Short- and long-term consequences of thermal variation in the larval environment of anurans

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Summary

1. To survive adverse or unpredictable conditions in the ontogenetic environment, many organisms retain a level of phenotypic plasticity that allows them to meet the challenges of rapidly changing conditions. Larval anurans are widely known for their ability to modify behaviour, morphology and physiological processes during development, making them an ideal model system for studies of environmental effects on phenotypic traits. Although temperature is one of the most important factors influencing the growth, development and metamorphic condition of larval anurans, many studies have failed to include ecologically relevant thermal fluctuations among their treatments.

2. We compared the growth and age at metamorphosis of striped marsh frogs *Linnodynastes peronii* raised in a diurnally fluctuating thermal regime and a stable regime of the same mean temperature. We then assessed the long-term effects of the larval environment on the morphology and performance of post-metamorphic frogs.

3. Larval *L. peronii* from the fluctuating treatment were significantly longer throughout development and metamorphosed about 5 days earlier. Frogs from the fluctuating group metamorphosed at a smaller mass and in poorer condition compared with the stable group, and had proportionally shorter legs.

4. Frogs from the fluctuating group showed greater jumping performance at metamorphosis and less degradation in performance during a 10-week dormancy. Treatment differences in performance could not be explained by whole-animal morphological variation, suggesting improved contractile properties of the muscles in the fluctuating group.

Key-words: development, metamorphic, plasticity, performance, temperature

Introduction

Environmental heterogeneity presents a challenge to developing organisms, as habitat conditions and resource levels define growth and differentiation rates (Hentschel 1999; Dmitriew & Rowe 2005). To ameliorate potential negative fitness consequences in a changing environment, some organisms can modify behavioural and physiological processes. Because tissues are rapidly generated and altered during development, small changes in the ontogenetic environment can result in dramatic changes in individual phenotypic traits, producing lasting effects on morphology, condition and performance (e.g. Thompson 1999; Johnston & Temple 2002). Many of the traits defined during ontogeny also have significant long-term consequences on fitness (Desai & Hales 1997; Lindström 1999), affecting the abilities of organisms to evade predation (Downes & Shine 1999; Broomhall 2004; Buckley, Michael & Irschick 2005) or breed successfully (Semlitsch, Scott & Pechmann 1988; Scott 1994) in adult life.

For some organisms, including most insects and amphibians, the ontogenetic form dramatically differs from that of adults, and plasticity in the phenotype during development influences the traits exhibited by the adult form. Such plasticity has facilitated the adaptation of anuran larvae to a wide variety of habitat types and makes them an ideal model system for examining the short- and long-term consequences of environmental variation (e.g. Morey & Reznick 2000; Van Buskirk & Saxer 2001; Relyea 2002; Kraft, Wilson & Franklin 2005). The prevalence of tadpoles in even the impermanent, unpredictable water bodies of xeric

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environments exemplifies their abilities to meet the challenges of a heterogenic environment through modifications at whole-animal or cellular levels. If the aquatic environment begins to deteriorate, larvae can speed maturation to the terrestrial phase and increase their chances of survival (Laurila & Kujasalo 1999; Merila, Laurila & Lindgren 2004; Morey & Reznick 2004).

Temperature is among the most powerful environmental cues to young ectotherms, due to its influence on the enzymatic and biochemical rates that drive development and growth (Hochachka & Somero 1984). Many anuran larvae inhabit shallow, ephemeral pools where diurnal temperature fluctuations are ubiquitous, and they experience high maximum temperatures and wide temperature fluctuations on a daily basis throughout development. High temperatures are associated with increased metabolic rates and accelerated maturation rates for larval anurans (Atkinson 1996) and produce earlier-metamorphosing young at a smaller body size (Smith-Gill & Berven 1979; Atkinson 1996; Merila *et al.* 2000; Laugen, Laurila & Merila 2003). When temperatures fluctuate daily, the physiological rates controlling anuran ontogeny also fluctuate daily – slowing in cold temperatures (i.e. night) and accelerating in hot temperatures (i.e. day) – but the overall effect of these diurnal rate changes on developing anurans is unknown.

