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Seasonal acclimatisation of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*)

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Summary

Reptiles living in heterogeneous thermal environments are often thought to show behavioural thermoregulation or to become inactive when environmental conditions prevent the achievement of preferred body temperatures. By contrast, thermally homogeneous environments preclude behavioural thermoregulation, and ectotherms inhabiting these environments (particularly fish in which branchial respiration requires body temperature to follow water temperature) modify their biochemical capacities in response to long-term seasonal temperature fluctuations. Reptiles may also be active at seasonally varying body temperatures and could, therefore, gain selective advantages from regulating biochemical capacities. Hence, we tested the hypothesis that a reptile (the American alligator *Alligator mississippiensis*) that experiences pronounced seasonal fluctuations in body temperature will show seasonal acclimatisation in the activity of its metabolic enzymes. We measured body temperatures of alligators in the wild in winter and summer (*N*=7 alligators in each season), and we collected muscle samples from wild alligators (*N*=31 in each season) for analysis of metabolic enzyme activity (lactate dehydrogenase, citrate synthase and cytochrome *c* oxidase). There were significant differences in mean daily body temperatures between winter (15.66±0.43°C; mean ± S.E.M.) and summer (29.34±0.21°C), and daily body temperatures fluctuated significantly more in winter compared with summer. Alligators compensated for lower winter temperatures by increasing enzyme activities, and the activities of cytochrome *c* oxidase and lactate dehydrogenase were significantly greater in winter compared with summer at all assay temperatures. The activity of citrate synthase was significantly greater in the winter samples at the winter body temperature (15°C) but not at the summer body temperature (30°C). The thermal sensitivity (Q_{10}) of mitochondrial enzymes decreased significantly in winter compared with in summer. The activity of mitochondrial enzymes was significantly greater in males than in females, but there were no differences between sexes for lactate dehydrogenase activity. The differences between sexes could be the result of the sex-specific seasonal demands for locomotor performance. Our data indicate that biochemical acclimatisation is important in thermoregulation of reptiles and that it is not sufficient to base conclusions about their thermoregulatory ability entirely on behavioural patterns.

Key words: thermoregulation, acclimatisation, reptile, *Alligator mississippiensis*, body temperature, lactate dehydrogenase, citrate synthase, cytochrome *c* oxidase, enzyme activity.

Introduction

The concept that reptiles regulate their body temperature by behavioural means, such as shuttling between sun and shade (Cowles and Bogert, 1944; Hertz et al., 1993), has become widely accepted in vertebrate thermal physiology. Behavioural adjustments enable many diurnal species of reptile to maintain high and stable body temperatures in the face of fluctuations in environmental temperatures (Avery, 1982; Seebacher et al., 1999). The importance of body temperature regulation is seen to lie in maximising the rates of temperature-sensitive physiological functions (Huey, 1982). The rates of chemical reactions, including those catalysed by enzymes, are dependent on the energetic state of the compounds involved, which in turn is strongly influenced by temperature. The rates of most physiological processes are, therefore, a direct function of the temperature of the organism. Thermoregulation that includes high metabolic heat production combined with effective insulation often allows endotherms to maintain an elevated body temperature within a narrow range (Lovegrove et al., 1991). The low metabolic rates of reptiles make metabolic heat production negligible, and regulation of body temperature is achieved by behavioural means such as microhabitat selection (Cowles and Bogert, 1944; Huey and Slatkin, 1976; Hertz et

al., 1993) and behavioural posturing (Muth, 1977; Seebacher, 1999). In addition, many reptiles can alter rates of heat exchange with the environment by increasing or decreasing heart rate and peripheral blood flow during heating or cooling, respectively (Bartholomew and Tucker, 1963; Robertson and Smith, 1979; Seebacher and Franklin, 2001). However, it is also possible that animals respond to changing thermal environments by changing their biochemical characteristics rather than attempting to maintain stable body temperatures (Crawford et al., 1999; Guderley and St Pierre, 2002). Phenotypic changes in response to variation in environmental conditions (acclimatisation) may confer selective advantages by counteracting environmentally induced declines in performance (Wilson and Franklin, 2002; Johnston and Temple, 2002). Acclimatisation is thought to occur particularly in response to long-term changes in environmental conditions, such as to seasonal or latitudinal variation (Scheiner, 1993; Wilson and Franklin, 2000). For example, many fish respond to seasonally changing water temperatures and, hence, body temperatures, by reversibly acclimatising the capacities of their enzyme-catalysed metabolic processes (Guderley, 1990; Segal and Crawford, 1994; Martinez et al., 1999). In addition, there may be differences in metabolic enzyme activities among closely related species living at different latitudes (Pierce and Crawford, 1997). Metabolic acclimation/acclimatisation may occur at the ultrastructural level, such as in mitochondrial numbers or cristae density (St Pierre et al., 1998; Guderley and St Pierre, 2002), or by changes in enzyme activity (Somero et al., 1998; Crawford et al., 1999). Enzyme activity may be altered in response to temperature by changing rates of transcription and enzyme concentrations (Crawford and Powers, 1989, 1992) or by expressing allozymes and isozymes with different thermal sensitivities (Lin and Somero, 1995; Fields and Somero, 1997). Ectotherms may also show biochemical changes during winter dormancy. For example, metabolic enzyme activities were downregulated during winter dormancy compared with the preceding, warmer activity period in a freshwater turtle (*Chrysemys picta marginata*) living at mid to high latitudes (Olson, 1987). Depression in enzyme activity during winter dormancy may result from a combination of low temperatures and anoxic conditions (Olson and Crawford, 1989; St Pierre and Boutilier, 2001).

