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Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*

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Summary

1. We examined the effect of thermal acclimation on fighting success and underlying performance traits in the crayfish *Cherax destructor*. We tested the hypothesis that animals will be more successful when fighting at their acclimation temperature than at a colder or warmer temperature, and that changes in metabolic capacity underlie differences in behavioural performance.

2. Thermal acclimation (to 20 C and to 30 C) had a significant effect on behavioural contests, and the likelihood of winning was significantly greater when individuals fought at their acclimation temperature against an individual from an alternate acclimation temperature.

3. The ratio of ADP stimulated respiration to proton leak (respiratory control ratio) of isolated mitochondria increased significantly in chelae muscle of the cold-acclimated group, and differences in respiratory control ratio between winners and losers were significantly correlated with the outcome of agonistic encounters. However, acclimation did not affect tail muscle mitochondria or the activity of pyruvate kinase in either chelae or tail muscle.

4. The force produced by closing chelae was thermally insensitive within acclimation groups, and there were no significant differences between acclimation treatments. None the less, differences in chelae width between contestants were significantly correlated with the outcome of agonistic encounters, but this perceived resource holding power did not reflect the actual power of force production.

5. Thermal acclimation in *C. destructor* has beneficial consequences for dominance and competitive ability, and the success of cold acclimated animals at the cold temperatures can be at least partly explained by concomitant up-regulation of oxidative ATP production capacity.

Key-words: acclimation, agonistic behaviour, fitness, mitochondria, phenotypic plasticity

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Introduction

An important goal within the discipline of evolutionary physiology is to understand both the physiological mechanisms underlying phenotypic variation and its ecological significance (Garland & Carter 1994; Feder, Bennett & Huey 2000). Studies of environmentally induced phenotypic variation (phenotypic plasticity) offer the opportunity to examine both the mechanisms underlying alternate phenotypic expression from a single genotype and the ecological implications of these modifications (Whiteley & Faulkner 2005; Lucassen *et al*. 2006). An important step in understanding phenotypic plasticity is the development of theoretical models predicting the conditions for its evolution (Via & Lande 1985; Van Tienderen 1991; DeWitt & Scheiner 1992; Gomulkiewicz & Kirkpatrick 1992), and empirical tests of the costs and benefits are mandatory to test hypotheses about its ecological significance (McCollum & Van Buskirk 1996; Relyea 2002). In particular, the relationships between the mechanistic function of traits and their selection in different environments (ecological implications) will determine the fitness of alternate phenotypic expression (Dudley 1996; Woods & Harrison 2002). Hence, knowledge of the mechanisms underlying plastic modifications can help identify the likely constraints and/or limits to the directions of phenotypic evolution and the possible lineages that are predispose to rapid diversification (Frankino & Raff 2004). In thermally varying environments, temperature-induced constraints of biochemical

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capacities may provide the proximate mechanisms that limit fitness of organisms (Portner *et al*. 2006), and biochemical capacities will therefore be under relatively strong selection (Woods & Harrison 2002).

Here we examine the nature of plastic responses by adopting an integrative approach that relates reversible modifications in physiological traits (acclimation responses; Guderley & St Pierre 2002; Johnston & Temple 2002), with the ecological implications and fitness consequences (Harvell 1990; Van Buskirk & McCollum 1999). This integrative approach will place reversible phenotypic plasticity of physiological traits within the broader context of the evolutionary significance of phenotypic plasticity.

The most pervasive environmental variable that affects biological functions is heat, and the thermodynamics of biochemical rate functions means that all, or nearly all, biological rates are influenced by the temperature of the organism. Many ectotherms possess the capacity to respond to variation in the thermal environment by phenotypic modifications that presumably allows the maintenance of physiological function across a diversity of environments. Thermal plasticity may manifest itself during development (developmental plasticity) or may occur as a reversible response (acclimation) in long-lived organisms (Huey *et al*. 1999; Wilson & Franklin 2002; Terblanche & Chown 2006). The adaptive significance of developmental acclimation is disputed (Leroi, Lenski & Bennett 1994; Zamudio, Huey & Crill 1995; Huey & Berrigan 1996; Bennett & Lenski 1997; Gibert, Huey & Gilchrist 2001), and although the physiological mechanisms underlying seasonal acclimation responses are well documented (Guderley 2004), their fitness benefits need to be confirmed empirically (Angilletta *et al*. 2006).