A burgeoning appreciation of ecological relevance in physiological studies has begun to show how natural thermal fluctuations influence developing organisms, particularly invertebrates (e.g. Dallwitz 1984; Hagstrum & Milliken 1991; Worner 1992; Scott 1994; Scholnick 1995) and reptiles (Georges, Limpus & Stoutjesdijk 1994; Shine & Harlow 1996; Shine 2004; Georges *et al.* 2005). Moderate thermal fluctuations may be associated with faster growth (Worner 1992; Georges *et al.* 1994; Liu & Meng 2000; Georges *et al.* 2005) and lower mortality rates (Scott & Grigarick 1978), but other studies have found either no effect (see Eubank, Atmar & Ellington 1973) or retardation of development in thermally fluctuating regimes (Hagstrum & Milliken 1991), particularly those encompassing extreme temperatures (Georges *et al.* 1994, 2005). To our knowledge, diurnally fluctuating thermal conditions have not yet been considered for larval anurans, despite the prevalence of these highly plastic organisms in naturally fluctuating habitats.

Thermal fluctuations in the larval environment are expected to affect more than just short-term growth and maturation for young anurans, as metamorphic morphology and condition influence their abilities to survive to reproductive maturity (Arnold & Wassersug 1978; Semlitsch *et al.* 1988). Larger frogs show greater locomotor performance in tests of both speed and endurance (Emerson 1978; John-Alder & Morin 1990; Beck & Congdon 2000; Tejedo, Semlitsch & Hotz 2000a) and an increased capacity for fuel storage (van Beurden 1980). Despite the obvious connection between tadpole environment and post-metamorphic life,

relatively few studies have attempted to describe this long-term link in terms of condition or performance (but see Smith 1987; Tejedo, Semlitsch & Hotz 2000b; Van Buskirk & Saxer 2001; Álvarez & Nicieza 2002a).

In this study, we exposed larval striped marsh frogs *Limnodynastes peronii* (Fitzinger) to a diurnally fluctuating thermal regime or a constant regime of the same mean and compared several short- and long-term phenotypic traits. *L. peronii* are common in eastern-coastal Australia and have been used extensively in studies of larval thermal and predator-mediated plasticity (e.g. Wilson & Franklin 1999; Kraft, Wilson & Franklin 2005). Larval *L. peronii* are found in a variety of aquatic habitats, from deep thermally stable ponds to shallow, thermally unstable pools, making it an ideal species for studies of thermal regimes. We used these characteristics of the thermal ecology of *L. peronii* to investigate the effects of a fluctuating and a stable temperature regime on growth and development. We then examined long-term effects of larval thermal regimes on metamorphic condition, locomotor abilities and performance in response to an environmental stress. For this latter measure, we simulated the first winter in order to determine how recent metamorphic young might fare in realistically cold, food-poor conditions. Other studies of vertebrates suggest that for moderate, natural temperature ranges like these, ontogeny in the fluctuating regime should be rapid as it is in a hot, stable temperature (Georges *et al.* 2005). Thus, we predicted rapid growth and development of tadpoles in the fluctuating thermal regime, culminating in earlier metamorphosis at a smaller body size, as found in hot environments (Atkinson 1996). Furthermore, we predicted that these smaller frogs would show reduced jumping distance and a reduced ability to withstand winter conditions.

Materials and methods

We collected eggs from four egg masses in south-eastern Queensland in May 2004 and mixed them together to randomize genetic effects on the treatments. Eggs were maintained at 25 °C until hatch (approximately 48 h), then 15 tadpoles were randomly allocated to each of six fluctuating or six stable temperature tanks. All tanks contained 45 L of aged tap water, a 2-cm base layer of fine gravel and an individual aquarium box filter containing filter-wool and activated carbon. Larvae were fed boiled lettuce *ad libitum*, and water quality was maintained by the regular removal of uneaten lettuce, in-tank filtration and a weekly 50% water change. After metamorphosis, frogs were housed individually at 25 °C, given ample water and fed crickets *ad libitum*. Photoperiod was set to 12 L/12D throughout the experiment.