Other than during winter dormancy, reptiles are thought not to acclimatise biochemically but, instead, to thermoregulate behaviourally or become inactive when environmental conditions preclude the attainment of ‘preferred’ body temperatures (e.g. Case, 1976; Bartholomew, 1982; Grant and Dunham, 1988; Grant, 1990). Many reptiles, however, are active at seasonally varying body temperatures (Christian et al., 1983; Van Damme et al., 1987; Seebacher and Grigg, 1997; Grigg et al., 1998), and it is conceivable that reptiles too could gain selective advantages from regulating biochemical capacities in response to changing environmental conditions. Semi-aquatic species, in particular, experience pronounced seasonal fluctuations in thermal conditions (Costanzo et al., 2000), and winter body temperatures, even of tropical crocodiles, for example, are several degrees below summer averages, with the animals nonetheless remaining active (Seebacher and Grigg, 1997; Grigg et al., 1998). Hence, it was the aim of the present study to investigate whether a reptile that experiences marked seasonal climatic variations, the American alligator *Alligator mississippiensis*, shows seasonal acclimatisation in metabolic enzyme activities to compensate for Q_{10}-related decreases in enzyme activity during winter.

Materials and methods

Field data and sample collection

Alligators *Alligator mississippiensis* (Daudin 1801) were captured by noose in the wild at the Rockefeller Wildlife Refuge, LA, USA (29°40′ N, 92°50′ W) in July 2001 (summer; *N*=31; 14 males and 16 females + 1 juvenile of undetermined sex) and February 2002 (winter; *N*=31; 17 males and 14 females). Alligator body mass ranged from 0.92 kg to 54.54 kg in summer (females, 1.65–39.63 kg; males, 1.27–54.54 kg; and one juvenile of 0.92 kg), and from 0.85 kg to 23.17 kg in winter (females, 1.26–22.66 kg; males, 0.85–23.17 kg). Tissue samples were collected from all captured animals by punch biopsy (using a Baxter, USA biopsy punch) at the side of the tail between the 5th and 6th rows of scales posterior to the vent. Tissue samples were transferred into liquid nitrogen immediately after collection.

In addition to tissue sampling, body temperature records were obtained from seven animals in each season. Body temperature data and details of body temperature data collection and analysis are given elsewhere (F. Seebacher et al., in press) but are summarised here to provide the ecological context. Briefly, 20 animals were implanted with temperature loggers (iButton thermochron; Dallas Semiconductor, Dallas, TX, USA) in each season, and seven of the implanted animals were recaptured and had their dataloggers removed in each season. Data were collected every 10 min or 15 min for an average of 10.3±1.3 days (mean ± S.E.M.; range 8–17 days) from each recaptured animal in summer, and for 7.6±1.3 days (range 5–13 days) in winter, excluding the first three days of data obtained after the release of the animals.

Biochemical assays

We measured the activities of lactate dehydrogenase (LDH), citrate synthase (CS) and cytochrome *c* oxidase (CCO), which are active in anaerobic glycolysis, the Krebs cycle and the electron transport chain, respectively (Voet and Voet, 1995). Enzyme activity was determined with a UV/visible spectrophotometer (Beckman DU 640 or Pharmacia Ultrospec III) equipped with a temperature-controlled cuvette holder. Assays were carried out in duplicate at experimental temperatures of 15°C and 30°C for summer samples and at 15°C, 22.5°C and 30°C for winter samples. Assay temperatures were chosen for their ecological relevance indicated by body temperature measurements of animals in the field (see Results). Calculations of enzyme activity were based on the linear portions of the reaction rates, and enzyme activity

was expressed as units g^{-1} wet tissue. One unit is equivalent to 1 μmol substrate transformed min^{-1}. Saturating substrate concentrations were determined in preliminary tests and were not limiting reaction rates; i.e. doubling homogenate concentration in the assays doubled activity, but doubling substrate concentrations did not alter reaction rates.

Muscle tissue (0.05–0.1 g) was homogenised in nine volumes of extraction buffer (pH 7.5), consisting of 50 mmol l^{-1} imidazole/HCl, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} ethylene diamine tetra-acetic acid (EDTA), 1 mmol l^{-1} reduced glutathione and 1% Triton X-100, and tissue was kept on ice during homogenisation. For LDH assays, tissue homogenates were further diluted by a factor of 10 in summer samples and a factor of 50 in winter samples.