The effect of temperature on the metabolic capacity and competitive behaviour of crayfish offers an excellent system for examining questions about the adaptive significance of thermal acclimation, and linking these with their underlying physiological mechanisms. Crayfish are highly aggressive animals that routinely engage in fierce competitive bouts for both territories and resources (Bergman *et al*. 2003; Gilmour, DiBattista & Thomas 2005), and metabolic capacity and physical strength are likely to be important determinants of their competitive ability. Additionally, many species of crayfish experience wide seasonal fluctuations in environmental temperature, so that it would be advantageous if their physiological capacity exhibited pronounced responses to thermal heterogeneity (Nguyen *et al*. 2004). The large chelae (claws) of crayfish are utilized as both defensive and offensive weapons against conspecifics and predators. Muscle metabolic capacity may limit chelae function, which can also influence an individual's dominance behaviour and subsequent position within a social hierarchy (Garenc *et al*. 1999; Sneddon, Taylor & Huntingford 1999; Guderley & Couture 2005).

In this study, we tested the hypothesis that social dominance is determined by metabolic attributes of individuals, and that thermal acclimation of metabolic capacity will be beneficial for the outcomes of intraspecific contests. We chose the Australian crayfish *Cherax destructor* as study organisms because both males and females of this species are very aggressive and routinely fight with conspecifics. Specifically, we propose the hypothesis that crayfish acclimated to a cool temperature will out-compete conspecifics that have acclimated to a warm environment when tested under cool conditions, and vice versa at warm temperatures. Given that *C. destructor* enter extended competitive bouts during territorial disputes and routinely utilize their chelae to hold and dislodge their competitor, we also hypothesized that thermal acclimation of chelae strength will provide a similar benefit. We measured metabolic capacity of the chelae and tail muscle to determine if changes in whole-animal and territorial performance were associated with underlying biochemical and physiological modifications with acclimation.

Materials and methods

EXPERIMENTAL TREATMENTS

Adults of the native Australian crayfish, *Cherax destructor* (20 males, 10 females, mean mass 42·6 g ± 2·2 SE), were obtained from a commercial supplier (Crazy Crays, NSW, Australia). Animals were housed in separate sex groups in plastic tanks (60 L, five animals per tank) with a gravel substrate and numerous shelters. Crayfish were randomly divided equally into two acclimation treatments and exposed to either 20 C or 30 C for 4 weeks. These temperatures were chosen because they are typical of *C. destructor* habitat but do not represent potentially harmful extremes, and temperature was maintained by placing tanks into controlled temperature chambers. Animals were fed commercially available crayfish pellets supplemented with fresh carrots. At the completion of the acclimation period, the fighting capacity, maximum claw strength, and several muscle metabolic characteristics were assessed for all individuals. During behavioural and performance experiments, the body temperature of the crayfish was changed at a rate that did not exceed 5 C h^{-1}.

BEHAVIOURAL OBSERVATIONS

The competitive dominance behaviour of each individual crayfish was determined during staged one-on-one competitive bouts. Competitive experiments involved competing one crayfish from each acclimation group against each other at both 20 C and 30 C. As both body size and sex are likely determinants of dominance and territorial success, crayfish from each acclimation group were first size- and sex-matched with an individual from the alternate acclimation group. Thus, 10 pairs of males and five pairs of females were identified and tested at both 20 C and 30 C. The order of test

temperatures was randomized for each pair of crayfish. Following each initial test for a pair, crayfish were returned to their host acclimation temperature and were retested at the next test temperature after 24 h.

Competitive bouts were conducted in an aquarium ($0{\cdot}34 \times 0{\cdot}20 \times 0{\cdot}23$ m) that contained aged tap-water (pH 7·0), a gravel base layer of 1 cm, and a small cylindrical retreat. The observation tank was maintained at the appropriate test temperature by conducting experiments in a controlled temperature room. Black plastic was taped around the sides and back of the observation aquarium to reduce any external stimuli. An aquarium light was also placed above the tank for adequate illumination and to reduce the ability of the animals to detect movement in the darkened observation room. Initially, the observation tank was also subdivided into three sections with plastic dividers. One crayfish from each acclimation group (competitive pair) was then randomly assigned and introduced to either the far left or right section of the aquarium. Following a 10-min settling period, the plastic dividers were simultaneously lifted remotely and the crayfish were given the opportunity to explore the tank and interact. The behaviour of each crayfish was then observed and recorded over a 10-min period. Observations were conducted remotely using a camera connected to a computer that was located behind a room partition so that the observers were never in direct visual contact with the focal animals.

