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RESEARCH ARTICLE

Striped marsh frog (*Limnodynastes peronii*) tadpoles do not acclimate metabolic performance to thermal variability

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SUMMARY

Human-induced climate change is predicted to affect not only the mean temperature of the environment but also the variability and frequency of extreme climatic events. Variability in an organism's developmental environment has the potential to markedly affect an individual's growth trajectory and physiological function, leading to impacts on individual fitness and population dynamics. Thus, it is important to consider the consequences of thermal variability on developing organisms and understand their capacity to respond to such increased variation. We investigated the capacity of larval striped marsh frogs (*Limnodynastes peronii*) to initiate a response to increases in the thermal variability of their developmental environment by reducing the sensitivity of their physiological rate functions to changes in temperature. In variable environments, we expected the thermal sensitivity of rate functions to decrease and their performance breadth to widen so as to buffer the effect of thermal variability. We raised larvae in stable (24°C), narrowly variable (22–26°C; mean 24°C) and widely variable (14–34°C; mean 24°C) thermal environments and measured the thermal sensitivity of their locomotor performance, heart rate, oxygen consumption and activities of two metabolic enzymes, lactate dehydrogenase and cytochrome *c* oxidase. We found that the temperature-dependent relationships of these physiological functions did not differ between tadpoles raised in stable or variable thermal conditions. Furthermore, the Q_{10} values of each response variable were virtually unaffected by treatment when measured over the entire thermal range. Our results reveal that larval amphibians exhibit little plasticity in metabolic traits to thermal variability. This lack of plasticity may have important implications for the growth and population dynamics of organisms in environments that are beginning to experience increased thermal variability.

Key words: acclimation, performance, tadpole, temperature.

INTRODUCTION

Temperature is the most pervasive abiotic characteristic of the environment influencing animal function because it affects performance at all levels of organisation (Hochachka and Somero, 2002). Almost all organisms experience changes in temperature during their lives, and this thermal variation can have important consequences for phenotypes, particularly for ectotherms. To compensate for long-term thermal variation, many organisms modify their morphology, physiology or behaviour to enhance performance in the new environment (DeWitt and Scheiner, 2004; Angilletta, 2009). Such environmentally induced physiological plasticity (acclimatisation or acclimation) is best known in response to seasonal thermal changes (Wilson and Franklin, 2002). Many taxa can modify the thermal properties of traits such as enzymatic activity (Guderley and St Pierre, 2002; Hochachka and Somero, 2002; Rogers et al., 2004), metabolic rate (Booth, 1998) or locomotor performance (Wilson and Franklin, 1999; Wilson et al., 2007) to maintain constant or near constant performance despite pronounced seasonal differences in the thermal environment. However, most environments also experience substantial daily thermal variation. Like other organismal responses to temperature, animals would benefit if physiological processes could be buffered against daily temperature fluctuations so that physiological rates are constant and predictable.

Diel variation may be particularly important during development when the period of daily variation in temperature is long relative to the animals' developmental rate. In some environments, temperatures vary by more than 20°C over a 24 h cycle (Kingsolver et al., 2004; Georges et al., 2005; Niehaus et al., 2006), which represents extreme instability that may cause disruption to the development of free-living embryos or larvae. Daily temperature cycles can either accelerate or retard growth of free-living larvae such as amphibian tadpoles (Niehaus et al., 2006), and can thereby influence metamorphic, hatchling or adult phenotypes (Schaeffer and Ryan, 2006; Dhillon and Fox, 2007). These developmental effects can determine how well the adult phenotype is matched to the environmental conditions experienced during its lifetime (Levins, 1968), and thereby influence individual fitness and population dynamics. A change in environmental variability during the ontogeny of the organism can cause a mismatch between developmental and adult environments, and therefore also a mismatch between adult phenotype and environmental conditions. Human-induced climate change has the potential to cause such a mismatch because in addition to increases in mean temperature, variability around the mean and extreme events are also increasing (Ganguly et al., 2009). Hence, determining the relative effect of stable and variable environments on developmental rate (Niehaus et al., 2006) and phenotypic expression of animals is important for

understanding the dynamics of natural populations (Kingsolver et al., 2004).