LDH activity was determined by following the absorbance of NADH at 340 nm. The assay medium was 100 mmol l^{-1} potassium phosphate (KH_2PO_4/K_2PO_4) buffer (pH 7.0), 0.16 mmol l^{-1} NADH and 0.4 mmol l^{-1} pyruvate. The millimolar extinction coefficient of NADH is 6.22. CS activity was measured as the reduction of DTNB [5,5′ dithiobis-(2-nitrobenzoic) acid] at 412 nm. The assay was conducted in 100 mmol l^{-1} Tris/HCl, pH 8.0, 0.1 mmol l^{-1} DTNB, 0.1 mmol l^{-1} acetyl CoA and 0.15 mmol l^{-1} oxaloacetate. Control assays (in which oxaloacetate was omitted) were performed to quantify any transfer of sulfhydryl groups to DTNB other than that caused by CS activity. The millimolar extinction coefficient of DTNB is 14.1. The oxidation of reduced cytochrome *c* by CCO was measured at 550 nm against a reference of 0.05 mmol l^{-1} cytochrome *c* oxidised with 50 μmol l^{-1} $K_2F(CN)_6$. The assays were performed in 100 mmol l^{-1} KH_2PO_4/K_2PO_4, pH 7.5 and 0.05 mmol l^{-1} cytochrome *c* reduced with sodium hydrosulphide ($Na_2S_2O_4$). Excess sodium hydrosulphide was removed by bubbling air through the solution. The millimolar extinction coefficient of cytochrome *c* is 19.1.

Thermal sensitivities of enzyme were expressed as Q_{10} values that were calculated as: $Q_{10}=(k_2/k_1)^{10/T2-T1}$, where k = reaction rate at temperatures 1 and 2, and T = temperature.

Statistical analysis

Enzyme activities were compared by a three-factor analysis of variance (ANOVA) with season (summer and winter), sex (male and female) and assay temperature (15°C and 30°C) as factors. Individual means were compared by Tukey's *post-hoc* tests. Linear model 1 regressions were performed to test for significant relationships between enzyme activities and body mass. Values are given as means ± S.E.M.

Results

Mean daily body temperatures of alligators in summer were 29.34±0.21°C and did not change with body mass (Fig. 1). In winter, mean daily body temperatures were 15.66±0.43°C, and mean body temperatures increased with body mass, M ($y=14.17M^{0.048}$, $r^2=0.72$; Fig. 1). Alligators in winter experienced significantly greater daily variations in body temperature compared with summer animals (one-way ANOVA on daily body temperature amplitudes using mass as a covariate: $F_{1,11}=90.84$, $P<0.0001$; Fig. 2).

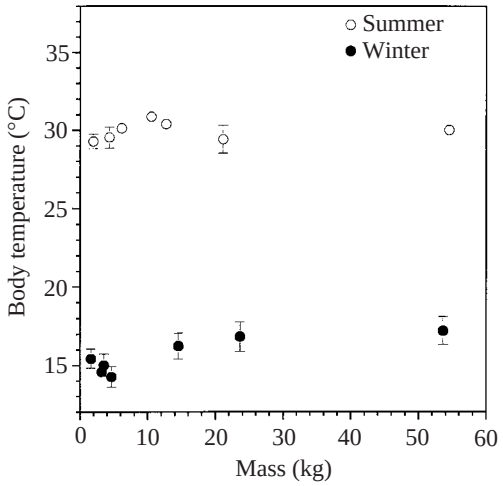

Fig. 1. Mean daily body temperatures of alligators were significantly lower in winter compared with in summer. In winter, body temperatures increased with body mass, but there were no mass-related differences between alligators in summer. Redrawn from F. Seebacher et al., in press.

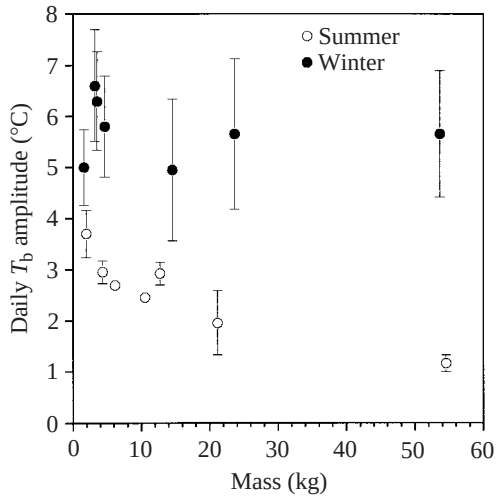

Fig. 2. Daily amplitudes of body temperatures (T_b). Alligators experienced significantly greater fluctuations in daily T_b in winter compared with in summer. Redrawn from F. Seebacher et al., in press.

As expected, all enzyme activities were significantly greater at an assay temperature of 30°C compared with 15°C (LDH, $F_{1,116}=133.72$, $P<0.0001$; CS, $F_{1,116}=97.68$, $P<0.0001$; CCO, $F_{1,116}=47.34$, $P<0.0001$; Fig. 3). Activities in winter samples were significantly greater than in summer samples for LDH ($F_{1,116}=1195.21$, $P<0.0001$) and CCO ($F_{1,116}=63.82$, $P<0.0001$) but not for CS ($F_{1,116}=0.84$, $P=0.36$). The interaction between assay temperature and season was, however, significant for CS ($F_{1,116}=6.00$, $P<0.02$), and activity

