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Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm

E. J. Glanville and F. Seebacher*
School of Biological Sciences A08, University of Sydney, NSW 2006, Australia
*Author for correspondence (e-mail: fseebach@bio.usyd.edu.au)

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Summary

Thermoregulating animals are thought to have evolved a preferred body temperature at which thermally sensitive performance is optimised. Even during thermoregulation, however, many animals experience pronounced variability in body temperature, and may regulate to different body temperatures depending on environmental conditions. Here we test the hypothesis that there is a trade-off between regulating to lower body temperatures in cooler conditions and locomotory and metabolic performance. Animals (estuarine crocodiles, *Crocodylus porosus*) acclimated to cold (N=8) conditions had significantly lower maximum and mean daily body temperatures after 33 days than warm-acclimated animals (N=9), despite performing characteristic thermoregulatory behaviours. Concomitant with behavioural changes, maximum sustained swimming speed (U_{crit}) shifted to the respective mean body temperatures during acclimation (cold=20°C, warm=29°C), but there was no difference in the maxima between acclimation groups. Mitochondrial oxygen consumption changed significantly during acclimation, and maximum respiratory control ratios coincided with mean body temperatures in liver, muscle and heart tissues. There were significant changes in the activities of regulatory metabolic enzymes (lactate dehydrogenase, citrate synthase, cytochrome *c* oxidase) and these were tissue specific. The extraordinary shift in behaviour and locomotory and metabolic performance shows that within individuals, behaviour and physiology covary to maximise performance in different environments.

Key words: phenotypic plasticity, thermal reaction norm, mitochondria, metabolism, reptile, acclimation.

Introduction

The thermal environment changes perpetually on a geological timescale, seasonally and daily. Hence, all organisms have evolved with thermal variability as a selection pressure, and environments that provide a constant thermal signal are rare (Osborn and Briffa, 2006). Evolutionary responses to thermally variable environments range from regulation of the internal environment to a constant or near-constant level to letting body temperature fluctuate proportionally to environmental fluctuations while compensating cellular functions for the thermodynamic effect on reaction rates (Guderley, 2004; Lovegrove, 2005).

Animals regulate their body temperature to a particular 'set-point' temperature that coincides with the ideal or optimal temperature for organism function (Cabanac, 2006). Body temperature set-points are controlled by temperature-sensitive neurons that link the animal core and periphery *via* the hypothalamus (Hammel, 1965; Boulant, 2006; Wechselberger et al., 2006), and thermoregulation may be influenced by endocrine factors that act on a daily or seasonal basis (Lutterschmidt et al., 2003). The behavioural mechanisms of thermoregulation are similar among terrestrial ectotherms (Muth, 1977; Seebacher, 1999; Samietz et al., 2005), although actual body temperature is determined by the thermal sensitivities of the biochemical components, particularly enzyme activities, of the organisms (Somero, 1995). The regulated or preferred body temperature of terrestrial ectotherms is thought to have co-evolved with performance optima in response to the dominant thermal signal in the environment (Huey and Bennett, 1987; Samietz et al., 2005). The current view is that temperature–performance curves are non-reversibly centred around the mean (preferred) body temperature of individuals (Angilletta et al., 2002). By contrast, many aquatic animals that have limited opportunity for thermoregulation possess a high degree of reversible phenotypic plasticity [i.e. phenotypic flexibility = plasticity within individuals in contrast to developmentally fixed plasticity within genotypes (Piersma and Drent, 2003)]. In particular, cellular responses to thermal change occur in metabolic pathways that supply ATP for essential functions such as maintenance of membrane potentials, protein synthesis and gluconeogenesis (Hulbert and Else, 2000), as well as for energetically expensive locomotor performance, reproduction and growth, which maximise fitness (Johnston and Temple, 2002; Guderley, 2004).

Most of the biochemical pathways of protein synthesis and metabolic ATP production are highly conserved among organisms (Smith and Morowitz, 2004). If the capacity for flexibility were an inherent quality of metabolic and other biochemical pathways, it could be expected that phenotypic flexibility is also seen in animals that regulate their body temperature (Seebacher, 2005). Here, we test the hypothesis that an ectotherm (*Crocodylus porosus*, Schneider) thermoregulates to a higher or lower body temperature when experiencing 'warm' or 'cool' environments, respectively (Seebacher and Grigg, 1997), and concomitantly shifts the thermal sensitivity of locomotor and metabolic functions.