Metabolism and locomotion are important fitness-related functions because their capacities underlie development, growth and behaviour (LeGillard et al., 2004; Wilson et al., 2007; Seebacher, 2009). Cells require ATP for maintenance of protein synthesis and membrane potentials at rest, for example (Hulbert and Else, 2000), as well as for growth and muscle contraction. Enzyme-catalysed metabolic pathways and muscle function are temperature sensitive, so that body temperature fluctuations in variable environments could cause substantial variation in ATP availability and locomotor performance. To overcome the resultant unpredictability in performance, organisms can gain a selective advantage by reducing the thermal sensitivity of physiological rate functions. In variable environments, the thermal sensitivity of rate functions should decrease to buffer the effect of temperature variability and to widen the performance breadth. In stable conditions, physiological processes should be thermally sensitive with performance maxima falling within the limited range of environmental temperatures (Gabriel, 2005; Gabriel et al., 2005). We tested these hypotheses by raising striped marsh frogs (*Limnodynastes peronii*) in different variable and stable thermal environments and measuring the thermal sensitivity of locomotor performance, heart rate, oxygen consumption and metabolic enzyme activities as response variables.

MATERIALS AND METHODS

Animals and thermal treatments

We collected freshly laid egg masses of *Limnodynastes peronii* Duméril and Bibron 1841 from pond habitats around southeast Queensland, Australia (28°S, 145°E). Different egg masses were used to examine the effects of thermal variability on enzyme activity, heart rate, oxygen consumption and locomotor performance. In each case, we mixed eggs from eight to nine egg masses and divided them randomly among thermal treatments to minimise genetic effects (Kraft et al., 2005). Eggs were housed in 4 l plastic tanks in the appropriate thermal conditions for each treatment (see below) until they hatched. At that time, tadpoles were randomly allocated to 1.25 l plastic tanks filled with dechlorinated aged water at densities of eight to nine individuals per litre. There were 12 replicate tanks for each thermal treatment and for each response variable (i.e. a total of 144 containers each containing eight to nine individuals); we set up more containers than needed for some of the experiments in case of mortality. After reaching the stage of yolk absorption (2–14 days after hatching, Gosner stage 25) (Gosner, 1960), larvae were fed with boiled spinach twice daily. Excess spinach was removed each day and partial water changes were conducted as necessary to inhibit water spoilage.

We raised eggs and larvae in three thermal treatments that were designed to reflect the range of daily thermal cycles experienced by *L. peronii* in the wild: stable (24±0.5°C), narrowly variable (22–26°C) and widely variable (14–34°C); all treatments had the same mean daily temperature (24°C). In previous studies, we had established that larvae grow rapidly at 24°C (Niehaus et al., 2006). Tadpoles of striped marsh frogs are known to inhabit the full range of aquatic environments represented by our experimental thermal protocols (Niehaus et al., 2006). For example, tadpoles that grow in shallow ephemeral pools exposed to full sun can experience temperature variations from less than 14°C to greater than 36°C in a day (R.S.W., personal observations). In contrast, those tadpoles that grow in deep and shaded ponds experience almost no daily fluctuations in temperature. Cycling water temperatures were controlled by aquatic heaters connected to electronic timers, which turned on at 06:00 h and off at 15:00 h (Fig. 1); water was cooled by bubbling air from the room (maintained at 14°C) into each water bath. Stable conditions were also maintained using aquatic heaters and airstones. After killing tadpoles (see below), we measured the length of tadpoles used subsequently for enzyme assays with electronic callipers (±0.01 mm) and we determined the mass of tadpoles subsequently used for heart rate measurements using an electronic balance (±0.0001 g) after removing excess water with a paper towel. All procedures were approved by The University of Queensland Animal Ethics Committee and the Queensland Department of Environment and Heritage.

