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Beneficial acclimation: sex specific thermal acclimation of metabolic capacity in the striped marsh frog (*Limnodynastes peronii*)

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Summary

Reproductive success in thermally varying environments will depend on maintaining metabolic capacity of tissues that are important in mating behaviours. Here we test the hypothesis that cold acclimation will occur in those tissues that are important for reproduction, and that acclimation will be sex specific, reflecting behavioural differences between the sexes. We used the frog *Limnodynastes peronii* as a model because anurans engage in energetically demanding reproductive behaviour, and many species, including *L. peronii*, are reproductively active across seasons. Additionally, reproductive behaviours such as calling and amplexus are sex specific. We acclimated animals to naturally occurring autumn (15°C, *N*=10) and summer (25°C, *N*=10) temperatures. Whole-animal resting oxygen consumption decreased with lowered temperature, but there was no difference in oxygen consumption between acclimation treatments or sexes. However, the respiratory control ratio (RCR) of mitochondria from the liver and external oblique calling muscle increased with cold acclimation. The increase in RCR with thermal acclimation was due to upregulation of state 3 respiration, and not to a decrease in state 4 respiration. Males had higher activity of citrate synthase, β-hydroxyacyl CoA dehydrogenase and cytochrome *c* oxidase than females in the calling (external oblique) muscle, and males also showed thermal acclimation of these enzymes while females did not. Additionally, males had greater activity of metabolic enzymes in the principal muscle (extensor carpi radialis) used during amplexus. However, there were no differences in metabolic capacity between sexes in the gastrocnemius muscle and in liver, and both sexes showed significant acclimation of lactate dehydrogenase and cytochrome *c* oxidase in the former and latter, respectively. In *L. peronii*, thermal acclimation of metabolic capacities is linked to reproductive success, and reversible phenotypic plasticity therefore confers a selective advantage by extending the temporal and spatial extent of the animals' fundamental niche.

Key words: temperature, reproduction, mitochondria, fitness, oxygen consumption, amphibia.

Introduction

Temperature is the most pervasive physical variable affecting biological processes (Somero, 1997). When temperature decreases, enzyme-catalysed rate processes and diffusion rates also decrease, thereby reducing the metabolic capacity of animals. Many species are able to overcome this effect by increasing the amount or type of metabolic enzymes expressed (Hochachka and Somero, 2002), and by altering the composition of cellular membranes to maintain the activity of membrane-bound enzymes (Wodtke, 1981; Guderley et al., 1997). These molecular changes will affect metabolism at tissue and whole animal levels so that physiological processes can proceed at a constant or near constant rate, despite pronounced temperature fluctuations.

Thermal compensation of ATP production may be beneficial by increasing components of fitness such as survival and reproductive success, and if acclimation of metabolic capacity is beneficial it should increase the relative fitness of the individual (Huey et al., 1999; Seebacher and Wilson, 2006). However, thermal acclimation of metabolism may also represent a cost that is incurred by the energy required for increased rates of transcription and translation (Guderley, 2004), and by the damage caused to membranes by the increase in reactive oxygen species (Guderley and St-Pierre, 2002; Brand et al., 2004). Hence, acclimation may vary according to relative benefits and costs, and it would be advantageous if acclimation occurred selectively in the tissues, pathways or sex where it is most beneficial. Detection of acclimation will also depend on choosing appropriate traits for investigation (Kingsolver and Huey, 1998). For example, resting oxygen consumption represents an energetic cost of living (Hulbert and Else, 2000; Clarke, 2003), and it may be more beneficial for ectotherms to downregulate resting metabolic rates (Seebacher, 2005). On the other hand, it may be beneficial to upregulate the capacity of processes or pathways that are linked to fitness, such as locomotor performance or ATP production, as environmental temperatures decrease.

The reproductive processes and behaviour of anurans are energetically demanding, particularly for males (Kaplan, 1987; Ryan, 1991; Searcy and Andersson, 1986). Calling by anurans is one of the most energetically demanding activities in ectotherms (Schwartz et al., 1995; Taigen and Wells, 1985;

Walsberg et al., 1986), and maintaining ATP-producing processes at reduced temperatures is essential for reproductive success. Males call to attract females, and to establish and mark territories. Rate, duration and intensity of calling influence attractiveness to females (Gerhardt et al., 2000; Klump and Gerhardt, 1987; Wells, 1977), with preference for energetically demanding call characteristics such as high frequency and volume (Arak, 1983; Sullivan, 1983). Additionally, males call to inhibit other males, and also engage in 'wrestling' with conspecifics to control calling sites and capture females already in amplexus with males (Wells, 1977). Temperature also influences the reproductive output of female frogs by its influence on clutch and egg size, and the survival and growth of the offspring (Kaplan, 1987; Parichy and Kaplan, 1995). 80% of total protein in amphibian eggs (Wallace, 1985) consists of vitellogenin that females produce in the liver.

