortality in TME was 10.9%, and the number of metamorphs from each experimental container varied between seven and ten (average 8.91). Northern and southern tadpoles did not differ in their responses to the low-temperature treatment in terms of survival ($P > 0.089$). However, a significant predator × temperature treatment interaction ($F_{1,89} = 20.08, P < 0.001$, see S4 for complete analysis) indicated that there were synergistic effects between these two treatments resulting in much lower survival of predator-exposed tadpoles at low temperature (12% lower on average, while low temperature had no significant effect on survival in tadpoles raised without predators (Fig. 4b).

Metamorphic traits

Control tadpoles metamorphosed earlier than cold-treated ones (c. 5 days earlier on average, $F_{1,89} = 196.8, P < 0.001$; Fig. 2a,b; see S1 for complete analysis), and these effects did not differ geographically ($P > 0.61$, Fig. 2b). Predator exposure increased developmental time (>4 days on average, $F_{1,89} = 174.59, P < 0.001$). There were no interaction effects on developmental time ($P > 0.072$; Fig. 2d). In contrast to FME, low-temperature treatment did not affect mass at metamorphosis in either area ($P = 0.74$; Fig. 2c,d), but predation risk increased mass at metamorphosis (5% on average, $F_{1,83.2} = 6.14, P = 0.015$, Fig. 2b).

Post-metamorphic traits

Size-corrected jump length was not affected by temperature ($P = 0.061$; Fig. 5c; see S5 for complete analysis). As in FME, predator exposure increased jump length (4% on average, $F_{1,87.9} = 5.86, P = 0.018$; Fig. 5a,c). However, we detected a significant predator × temperature interaction ($F_{1,87.9} = 6.15, P = 0.015$), because in froglets exposed to predators, those reared at low temperature jumped shorter distances than those maintained at high temperature throughout the larval period (6% shorter on average). No such effect was found when tadpoles were reared in the absence of predators (Fig. 5c).

In contrast to FME, in TME, northern froglets had higher levels of lipids than southern tadpoles (17% higher on average, $F_{1,90} = 28.92, P < 0.001$; Fig. 5d). Predator-exposed animals had lower amounts of lipids than non-exposed individuals (21% lower on average, 1239
$F_{1, 90} = 51.84, P < 0.001$; Fig. 5d), However, temperature did not affect lipid levels ($P = 0.332$, Fig. 5d), nor were any of the interactions significant ($P > 0.116$).

Discussion

TREATMENT EFFECTS ON GROWTH AND ACTIVITY

Our manipulations of food level or temperature were successful in reducing tadpole growth so that at the end of the treatments tadpoles had only half the mass of control tadpoles. In general, northern tadpoles suffered a greater relative growth loss in response to low food (in the absence of predators) and cold temperatures as compared to southern tadpoles. The slowest growing group, southern tadpoles raised with predators, was the least affected in terms of mass loss by starvation, but experienced higher mortality. A similar pattern with slow-growing animals showing less mass loss has been documented in insects (Gotthard, Nylin & Wiklund 1994; Stoks, De Block & McPeek 2006). As the southern tadpoles do not have lower basal metabolic rate (Lindgren & Laurila 2009), which otherwise could explain their slower depletion of energy reserves (Gotthard 2001), this pattern may be due to their lower activity level which may result in lower energy expenditure (see below).

We found that low food level increased activity while low temperature decreased activity. Predator presence decreased activity rates in all treatments, and northern tadpoles were more active than southern tadpoles in all treatment combinations, suggesting that the higher growth and development rates in northern populations are associated with higher foraging activity (Laurila, Lindgren & Laugen 2008). The most prominent difference in activity between northern and southern tadpoles was found in the predator-exposed treatment at the beginning of the low food rations; while predator-exposed southern tadpoles were only slightly more active under food stress, northern tadpoles increased their activity to rates similar to tadpoles raised in the absence of predators. Theory predicts that foragers should accept higher foraging risks when food levels are low, because the benefits of active foraging outweigh its potential costs (Lima 1998). The increased risk-taking behaviour in northern tadpoles can be explained by the fact that because of the more stringent time constraints, delayed growth and development are particularly costly for them. The high activity in this group seized during the course of the low-food period, possibly because the cost/benefit assessment changed with prolonged food stress, and high activity rate became energetically expensive for the energy-deprived tadpoles.

CATCH-UP GROWTH RESPONSES

Our analyses of compensatory responses focused on the effects of growth stress in two traits – size at and timing of metamorphosis – closely related to fitness in amphibi-
compensatory responses in cooled damselfly larvae as compared to starved larvae. If increased foraging allows for compensation after low food but not after low temperature, compensating after low food would likely carry greater costs in terms of predation and hence be more risky at lower latitudes (Laurila, Lindgren & Laugen 2008). However, we found that activity increased to above control levels only following a period of low temperature, and only in the absence of predators. The lack of changes in activity after low food indicates that tadpoles use other mechanisms to compensate for low food. Possible mechanisms include physiological modifications like increased growth efficiency (Lindgren & Laurila 2005; Stoks et al. 2005).

**Costs Following Low Food/Temperature**

We did not detect any increase in activity in the presence of predators during the compensatory period in either experiment, suggesting that behaviourally mediated increase in predation risk is a minor cost for compensatory responses in this system. In a similar vein, and contrary to our prediction, the compensatory responses were not weaker in the presence of a predator.