Based on preliminary trials using crayfish not subsequently used in any experiments, we developed a scoring system that represented competitive and territorial success during our staged encounters. The two most prevalent and easily recognized behaviours were 'fights' and 'retreats'. Fights were defined as the two animals facing one another with each attempting to hold and unbalance the other. Crayfish typically used their chelae to push their opponent and to take hold of the adversary's chelae. Eventually, one of the contestants would give in and move away, and the animal that remained was scored as the winner of the fight. In a 'retreat', upon encountering one another one of the animals would turn and retreat immediately without engaging in a fight, and the animal that remained was scored as the winner of the retreat. We scored victory in a fight as 2 points, and a retreat as 1 point for the winner. No animals were physically harmed during any of the observed encounters.

CHELAE FORCE MEASUREMENTS

Following behavioural observations, the maximum closing force of the chelae for each individual crayfish was measured at 20 C and 30 C. The order of testing for each individual at each temperature was kept identical to that used in the behavioural experiments and trials were at least 24 h apart. Force measurements were recorded using a custom-built sensor that consisted of two metal plates ($25 \times 5 \times 1$ mm) separated by a third metal plate (of 4 mm thickness) acting as a pivot, with all three plates mounted in a block of wood. The former two plates protruded by 12 mm beyond the pivot plate, and each had a strain gauge (RS Electronics, Sydney, Australia) attached to it with epoxy resin. The outputs from each strain gauge were connected to a custom made wheatstone bridge each linked to a bridge amplifier (AD Instruments, Sydney, Australia). Output from the bridge amplifiers was monitored with a computerized recording system (PowerLab, AD Instruments, Australia). Each strain gauge was calibrated so that the voltage output from each bridge amplifier could be converted to Newtons (N) of force. When presented with the device, crayfish readily closed their chelae on the two plates carrying the strain gauges so that the total force produced by the closing of the chelae could be measured. The greatest force (the sum of both force transducers) produced by three to five bites at each temperature was defined as their maximum chelae force. Chelae width and length of each individual was measured using calipers.

MITOCHONDRIAL OXYGEN CONSUMPTION

Aerobic metabolic capacity was measured as oxygen consumption of isolated mitochondria from tail muscle and chela (claw) muscle. Animals were euthanased by decapitation, and tissue (0·1 g) was immediately collected and transferred on to ice, where it was finely diced, weighed and homogenized in 4 volumes of ice-cold isolation buffer [140 mM KCl, 10 mM EDTA, 5 mM $MgCl_2$, 20 mM HEPES, 0·5% bovine serum albumin (BSA), pH 7·3 at 20 C] by five gentle passes in a glass homogenizer. 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 l of isolation medium, which was further diluted in 2250 l of ice cold assay medium (140 mM KCl, 20 mM HEPES, 5 mM Na_2HPO_4, and 0·5% BSA, pH 7·3 at 20 C).

Oxygen consumption of the mitochondrial solution (250 l per assay) obtained from each animal was measured in a temperature controlled respiration chamber (Mitocell, Strathkelvin Instruments, Glasgow, Scotland) at 20 C and at 30 C. The oxygen concentration of the solution was measured with a calibrated oxygen electrode connected to an oxygen meter (model 782, Strathkelvin Instruments, Scotland). After the oxygen concentration of the solution has stabilized, 5 l each of 10 mM malate and 5 mM pyruvate were added to obtain the State 2 rate of oxygen consumption (Skorkowski 1988; Blier & Guderley 1993; Johnston *et al*. 1994). State 3 oxygen consumption rate was obtained from the rate of decrease in oxygen concentration after the addition of 10 l of 50 mM ADP neutralized with KOH. State 3 represents the oxygen consumption rate stimulated by

the presence of ADP and its phosphorylation to ATP. State 4 oxygen consumption rate was measured after all ADP was consumed (Johnston *et al*. 1994); State 4 oxygen consumption rates represent the energy lost as a result of proton leak across the mitochondrial membrane. The respiratory control ratio (RCR) was calculated as the ratio between State 3 and State 4 rates to express the coupling of oxidative metabolic pathways to energy (ATP) production (Johnston *et al*. 1994).