The marsh frog *Limnodynastes peronii* is widespread in Australia, ranging from tropical to temperate regions (Cogger, 2000). Even in temperate areas of New South Wales, reproductive activities occur for 9 months in the year and males maintain their calling performance across pronounced seasonal temperature fluctuations. Hence, the species is ideal for testing the hypotheses that acclimation of metabolic capacity occurs in traits that are linked to reproductive success, and that the response is different between the sexes.

Materials and methods

Collection and maintenance

Twenty adult *Limnodynastes peronii* Duméril and Bibron 1841 (10 males and 10 females) were collected in summer (January) in Sydney, Australia (34°10′S, 151°30′E). Animals were maintained in containers with peat moss substrate, access to water, and kept on a 12 h:12 h L:D cycle. Animals were fed twice a week on crickets, cockroaches and mealworms, supplemented with vitamin powder. Frogs were split into two groups, one maintained at 15°C and the other at 25°C, with equal numbers of males and females in each treatment. These temperatures were chosen to simulate the range of air temperature experienced by this species when active in Sydney between August and May. Temperature of each treatment group was changed by 1°C every day until the acclimation temperature was reached. All animals were collected in January, and acclimated for at least 60 days before sampling. The increase in body mass (% change in body mass from capture to sampling) in the 15°C acclimated group (13.8±3.2%) was similar to that of the 25°C acclimated group (14.3±3.28%).

Measurement of resting metabolic rate

Resting metabolic rate (RMR) was measured by closed-system respirometry. Frogs were fasted for 5 days before respirometry. Each frog was placed in a respirometry container (550 ml) with 1 ml of water, and the chamber was sealed. After 30 min, an oxygen sample was taken from the container using a syringe with stopcock. The gas sample was drawn through Drierite® to absorb moisture, and then Carbosorb® to absorb CO_2, and again through Drierite®. The oxygen concentration of the sample was determined with an Amtek N-37M oxygen sensor attached to an Amtek S-3A/11 oxygen analyser (Pittsburgh, PA, USA). Oxygen consumption rate of each frog was measured at 15°C and at 25°C in controlled temperature environments (4 h between measurement at each temperature), and the order of test temperatures was randomised for each individual. Resting metabolic rate (RMR) was calculated according to Vleck (Vleck, 1987). To ensure that measurements reflected the resting metabolic rate, respirometry trials were repeated 3–4 times for each individual (with 1 week between measurements) until animals reached a stable resting metabolic rate (Iglesias et al., 2003).

Tissue collection

After RMR measurements, animals were anaesthetised in a 0.5% solution of MS222 (neutralised to pH 7), and euthanised by double pithing. Each animal was weighed to 0.1 g, and snout–vent length was measured. The liver, gastrocnemius muscle, extensor carpi radialis muscle and external oblique muscle were dissected from the animal and frozen at –80°C. Additional samples of liver, external oblique muscle and gastrocnemius muscle were collected to isolate mitochondria. The remaining portion of each individual was weighed again, and then dried at 60°C for 72 h, to provide an estimate of total dry mass. We removed the eggs from gravid females to be weighed separately.

Mitochondrial respiration

Tissue was homogenised in a glass homogeniser in 9 volumes of isolation buffer (pH 7.3; 140 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes, 10 mmol l^{-1} EDTA, 5 mmol l^{-1} $MgCl_2$, 0.5% BSA). The homogenate was centrifuged at 1400 ***g*** for 5 min, and the supernatant was removed and centrifuged again at 9000 ***g*** for 7 min. The pellet was resuspended in assay buffer (10:1 buffer: initial tissue mass, pH 7.3; 140 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes, 5 mmol l^{-1} Na_2HPO_4, 0.5% BSA) to make up a stock solution. The stock solution was further diluted by 9 volumes of assay buffer for measurements of oxygen consumption in a temperature controlled respiration chamber (Mitocell 200A, Strathkelvin, Scotland). Oxygen consumption was measured with a microelectrode connected to an oxygen analyser (model 1302, Strathkelvin). State 2 oxygen consumption rate was measured by adding pyruvate (2.5 mmol l^{-1} final concentration), and malate (5 mmol l^{-1} final concentration) to spark the TCA cycle. Maximal oxidation rate (state 3) was measured with the addition of ADP to the chamber (3.8 mmol l^{-1} final concentration). The respiratory control ratio (RCR) was calculated by the ratio of state 3 oxygen consumption to state 4 oxygen consumption when all ADP was consumed.

To determine protein concentration, 50 μl of mitochondrial solution was resuspended in 1 ml of BSA-free buffer, and centrifuged at 12000 ***g*** for 10 min. The pellet was retained and resuspended, and this process was repeated twice. The resulting solution was used to determine the protein concentration using the bicinchoninic acid method (Sigma, Sydney, Australia).