Enzyme activities

Tadpoles (10–11 days after hatching) were killed in a solution of MS-222 (Sigma-Aldrich, Castle Hill, NSW, Australia). We recorded total body length using digital callipers, and dissected out the tail muscle. Because of the small size of the tadpoles, we combined the tissues of seven to eight randomly selected tadpoles from a single housing tank to form each replicate (N=8 replicate tanks per thermal treatment). Muscle tissues were dissected on ice and immediately stored in liquid nitrogen until assays were conducted. We determined activities of lactate dehydrogenase (LDH) and cytochrome *c* oxidase (CCO) as indicators of anaerobic and oxidative ATP production capacities, respectively. Tissue homogenisation and enzyme assays were conducted according to published protocols (Seebacher et al., 2003). Enzyme activities were determined at 14, 19, 24, 29 and 34°C.

Heart rate

We randomly selected one tadpole (10–14 days after hatching) from each replicate container (N=12) to be tested at each temperature (10, 14, 19, 24, 29, 34 and 38°C). We placed individual tadpoles into 50 ml plastic tubes filled with dechlorinated aged water and allowed them 1 h to adjust to the test temperature. We then transferred tadpoles into 50 ml glass jars and allowed them to settle in the testing environment. Typically, tadpoles rested on the bottom of their jar within a few minutes. The glass jars were placed on the bottom of a shallow, temperature-controlled water bath (±0.5°C) set to the appropriate test temperature, and observations were conducted *via* a mirror angled at 45 deg under the water bath. At this early stage of development, the tadpoles' hearts could be readily observed through their transparent abdomens.

To obtain resting heart rates, we counted the number of heartbeats in 20 s for each tadpole, alternating between individuals from the three thermal treatments to avoid any treatment-time bias. We measured resting heart rate three times for each tadpole, and used the mean of these measures in our analyses. To obtain an estimate of maximum heart rate, we moved each tadpole container from side-to-side at a frequency of 2 Hz for 20 s and immediately counted heartbeats for 10 s. This was referred to as 'post-exercise' heart rate. As this procedure was stressful for the tadpoles, it was done only once per individual. We calculated factorial scope for heart rate as maximum resting heart rate divided by mean resting heart rate and used this measure in our analyses.

Oxygen consumption

We measured rates of oxygen consumption of newly hatched tadpoles (2 days after hatching) at 10, 14, 19, 24, 34 and 38°C using closed-system respirometry (Sinclair et al., 2006). Tadpoles were selected for the experimental trials and equilibrated to the appropriate test temperature as described above for heart rate. We then placed individual tadpoles into 2 ml syringes filled with dechlorinated aged

water. Water was saturated with air before the experiment, and the syringes were sealed for oxygen consumption measurements. Syringes were floated in a water bath set to the test temperature (±0.5°C), and tadpoles were left in the respirometers for 10–120 min, depending on the test temperature (higher temperatures required less time). Water samples from the respirometer were analysed using a Clarke-type oxygen electrode connected to an oxygen analyser. We ran four control assays with a syringe filled with water only for each test temperature. After obtaining rates of oxygen consumption, we killed and measured the mass of each tadpole as described above for heart rate. We successfully measured oxygen consumption for nine to 12 tadpoles from each treatment at each temperature.