Aliquots of isolated mitochondria were saved to determine mitochondrial protein concentration. Duplicate aliquots (50 L) were washed in 1500 L assay medium without BSA by centrifuging at 12 000 *g* for 10 min. The pellet was resuspended and centrifuged three times to remove BSA. Protein concentration of the washed mitochondrial solution was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

ENZYME ASSAYS

We determined activities of pyruvate kinase and lactate dehydrogenase from claw and tail muscle samples. Pyruvate kinase catalyses the transphosphorylation of phosphoenolpyruvate and ADP to pyruvate and ATP, and it is a control point for glycolytic activity (Muños & Ponce 2003). Lactate dehydrogenase reflects anaerobic capacity by catalysing the reduction of pyruvate to lactate (Somero 2004).

Tissue samples (0·05–0·1 g) were collected immediately following euthanasia of crayfish and frozen at –80 C for later analysis. Tissues were homogenized in a glass homogenizer in 9 volumes of ice cold extraction buffer (50 mM imidazole, 2 mM $MgCl_2$, 5 mM EDTA, 0·1% Triton and 1 mM glutathione, pH 7·5 at 20 C). Activity of both enzymes was assayed as the disappearance of NADH at 340 nm in a UV/Vis spectrophotometer with a temperature controlled cuvette holder (Ultrospec 2100pro, Amersham, Sydney, Australia) at 20 C and at 30 C. Pyruvate kinase activity was assayed in 75 mM Tris/HCl buffer (pH 7·5) with 75 mM KCl, 1 mM EDTA, 10 mM $MgCl_2$, 0·12 mM NADH, 2 mM ADP, 1 mM phosphoenolpyruvate, and excess lactate dehydrogenase. Lactate dehydrogenase was assayed in KH_2PO_4/K_2HPO_4 buffer (pH 7·0) containing 0·16 mM NADH and 0·4 mM pyruvate.

STATISTICAL ANALYSIS

Behavioural scores were analysed by chi-square contingency tables. Each individual received a total score for each trial ('fights' + 'retreats'), and at each assay temperature the null-hypothesis (expected numbers) was that animals from cold and warm acclimation treatments will be equal in their success (score) of winning encounters. Hence, for each test temperature we performed a chi-square test with acclimation group (20 or 30 C) as variable, and with expected numbers = 0·5*total points scored.

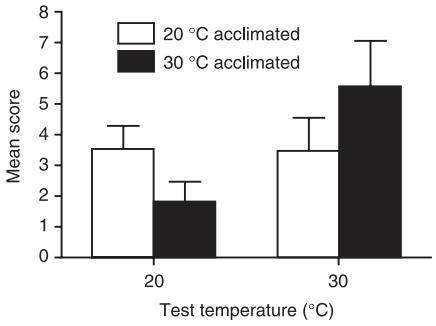

Fig. 1. Effect of temperature on the competitive territorial performance of crayfish (*Cherax destructor*) following acclimation to either 20 or 30 C for 4 weeks. Crayfish scored significantly higher when test temperatures coincided with their acclimation temperature. Data represent means ± SE. Significant differences were detected between acclimation groups at the level of $P < 0{\cdot}05$.

Chelae force, State 3 and State 4 oxygen consumption rates, RCRs, and enzyme activities were analysed by ANOVA with 'acclimation treatment' and 'sex' as factors, and 'test temperature' as a repeated measure. When 'sex' was not significant, this factor was dropped and the analysis was repeated with 'acclimation treatment' as the main effect, and 'test temperature' as the repeated measure. In the analysis of chelae force and enzyme activities, chelae width and body mass were used as covariates, respectively. Where appropriate, means were compared by Tukey *post hoc* test.

To determine significant relationships between behavioural scores and changes in any of the independent variables measured, we performed nonlinear regression analyses (using the Levenberg–Marquhart algorithm in CurveExpert 1·3). We regressed each variable (mass, chelae width, chelae force, chelae force/width, RCRs of chelae and tail muscle, and LDH and PK activities of chelae and tail muscle) separately against behavioural scores at each test temperature (20 C and 30 C). Our interest was in the relative performance of contestants in a pair so that in the analysis we used contrasts of the variables (Y1–Y2; Zamudio *et al.* 1995) and contrasts in behavioural scores, i.e. score individual 1–score individual 2, where 1 signifies the individual that has been acclimated at the test temperature. To fit power or logarithmic functions to data sets that include negative values, we added a constant to all data points, and we used the correlation coefficient, *r*, to determine significant relationships.