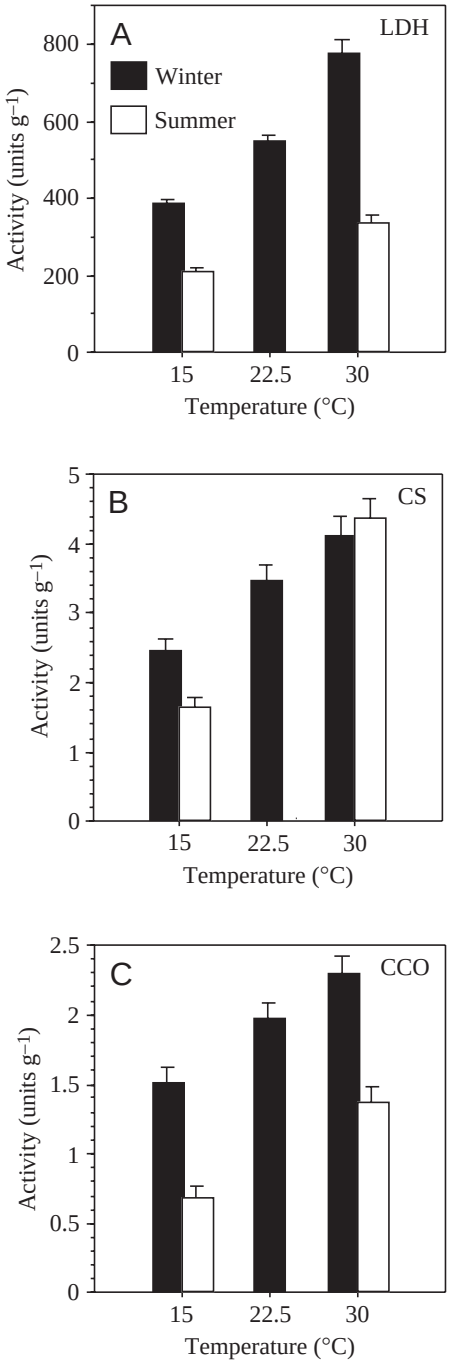

Fig. 3. Metabolic enzyme activities of alligators in winter and summer at different assay temperatures. There were significant differences between seasons and assay temperatures in all enzymes: (A) lactate dehydrogenase (LDH), (B) citrate synthase (CS) and (C) cytochrome *c* oxidase (CCO). Note that the activity of LDH and CCO does not differ between winter animals at 15°C and summer animals at 30°C and that CS activity is significantly elevated at 15°C in winter compared with in summer.

at 15°C was significantly greater in winter compared with summer but did not vary between seasons at 30°C. Interestingly, activity at 15°C in winter alligators was not significantly different from activity at 30°C in summer

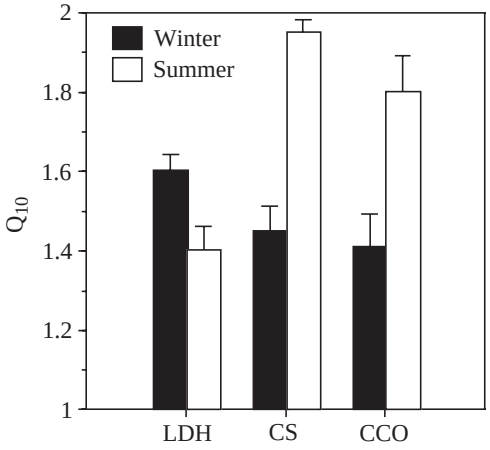

Fig. 4. Q_{10} values for each enzyme in winter and in summer. The thermal sensitivity of mitochondrial enzymes – citrate synthase (CS) and cytochrome *c* oxidase (CCO) – decreased significantly in winter, but Q_{10} values for lactate dehydrogenase (LDH) increased in winter compared with in summer.

alligators for LDH and CCO (Fig. 3), suggesting complete thermal compensation.

The thermal sensitivity of enzyme activity, expressed as Q_{10} values calculated between 15°C and 30°C, changed with season in all enzymes (Fig. 4). Q_{10} values were significantly lower in winter compared with in summer for the mitochondrial enzymes (two sample *t*-test; CS, t=7.57, P<0.0001; CCO, t=3.26, P<0.002). By contrast, Q_{10} values for LDH were greater in winter than in summer (t=–2.57, P<0.02; Fig. 4). In winter samples, Q_{10} values were similar between 15–22.5°C (LDH, 1.62±0.047; CS, 1.52±0.055; CCO, 1.41±0.12) and 22.5–30°C (LDH, 1.55±0.091; CS, 1.41±0.11; CCO, 1.36±0.092).

The activity of the mitochondrial enzymes was significantly greater in males than in females (CS, $F_{1,116}$=9.14, P<0.01; CCO, $F_{1,116}$=8.89, P<0.01; Fig. 5), but sex did not interact with either season or assay temperature (all $F_{1,116}$<2.50, all P>0.1), indicating that, although absolute activities varied, the seasonal and thermal responses were similar in males and females (Fig. 5). There was no difference between the sexes in LDH activity ($F_{1,116}$=1.56, P=0.21).

Enzyme activities did not change with body mass at any season or assay temperature (linear regression: all $F_{1,30}$<4.0, all P>0.05, all r^2<0.1; Fig. 6).