Reptiles, including crocodilians, regulate their body temperature behaviourally (Seebacher and Grigg, 1997). The efficacy of behavioural thermoregulation depends on the operative temperature experienced by the animal: the lesser the difference between operative temperature and the 'preferred' body temperature to which animals regulate, the greater the behavioural cost of thermoregulation (Huey, 1974; Huey and Slatkin, 1976). Animals could therefore decrease the behavioural cost of thermoregulation by regulating to a lower temperature in cold conditions. There may be a trade-off between minimising the time spent on thermoregulation (behavioural cost) and the thermodynamically decreased performance in locomotion and cellular rate functions at the lower body temperature. Alternatively, fitness could be maximised if performance was compensated at lower body temperatures.

Materials and methods

Animal maintenance

Juvenile estuarine crocodiles (*Crocodylus porosus*, Schneider; N=18) were obtained from a crocodile farm (Wildlife International, Darwin, NT, Australia). Mean body mass of crocodiles before experimentation was 172.9±6.7 g and mean snout–tail length was 422.0±3.0 mm (mean ± s.e.m.). Crocodiles were housed in plastic tanks (830×620×1250 mm; three animals per tank), which were designed so that animals could thermoregulate behaviourally. Shelter and water (up to 200 mm depth) were provided at one end, and a dry basking space at the other end. A full-spectrum light source (Reptiglo; Exo Terra, Montreal, Canada) was suspended above each tank, and a constant 12 h:12 h L:D light cycle was maintained for all treatments. In addition, an infrared heat lamp delivered 400 W m^{-2} to the dry basking area in the tank, which is comparable to solar radiation received while basking in spring (Seebacher, 1999). For four weeks before acclimation treatments, all animals were maintained at an air temperature of 26.7±0.03°C and a water temperature of 26.0±0.03°C, and radiant heat was available for 7.5 h per day. Throughout the experimental period, crocodiles were fed *ad libitum* on live crayfish (farmed *Cherax destructor*) and insects, which closely resembles their natural diet (Webb et al., 1991).

Acclimation treatments

Thermal conditions for two acclimation treatments were chosen to resemble winter and summer in *C. porosus*' natural habitat in North Queensland, Australia (Seebacher and Grigg, 1997). Treatments were performed in controlled environment rooms (one tank per room): in the cold treatment (winter, N=8 animals, note that one animal did not feed and lost condition and was therefore excluded from the treatment) mean air temperature was 20.2±0.03°C, resulting in slightly lower water temperatures (19.5±0.03°C), and basking opportunity was provided for 6 h day^{-1}. In the warm treatment (summer, N=9) air temperature was 29.5±0.01°C, water temperature was 29.2±0.04°C and basking opportunity was provided for 9 h day^{-1}. Crocodiles were acclimated for 33 days (Bouchard and Guderley, 2003).

All animals in the cold and warm treatments grew slightly during the period of acclimation (warm, start=166.4±11.0 g, end=171.8±12.6 g; cold, start=179.3±7.6 g, end=182.5±7.1 g) and there were no significant differences in body mass between the treatments either before (t_{15}=–1.34, P=0.20) or after (t_{15}=–1.25, P=0.23) the experimental period.

Body temperature

Data loggers (iButton thermochron; Dallas Semiconductor, Dallas, TX, USA; accurate to ±0.5°C) were surgically implanted into the peritoneal cavity of five crocodiles per treatment. Surgery was performed in sterile conditions under a local anaesthetic [Lignocaine (Seebacher and Grigg, 1997)], and data loggers weighed less than 3% of the smallest crocodile's body mass (3.1 g). Temperature was recorded every 35 min to enable recording throughout the acclimation period, and acclimation treatments did not begin until 10 days after the operation. At the conclusion of all experiments, animals were euthanized (120 mg kg^{-1} sodium pentabarbitone injected into the occipital sinus) and the data loggers were recovered.

Locomotor performance

Sustained swimming performance [critical sustained swimming speed, U_{crit} (Brett, 1965)] was measured in a custom-made oval-shaped flume (2.0×1.3×0.7 m). Animals were placed individually into a confined space within the flume where they could be observed without disturbance through a one-way viewing panel. Water flow was generated by an electric outboard motor (Riptide RT40; Minn Kota, Bob Littler Agencies, Hemmant, QLD, Australia) regulated with a DC power supply (MP3090; Powertech, Osborne Park, WA, Australia) that was operated several metres distant from the flume. The settings on the power supply were calibrated for water flow with a flow probe (FP101; Global Enviroequip Systems, Brisbane, QLD, Australia), and turbulence in the experimental area was minimised by two sets of baffles (Elsworth et al., 2003).

The flume was situated in a temperature-controlled experimental room (minimum of 15°C), and the water was heated with two immersion heaters (Thermomix; Julabo, Seelbach, Germany). Water temperature was monitored throughout the experiments with a calibrated digital thermometer (accurate to 0.05°C; QM-1600; Digitech, Hounslow, UK).