The fluctuating thermal regime was based on data collected from ephemeral pools in south-eastern Queensland midway through the breeding season, which fluctuated between 18 and 32 °C over typical

24-h periods. These temperatures are within the non-lethal range for this species (Wilson 2001). The fluctuating regime was designed to simulate natural conditions as closely as possible, with heating occurring more rapidly than cooling. A 200-W Jaeger aquarium heater was used to increase the water temperature from ambient (18 °C) to 32 °C throughout the morning period and maintained this temperature for 4 h. The heater was then switched off to allow the water to cool down to 18 °C overnight. Because temperatures in this treatment did not rise and fall uniformly, the mean daily temperature of the fluctuating treatment was calculated as 24 °C based on measures taken at 2-min intervals over 3 days. For the stable treatment tanks, temperature was maintained at a constant 24 °C using identical aquarium heaters. Small aerators were placed adjacent to each heater to promote even distribution of heat throughout each tank. During larval development, one stable temperature tank was removed due to unknown mortality effects.

To assess the influence of treatment on tadpole growth, we measured the total body lengths (tip of snout to end of tail; ± 0·1 mm) of three randomly selected larvae from each tank using a Leica digital camera setup (Leica AG, Solms, Germany) at 18, 25 and 32 days after hatching. Photos were analysed using SIGMASCAN PRO 5·0 (Systat Software, California). When all four limbs had emerged, tadpoles were placed into individual containers, and metamorphosis was defined as complete tail reabsorption. The total amount of time between hatch (day 0) and metamorphosis was recorded for each individual and used to calculate age at metamorphosis.

Immediately upon metamorphosis, and again before and after dormancy, we measured the mass (with empty bladder), body and leg lengths and jumping performance for most individuals. Some measurements were not collected from all individuals due to equipment malfunction or behavioural issues with the animals, but these were not related to treatment. We estimated body condition using a standard index, defined as $10^4*(mass/body\ length^3)$. Maximum jumping lengths are commonly used as an indicator of performance capabilities in frogs (e.g. Wilson & Franklin 2000; Van Buskirk & Saxer 2001; Wilson 2001). Maximum jumping capacity was recorded for each individual on a non-slip mat at 25 °C, a temperature that approximates the optimum for maximum jumping performance in this species (Wilson 2001). Jumping was initiated by lightly touching the animal's urostyle, and a minimum of three uninterrupted jumps were demarcated on the jumping surface. The longest jump for each individual was then used as the maximum jumping performance.

At 9 weeks of age, all metamorphic frogs were transferred to individual containers filled three-quarters full with damp sand to begin a simulated winter. In the wild young *L. peronii* typically remain stationary under leaf litter during cold, dry periods, so each frog was also provided with a small shelter. Winter conditions were initiated by gradually decreasing the

ambient temperature in a controlled-temperature room to 10 °C over 2 weeks. Because rates of evaporative water loss were high in the room, we dampened the sand with small amounts of water every 5–10 days, as required. After 10 weeks of dormancy, we re-measured the mass (with empty bladder) and maximum jumping performance of each frog to determine the effects of the simulated winter dormancy. Prior to assessment of jumping performance, each individual metamorph was placed at 20 °C overnight and then transferred to the test temperature (25 °C) for 2 h prior to measurement.