Note that some tissue samples were lost so that there are reduced sample sizes in some of the data for mitochondrial oxygen consumption and enzyme assays.

Results

BEHAVIOURAL OBSERVATIONS

Crayfish have significantly greater success in winning encounters with conspecifics when tested at their own acclimation treatment temperature (Fig. 1). Hence,

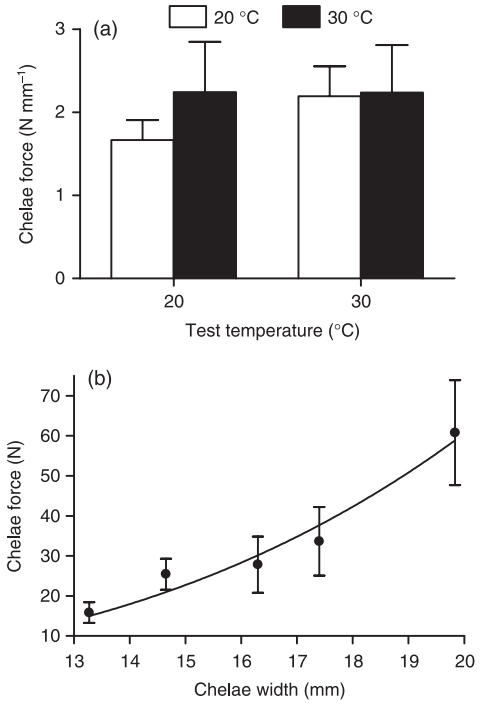

Fig. 2. Effect of temperature on the maximum chelae force (mean force per mm width ± SE) of crayfish (*Cherax destructor*) acclimated to either 20 or 30 C for 4 weeks. (a) No significant differences were detected between acclimation groups at both test temperatures ($P > 0{\cdot}05$). However, chelae force increased allometrically with chelae width (Force = 0·0022 Width$^{3{\cdot}41}$, $R^2 = 0{\cdot}96$, solid line, Fig. 2b).

cold-acclimated animals have significantly higher scores than warm acclimated animals at 20 C ($\chi^2 = 8{\cdot}45$, $P < 0{\cdot}005$) and, vice versa when tested at 30 C ($\chi^2 = 7{\cdot}12$, $P < 0{\cdot}01$; Fig. 1).

CHELAE FORCE

Maximum chelae force varies significantly with test temperature ($F_{1,27} = 4{\cdot}45$, $P = 0{\cdot}044$; Fig. 2a), but chelae force does not differ between acclimation groups ($F_{1,27} = 1{\cdot}21$, $P = 0{\cdot}28$). Within acclimation groups, there are no differences in chelae force between test temperatures (Tukey test $P > 0{\cdot}10$), and the only significant difference exists between cold- and warm-acclimated animals at 20 C ($P < 0{\cdot}05$). Maximum chelae force does not vary significantly with sex ($F_{1,25} = 3{\cdot}34$, $P = 0{\cdot}079$), and sex does not interact with acclimation treatment ($F_{1,25} = 1{\cdot}27$, $P = 0{\cdot}27$; Fig. 2a). In contrast, maximum chelae force increases significantly with increasing chelae width ($F_{1,27} = 19{\cdot}51$, $P < 0{\cdot}0001$; Fig. 2b).

MITOCHONDRIAL OXYGEN CONSUMPTION

Sex does not affect either State 3 or State 4 oxygen consumption rates, nor RCRs in mitochondria from chelae muscle (all $F_{1,20} < 1{\cdot}02$, $P > 0{\cdot}33$; Fig. 3a,b). However, both State 3 and State 4 oxygen consumption rates increase significantly with increasing assay temperature (both $F_{1,22} > 6{\cdot}93$, $P < 0{\cdot}02$; Fig. 3a). Additionally, both State 4 rates ($F_{1,22} = 5{\cdot}19$, $P < 0{\cdot}04$) and