Discussion

Our data show that during winter acclimatisation, alligators are capable of increasing the activity of muscle enzymes, presumably to compensate for the depressive effect of lower body temperatures. Alligators did not undergo winter dormancy during the study, and animals were observed to feed, move in water and on land and were in very good condition, with considerable subcutaneous fat stores (observed during surgery). Instead, the study animals showed thermal compensation of the

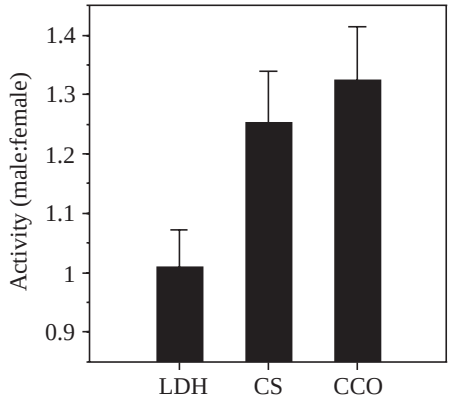

Fig. 5. Ratio of enzyme activity of males to females. The activity of mitochondrial enzymes [citrate synthase (CS) and cytochrome *c* oxidase (CCO)] was significantly greater in males compared with in females, but there was no difference between sexes in the activity of lactate dehydrogenase. There was no interaction between sex and assay temperature or season, and pooled data for each enzyme are shown.

activities of lactate dehydrogenase and cytochrome *c* oxidase, such that effective activities did not differ between winter and summer despite a difference of 15°C in body temperature (i.e. activities in winter at 15°C were not different from activities in summer at 30°C). The activity of citrate synthase was significantly elevated in winter at 15°C, although it was less than in summer samples at the summer acclimatisation temperature.

These data may have important implications for reptilian thermal physiology, because acclimatisation of enzyme activity indicates that performance in reptiles may be less dependent on the animals attaining a 'preferred' body temperature range than previously thought. The notion of 'preferred' or 'selected' body temperatures should be employed with caution, because there may not be a single species-specific optimal body temperature (e.g. Hertz et al., 1993; Christian and Weavers, 1996; Andrews et al., 1999). On the contrary, optimal body temperatures may be plastic and change with acclimatisation, reflecting a shift in the thermal dependency of physiological processes, so that, as demonstrated for alligators in this study, the *a priori* assumption that 'warm is always better' may not always be true. Thermoregulation in reptiles is often interpreted as the ability of animals to behaviourally maintain near-constant body temperatures in the face of biotic and abiotic constraints (Christian and Tracy, 1981; Angiletta, 2001; Grbac and Bauwens, 2001; Seebacher and Grigg, 2001). Our data indicate, however, that it may not be sufficient to base conclusions about thermoregulatory ability of reptiles entirely on behavioural patterns and that comparisons of field body temperatures with single 'selected' body temperatures may be temporally confounded because biochemical acclimatisation may change thermal optima.

The decrease in thermal sensitivity of citrate synthase activity in winter may explain the greater activity of this enzyme in winter compared with in summer at 15°C but not at 30°C. By contrast, cytochrome *c* oxidase activity was

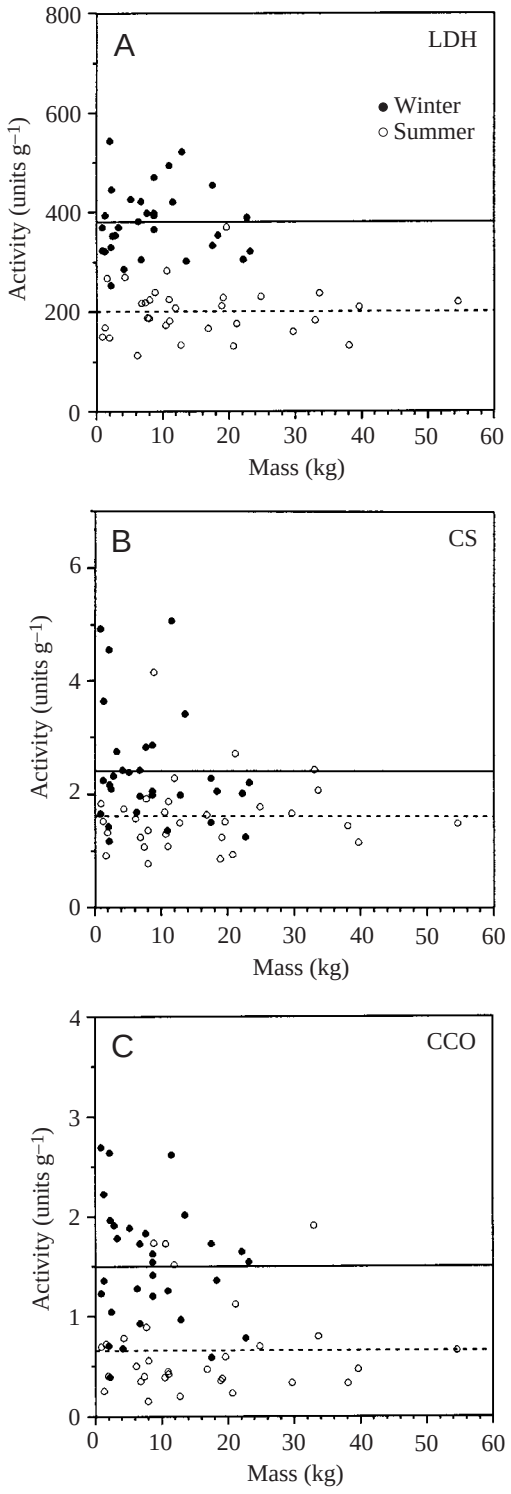

Fig. 6. Enzyme activities (means ± S.E.M.) plotted against body mass. Activities did not change with body mass for any enzyme, at any season or at any assay temperature. Examples shown here are from winter (solid circles) and summer (open circles) at 15°C. Solid lines indicate mean activities in winter; broken lines indicate mean activities in summer.