Enzyme assays

Tissue was homogenised in 99 volumes of extraction buffer (pH 7.5; 50 mmol l^{-1} imidazole, 2 mmol l^{-1} $MgSO_4$, 5 mmol l^{-1} EDTA, 1 mmol l^{-1} glutathione, 0.1% Triton). The homogenate was further diluted to 1:200 for lactate dehydrogenase (LDH) assays. Enzyme activity was determined in a spectrophotometer

(Ultrospec 2100pro, Amersham, Australia) with a temperature controlled cuvette holder. Assays were conducted at 15°C and 25°C according to published methods (Seebacher et al., 2003). All chemicals were supplied by Sigma (Australia) and ICN Biochemicals (Australia), and saturating substrate concentrations were determined before assays of experimental tissues.

LDH activity (forward direction: pyruvate to lactate) was determined by monitoring the absorbance of NADH at 340 nm. This assay was completed in potassium phosphate buffer (pH 7.0) containing 0.16 mmol l^{-1} NADH and 0.4 mmol l^{-1} pyruvate. The activity for the reverse direction (lactate to pyruvate) was measured in a Tris buffer (pH 9.3) with 50 mmol l^{-1} lactate, 10 mmol l^{-1} NAD^+, as the appearance of NADH at 340 nm.

Citrate synthase (CS) activity was monitored by the change in absorbance of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) at 512 nm, in Tris buffer (pH 8.0) containing 0.1 mmol l^{-1} DTNB, 0.1 mmol l^{-1} acetyl-CoA, and 0.15 mmol l^{-1} oxaloacetate (omitted for controls). Any activity detected in control assays was subtracted from the experimental assays.

Cytochrome *c* oxidase (CCO) activity was assayed by monitoring the change in absorbance of reduced cytochrome *c* (0.05 mmol l^{-1}) in potassium phosphate buffer (pH 7.5) at 550 nm. Cytochrome was reduced by addition of sodium thiosulfate, and excess sodium thiosulfate was removed by bubbling with air.

β-hydroxyacyl CoA dehydrogenase (HOAD) activity was assayed in imidazole buffer (50 mmol l^{-1} imidazole, 1 mmol l^{-1} EDTA, pH 8) containing 0.16 mmol l^{-1} NADH, 0.1 mmol l^{-1} aceto-acetyl-CoA, and 1 mmol l^{-1} KCN. The activity of HOAD was measured by the depletion of NADH at 340 nm.

Statistical analysis

Oxygen consumption was $\log_{10}$ transformed and analysed using a repeated-measures analysis of covariance (RM-ANCOVA) with sex, test temperature and acclimation treatment as factors, and dry mass ($\log_{10}$ transformed) as a covariate. A power analysis was used to estimate the probability of detecting a 20% difference between treatments, to ensure that lack of differences between acclimation treatments is not due to lack of statistical power. Mitochondrial respiration data were analysed in a repeated-measures multivariate analysis of variance (RM-MANOVA) for each respiration state, with acclimation group, sex and test temperature (repeated) as factors. Respiratory control ratio was analysed in a RM-MANOVA, with acclimation group, sex and test temperature (repeated) as factors. Enzyme data were analysed with a RM-MANOVA for each enzyme, with acclimation group and test temperature (repeated) as factors. Fisher's protected LSD (corrected by sequential Bonferroni test) was used to separate the effects of acclimation and sex from each MANOVA and ANCOVA. The proportion of egg mass to gravid female mass was calculated as a proportion of total dry body mass (including eggs). Assumptions of normality and homogeneity of variance were tested, and $\log_{10}$ and square-root transformations were used to correct non-normal data and differences in variance between groups, but non-transformed data are shown. Significance was set at $P<0.05$, where Bonferroni correction was applied, the original F value is shown but P is corrected for multiple tests. Analyses were performed in Genstat© (VSN International Ltd., Hemel Hempstead) or R© (R Foundation for Statistical Computing, Vienna, Austria).

Results

Resting metabolic rate

Resting metabolic rate (ml O_2 h^{-1} g^{-1} dry mass, mean ± s.e.m.) increases significantly with test temperature ($F_{1,18}=84.37$, $P<0.001$; Fig. 1), but there are no differences between acclimation groups ($F_{1,16}=0.93$, $P>0.05$) or sex ($F_{1,16}=0.87$, $P>0.05$; Fig. 1).