Swimming performance of larvae

We measured the swimming performance of larval *L. peronii* at 10, 14, 19, 24, 29, 34 and 38°C. We randomly selected one tadpole from each replicate container to be tested at one of the temperatures, so each container had a tadpole tested at each temperature. To prevent thermal shock, tadpoles were brought to each test temperature from their treatment environment at a rate of 4°C h^{-1}. Swimming performance was tested in a glass aquarium (30×15×7 cm) filled with dechlorinated aged tap water. The aquarium was suspended over a mirror set at a 45 deg angle, and a high-speed digital camera (Redlake Imaging Corporation, Tallahassee, FL, USA) was used to record the mirror images of larvae at 200 Hz. Recordings were analysed using the Redlake Motionscope Media Player package (Redlake Imaging Corporation). We elicited burst swims by touching the tadpole's head with a fine wire, and recorded both the maximal instantaneous swimming speed (U_{max}) and mean swimming speed over 160 ms ($\overline{U}$) for five consecutive responses. Although we arbitrarily selected 160 ms as the duration for calculating the mean swimming speed, this duration typically enabled greater than five tail beats. Sequences were replayed frame-by-frame and the start of the analyses of the swimming response was taken as the frame preceding when movement was first detected. Because tadpoles show some side-to-side motion during swimming, we estimated distance and speed of swimming using the midpoint of the tadpole's head, as determined by averaging values calculated from the snout and the back of the head (Wilson et al., 2005). Instantaneous measures of velocity were then calculated by differentiating distance data that was previously subjected to a three-point moving average filter (Wilson and Franklin, 1999), where each datum point is derived from the mean of three successive raw velocity measurements. Thus, U_{max} was defined as the peak instantaneous velocity attained by an individual in any of the analysed swims. We used only the fastest swim for each individual as an estimate of maximal swimming performance (Wilson et al., 2000). Because mean and maximal swimming speeds were highly correlated, we used a principal components analysis (PCA) to create a single factor describing the covariation between these two variables. We analysed scores for the first principal component (PC1) because this component described 98% of the variation in swimming performance for both variables. All analyses were conducted using the composite measure of performance based on PCA1 (U_P).

Statistical analyses

We used the same tissue sample across all test temperatures for enzyme assays, and therefore analysed the data using a repeated-measures analysis of covariance (ANCOVA) with treatment as a categorical factor and test temperature as a repeated measure, and body length as a continuous covariate. Differences in body length and body mass were compared between treatments using a one-way ANOVA. We analysed differences in factorial heart rates, locomotor performance, mass-specific rates of oxygen consumption and Q_{10} values (see below) using an ANCOVA with treatment and test temperature as independent factors, and mass as a covariate.

We calculated thermal sensitivity of activity rates as: $Q_{10}=(R_1/R_2)^{(10/T2-T1)}$, where R represents rates at temperatures (T) 1 and 2. We calculated thermal sensitivities for the entire thermal range, as well as low temperatures (14–24°C) and high temperatures (24–34°C). All analyses were conducted using STATISTICA version 9 (StatSoft, North Melbourne, VIC, Australia), and significance was assigned at $P<0.05$. Errors around means are shown as standard errors in the text and 95% confidence intervals in the figures.

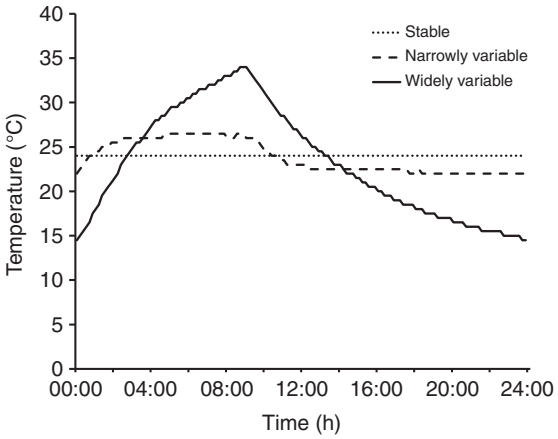

Fig. 1. Daily variation in temperature experienced by larval striped marsh frogs (*Limnodynastes peronii*) from the three different treatments. Embryos were randomly allocated to a stable (24°C), narrowly variable (22–26°C) or widely variable (14–34°C) thermal treatment. All three treatments had the same mean temperature over the daily cycle (24°C).

RESULTS

Body size

By 10–11 days of age, the body length of tadpoles raised in widely variable temperatures (14.84±0.20 mm) was significantly ($F_{2,212}=50.0$, $P<0.0001$) shorter than that of tadpoles raised in narrowly variable (18.35±0.39 mm) or stable temperatures (18.20±0.36 mm). Body lengths did not significantly differ between stable and narrowly variable temperatures. Body masses of tadpoles in the widely variable treatment (0.099±0.006 g) were significantly lower ($F_{2,251}=8.50$, $P<0.0001$) than those in the narrowly variable (0.133±0.010 g) and stable treatments (0.122±0.007 g). Tadpole mass did not differ between the stable and narrowly variable treatments.