Longer larval period was a prominent cost of food and temperature stress in both areas, with both low food and low temperature increasing developmental time on average by five days. Time of metamorphosis is directly related to survival and fitness in many amphibians (Smith 1987; Semlitsch, Scott & Pechmann 1988; Altweg & Reyer 2003), and delaying metamorphosis can increase mortality by pond desiccation and decrease the chance of surviving hibernation because of the reduced time available for post-metamorphic growth before the onset of winter. While prolonged larval period should be particularly costly for the more time-constrained high-latitude tadpoles, we did not detect area × food or area × temperature interactions in development time.

In general, we found no evidence for our prediction that costs of compensation should be higher in the populations showing stronger compensatory responses. On the contrary, exposure to low food levels increased mortality in the southern but not in the northern populations. This result is in contrast to previous studies on insects showing that populations with low individual growth rates have higher starvation tolerance (Gotthard, Nylin & Wiklund 1994; Stoks, De Block & McPeek 2006). Exposure to predator presence under food stress further increased mortality in the southern populations, suggesting that northern populations were better adapted than the combination of food and predation stress. By contrast, exposure to low temperature increased mortality similarly both in northern and in southern tadpoles under predation stress, but not in the presence of predators. Several studies have shown that, when presented with other stressors, both temperature and predation risk can act synergistically and increase the negative fitness effects (e.g. Sih, Bell & Kerby 2004; Crain, Kroeker & Halpern 2008; Rogell et al. 2009). Our results indicate that even a short-term exposure to low temperature, followed by a catch-up growth response, can dramatically increase mortality of tadpoles in the presence of predators. As such conditions are likely to be common in nature, these results highlight the importance of investigating combinations of factors in studies focusing on individual performance.

Jump length can influence post-metamorphic fitness by affecting feeding performance (Walton 1988) and ability to escape from predators (Wassersug & Sperry 1977). We found effects on jumping performance in response to both transient periods of low temperature and low food, even when juveniles had fully compensated in terms of size. However, this response depended on the geographic origin (in FME) and whether tadpoles had been exposed to predators (in TME). Southern juveniles made shorter jumps if they had been exposed to a transient period of low food, whereas low-food treatment tended to have a positive effect on jumping performance of northern juveniles. Hence, northern tadpoles were better able to compensate after a period of low food, both in terms of metamorphic size and post-metamorphic locomotor performance. In a previous study, Capellán & Nicieza (2007) found no effect of compensatory growth on jumping performance.

Predator presence increased size-corrected jump length in both experiments. Previous studies have found that predator-induced tadpoles may have longer or more muscular legs as juveniles compared with those raised without predators (Relyea 2001; Van Buskirk & Saxer 2001), which may increase their jumping performance (Tejedo et al. 2010). Furthermore, the lower lipid levels of the predator-exposed froglets (see below) may have increased their jump length. In TME, however, the predator-induced enhancement of jumping performance was counteracted by the low temperature, which had a negative effect on jumping performance in predator-exposed froglets. These results again indicate that even a short-term exposure to low temperature, when combined with compensatory responses and additional stressors can strongly influence performance.

In amphibians, whole body lipid levels at metamorphosis have been shown to correlate with juvenile survival (Scott et al. 2007), and they are an important indicator of overwintering capacity as lipids are the main energy source during hibernation (Feder & Burggren 1992). Contrary to our prediction, we did not find that the stress treatments decreased froglet lipid levels. In fact, low food level increased the amount of lipid reserves. Although not shown previously in amphibians, it is likely that elevated lipid levels can be a strategic response to transiently low food levels. For example, Miglavs & Jobling (1989) found higher lipid levels in starved and refed fish as compared to constantly fed fish, and many bird species maintain higher fat levels when exposed to fluctuating food environments (Ekman & Hake 1990). Studies on compensatory growth effects on lipid stores have found that lipid
levels are restored during realimentation or after cold temperatures (De Block, McPeek & Stoks 2008b), yet others have found that lipid levels are restored but there are deferred costs in terms of lower lipid levels later in life (Morgan & Metcalfe 2001; Stoks, De Block & McPeek 2006), suggesting considerable variation across systems. We found variation in lipid storage as the southern frogs had higher lipid levels than the northern ones in FME, but the situation was the opposite in TME. The lipid levels of the northern frogs remained relatively constant across the experiments. As the experimental conditions for the control individuals were identical, the variation in the lipid levels of southern populations may indicate a carry-over effect from the parental environment. We also found that predator-exposed tadpoles in general had lower lipid levels suggesting that decreased energy storage can be a significant cost of predator-induced defences in *R. temporaria*. The only previous comparable study on amphibians found that lipid levels of juvenile *Discoglossus galganoi* were not affected by diet quality or predators (Nicieza, Álvarez & Atienza 2006).

In summary, our results show that catch-up growth responses can depend on the life-history strategies of the individuals in the populations studied, as well as on the type of stressors the organisms are exposed to. We found that while northern tadpoles were better at handling a period of low food compared with southern tadpoles, both northern and southern tadpoles were able to show full catch-up growth responses after a period of low temperature. Compensation in metamorphic mass occurred partly by delaying metamorphosis and, after temperature stress, by increasing foraging activity. Low food levels increased mortality and decreased locomotor performance only in the southern populations, which, together with the poorer compensatory capacity, suggests that low food is a less common stressor for the southern tadpoles than low temperature. Predator presence increased mortality in low temperature suggesting that the presence of additional stressors will increase the costs of compensatory responses. It is also possible that some of the costs will appear later in life, for example, in terms of increased oxidative stress, reduced immune function or reduced reproductive output (e.g. De Block & Stoks 2008; Auer et al. 2010). However, we note that separating the effects of compensatory responses from potential delayed effects of the stress treatment *per se* is difficult. Hence, understanding the fitness consequences of compensatory responses continues to be a challenge for evolutionary ecologists.

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