Mitochondrial oxygen consumption

State 3 and state 4 oxygen consumption of mitochondria (nmol O_2 min^{-1} mg^{-1} mitochondrial protein, mean ± s.e.m.) increase significantly in all tissues and treatments with increasing test temperature (liver: $F_{1,14}=10.12$; external oblique muscle: $F_{1,14}=9.83$; gastrocnemius: $F_{1,14}=8.31$, all $P<0.05$. Table 1). Mitochondria from the liver and external oblique muscle of cold-acclimated frogs have significantly higher state 3 rates than warm-acclimated frogs (Table 1; Bonferroni-corrected LSD, $P<0.05$). There is no difference in state 3 respiration in the gastrocnemius muscle between acclimation treatments (Bonferroni-corrected LSD, $P<0.05$). State 4 respiration does not change with acclimation treatment in any tissue (Table 1; $P>0.05$). There are no differences between males and females in either state 3 or 4 respiration (liver: $F_{1,14}=3.23$, external oblique muscle: $F_{1,14}=3.92$, gastrocnemius: $F_{1,14}=1.45$, $P<0.05$).

The respiratory control ratio (RCR) differs significantly between acclimation groups in liver at both 15°C and 25°C, and in external oblique muscle at 15°C (Bonferroni-corrected LSD, $P<0.05$; Table 1). There is no change in RCR with acclimation in the gastrocnemius muscle at either 15°C or 25°C (Bonferroni-corrected LSD, $P<0.05$; Table 1).

Metabolic enzymes

In the gastrocnemius muscle, acclimation treatment does not have a significant effect on enzyme activities, except for LDH where activity (pyruvate→lactate) is greater in cold-acclimated

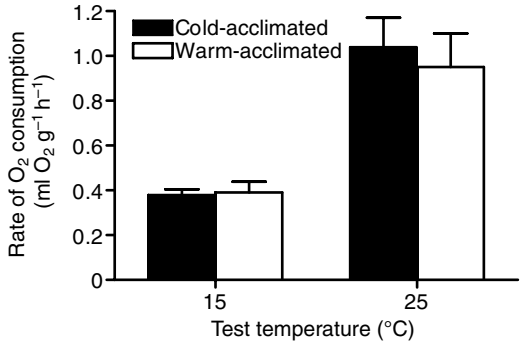

Fig. 1. Rates of resting oxygen consumption (ml O_2 h^{-1} g^{-1} dry mass, mean ± s.e.m.) of cold (15°C, black bars) and warm (25°C, white bars) acclimated frogs at test temperatures of 15 and 25°C. Oxygen consumption increased with increasing test temperature, but there was no difference between the two acclimation groups.

Table 1. *State 3 and state 4 rates of oxygen consumption by isolated mitochondria and respiratory control ratios (RCR) from gastrocnemius muscle, external oblique muscle, and liver of cold- and warm-acclimated frogs at 15°C and 25°C*

	Gastrocnemius				External oblique				Liver			
Acclimation	Cold		Warm		Cold		Warm		Cold		Warm	
Assay temperature (°C)	15	25	15	25	15	25	15	25	15	25	15	25
State 3	0.10±0.05	0.16±0.14	0.10±0.03	0.14±0.09	0.066±0.093*	0.11±0.04	0.048±0.008*	0.11±0.04	0.043±0.007*	0.089±0.023*	0.035±0.007*	0.071±0.023*
State 4	0.008±0.002	0.021±0.007	0.006±0.001	0.014±0.003	0.008±0.002	0.017±0.006	0.007±0.001	0.017±0.007	0.005±0.001	0.008±0.005	0.005±0.001	0.010±0.003
RCR	12.2±2.3	8.0±1.0	13.7±2.4	7.6±1.1	9.3±0.5*	7.3±1.3	7.0±0.7*	8.7±1.5	9.5±1.0*	10.5±1.0*	6.1±0.9*	5.9±1.1*

Rates of oxygen consumption (μmol O_2 min^{-1} mg mitochondrial $protein^{-1}$) are means ± s.e.m. (N=5).
RCR, respiratory control ratio.
Asterisks indicate differences between acclimation treatments; there were no significant differences between sexes.

than in warm-acclimated frogs at 15°C (Bonferroni-corrected LSD, P<0.05; Table 2). Similarly, enzyme activities in liver are not affected by acclimation treatment, except that cold-acclimated frogs have significantly greater CCO activity at 15°C, than warm-acclimated frogs (Bonferroni-corrected LSD, P<0.05), but there are no differences between sexes (Bonferroni-corrected LSD, P>0.05; Table 2).