Enzymatic activity

There were no interactive effects of thermal treatment and test temperature on the activity rates of LDH (repeated-measures ANCOVA: $F_{8,34}=1.67$, $P=0.14$) or COX ($F_{8,34}=1.52$, $P=0.19$; Fig. 2). Activity of LDH increased significantly with increasing test temperature (ANOVA: $F_{4,104}=25.3$, $P<0.0001$; Fig. 2). However, activity of the enzyme COX did not change significantly with test temperature (ANOVA: $F_{4,104}=1.74$, $P=0.18$).

The thermal sensitivities (Q_{10}) of both enzymes were not significantly different between treatments when measured over the

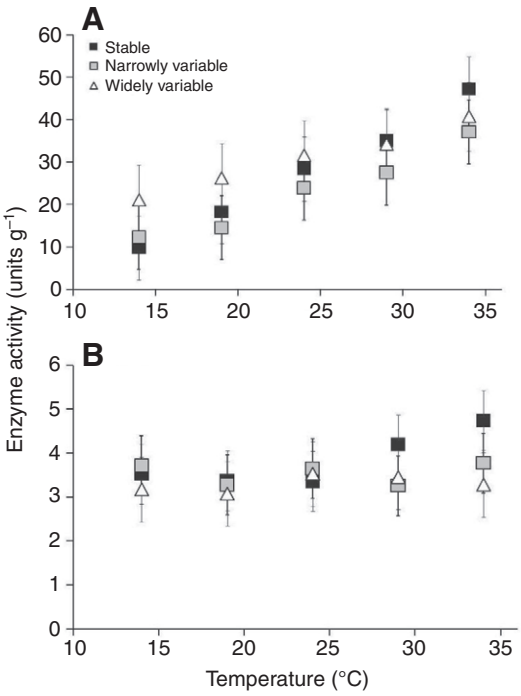

Fig. 2. The effects of acute changes in temperature on the size-specific rates of enzyme activity for (A) lactate dehydrogenase (LDH) and (B) cytochrome *c* oxidase (CCO) in larval *Limnodynastes peronii* from stable, narrowly variable and widely variable thermal treatments. No significant differences were detected among the thermal treatments in either enzyme. Data are means ± 95% confidence intervals.

entire range of test temperatures, between 14 and 34°C (repeated-measures ANCOVA: LDH, $F_{2,23}$=0.63, P=0.55; COX, $F_{2,23}$=2.6, P=0.10). However, at high test temperatures, activities of both LDH ($F_{2,23}$=3.9, P=0.04) and COX ($F_{2,23}$=3.7, P=0.04) were more sensitive to temperature change in tadpoles raised in stable environments. Between 24 and 34°C, tadpoles raised in stable temperatures had higher LDH Q_{10} values (1.76±0.25) than those raised in narrowly (Q_{10}=1.57±0.17) or widely variable (Q_{10}=1.39±0.18) temperatures. Similarly, Q_{10} of COX was higher in tadpoles raised in stable temperatures (Q_{10}=1.47±0.18) than those raised in narrowly (Q_{10}=1.03±0.12) or widely variable (Q_{10}=0.95±0.07) temperatures.