We examined most effects of the larval thermal environment on morphological traits (body length or mass, condition) and age using analysis of variance (ANOVA), with tanks nested within treatments. Because body mass at metamorphosis was potentially affected by age we used an analysis of covariance (ANCOVA) model to compare these traits between treatments. To determine whether frogs from the two treatments differed in relative leg lengths, and whether these differences contributed to differences in jumping performance, we compared the body-length calculated residuals of these traits using Pearson product moment correlations. Changes in mass and jumping distance of individual frogs occurring over the 10-week 'winter' were then compared using a two-factor repeated measures analysis of variance, with treatment and time as factors (rmANOVA). Results are presented as treatment mean ± SEM, and statistical significance is at the level of $P < 0.05$. Significance levels were adjusted for multiple comparisons using the Bonferroni method. Statistics were conducted using SIGMASTAT 3·0 (Systat Software, California) and SAS 8·0 (SAS Institute, Inc., North Carolina).

Results

The body lengths of larval *L. peronii* were compared between a stable 24 °C temperature regime and a diurnally fluctuating regime that varied between 18 and 32 °C. Larval *L. peronii* from the fluctuating treatment were significantly longer at 18 days ($F = 339.5$, d.f. = 1,11, $P < 0.0001$), 25 days ($F = 376.2$, d.f. = 1,11, $P < 0.0001$) and 32 days ($F = 30.4$, d.f. = 1,11, $P < 0.0001$) post-hatch (Fig. 1). At day 18, tadpoles from the fluctuating treatment were already 2·5 times longer than those from the stable regime (316·5 ± 14·3 vs. 130·0 ± 5·6 mm).

Tadpoles from the fluctuating group reached metamorphosis on average 5 days before the stable treatment (48·6 ± 0·7 vs. 54·7 ± 0·8 days), and there was a highly significant interaction between age and treatment on metamorphic mass ($F = 21.1$, d.f. = 1,11, $P < 0.0001$) (Fig. 2). Overall, frogs from the fluctuating treatment metamorphosed at a smaller body mass (0·51 ± 0·02 vs. 0·55 ± 0·03 g; $F = 2.3$, d.f. = 1,98, $P = 0.02$) and in poorer body condition (0·195 ± 0·004 vs. 0·2 ± 0·005 g mm^{-3}; $F = 2.0$, d.f. = 1,64, $P = 0.05$), but total body length or leg length did not differ significantly between treatments.
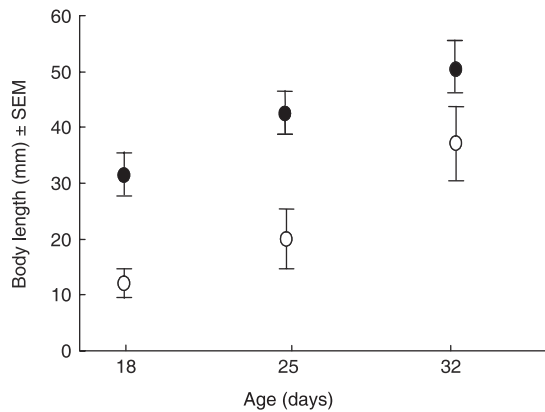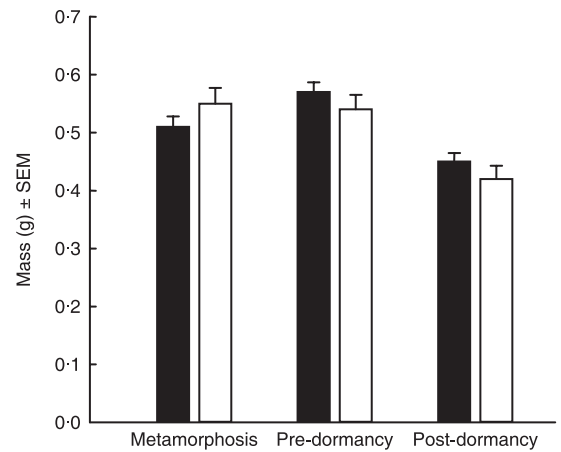
Fig. 1. Mean body lengths of *Limnodynastes peronii* tadpoles raised in fluctuating (closed circles) and stable (open circles) thermal regimes at 18, 25 and 32 days post-hatch. Age is calculated with hatch = 0.
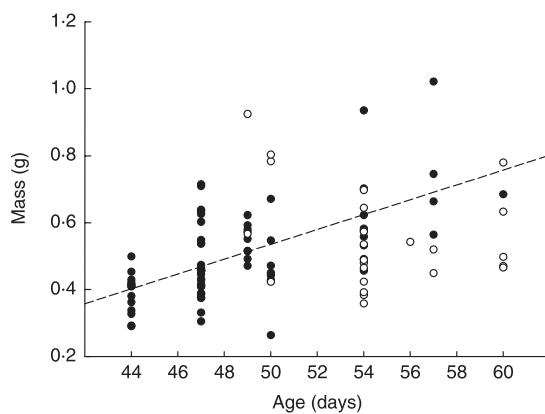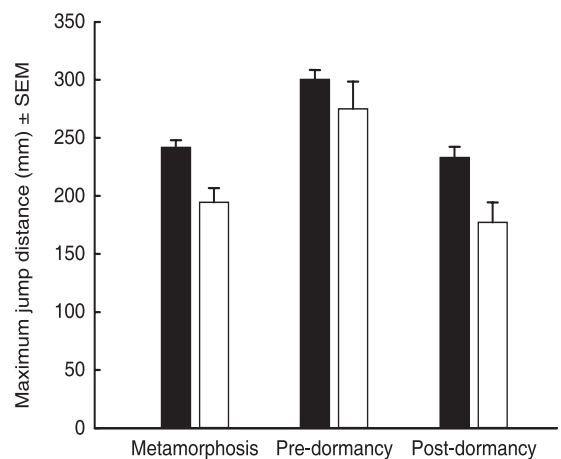