In outer oblique (calling) muscle, cold-acclimated frogs have higher activity of CCO ($F_{1,18}$=10.73, P<0.05), CS ($F_{1,18}$=8.53, P<0.05), and HOAD ($F_{1,18}$=11.99, P<0.01; Table 3). Additionally, sex has a significant effect on the activity of these enzymes (CCO: $F_{1,18}$=12.42, P<0.05. CS: $F_{1,18}$=7.87, P<0.05. HOAD: $F_{1,18}$= 54.93, P<0.05), and there is an interaction between sex and acclimation groups (CCO: $F_{1,9}$=13.13, P<0.05. CS: $F_{1,9}$=11.59, P<0.05. HOAD: $F_{1,9}$=20.88, P<0.01). Male frogs have significantly greater CCO and CS activities than females, and activity of these enzymes increases with cold acclimation in males (Bonferroni-corrected LSD, P<0.05). There is no difference in CCO or CS activity between cold- and warm-acclimated females (Bonferroni corrected LSD, P>0.05). Males have significantly higher HOAD activity than females (Bonferroni-corrected LSD, P<0.05), and there are significant differences between acclimation treatments in males (Bonferroni-corrected LSD, P<0.05). Thermal acclimation has no effect on the activity of HOAD in outer-oblique muscle in females (P>0.05).

There is no effect of acclimation treatment on enzyme activity in the extensor carpi radialis muscle (Table 4), but there are differences between sexes in the activity of enzymes. Males have higher LDH activity than females for both pyruvate reduction ($F_{1,18}$=9.54, P<0.05) and lactate oxidation ($F_{1,18}$=34.78, P<0.05). Males also have significantly higher CS activity than females ($F_{1,18}$=14.78, P<0.05).

Egg mass

There is no significant difference in the percentage of body mass of females comprising eggs between the two acclimation treatments (cold: 26.4±6.7%, warm: 22.3±2.0%; $F_{1,6}$=1.04, P>0.05).

Discussion

Sex-specific acclimation of ATP-producing pathways in tissues that are linked directly to reproductive success indicates that thermal acclimation is a response selected for the fitness benefits it confers on individuals. In external oblique (calling) muscle, females show no acclimation of metabolic enzymes, but males show perfect temperature compensation in the activity of these enzymes with acclimation. The external oblique muscle is important in calling (Girgenrath and Marsh, 1997; Martin and Gans, 1972), and male anurans often have higher activities of metabolic enzymes in this muscle than females (Given and McKay, 1990; Marsh and Taigen, 1987). Additionally, citrate synthase activity is a correlate of calling ability in *Pseudacris crucifer* (Zimmitti, 1999). The calling muscles are highly aerobic muscles that can contract repeatedly over long periods (Marsh, 1999), so that temperature compensation in oxidative pathways ensures the supply of ATP used in muscular contraction. Upregulation of metabolic capacity of this muscle maximises call volume and frequency,

Table 2. *Metabolic enzyme activities measured at 15°C and 25°C in gastrocnemius muscle and in liver in cold and warm acclimated frogs*

		Gastrocnemius		Liver	
Enzyme	Temperature (°C)	Cold	Warm	Cold	Warm
LDH					
Forward	15	222.0±14.8*	162.0±15.2*	35.0±3.5	36.0±3.6
	25	327.0±14.1*	237.0±15.4*	55.0±5.7	61.0±5.7
Reverse	15	94.0±4.8	74.0±6.7	16.0±1.7	18.0±1.7
	25	125.0±5.9	106.0±9.0	20.0±1.1	25.0±2.3
CCO	15	4.0±0.5	3.5±0.4	5.6±0.3*	3.0±0.3*
	25	4.6±0.5	4.3±0.5	5.8±0.6*	4.8±0.6*
CS	15	7.0±0.4	6.0±0.5	4.0±0.3	6.0±0.6
	25	8.0±0.7	9.0±0.8	6.0±0.5	9.0±1.2

Metabolic enzyme activities (U g^{-1} wet tissue mass) are means ± s.e.m. (N=5).

LDH, lactate dehydrogenase (forward, pyruvate reduction; backward, lactate oxidation); CCO, cytochrome *c* oxidase; CS, citrate synthase.

There were no significant differences between sexes, and mean data from both sexes are shown. An asterisk indicates significant differences between acclimation treatments.

Table 3. *Metabolic enzyme activities measured at 15°C and 25°C in external oblique calling muscle in cold- and warm-acclimated frogs*

		Cold acclimated		Warm acclimated	
Enzyme	Temperature (°C)	Male	Female	Male	Female
LDH					
Forward	15	134.0±13.3		107.0±13.8	
	25	194.0±12.2		160.0±16.3	
Reverse	15	56.0±6.2		73.0±25.1	
	25	77.0±12.9		88.0±23.2	
CCO	15	11.6±0.8*	5.0±1.2	6.4±0.7*	3.5±0.6
	25	13.2±2.7	6.2±1.3	12.4±2.4	8.2±3.0
CS	15	36.9±6.3*	6.8±1.1	14.5±4.3*	6.8±1.0
	25	53.3±8.9*	11.3±3.5	26.3±3.9*	9.3±1.8
HOAD	15	6.4±2.1*	2.1±0.2	3.4±0.3*	2.0±0.3
	25	12.6±3.0*	3.1±0.5	5.3±1.0*	3.2±0.4

Metabolic enzyme activities (U g^{-1} wet tissue mass) are means ± s.e.m. (N=5).