significantly elevated in winter animals at all test temperatures, as well as being less thermally sensitive in winter compared

to in summer. The mechanisms responsible for the acclimatisation response in mitochondrial enzymes appear, therefore, to differ for citrate synthase and cytochrome *c* oxidase. In so far as changes in Q_{10} values reflect protein characteristics, it is possible that modifications in citrate synthase in muscle explain the seasonal changes in activity. On the other hand, cytochrome *c* oxidase is a membrane-bound enzyme, so seasonal changes in membrane properties could explain the modifications in activity. Marked modifications of membrane lipids are known to occur during seasonal acclimatisation of ectotherms (Hazel, 1995), and such changes are likely to modify the activity and thermal sensitivity of membrane-bound enzymes (St Pierre et al., 1998; Guderley and St Pierre, 2002). An increase in enzyme concentration (Pierce and Crawford, 1997) or changes in mitochondrial density and/or characteristics (St Pierre et al., 1998; Guderley and St Pierre, 2002) could also intervene. As for cytochrome *c* oxidase, the activity of lactate dehydrogenase was significantly elevated in winter animals, but lactate dehydrogenase also had a greater Q_{10} value in winter than in summer. The latter finding is somewhat baffling because it would be expected that a decrease in thermal sensitivity would be advantageous at a time when the animals experienced significantly greater fluctuations in body temperature as well as significantly lower body temperatures.

The fact that male alligators had significantly greater aerobic enzyme activities is interesting in the context of ecological differences between male and female crocodilians. Male crocodiles travel significantly further than females during periods of dispersal (Tucker et al., 1998), and males must establish territories in preparation for courtship and breeding (Vliet, 2001) in spring (Seebacher and Grigg, 2001). Both dispersal and territoriality require sustained activity likely to be fuelled by aerobic metabolism (Elsworth et al., in press), so selection pressures may favour higher aerobic metabolic capacity in males compared with females. Hence, although the phenotypic responses of enzyme activities to seasonal climatic changes were similar in males and females (no interaction between sex and other variables), the seasonal phenotypic differences appear to be superimposed on genotypic gender-based differences.

The lack of a scaling relationship in metabolic enzyme activity does not reflect the typical mass-related decrease in oxygen consumption observed in crocodilians (Grigg, 1978; Wright, 1986; Emshwiller and Gleeson, 1997). It may be that scaling of oxygen consumption is caused by oxygen transport constraints rather than by mass-specific changes in oxygen demand (Goolish, 1991; Bejan, 1997). The lack of constant scaling of metabolic enzyme activity is not uncommon among ectotherms (Baldwin et al., 1995; Norton et al., 2000), as scaling of enzyme activity may be a function of several biotic factors such as developmental stage (Garenc et al., 1999) and size-specific demands for locomotory performance (Somero and Childress, 1980). Hence, more detailed experimental studies are needed to determine the nature of the scaling relationship, or lack thereof, of metabolic enzyme activity in alligators, particularly considering the relatively narrow body mass range of our study animals in winter. Moreover, it would be useful to assay more metabolically active organs, such as heart and liver, in addition to muscle.

Many aquatic ectotherms change biochemical capacities with seasonal acclimatisation or thermal acclimation (e.g. see Guderley and St Pierre, 2002), and this ability may be the result of their inability to compensate behaviourally for environmental variation in homogeneous marine environments. Body temperatures of aquatic and semi-aquatic ectotherms are often closely tied to water temperature fluctuations, particularly to long-term, seasonal fluctuations (Seebacher and Grigg, 1997), as a result of the high rates of convective heat exchange in water. Hence, aquatic or semi-aquatic habits may provide the context within which acclimatisation is advantageous. The notion that acclimatisation is restricted to aquatic species (Wilson and Franklin, 2000), however, may be a little simplistic because the proximate cause for biochemical/physiological acclimatisation is body temperature, but body temperature is determined by a complex suite of parameters such as behaviour, heat transfer characteristics and body mass, as well as environmental conditions. It is conceivable, therefore, that terrestrial species experience similar seasonal fluctuations in body temperature and could gain similar advantages from biochemical acclimatisation as aquatic animals despite the fact that they are able to thermoregulate on a daily basis. Acclimatisation may be less pronounced, however, in animals that experience large daily fluctuation in body temperature, because selection would decrease the thermal sensitivity of biochemical traits (Wilson and Franklin, 2000). In addition, metabolic acclimatisation may be energetically expensive, for example with respect to ATP used during increased rates of transcription, so that the benefits of maintaining biochemical/physiological performance may by outweighed by the increased energetic costs, and dormancy becomes the more advantageous response, particularly in extreme climates (e.g. St Pierre and Boutilier, 2001).

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References

Andrews, R. M., Méndez-de la Cruz, F. R., Villagrán-Santa Cruz, M. and Rodriguez-Romero, F. (1999). Field and selected body temperatures of the lizards *Sceloporus aeneus* and *Sceloporus bicanthalis*. *J. Herpetol.* **33**, 93-100.

Angilletta, M. J. (2001). Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* **82**, 3044-3056.