Fig. 2. Mass at metamorphosis for *Limnodynastes peronii* raised in fluctuating (closed circles) or stable (open circles) groups, accounting for metamorphic age. The dotted line represents the best linear fit for the data.

Fig. 3. Mean mass of young *Limnodynastes peronii* raised in either a fluctuating (closed bars) or stable (open bars) thermal regime at metamorphosis and before and after a 10-week 'winter' dormant period.

Fig. 4. The mean maximum jumping distance of young *Limnodynastes peronii* raised in either a fluctuating (closed bars) or stable (open bars) thermal regime at metamorphosis and before and after a 10-week 'winter' dormant period.

After metamorphosis, the performance of young frogs from the fluctuating and stable treatment groups was compared in two tests: jumping ability and response to an environmental stress (simulated winter). At metamorphosis, frogs from the fluctuating regime jumped an average of 24% farther ($245 \pm 6 \cdot 2$ vs. $185 \cdot 9 \pm 10 \cdot 1$ mm; $F = 4 \cdot 6$, d.f. = 1,98, $P < 0 \cdot 001$) despite having lower mass, poorer body condition and similar body lengths to frogs from the stable treatment. A multiple regression was used to compare the predictive value of mass, body condition, leg length and total body length on the jumping performance of recently metamorphosed individuals, and only mass was a significant predictor of jumping performance ($r^2 = 0 \cdot 13$, d.f. = 45, $P = 0 \cdot 003$). However, when body size was accounted for in analyses, the relative leg-to-body length was found to be different between treatments ($t = -3 \cdot 9$, $P < 0 \cdot 001$), with frogs from the fluctuating treatment having short legs for their body size. Only among the fluctuating group was there a relationship between residual leg length and jumping performance ($r_p = 0 \cdot 39$, d.f. = 45, $P = 0 \cdot 01$).