LDH, lactate dehydrogenase (forward, pyruvate reduction; backward, lactate oxidation); CCO, cytochrome *c* oxidase; CS, citrate synthase; HOAD, β-hydroxyacyl CoA dehydrogenase.

There were significant differences between sexes where values for males and females are shown separately, otherwise the grand mean for both sexes are shown in the 'male' column. An asterisk indicates significant differences between acclimation treatments.

both of which are determinants of reproductive success in male anurans (Arak, 1983; Klump and Gerhardt, 1987; Gerhardt et al., 2000). The increases in aerobic metabolic enzyme activities in males provide the capacity to maintain calling despite seasonally lowered temperature. Female anurans prefer males whose calls reflect increased energetic costs (Arak, 1983; Sullivan, 1983), so that males that do not undergo acclimation in the calling muscle may lose the opportunity to mate when environmental temperature decreases. It seems likely that sex hormones may regulate the difference in acclimation response between male and females, given that testosterone increases muscle contractile abilities in *Hyla chrysoscelis* (Girgenrath and Marsh, 2003).

In contrast, there were no sex-specific differences in acclimation of state 3 oxygen consumption rate of mitochondria and enzyme activities in liver. State 3 rates and LDH activities also increased in external oblique and gastrocnemius muscles of females, respectively. Thermal acclimation of metabolism in females will be advantageous to maintain ATP supply for locomotion and growth of reproductive tissues in thermally variable environments (Guderley and Johnston, 1996; St-Pierre et al., 1998). Skeletal muscle has limited ability to store glycogen, and stores of glycogen in muscle can limit activity (Guppy, 1988). The liver is essential in the synthesis and release of glucose to other parts of the body (Bollen et al., 1998), and upregulation of metabolic capacity in the liver may be important to support calling, locomotion and growth. Gravid female *L. peronii* produce up to 25% of dry body mass in eggs, and exposure to cold does not affect the egg mass produced. The synthesis of vitellogenin represents an energetic cost that can be met at lowered temperature by upregulating metabolic capacity in the liver (Wallace, 1985), so that thermal compensation of metabolism in females may have fitness benefits by maintaining egg production in cooler environments.

Table 4. *Metabolic enzyme activity in extensor carpi radialis, the principal muscle used by males during amplexus*

Enzyme	Temperature (°C)	Males	Females
LDH			
Forward	15	256.5±13.7*	144.0±22.8*
	25	337.5±25.4*	205.0±24.9*
Reverse	15	96.5±5.2*	49.5±4.2*
	25	119.0±9.1*	60.0±4.5*
CS	15	14.5±1.8*	6.5±0.9*
	25	21.5±3.1*	8.5±1.0*
CCO	15	4.1±0.7	3.0±1.1
	25	4.2±1.1	4.5±1.2

Metabolic enzyme activities (U g^{-1} wet tissue mass) are means ± s.e.m. (*N*=5).

LDH, lactate dehydrogenase (forward, pyruvate reduction; backward, lactate oxidation); CCO, cytochrome *c* oxidase; CS, citrate synthase.

Acclimation treatment had no effect on any enzyme activity, and pooled values for both acclimation treatments are shown. Significant differences between males and females are indicted by an asterisk.

Surprisingly, the activity of LDH increases in the gastrocnemius muscle with cold acclimation, although *Limnodynastes peronii* shows no acclimation of either burst swimming or jumping performance (Wilson and Franklin, 2000), both of which rely on force production by the gastrocnemius muscle. Animals used in the current study were from the same population as those used by Wilson and Franklin (Wilson and Franklin, 2000). Hence, *L. peronii* from the same population undergo thermal acclimation of LDH activity in the gastrocnemius muscle, but not acclimation of locomotor performance, which indicates that LDH activity is not related to jumping and burst swimming performance.

The size (Kirby, 1983; Yekta and Blackburn, 1992) and contractile properties (Peters and Aulner, 2000) of extensor carpi radialis muscle of male anurans are enhanced compared to females, and metabolic capacity (LDH and CS activity) of extensor carpi muscle is higher in male *L. peronii* than in females. This muscle is used in amplexus (Peters and Aulner, 2000) and for bouts of 'wrestling' between males competing for mates or calling positions (Clyne, 1967; Schäuble, 2004). The increased enzyme activity in this muscle in males is another specialisation of muscle phenotype for sexual selection. One of the assumptions of previous calculations of the energetic costs of amplexus is that anaerobic metabolic pathways do not contribute to the energetics of amplexus (James, 2003). However, the difference between males and females in LDH activity means that glycolysis does have a significant role in amplexus, although it may be important only for male–male physical competition.