Crocodiles were trained to swim in the flume on three occasions before the start of the experiment (Elsworth et al., 2003), and after training all animals swam continuously during the trials. Critical sustained swimming speed was determined as $U_{crit}=U_f+[(t_f/t_i)\times U_i]$, where U_f is the greatest swimming speed maintained for a whole time interval, t_f is time spent at the final speed, t_i is the time interval between speed increments and U_i is the speed increment (Brett, 1965). Pilot studies on two crocodiles not used in the acclimation experiment were performed to determine t_i (150 s), U_i (0.04 m s^{-1}) and the initial flow (0.16 m s^{-1}) (Brett, 1965; Elsworth et al., 2003), and crocodiles were allowed 15 min to equilibrate to water temperature before swimming trials (Seebacher, 1999). Animals were swum until fatigued, which was defined as the time when crocodiles could no longer hold position in the water column (Brett, 1965). Each crocodile was swum at 17°C, 20°C, 25°C, 30°C and 32.5°C in random order, with at least 40 h between swimming trials (Elsworth et al., 2003). U_{crit} is reported as body length (*BL*) s^{-1}.

Mitochondrial oxygen consumption

Oxygen consumption of isolated mitochondria was measured in tail muscle (longissimus dorsi), liver and heart (ventricle) tissue. After euthanasia, tissue (0.1 g) was immediately transferred onto ice, where it was finely diced, weighed and homogenised in four volumes of ice-cold isolation buffer [140 mmol l^{-1} KCl, 10 mmol l^{-1} EDTA, 5 mmol l^{-1} $MgCl_2$, 20 mmol l^{-1} Hepes, 0.5% bovine serum albumin (BSA), pH 7.3 at 20°C] by five gentle passes in a glass homogeniser. The homogenate was centrifuged at 1400 ***g*** for 5 min at 4°C, and the supernatant was removed and placed in fresh Eppendorf tubes. Mitochondria were separated by centrifuging the supernatant for 7 min at 9000 ***g***, and the supernatant was discarded. The mitochondrial pellet was resuspended in 150 μl of isolation medium, which was further diluted in 2250 μl of ice-cold assay medium (140 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes, 5 mmol l^{-1} Na_2HPO_4 and 0.5% BSA, pH 7.3 at 20°C).

Oxygen consumption of the resulting mitochondrial solution (250 μl per assay) was measured in a temperature-controlled respiration chamber (Mitocell; Strathkelvin Instruments, Glasgow, UK). The oxygen concentration of the solution was measured with a calibrated oxygen electrode connected to an oxygen meter (model 782; Strathkelvin Instruments). After the oxygen concentration of the solution had stabilised, 5 μl each of 10 mmol l^{-1} malate and 5 mmol l^{-1} pyruvate were added to obtain the State 2 rate of oxygen consumption (Blier and Guderley, 1993; Johnston et al., 1994). The State 3 rate of oxygen consumption was obtained from the rate of decrease in oxygen concentration after the addition of 10 μl of 50 mmol l^{-1} ADP neutralised with KOH. The uncoupled, State 4 oxygen consumption rate was measured after all ADP was consumed (Johnston et al., 1994). Oxygen consumption assays were performed in duplicate for each sample at 20°C, 25°C and 30°C. The respiratory control ratio (RCR) was calculated as the ratio between State 3 and State 4 rates to express the coupling of electron transport to ATP production.

Samples of isolated mitochondria were saved to determine mitochondrial protein concentration. Duplicate samples (50 μl) were washed in 1500 μl of BSA-free assay medium by centrifuging at 12 000 ***g*** for 10 min. The pellet was resuspended and centrifuged three times to remove BSA.