Most *L. peronii* metamorphs remained inactive on the sand surface for the duration of the simulated winter. Across both thermal treatments, the average body mass decreased significantly during dormancy ($F = 415 \cdot 7$, d.f. = 1,149, $P < 0 \cdot 001$), with metamorphs from both treatments losing 20–22% of pre-dormancy body mass (Fig. 3). There was a significant interaction between time and treatment ($F = 4 \cdot 43$, d.f. = 1,149, $P = 0 \cdot 04$), as frogs from the stable group lost a higher proportion of their body mass over 'winter'. Although post-dormant metamorphs were typically lighter than they were at metamorphosis, they appeared healthy and immediately became active when returned to warmer temperatures.

Across treatments, maximum jump distance decreased significantly during the 10-week dormancy ($F = 99 \cdot 1$, d.f. = 1,149, $P < 0 \cdot 001$) (Fig. 4), by an average of 22% compared with pre-dormancy distances. Although

frogs from the fluctuating treatment always jumped farther than those from the stable treatment, this difference was most dramatic at metamorphosis and after dormancy. Treatment had no effect on the pre- and post-dormancy jumping performance, but there was a significant interaction between time and treatment on performance ($F = 6·8$, d.f. = 1,149, $P = 0·01$). Frogs from the stable treatment suffered a loss in performance of 38%, nearly double that shown for the fluctuating treatment (21%).

There was no direct correspondence between individual variation in change in mass and change in performance after a 10-week dormancy. Mass loss was ubiquitous, varying between 5 and 35% of body mass lost during the dormant period. In contrast, changes in jumping performance varied widely between a loss of 80% of original maximum distance to a gain of more than 40%. Although frogs in better condition may catabolize less muscle for fuel during food-poor periods (van Beurden 1977), individuals retaining better jumping performance did not necessarily begin dormancy in better condition ($F = 3·2$, d.f. = 1,149, $P = 0·08$) and were evenly distributed between the stable and fluctuating treatments ($P = 0·5$).

Discussion

L. peronii raised in fluctuating or stable thermal regimes differed in both short- and long-term phenotypic traits. As predicted, tadpoles in the fluctuating treatment grew more rapidly and metamorphosed earlier than those in the stable treatment. Although frogs metamorphosed from the fluctuating treatment at a smaller mass, they possessed greater jumping performance at metamorphosis and following a 10-week dormant period.

The accelerated growth and maturation rates that we observed in the fluctuating group were similar to those reported for larval anurans raised in high, constant temperatures (Atkinson 1996; Merila *et al.* 2000; Laugen *et al.* 2003) and for some invertebrates (Hagstrum & Hagstrum 1970; Scott & Grigarick 1978; Hagstrum & Milliken 1991) and reptiles (Shine & Harlow 1996) in fluctuating regimes. Although larval *L. peronii* from the fluctuating regime were longer than those from the stable regime at each sampling interval, the greatest differences occurred early in development (i.e. prior to day 18). Similarly, Scholnick (1995) reported that tadpole shrimp *Triops longicaudatus* (Leconte) raised in a high daily fluctuating temperature regime were heavier than those from a low regime, but growth differences were restricted to early stages of development. Like *T. longicaudatus*, *L. peronii* is known to inhabit ephemeral water bodies where desiccation is a serious threat to survival, and individuals must reach metamorphosis before the water disappears. Thermal fluctuations early in ontogeny may trigger physiological mechanisms for fast growth in the event of pool desiccation (Newman 1989; Laurila & Kujasalo 1999).

We predicted that the high temperatures experienced in the fluctuating regime would produce a smaller body size at metamorphosis (Atkinson 1996; Blouin & Brown 2000; Laugen *et al.* 2003; Georges *et al.* 2005). Differences in body length diminished over the larval phase and were significant but small by metamorphosis. In addition, larvae from the fluctuating regime reached metamorphosis only a few days prior to those from the stable regime. Previous studies have shown that metamorphic phenotypes may be related to recent growth history in environments varying in food resources (Tejedo & Reques 1994), but we are unaware of studies considering such phenotypic development in ecologically relevant thermal fluctuations. In our study, it appears that the short lag in development between our stable and fluctuating thermal groups was sufficient to make up the growth differences of the early post-hatch period. We hypothesize that this 'catch-up' results from an interaction between body mass and temperature for individuals in the fluctuating group. When individuals are small, their mass-specific metabolic rates are higher and increases in temperature may show a more marked effect than later in larval development, when the tadpoles are larger. The metabolic changes during ontogeny in fluctuating and stable regimes remain to be tested for larval anurans.