A single measurement of metabolism is unlikely to be adequate to test for the occurrence of thermal acclimation, and measurements of resting oxygen consumption may be particularly unsuited. Despite significant acclimation of oxidative phosphorylation in mitochondria, and acclimation of several metabolic enzymes, there was no change in resting oxygen consumption. Resting oxygen consumption does not reflect metabolic capacity, but is a measure of the 'cost of living' incurred by the energetic cost of proton leak, protein synthesis and Na^+,K^+-ATPase activity to maintain membrane potentials (Hulbert and Else, 2000). Although there may be a correlation between resting oxygen consumption and metabolic capacity (Gomes et al., 2004), ideally selection should minimise the former while maximising the latter. Hence, it is inappropriate to use resting oxygen consumption as a measure of acclimation or adaptive response to environmental change.

Acclimation of metabolic capacity will permit reproductive activity in varying thermal environments, which allows ectotherms such as *L. peronii* to extend their 'fundamental' ecological niche (Kearney and Porter, 2004) both temporally and spatially. Hence, at least in *L. peronii*, the capacity for reversible phenotypic plasticity should be viewed as a trait that is under selection for the fitness benefits it confers. Additionally, the potential for plasticity must be considered in models that attempt to predict species' responses to climate change. Climate has never been stable during evolutionary history so that selection would favour plastic rather than fixed phenotypes, even in environments that are relatively stable at present (Seebacher et al., 2005).

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References

Arak, A. (1983). Sexual selection by male male competition in natterjack toad choruses. *Nature* **306**, 261-262.

Bollen, M., Keppens, S. and Stalmans, W. (1998). Specific features of glycogen metabolism in the liver. *Biochem. J.* **336**, 19-31.

Brand, M. D., Affourtit, C., Esteves, T. C., Green, K., Lambert, A. J., Miwa, S., Pakay, J. L. and Parker, N. (2004). Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic. Biol. Med.* **37**, 755-767.

Clarke, A. (2003). Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* **18**, 573-581.

Clyne, D. (1967). *Australian Frogs*. Melbourne: Periwinkle Books.

Cogger, H. G. (2000). *Reptiles and Amphibians of Australia*. Sydney: Reed New Holland.

Gerhardt, H. C., Tanner, S. D., Corrigan, C. M. and Walton, H. C. (2000). Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behav. Ecol.* **11**, 663-669.

Girgenrath, M. and Marsh, R. L. (1997). *In vivo* performance of trunk muscles in tree frogs during calling. *J. Exp. Biol.* **200**, 3101-3108.

Girgenrath, M. and Marsh, R. L. (2003). Season and testosterone affect contractile properties of fast calling muscles in the gray tree frog *Hyla chrysoscelis*. *Am. J. Physiol.* **284**, R1513-R1520.

Given, M. F. and McKay, D. M. (1990). Variation in citrate synthase activity in calling muscles of carpenter frogs, *Rana virgatipes*. *Copeia* **1990**, 863-867.

Gomes, F. R., Chaui-Berlinck, J. G., Bicudo, J. E. P. W. and Navas, C. A. (2004). Intraspecific relationships between resting and activity metabolism in anuran amphibians: influence of ecology and behavior. *Physiol. Biochem. Zool.* **77**, 197-208.

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* **79**, 409-427.

Guderley, H. and Johnston, I. A. (1996). Plasticity of fish muscle mitochondria with thermal acclimation. *J. Exp. Biol.* **199**, 1311-1317.

Guderley, H. and St-Pierre, J. (2002). Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *J. Exp. Biol.* **205**, 2237-2249.

Guderley, H., Pierre, J. S., Couture, P. and Hulbert, A. J. (1997). Plasticity of the properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. *Fish Physiol. Biochem.* **16**, 531.

Guppy, M. (1988). Limiting carbohydrate stores: alleviating the problem in the

marathon runner, a hibernating lizard, and the neonate brain. *Can. J. Zool*. **66**, 1090-1097.

Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.

Huey, R. B., Berrigan, D., Gilchrist, G. W. and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool*. **39**, 323-338.

Hulbert, A. J. and Else, P. L. (2000). Mechanisms underlying the cost of living in animals. *Annu. Rev. Physiol.* **62**, 207-235.

Iglesias, S., Thompson, M. B. and Seebacher, F. (2003). Energetic cost of a meal in a frequent feeding lizard. *Comp. Biochem. Physiol.* **135A**, 377-382.

James, D. M. (2003). The metabolic cost of amplexus in the grey tree frog (*Hyla versicolor*): assessing the energetics of male mating success. *Can. J. Zool.* **81**, 388.

Kaplan, R. H. (1987). Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* **71**, 273.