Heart rate

The relationship between test temperature and resting heart rate was unaffected by treatment ($F_{12,252}$=0.53, P=0.55). However, resting heart rate was highly influenced by acute changes in test temperature ($F_{6,252}$=104.7, P<0.0001). Across all treatments, resting heart rate increased from 34.8±2.0 beats min^{-1} at 10°C up to a peak of 209.7±2.1 beats min^{-1} at 38°C (Fig. 3). Similarly, the relationship between test temperature and both maximum heart rate ($F_{12,252}$=0.70, P=0.75) and heart rate scope ($F_{12,252}$=0.59, P=0.85) was unaffected by treatment. Acute changes in test temperature markedly affected maximum heart rate ($F_{6,252}$=235.1, P<0.0001) and increased from 65.1±3.1 at 10°C up to a peak of 192.0±3.2 at 38°C (Fig. 3). Heart rate scope steadily decreased with acute increases in temperature from 1.89±0.04 at 10°C to a low of 0.97±0.04 at 38°C ($F_{6,252}$=84.9, P<0.0001) (Fig. 4). Resting heart rates were actually marginally greater than maximum heart rates at temperatures above 34°C,

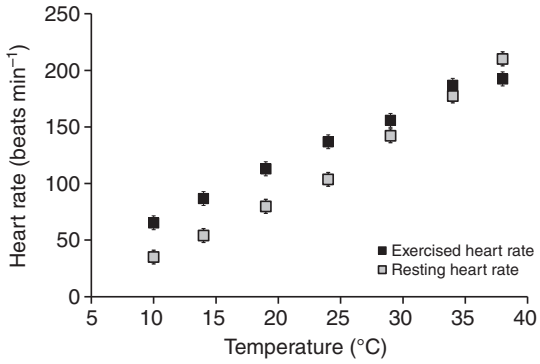

Fig. 3. The effect of temperature on the resting and exercised heart rates for larval *Limnodynastes peronii* from stable, narrowly variable and widely variable thermal treatments. No significant differences among thermal treatments were detected so data were pooled. However, resting ($F_{6,230}$=1043.1, P<0.0001) and maximum ($F_{6,230}$=235.0, P<0.0001) heart rates were significantly greater at higher temperatures. Data are means ± 95% confidence intervals.

suggesting that any activity at these extreme temperatures may induce a stress response that marginally reduces heart rate.

Across the entire range of temperatures, mean Q_{10} values for resting heart rate did not differ between treatments (Q_{10}=1.88±0.02, 1.88±0.03 and 1.93±0.03 for stable, narrowly variable and widely variable treatments, respectively; ANCOVA: $F_{3,32}$=1.05, P=0.36). However, across all three treatments, Q_{10} values of resting heart rate were higher between 10 and 24°C (Q_{10}=2.08±0.06, 2.08±0.06 and 2.25±0.05 for stable, narrowly variable widely variable treatments, respectively) than between 24 and 38°C (Q_{10}=1.72±0.06, 1.64±0.03 and 1.65±0.04 for stable, narrowly variable and widely variable treatments, respectively). At low temperatures, resting heart rates of tadpoles from widely variable temperatures had significantly higher Q_{10} values ($F_{3,32}$=4.03, P=0.03), but there were no differences between treatments at high temperatures ($F_{3,32}$=2.3, P=0.12). Maximum heart rates were constant across test temperatures, and at the low and high temperature ranges (Q_{10} for all temperature ranges varied from 1.02±0.07 to 1.12±0.08), and there were no differences between treatment groups ($F_{2,31}$=1.4, P=0.25).

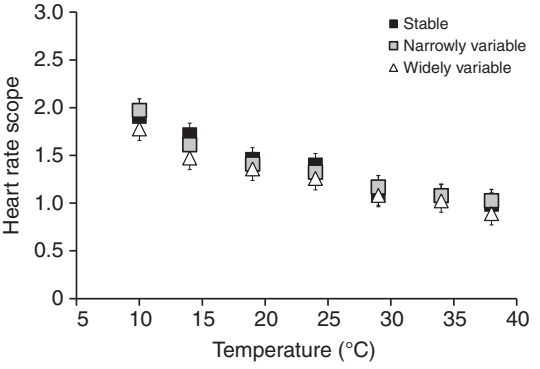

Fig. 4. The effects of acute changes in temperature on the heart rate scope (ratio of exercised to resting heart rates) for larval *Limnodynastes peronii* from stable, narrowly variable and widely variable thermal treatments. No significant differences were detected among the thermal treatments. Data are means ± 95% confidence intervals.