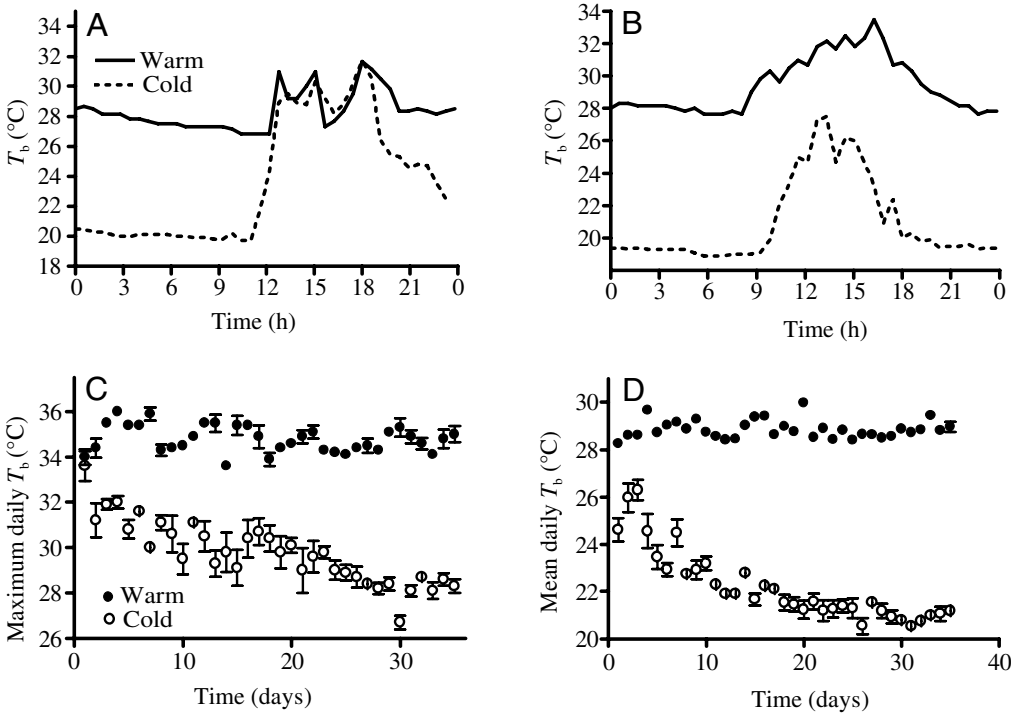

Fig. 1. Changes in thermoregulatory behaviour of *Crocodylus porosus* during acclimation. At the start of acclimation, all animals heated to the same maximum body temperature (T_b) during the day (A; means are shown, and all means ± s.e.m. <0.3°C). At the end of the acclimation period, crocodiles still displayed their characteristic thermoregulatory pattern, but maximum body temperatures of cold-acclimated animals (broken line) were significantly lower (B). During cold acclimation, maximum body temperatures (C; means ± s.e.m.) and mean daily body temperatures (D; means ± s.e.m.) decreased significantly.

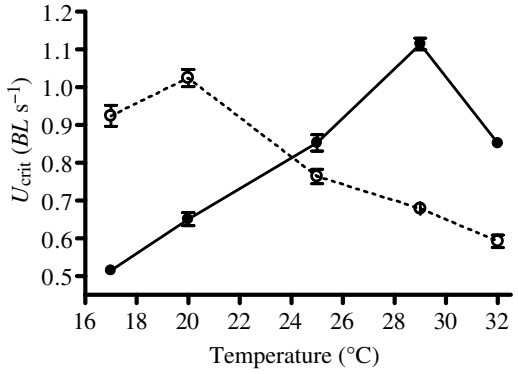

Fig. 2. Maximal sustained swimming performance shifts significantly with acclimation and coincides with mean body temperatures within each treatment (broken line, cold acclimation; solid line, warm acclimation). *BL*, body length.

Protein concentration of the washed mitochondrial solution was determined by the bicinchoninic acid protein assay (Sigma Aldrich, Sydney, NSW, Australia) according to the manufacturer's instructions.

Enzyme activity

Tissues (tail muscle, liver and heart) for lactate dehydrogenase (LDH), citrate synthase (CS) and cytochrome *c* oxidase (CCO) assays were collected at the same time as tissue used for mitochondrial oxygen consumption assays, but were transferred into liquid nitrogen immediately after collection and stored at –80°C for later analysis. Tissue samples (0.05–0.1 g) were homogenised in a glass homogeniser in 9 vols of ice-cold extraction buffer (50 mmol l^{-1} imidazole, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EDTA, 0.1% Triton and 1 mmol l^{-1} glutathione, pH 7.5 at 20°C). Enzyme activity was determined according to published protocols (Seebacher et al.,