Kearney, M. and Porter, W. P. (2004). Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* **85**, 3119-3131.

Kingsolver, J. G. and Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Am. Zool*. **38**, 545-560.

Kirby, A. C. (1983). Physiology of the sternoradialis muscle: sexual dimorphism and role in amplexus in the leopard frog (*Rana pipiens*). *Comp. Biochem. Physiol.* **74A**, 705-709.

Klump, G. M. and Gerhardt, H. C. (1987). Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature* **326**, 286.

Marsh, R. L. (1999). Contractile properties of muscles used in sound production and locomotion in two species of gray tree frog. *J. Exp. Biol.* **202**, 3215-3223.

Marsh, R. L. and Taigen, T. L. (1987). Properties enhancing aerobic capacity of calling muscles in gray tree frogs *Hyla versicolor*. *Am. J. Physiol.* **252**, R786-R793.

Martin, W. F. and Gans, C. (1972). Muscular control of the vocal tract during release signaling in the toad *Bufo valliceps*. *J. Morphol.* **137**, 1-28.

Parichy, D. M. and Kaplan, R. H. (1995). Maternal investment and developmental plasticity: functional consequences for locomotor performance of hatchling frog larvae. *Funct. Ecol*. **9**, 606-617.

Peters, S. E. and Aulner, D. A. (2000). Sexual dimorphism in forelimb muscles of the bullfrog, *Rana catesbeiana*: a functional analysis of isometric contractile properties. *J. Exp. Biol.* **203**, 3639-3654.

Ryan, M. J. (1991). Sexual selection and communication in frogs. *Trends Ecol. Evol.* **6**, 351.

Schäuble, C. S. (2004). Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynates tasmaniensis* and *L. peronii*. *Biol. J. Linn. Soc. Lond.* **82**, 39-56.

Schwartz, J. J., Ressel, S. J. and Bevier, C. R. (1995). Carbohydrate and calling: depletion of muscle glycogen and the chorusing dynamics of the neotropical treefrog *Hyla microcephala*. *Behav. Ecol. Sociobiol.* **37**, 125.

Searcy, W. A. and Andersson, M. (1986). Sexual selection and the evolution of song. *Annu. Rev. Ecol. Syst.* **17**, 507-533.

Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *J. Comp. Physiol. B* **175**, 453-461.

Seebacher, F. and Wilson, R. S. (2006). Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*. *Funct. Ecol.* **20**, 1045-1053.

Seebacher, F., Guderley, H., Elsey, R. M. and Trosclair, P. L., III (2003). Seasonal acclimatisation of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*). *J. Exp. Biol.* **206**, 1193-2000.

Seebacher, F., Davison, W., Lowe, C. J. and Franklin, C. E. (2005). A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fishes. *Biol. Lett.* **1**, 151-154.

Somero, G. N. (1997). Temperature relationships: from molecules to biogeography. In *Handbook of Physiology*. Vol. 2 (ed. W. H. Dantzler), pp. 1391-1444. Oxford: Oxford University Press.

St-Pierre, J., Charest, P. and Guderley, H. (1998). Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatisation of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **201**, 2961-2970.

Sullivan, B. K. (1983). Sexual selection in the great plains toad (*Bufo cognatus*). *Behaviour* **84**, 258-264.

Taigen, T. and Wells, K. (1985). Energetics of vocalization by an anuran amphibian *Hyla versicolor*. *J. Comp. Physiol. B* **155**, 163.

Vleck, D. (1987). Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. *J. Appl. Physiol.* **62**, 2103-2106.

Wallace, R. A. (1985). Vitellogenesis and oocyte growth in non-mammalian vertebrates. *Dev. Biol. N. Y.* **1**, 127-177.

Walsberg, G. E., Lea, M. S. and Hillman, S. S. (1986). Individual variation in maximum aerobic capacity: cardiovascular and enzymatic correlates in *Rana catesbeiana*. *J. Exp. Zool.* **239**, 1-5.

Wells, K. D. (1977). Social-behavior of anuran amphibians. *Anim. Behav.* **25**, 666-693.

Wilson, R. S. and Franklin, C. E. (2000). Inability of adult *Limnodynastes peronii* (Amphibia: Anura) to thermally acclimate locomotor performance. *Comp. Biochem. Physiol.* **127A**, 21-28.

Wodtke, E. (1981). Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome c oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). *Biochim. Biophys. Acta* **640**, 710-720.

Yekta, N. and Blackburn, D. (1992). Sexual dimorphism in mass and protein content of the forelimb muscles of the northern leopard frog *Rana pipiens*. *Can. J. Zool.* **70**, 670-674.

Zimmitti, S. J. (1999). Individual variation in morphological, physiological, and biochemical features associated with calling in spring peepers (*Pseudacris crucifer*). *Physiol. Biochem. Zool.* **72**, 666-676.