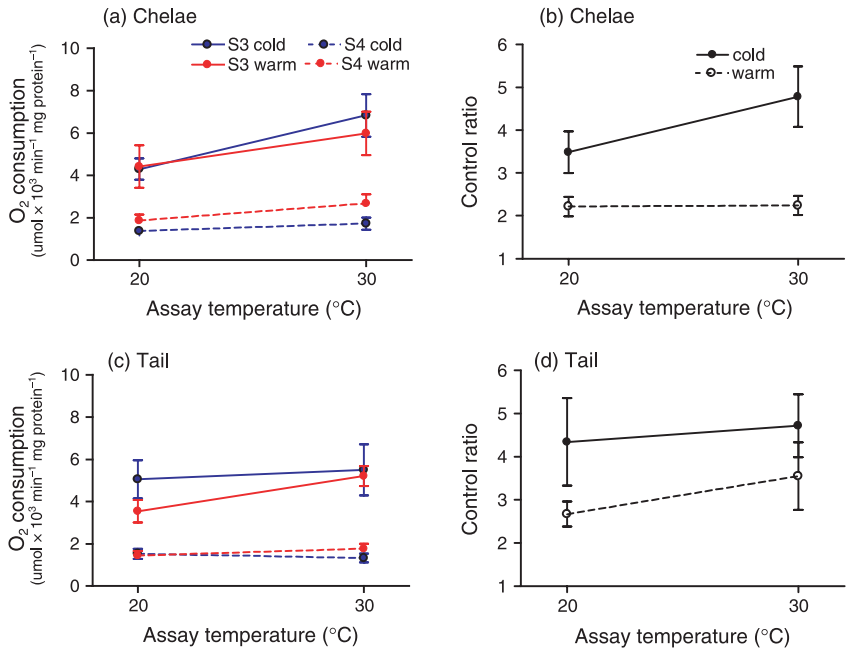

Fig. 3. State 3 (solid lines; mean ± SE) and State 4 (broken lines; mean ± SE) rates of mitochondrial oxygen consumption of chelae (a) and tail (c) muscle from warm (30 C, red) and cold (20 C, blue) acclimated crayfish (*Cherax destructor*) measured at different assay temperatures. Respiratory control ratios for chelae (b) and tail (d) muscle represent the efficacy of mitochondria to produce ATP.

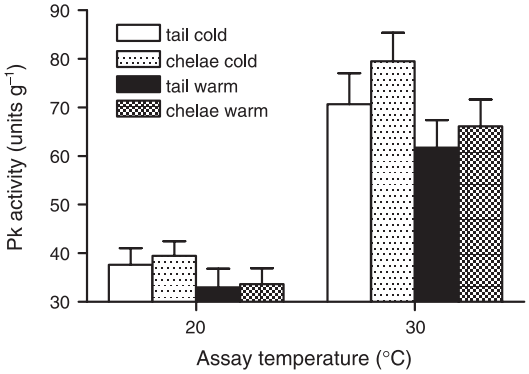

Fig. 4. The effect of temperature on the pyruvate kinase activity of tail and chelae muscle from crayfish (*Cherax destructor*) acclimated to either 20 C or 30 C for 4 weeks. No significant differences were detected between acclimation treatments or sexes.

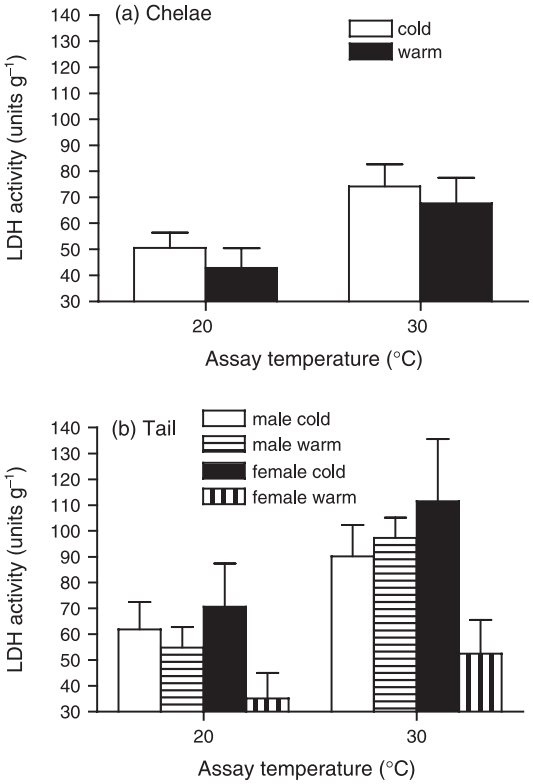

Fig. 5. The effect of temperature on the lactate dehydrogenase activity of chelae (a) and tail (b) muscle from crayfish (*Cherax destructor*) acclimated to either 20 C or 30 C for 4 weeks. Activity in tail muscle differed interacted significantly between sex, acclimation treatment and assay temperature.