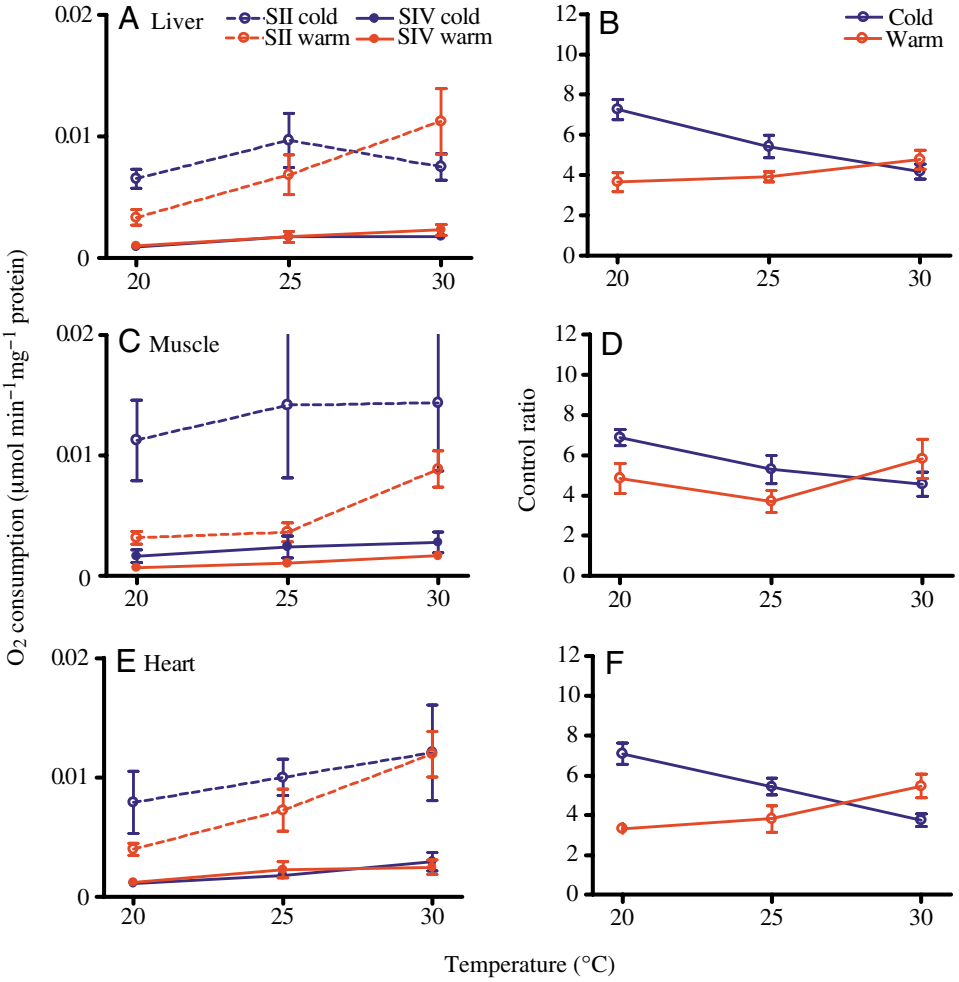

Fig. 3. Mitochondrial oxygen consumption rates (left panels) and respiratory control ratios (RCRs) (right panels) for liver (A,B), tail muscle (C,D) and heart (E,F) at different assay temperatures. State 3 (SIII) respiratory rates are shown as broken lines and state 4 (SIV) rates as solid lines; cold-acclimation treatments are represented by blue and warm-acclimation treatments by red. There are significant differences between acclimation treatments in SIII rates and RCRs of liver (A,B) and muscle (C,D), and RCRs of heart mitochondria differ significantly between acclimation treatments (F).

2003) in a spectrophotometer with a temperature-controlled cuvette holder (Ultrospec 2100 pro UV; Amersham Pharmacia, Sydney, NSW, Australia). Enzyme activity was expressed as units g^{-1} wet tissue mass and all assays were performed in duplicate at 20°C, 25°C and 30°C.

Statistical analysis

U_{crit}, mitochondrial oxygen consumption (State 3 and State 4 rates and RCRs) and enzyme activities were analysed separately as dependent variables in repeated-measures analyses of variance (RM-ANOVA), with 'temperature' as the repeated measure and 'acclimation treatment' and 'constant temperature room' as factors. Constant temperature room was not significant in any of the analyses except for State 3 oxygen consumption of liver and muscle mitochondria (both $F_{2,11}>5.6$, $P<0.025$). Repeated-measures ANOVA data were tested for the assumption of sphericity, measured by Mauchly's test. When the assumption of sphericity was violated, multivariate output was used because univariate outputs are only correct when the assumption of a spherical common covariance matrix is met and multivariate statistics are not affected by the sphericity assumption (Quinn and Keough, 2004). Hence, multivariate output was used for analysis of results from muscle and liver CCO activity, heart CS and LDH activity and U_{crit}. All data were also tested for normality of distribution and homogeneity of variance. Levene's test was used to test equality of variances. Thermal sensitivities were expressed as $Q_{10}=(k_2/k_1)^{10/T2-T1}$, where k_1 and k_2 are the reaction rates at temperatures T_1 and T_2, respectively. Values of Q_{10} were compared between treatments by one-way ANOVA.

One-way ANOVA was also used to determine significant differences in the mean daily body temperature (T_b) between individuals in the cold and warm treatments.