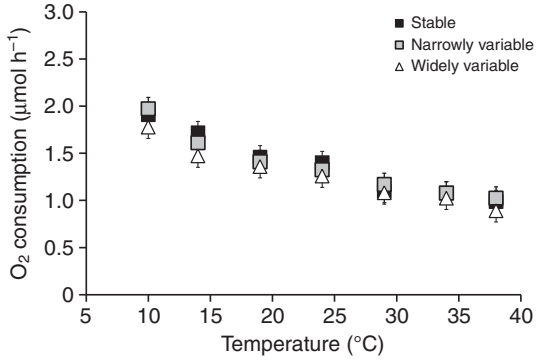

Fig. 5. The effects of temperature on the mass-specific rates of oxygen consumption for larval *Limnodynastes peronii* from stable, narrowly variable and widely variable thermal treatments. No significant differences were detected among the thermal treatments. Data are means ± 95% confidence intervals.

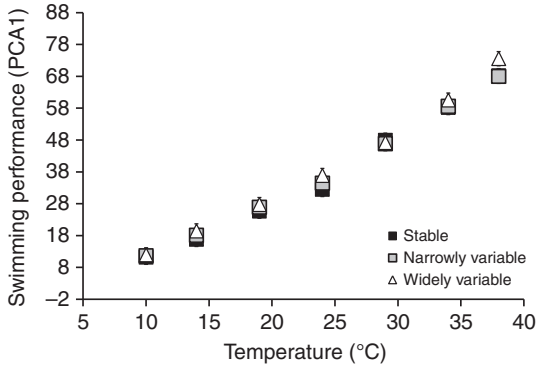

Fig. 6. The effect of temperature on the maximum swimming performance of larval *Limnodynastes peronii* from stable, narrowly variable and widely variable thermal treatments. No significant differences were detected among the thermal treatments in either enzyme. Swimming performance (PCA1) is the first dimension of a principal components analysis of maximum instantaneous swimming speed and mean swimming speed over the first 160 ms of a startle response. Data are means ± 95% confidence intervals.

Oxygen consumption

There was no interaction between thermal treatments and test temperatures ($F_{10,216}$=1.74, P=0.07; Fig. 5), although the near-significance was suggestive of a difference in rates among the highest two temperatures (Fig. 5). Temperature itself had a significant effect on oxygen consumption ($F_{5,216}$=131.7, P<0.0001), with tadpoles consuming more than 14 times more oxygen at 38°C than at 10°C.

The Q_{10} values over the range of 10–38°C did not differ among treatments ($F_{2,22}$=0.72, P=0.50). Thermal sensitivities were much higher at low temperatures (Q_{10}=5.60±0.89, 9.30±0.89 and 6.00±0.88 for stable, narrowly variable and widely variable treatments, respectively) than at high temperatures (Q_{10}=1.10±0.05, 1.70±0.07 and 1.20±0.07 for stable, narrowly variable and widely variable treatments, respectively). However, there were no differences between treatments (10–24°C: $F_{2,26}$=0.39, P=0.68; 24–38°C: $F_{2,26}$=2.66, P=0.10).

Swimming performance of larvae

The relationship between temperature and swimming performance was unaffected by differing levels of thermal instability (Fig. 6). We found that U_P was significantly influenced by both temperature ($F_{6,193}$=49.2, P<0.0001) and treatment ($F_{2,193}$=9.61, P<0.0001). However, the relationship between temperature and U_P (interaction effect) was not influenced by acclimation treatment ($F_{12,193}$=0.86, P=0.59) (Fig. 6).

DISCUSSION

We predicted that performance of tadpoles would acclimate in response to widely variable temperatures, so that thermal performance breadth increased while thermal sensitivity decreased. This prediction was true for a small range of ambient temperatures. However, over a broader temperature range, the temperature–rate relationships of two metabolic enzymes, heart rate, locomotor performance and oxygen consumption did not differ between tadpoles raised in stable or variable thermal conditions. Our results show that larval amphibians exhibit limited plasticity in metabolic traits to fluctuating temperatures, at least during early stages of development. This lack of plasticity may have important implications for growth in variable environments.