RCRs ($F_{1,22} = 11{\cdot}07$, $P < 0{\cdot}01$) differ significantly between acclimation treatments (Fig. 3b), but RCRs do not vary with temperature ($F_{1,22} = 1{\cdot}93$, $P = 0{\cdot}18$). There are no significant interactions between assay temperature and acclimation treatment in any of the claw muscle measurements (all $F_{1,22} < 1{\cdot}83$, $P > 0{\cdot}19$).

In tail muscle mitochondria, State 3 oxygen consumption rates do not vary significantly with sex ($F_{1,21} = 1{\cdot}36$, $P = 0{\cdot}26$) or acclimation treatment ($F_{1,23} = 0{\cdot}47$, $P = 0{\cdot}50$). State 3 rates increase significantly with assay temperature ($F_{1,23} = 6{\cdot}78$, $P = 0{\cdot}016$), but there is no significant interaction between assay temperature and acclimation treatment ($F_{1,231} = 2{\cdot}31$, $P = 0{\cdot}14$). State 4 oxygen consumption rates of tail muscle mitochondria differ significantly between the sexes ($F_{1,21} = 5{\cdot}09$, $P = 0{\cdot}035$), but none of the other factors or interactions is significant (all $F_{1,21} < 2{\cdot}05$, $P > 0{\cdot}17$; Fig. 3c). Similarly, neither sex ($F_{1,21} = 0{\cdot}85$, $P = 0{\cdot}37$), nor acclimation treatment or assay temperature significantly affect RCRs in tail muscle (all $F_{1,23} < 2{\cdot}42$, $P > 0{\cdot}13$; Fig. 3d).

ENZYME ACTIVITIES

Pyruvate kinase activity is not affected by sex in either chelae or tail muscle (both $F_{1,20} < 0{\cdot}19$, $P > 0{\cdot}67$), but activity increases significantly with increasing temperature in both chelae ($F_{1,22} = 17{\cdot}96$, $P < 0{\cdot}0001$) and tail ($F_{1,22} = 38{\cdot}48$, $P < 0{\cdot}0001$) muscle. However, there is no effect of acclimation treatment on pyruvate kinase activity in either muscle tissue (both $F_{1,22} < 2{\cdot}2$, $P > 0{\cdot}15$; Fig. 4).

Lactate dehydrogenase activity of chelae muscle is not affected by sex ($F_{1,20} = 0{\cdot}34$, $P = 0{\cdot}56$), acclimation treatment, or assay temperature (both $F_{1,22} < 1{\cdot}94$, $P > 0{\cdot}18$; Fig. 5a). In contrast, in tail muscle, lactate dehydrogenase activity increases significantly with increasing assay temperature ($F_{1,20} = 12{\cdot}41$, $P < 0{\cdot}002$). Additionally, there is a significant interaction between assay temperature, acclimation treatment, and sex ($F_{1,20} = 7{\cdot}97$, $P < 0{\cdot}02$), and activity of cold acclimated females is greater than that of warm acclimated animals at both test temperatures (Tukey $P < 0{\cdot}001$; Fig. 5b).

INDEPENDENT VARIABLES AND BEHAVIOURAL SCORES

At 20 C-test temperature, differences in RCR between contestants from different acclimation treatments are positively related to differences in behavioural score ($Y + 1 = 2{\cdot}74/(1 + 3{\cdot}81e^{-1{\cdot}17x})$, $r = 0{\cdot}65$, $P < 0{\cdot}05$; Fig. 6a). Similarly, there is a positive correlation between behavioural scores and chelae width at both 20 C ($Y + 2 = 1{\cdot}90e^{0{\cdot}090x}$, $r = 0{\cdot}5$, $P < 0{\cdot}05$; Fig. 6b) and at 30 C ($Y + 2 = 2{\cdot}44e^{0{\cdot}033x}$, $r = 0{\cdot}48$, $P < 0{\cdot}05$; Fig. 6c). However, none of the other variables is significantly related to differences in scores between competing pairs at 20 C or at 30 C (all $P > 0{\cdot}05$).