Avery, R. A. (1982). Field studies of body temperatures and thermoregulation. In *Biology of the Reptilia*, vol. 12 (ed. C. Gans and F. H. Pough), pp. 93-166. New York: Academic Press.

Baldwin, J., Seymour, R. S. and Webb, G. J. W. (1995). Scaling of metabolic metabolism during exercise in the estuarine crocodile (*Crocodylus porosus*). *Comp. Biochem. Physiol. A* **112**, 285-293.

Bartholomew, G. A. (1982). Physiological control of body temperature. In *Biology of the Reptilia*, vol. 12 (ed. C. Gans and F. H. Pough), pp. 167-211. New York: Academic Press.

Bartholomew, G. A. and Tucker, V. A. (1963). Control of changes in body temperature, metabolism, and circulation by the agamid lizard, *Amphibolurus barbatus*. *Physiol. Zool.* **37**, 199-218.

Bejan, A. (1997). Constructal tree network for fluid flow between a finite-size volume and one source or sink. *Rev. Gén. Therm.* **36**, 592-604.

Case, T. J. (1976). Seasonal aspects of thermoregulatory behavior in the Chuckawalla, *Sauromalus obesus* (Reptilia, Lacertilia, Iguanidae). *J. Herpetol.* **10**, 85-95.

Christian, K. A. and Tracy, C. R. (1981). The effects of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia* **49**, 218-223.

Christian, K. A., Tracy, C. R. and Porter, W. P. (1983). Seasonal shifts in body temperature and use of microhabitats by Galapagos land iguanas (*Conolophus pallidus*). *Ecology* **64**, 463-468.

Christian, K. A. and Weavers, B. W. (1996). Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecol. Monogr.* **66**, 139-157.

Costanzo, J. P., Litzgus, J. D., Iverson, J. B. and Lee, R. E., Jr (2000). Seasonal changes in physiology and development of cold hardiness in the hatchling painted turtle *Chrysemys picta*. *J. Exp. Biol.* **203**, 3459-3470.

Cowles, R. B. and Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* **83**, 261-296.

Crawford, D. L., Pierce, V. A. and Segal, J. A. (1999). Evolutionary physiology of closely related taxa: analyses of enzyme expression. *Am. Zool.* **39**, 389-400.

Crawford, D. L. and Powers, D. A. (1989). Molecular basis of evolutionary adaptation at the lactate dehydrogenase-B locus in the fish *Fundulus heteroclitus*. *Proc. Natl. Acad. Sci. USA* **86**, 9365-9369.

Crawford, D. L. and Powers, D. A. (1992). Evolutionary adaptation to different thermal environments via transcriptional regulation. *Mol. Biol. Evol.* **9**, 806-813.

Elsworth, P. G., Seebacher, F. and Franklin, C. E. (in press). Sustained swimming performance in crocodiles (*Crocodylus porosus*): effects of body size and temperature. *J. Herpetol.*

Emshwiller, M. G. and Gleeson, T. (1997). Temperature effects on aerobic metabolism and terrestrial locomotion in American alligators. *J. Herpetol.* **31**, 142-147.

Fields, P. A. and Somero, G. N. (1997). Amino acid sequence differences cannot fully explain interspecific variation in thermal sensitivities of gobiid fish A_4-lactate dehydrogenases (A_4-LDHS). *J. Exp. Biol.* **200**, 1839-1850.

Garenc, C., Couture, P., Laflamme, M.-A. and Guderley, H. (1999). Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*). *J. Comp. Physiol. B* **169**, 113-122.

Goolish, E. M. (1991). Aerobic and anaerobic scaling in fish. *Biol. Rev.* **66**, 33-56.

Grant, B. W. (1990). Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology* **71**, 2323-2333.

Grant, B. W. and Dunham, A. E. (1988). Thermally imposed time constraints on the activity of the desert lizards, *Sceloporus merriami*. *Ecology* **69**, 167-176.

Grbac, I. and Bauwens, D. (2001). Constraints on temperature regulation in two sympatric Podarcis lizards during autumn. *Copeia* **2001**, 178-186.

Grigg, G. C. (1978). Metabolic rate, Q_{10} and respiratory quotient (RQ) in *Crocodylus porosus*, and some generalizations about low RQ in reptiles. *Physiol. Zool.* **51**, 354-360.

Grigg, G. C., Seebacher, F., Beard, L. A. and Morris, D. (1998). Thermal relations of very large crocodiles, Crocodylus porosus, free-ranging in a naturalistic situation. *Proc. R. Soc. Lond. B* **265**, 1793-1799.

Guderley, H. (1990). Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am. J. Physiol.* **259**, R245-R252.

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.

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**, 19-42.

Hertz, P. E., Huey, R. B. and Stevenson, R. D. (1993). Evaluating temperature regulation by field active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**, 796-818.

Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia*, vol. 12 (ed. C. Gans and F. H. Pough), pp. 25-91. New York: Academic Press.

Huey, R. B. and Slatkin, M. (1976). Cost and benefit of lizard thermoregulation. *Q. Rev. Biol.* **51**, 363-384.

Johnston, I. A. and Temple, G. K. (2002). Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *J. Exp. Biol.* **205**, 2305-2322.

Lin, J.-J. and Somero, G. N. (1995). Thermal adaptation of cytoplasmic malate dehydrogenases of eastern pacific barracuda (*Sphyraena* spp): the role of differential isoenzyme expression. *J. Exp. Biol.* **198**, 551-560.