Results

Body temperature

Crocodiles in both treatments displayed characteristic body temperature peaks and troughs during the day, indicating shuttling between radiant heating and the water (Fig. 1A,B). During the first 72 h of acclimation, crocodiles in cold treatments heated to the same maximum temperatures as the warm animals ($F_{1,9}=1.965$, $P=0.199$), but cooled to the much lower water temperature at night (Fig. 1A). During the final 72 h of acclimation, maximum body temperatures chosen by

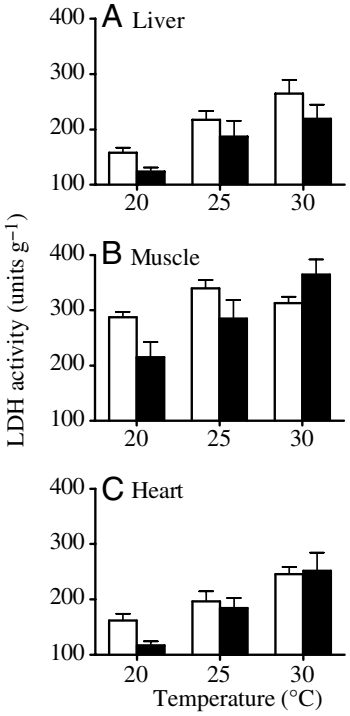

Fig. 4. Activities (mean ± s.e.m.) of lactate dehydrogenase (LDH) (units g^{-1} wet tissue) in liver (A), tail muscle (B) and heart (C). Open boxes indicate cold-acclimation treatment and solid boxes indicate warm-acclimation treatment. LDH activity is significantly greater at low temperatures in cold-acclimated animals in muscle and heart, but not in liver.

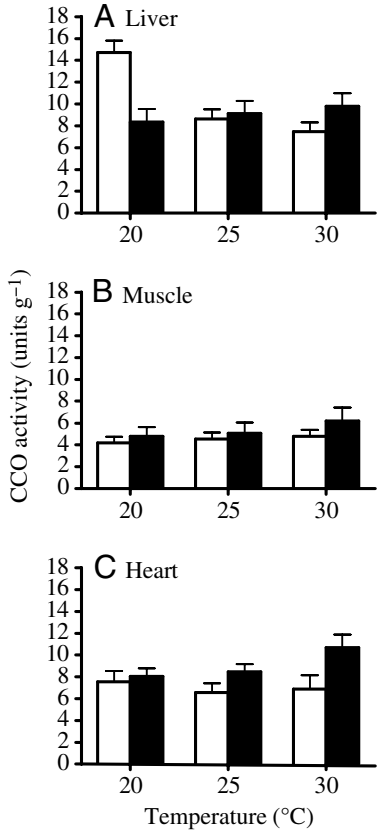

Fig. 5. Activities (mean ± s.e.m.) of cytochrome *c* oxidase (CCO) (units g^{-1} wet tissue) in liver (A), tail muscle (B) and heart (C). Open boxes indicate cold-acclimation treatment, and solid boxes indicate warm-acclimation treatment. There are significant interactions between acclimation treatment and test temperature in liver and heart, but not in muscle.

cold-acclimated animals were significantly lower than those of warm-acclimated animals ($F_{1,9}$=267.6, P<0.0005; Fig. 1B). Maximum daily body temperatures and mean daily body temperatures decreased steadily over the acclimation period in cold-acclimated animals (from 32.2±0.6°C to 28.3±0.3°C, and from 25.2±1.2°C to 20.9±0.7°C, respectively; Fig. 1C,D).

Swimming performance

Optimal swimming performances shifted with cold acclimation so that cold-acclimated crocodiles at 20°C performed as well as warm-acclimated animals at 30°C (cold at 20°C: 1.02±0.02 *BL* s^{-1}; warm at 30°C: 1.11±0.02 *BL* s^{-1}; Fig. 2). There was a significant effect of test temperature on U_{crit} ($F_{4,8}$=79.77, P<0.001), and there was a significant interaction between test temperature and acclimation treatment ($F_{4,8}$=134.215, P<0.001).

Mitochondrial oxygen consumption

In liver, State 3 and State 4 rates increased significantly with increasing temperature (both $F_{2,22}$>7.1, P<0.01; Fig. 3A), but acclimation treatments did not have a significant effect on either (both $F_{1,11}$<1.1, P>0.3). However, the interaction between acclimation treatment and temperature was significant for State 3 rates of oxygen consumption in liver mitochondria ($F_{2,22}$>4.62, P<0.03). RCRs in liver differed significantly between acclimation treatments (main effect: $F_{1,11}$=19.36, P<0.001; acclimation × temperature: $F_{2,22}$=15.16, P<0.0001), and also varied significantly with temperature ($F_{2,22}$=4.22, P<0.03; Fig. 3B).

In tail muscle, acclimation treatment had a significant effect on State 3 rates of mitochondrial oxygen consumption ($F_{1,11}$=6.16, P<0.03), but there were no other significant effects of temperature or acclimation treatment on either State 3 or State 4 rates (P>0.05; Fig. 3C). Nonetheless, RCRs of tail-muscle mitochondria varied significantly with temperature, and there was a significant interaction between temperature and acclimation treatment (both $F_{2,22}$>4.2, P<0.03; Fig. 3D).