Variation in anuran jumping performance is usually attributable to body size or condition, with larger individuals generally showing greater burst and endurance performance (Emerson 1978; John-Alder & Morin 1990; Beck & Congdon 2000; Tejedo *et al.* 2000a). In most species, this results from longer legs acting as extended levers to propel the animal forward during jumping (Zug 1972; Tejedo *et al.* 2000a,b), although in some cases musculature or energy storage directly in muscle tissues may contribute to jumping patterns (Emerson 1978). In contrast with predictions, the stable group (that showed poorer performance) were in better condition, had greater mass, and longer relative leg lengths – all factors that should relate with greater jump distance – compared with the fluctuating group. Therefore, we suggest that differences in jumping performance between treatments are most likely related to either the behavioural motivation to jump or the contractile properties of muscle. Possibly, frogs from the stable treatment achieved the same average velocity as those from the fluctuating treatment, using a series of shorter, faster jumps. This pattern has been suggested to explain differences in the interaction between morphology and jumping performance among species that hop rather than jump (Emerson 1978), but unfortunately our methods did not include a measure of time or total distance covered, precluding such an analysis. We do not believe that jumping performances were attributable to behaviour, however, because frogs from the fluctuating group consistently jumped farther at each of the three measurement periods. Alternatively, if high temperatures during development result in the production of larger or more numerous anaerobic muscle fibres, as they do in some fish (Johnston, Vieira & Hill 1996), then frogs encountering higher temperatures

may develop muscle that is better suited for burst locomotor function. Individual variation in muscle structure has been shown to affect the contractile properties of muscle tissues and whole-animal locomotor performance for a number of vertebrate species (Gleeson & Harrison 1988; Wilson *et al.* 2004). In this case, the high temperatures in the fluctuating group may have made them better jumpers.

Metamorphs with greater lipid stores reduce the need to metabolize muscle proteins to survive the first winter, as lipids are the preferred energy source during this time (van Beurden 1977). Álvarez & Nicieza (2002b) found that even after accounting for mass, frogs raised in higher-temperature treatments had lower postmetamorphic lipid levels. Although we did not directly measure body composition in this study, we would expect that if frogs from the thermally fluctuating treatment had fewer lipids, they would show greater degradation in jumping abilities after dormancy due to use muscle proteins for energy. However, we found the opposite, as the treatment group in better condition at metamorphosis (stable) was also the group that experienced the greatest loss in jumping performance. Thus, naturally fluctuating thermal treatment produced metamorphic frogs that showed greater abilities to escape predators (jumping performance) and survive their first winter. Increased survival abilities have also been shown for some shrimp (Scott & Grigarick 1978) and beetles (Renault *et al.* 2004) developing in fluctuating larval regimes. Precisely how fluctuating thermal regimes alter phenotypic traits at the mechanistic level remains to be tested.

In summary, we compared the developmental plasticity and subsequent phenotypic attributes of anurans exposed to a diurnally fluctuating regime with a constant regime of the same mean. In the short term, growth and development were accelerated in the fluctuating environment in a similar manner to that expected for a high-temperature, stable environment. Contrary to expectations, metamorphic morphology was similar between treatments, suggesting that the rapidly developing larvae in the fluctuating regime may not incur the same costs as those from a hot, stable thermal regime. In fact, long-term effects on individual locomotor performance suggest that larvae raised in environments reflecting natural conditions may even show improved contractile dynamics of hindlimb muscles at metamorphosis. Our results emphasize the need to place studies of anuran development in an ecological context, with consideration of the effects of diurnal or seasonal temperature fluctuations.

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