Lovegrove, B. G., Heldmaier, G. and Ruf, T. (1991). Perspectives of endothermy revisited: the endothermic temperature range. *J. Therm. Biol.* **15**, 185-197.

Martinez, M., Couture, P. and Guderley, H. (1999). Temporal changes in tissue metabolic capacities of wild Atlantic cod *Gadus morhua* (L.), from Newfoundland. *Fish Physiol. Biochem.* **20**, 181-191.

Muth, A. (1977). Body temperatures and associated postures of the zebra-tailed lizard (*Callisaurus draconoides*). *Copeia* **1977**, 122-125.

Norton, S. F., Eppley Z. and Sidell, B. D. (2000). Allometric scaling of maximal enzyme activities in the axial musculature of striped bass, *Morone saxatillis* (Walbaum). *Physiol. Biochem. Zool.* **73**, 819-828.

Olson, J. M. (1987). The effects of seasonal acclimatization on metabolic-enzyme activities in the heart and pectoral muscle of painted turtles *Chrysemys picta marginata*. *Physiol. Zool.* **60**, 149-158.

Olson, J. M. and Crawford, K. M. (1989). The effect of seasonal acclimatization on the buffering capacity and lactate dehydrogenase activity in tissues of the freshwater turtle *Chrysemys picta marginata*. *J. Exp. Biol.* **145**, 471-476.

Pierce, V. A. and Crawford, D. L. (1997). Phylogenetic analysis of glocolytic enzyme expression. *Science* **275**, 256-259.

Robertson, S. L. and Smith, E. N. (1979). Thermal indications of cutaneous blood flow in the American alligator. *Comp. Biochem. Physiol. A* **62**, 569-572.

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Ann. Rev. Ecol. Syst.* **24**, 35-68.

Seebacher, F. (1999). Behavioural postures and the rate of body temperature change in wild freshwater crocodiles, *Crocodylus johnstoni*. *Physiol. Biochem. Zool.* **72**, 57-63.

Seebacher, F., Elsey, R. M. and Trosclair, P. L., III (in press). Seasonal changes and regulation of body temperature in the American alligator (*Alligator mississippiensis*). *Physiol. Biochem. Zool.* **76**.

Seebacher, F. and Franklin, C. E. (2001). Control of heart rate during thermoregulation in the heliothermic lizard *Pogona barbata*: importance of cholinergic and adrenergic mechanisms. *J. Exp. Biol.* **204**, 4361-4366.

Seebacher, F. and Grigg, G. C. (1997). Patterns of body temperature in wild freshwater crocodiles, *Crocodylus johnstoni*: thermoregulation versus thermoconformity, seasonal acclimatisation, and the effect of social interactions. *Copeia* **1997**, 549-557.

Seebacher, F. and Grigg, G. C. (2001). Social interactions compromise thermoregulation in crocodiles *Crocodylus johnstoni* and *Crocodylus porosus*. In *Crocodilian Biology and Evolution* (ed. G. C. Grigg, F. Seebacher and C. E. Franklin), pp. 310-316. Chipping Norton: Surrey Beatty and Sons.

Seebacher, F., Grigg, G. C. and Beard, L. A. (1999). Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *J. Exp. Biol.* **202**, 77-86.

Segal, J. A. and Crawford, D. L. (1994). LDH-B enzyme expression: the mechanisms of altered gene expression in acclimation and evolutionary adaptation. *Am. J. Physiol.* **36**, R1150-R1153.

Somero, G. N. and Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in large-size fish. *Physiol. Zool.* **53**, 322-337.

Somero, G. N., Fields, P. A., Hofman, G. E., Weinstein, R. B. and Kawall, H. (1998). Cold adaptation and stenothermy in Antarctic notothenioid fishes: what has been gained and what has been lost? In *Fishes of Antarctica. A Biological Overview* (ed. G. di Prisco, E. Pisano and A. Clarke), pp. 97-109. Milano: Springer Verlag Italia.

St Pierre, J. and Boutilier, R. G. (2001). Aerobic capacity of frog skeletal muscle during hibernation. *Physiol. Biochem. Zool.* **74**, 390-397.

St Pierre, J., Charest, P.-M. 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.

Tucker, A. D., McCallum, H. I., Limpus, C. J. and McDonald, K. R. (1998). Sex-biased dispersal in a long-lived polygynous reptile (*Crocodylus johnstoni*). *Behav. Ecol. Sociobiol.* **44**, 85-90.

Van Damme, R., Bauwens, D. and Verheyen R. F. (1987). Thermoregulatory resposes to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica* **43**, 405-415.

Vliet, K. A. (2001). Courtship behaviour of American alligators *Alligator mississippiensis*. In *Crocodilian Biology and Evolution* (ed. G. C. Grigg, F. Seebacher and C. E. Franklin), pp. 383-408. Chipping Norton: Surrey Beatty and Sons.

Voet, D. and Voet, J. G. (1995). *Biochemistry*. New York: Wiley & Sons.

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

Wilson, R. S. and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**, 66-70.

Wright, J. C. (1986). Effects of body temperature, mass, and activity on aerobic and anaerobic metabolism in juvenile *Crocodylus porosus*. *Physiol. Zool.* **59**, 505-513.