State 3 and State 4 rates of oxygen consumption of heart mitochondria increased significantly with temperature (both $F_{2,22}$>6.5, P<0.01; Fig. 3E), but acclimation treatment did not have a significant effect, nor were there significant interactions between acclimation and temperature (all P>0.25). However, acclimation treatment significantly affected RCRs of heart mitochondria ($F_{1,11}$=5.94, P<0.03), and there was a significant interaction between temperature and acclimation treatment ($F_{2,22}$=22.64, P<0.0001).

Metabolic enzyme activity

In all tissues and treatments, LDH activity increased significantly with temperature (all $F_{2,22}$>20.55, P<0.001; Fig. 4A–C), but there was no significant effect of acclimation on LDH activity in any tissue (all $F_{1,11}$<3.6, P>0.08). However, LDH activity of cold-acclimated animals was greater at 20°C compared with warm-acclimated crocodiles, causing a significant interaction between temperature and acclimation treatment in muscle and heart (both $F_{2,22}$>8.90, P<0.001; Fig. 4B,C), but not in liver ($F_{2,22}$=0.077, P=0.93; Fig. 4A).

Temperature had a significant effect on the activity of CCO in all tissues and treatments (all $F_{2,22}$>4.12, P<0.03; Fig. 5A–C), but there was no effect of acclimation treatment in any tissue (all $F_{1,11}$<2.2, P>0.1; Fig. 5A–C). The interaction between acclimation treatment and test temperatures, however, was significant in liver and heart (all $F_{2,22}$>4.0, P<0.03).

Citrate synthase activity changed significantly with temperature in all tissues (all $F_{2,22}$>9.3, P<0.005; Fig. 6A–C), but acclimation did not have a significant effect on CS activity in any tissue (all $F_{1,11}$<4.2, P>0.06). However, there was a significant interaction between acclimation treatment and temperature in muscle and heart (both $F_{2,22}$>5.6, P<0.01; Fig. 6B,C), but not in liver ($F_{2,22}$=2.87, P=0.10; Fig. 6A).

When the thermal sensitivity of enzyme activities differed between treatments, Q_{10} values were significantly lower in cold- compared with warm-acclimated animals (Table 1).

Discussion

Animals that regulate their body temperature are thought to have evolved performance optima that coincide with the

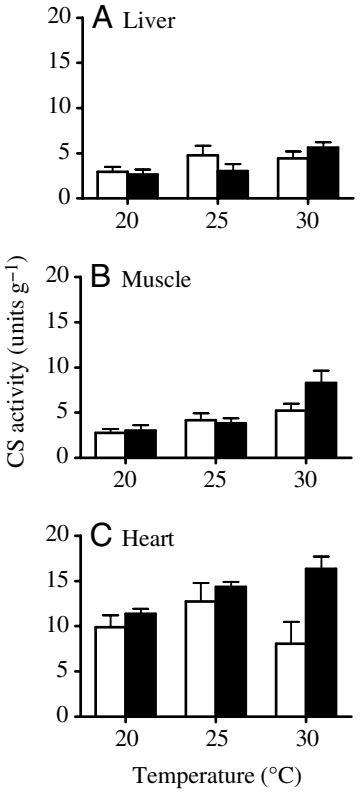

Fig. 6. Activities (mean ± s.e.m.) of citrate synthase (CS) (units g^{-1} wet tissue) in liver (A), tail muscle (B) and heart (C). Open boxes represent cold-acclimation treatment, and solid boxes represent warm-acclimation treatment. There are significant interactions between acclimation treatment and test temperature in muscle and heart, but not in liver.

regulated temperature range, sometimes referred to as the 'preferred body temperature'. Endothermic mammals, in particular, regulate body temperature very narrowly, and departure from that narrow range will often have negative consequences, and may even result in death (Harjunpää and Rouvinen-Watt, 2004). Similarly, many terrestrial ectotherms have evolved behavioural patterns that result in narrow body-temperature ranges compared with operative temperature fluctuations, at least when environmental heterogeneity is sufficient to allow thermoregulation (Hertz et al., 1993; Wills and Beaupre, 2000; Seebacher and Shine, 2004). The thermal sensitivity of performance may have co-evolved with preferred body temperatures, and the reaction norm of a genotype may include different phenotypes that have evolved in different thermal environments, such as at different latitudes (Angilletta et al., 2002).

The extraordinary shift in behaviour and locomotory and metabolic performance of *C. porosus* illustrates that the phenotypic response is composed of a series of temperature–performance curves that are defined by individual's capacity for plasticity (Piersma and Drent, 2003). Consequently, temperature selection during thermoregulation is a plastic variable, and the general validity of the 'final preferendum' paradigm (Reynolds and Casterlin, 1979; Jobling, 1981; Diaz et al., 2002) is questionable.