Wide diel fluctuations may interfere with the ability to adjust metabolism in response to seasonal temperature changes. For example, the unpredictable and unstable temperatures experienced by intertidal gastropods on a daily basis inhibit their responses to long-term thermal variability (McMahon et al., 1995). Theory predicts that seasonal or long-term acclimation in adults should be reduced in environments that are variable at shorter time scales (e.g. daily) because the environmental signals that trigger an acclimation response are obscured (Gabriel, 2005; Gabriel et al., 2005; Angilletta, 2009). Rather than shifting the optima of thermal performance curves, organisms in unstable environments may instead decrease thermal sensitivity of performance over the range of temperatures encountered. For example, the metabolism of intertidal limpets living in open, thermally variable habitats on rocky shores was much less sensitive to temperature fluctuation than that of limpets living in sheltered, thermally stable microhabitats (Sinclair et al., 2006). Similarly, we observed a reduction in thermal sensitivity over a reduced part of the thermal range in tadpoles of *L. peronii*, although the thermal sensitivity of enzyme activities, heart rate and oxygen consumption did not differ across a wide temperature range. Importantly, however, the pattern of thermal sensitivity differed between traits, and did not always match our predictions. For example, at high temperatures, both metabolic enzymes (LDH and CCO) were thermally less sensitive in tadpoles raised in variable conditions. This depression of metabolism at high temperatures may indicate a biochemical limitation that constrains maximal metabolic rates, or a regulated acclimation response to maintain maximal metabolic rates within functional limits (Seebacher et al., 2010). In contrast, at low temperatures, resting heart rates of tadpoles from the widely variable treatment were more sensitive to temperature changes, which indicates that mechanisms other than metabolic processes, such as myosin activities, Ca^{2+} transport or crossbridge formation (Johnston and Temple, 2002; Galli et al., 2009), determine thermal sensitivity in heart muscle. A possible advantage of increased thermal sensitivity of heart rates at lower temperature is that cardiac output and blood flow are maximised with even small increases in temperature, which could facilitate oxygen transport and locomotion early in the day.

Pronounced and rapid changes in temperature are likely to be stressful to developing organisms, and wide diel cycles are associated with reductions in body size for amphibians and fish (Schaefer and

Ryan, 2006; Dhillon and Fox, 2007). Here, we found that *L. peronii* larvae raised in widely variable temperatures were significantly smaller at 10–14 days of age than those raised in more stable conditions. High thermal sensitivity of metabolism could be responsible for small body sizes in widely variable (or stressful) temperatures, as individuals cannot acquire enough energy through feeding to balance the high metabolic demands of their cells and are thus forced to catabolise tissues (West et al., 2001; Gillooly et al., 2001; Gillooly et al., 2002; Makarieva et al., 2004). Chomsky et al. found that some species of sea anemone could acclimate metabolically in high temperatures, but not enough to counter the negative effects of high metabolism on growth (Chomsky et al., 2004); this may also be the case for tadpoles of *L. peronii*, where reduced thermal sensitivity at high temperatures does not completely offset the increased energy demand.

High temperatures are detrimental for tadpoles of *L. peronii* because cardiac scope is dramatically reduced, and responses to conditions experienced during development do not offset this effect. *Limnodynastes peronii* embryos and larvae are not able to survive constant 34°C temperatures for more than 24–48 h, but can complete metamorphosis in fluctuating conditions in which they spent 4 h at this temperature every day (A.C.N., unpublished data). If environmental temperatures increased to reach 34°C for longer periods of time, developmental rates of tadpoles could be compromised at the population level. Thus, human-induced changes in global thermal variation may cause – or already have caused – amphibian population declines *via* the negative effect of high temperatures on cardiac performance and development.

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