Biological responses of *C. porosus* to chronic temperature change occur at all levels of organisation. The shift in thermoregulatory behaviour and preferred body temperature indicates that animals can sense their environment and that there is an integrated response between behaviour and biochemical compensation. Clearly, at least in juvenile crocodiles, there is no trade-off between lower regulated body temperature and animal performance. The perfect compensation of swimming performance for lower body temperatures indicates that muscle function is remodelled to be optimised at the 'new' lower body temperature (Johnston and Temple, 2002; Guderley, 2004). Both aerobic and anaerobic ATP production capacity is compensated significantly with cold acclimation, although the response is tissue specific. The upregulation of CCO during cold acclimation in liver and heart reflects the high demand for aerobic ATP. The liver uses some of the ATP produced by oxidative phosphorylation to reconvert lactate to glucose *via* the LDH-catalysed synthesis of pyruvate (Garcia et al., 1994). In addition, the thermal sensitivity of CCO decreased with cold acclimation in muscle and liver, which may result from upregulation at lower temperatures and from changes in membrane fatty acid composition (Wu et al., 2004).

The fact that each acclimation group maximised RCRs at their mean body temperature indicates that oxidative phosphorylation is thermally plastic, and that ATP production is optimised in parallel with temperature selection. Production of ATP will depend on electron transport *via* mitochondrial

Table 1. *Thermal sensitivity (Q_{10}) of enzyme activities in different tissues of cold- and warm-acclimated animals*

Enzyme	Tissue	Acclimation	20–25°C	*P*	25–30°C	*P*
LDH	Muscle	Cold	1.5±0.2		1.0±0.1	
		Warm	1.9±0.2	ns	1.9±0.4	0.028
	Liver	Cold	2.2±0.4		1.7±0.4	
		Warm	2.5±0.7	ns	1.7±0.3	ns
	Heart	Cold	2.1±0.7		1.7±0.2	
		Warm	2.6±0.5	ns	1.9±0.3	ns
CCO	Muscle	Cold	1.4±0.4		1.1±0.1	
		Warm	1.1±0.1	ns	1.5±0.1	<0.02
	Liver	Cold	0.7±0.2		1.0±0.2	
		Warm	1.3±0.1	<0.03	1.2±0.03	ns
	Heart	Cold	0.8±0.1		1.2±0.2	
		Warm	1.1±0.09	ns	1.6±0.2	ns
CS	Muscle	Cold	2.2±0.8		2.0±0.5	
		Warm	1.7±0.7	ns	4.9±1.0	<0.03
	Liver	Cold	2.2±1.1		1.3±0.2	
		Warm	1.1±0.3	ns	3.3±0.8	<0.04
	Heart	Cold	2.1±0.0		0.5±0.2	
		Warm	1.5±0.1	ns	1.4±0.2	<0.002

Values are means ± s.e.m.

ANOVA results (*P*) indicate whether there were significant differences between cold- and warm-acclimation groups (all d.f.=1,15; ns, not significant; for significant results all $F>5.0$).

CCO, cytochrome *c* oxidase; CS, citrate synthase; LDH, lactate dehydrogenase.

complexes, in particular on complex IV (CCO), and on F_0F_1-ATPase activity that is responsible for phosphorylation of ADP. Both CCO and ATP synthase activities may be modified by changes in the fatty acid composition of the mitochondrial membrane (Yamaoka et al., 1988; Hulbert and Else, 1999). Response to temperature variation may therefore be partly controlled by membrane restructuring and this will be an important area for future research.

The potential for biochemical and physiological systems to be plastic, or flexible, within individuals exists within most animal groups. For example, there is a parallel shift in regulated body temperature and metabolic performance in response to environmental change in alligators (*Alligator mississippiensis*) (Seebacher et al., 2003). Facultative hypothermic responses [e.g. torpor and hibernation (St. Pierre and Boutilier, 2001; McKechnie and Lovegrove, 2002; Storey and Storey, 2004)] also represent reversible plasticity in the sense that biochemical systems shift in a functional way, in this case to minimise energy expenditure (Ramnanan and Storey, 2006) rather than maintaining constant or near-constant metabolic capacity.

The capacity even of thermoregulating animals for reversible plasticity means that the concept of thermal reaction norms as fixed (non-reversible) phenotypes (Via et al., 1995) is not sufficient to explain the evolution of thermal physiology. We suggest that the focus in thermal biology should be on the evolution of reversible plasticity (Wilson and Franklin, 2002; Piersma and Drent, 2003). Accordingly, a reaction norm exists if there are differences in mean trait values between populations and if the reversible plasticity surrounding each mean trait value does not completely encompass the plastic range of all other mean trait values (Seebacher, 2005).

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