Discussion

Dominance in a social hierarchy directly influences reproductive success by increasing access to resources, including mates (Maynard-Smith 1974; Parker 1974; Bergman & Moore 2005). Thermal acclimation influences the dominance and competitive ability of *C. destructor*, and individuals are more successful at their host acclimation temperature when fighting against

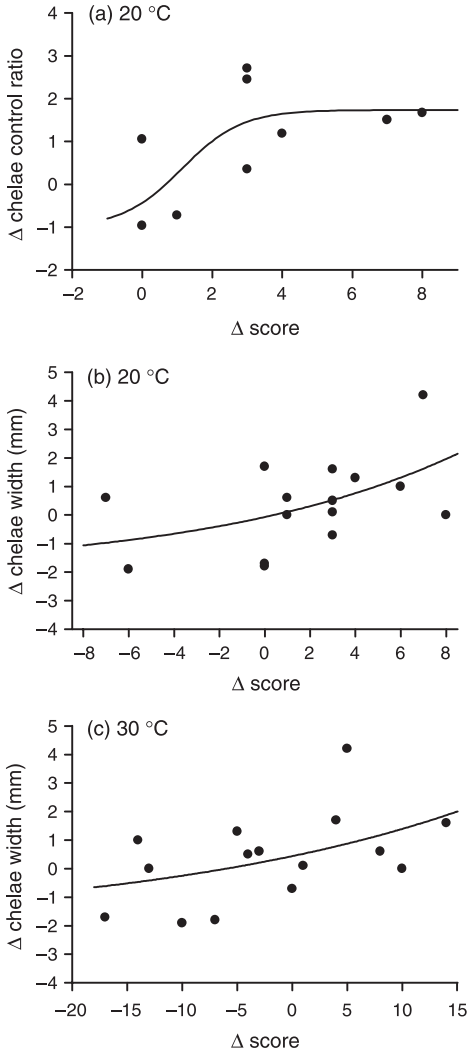

Fig. 6. When tested at 20 C, there were significant relationships between differences in respiratory control ratio in chelae muscle (Δ chelae control ratio) and differences in score (Δ score) between contestants (a). Differences in chelae width (Δ chelae width) were significantly related to differences in score at 20 C (b) and at 30 C (c).

opponents from a different acclimation environment. Interestingly, previous history of encounters between individuals, which may be one of the main aspects that characterizes winners and losers (Rutte, Taborsky & Brinkhof 2006), does not affect the outcomes of fights. Each pair in our experiments encountered one another twice: once at each individual's acclimation temperature. In the second encounter, at the reciprocal test temperature, acclimation temperature was the main determinant of the outcome. Hence, previous experience is unimportant relative to the effect of thermal acclimation over the time frame of the experiment. None the less, in animals acclimated to the same thermal conditions, behavioural or chemical cues may mark an individual as a winner or loser, and previous experience may modify the cues expressed by individuals (Bergman *et al*. 2003).

Chelae width was significantly related to outcomes of agonistic encounters independently of the closing force produced by the chelae, indicating that the size of the chelae acts as a visual cue determining outcomes of fights. Visual cues are common in agonistic encounters of animals (Earley, Tinsley & Dugatkin 2003; Zeil & Hemmi 2006). In the case of *C. destructor*, it appears that 'Resource Holding Power' (Parker 1974; Rutte *et al*. 2006) is assessed on the basis of chelae width, but this assessment is not an honest refection of the actual power, i.e. chelae closing force. It is surprising that chelae closing force is thermally insensitive given the marked effects of temperature on muscle performance (Johnston & Temple 2002). Selection may have favoured constant, thermally insensitive muscle performance in chelae, thereby minimizing the differences in the winning potential between individuals, and maximizing the potential fitness of the individual.

Metabolic capacity changes with thermal acclimation in *C. destructor*, and it is at least a correlate to winning or losing fights. The efficacy of oxidative ATP production (RCRs) of both sexes is significantly greater in cold acclimated animals, and the difference in RCRs between contestants is positively related to the outcome of agonistic encounters at the cold temperature. At the onset of muscular activity in crayfish, arginine is hydrolysed by arginine kinase to yield ATP (England & Baldwin 1983). Prolonged activity is subsequently fuelled by either glycolytic ATP production via pyruvate reduction by lactate dehydrogenase (Somero 2004), and by oxidative pathways. Pyruvate kinase represents the final step in glycolysis that controls substrate (pyruvate) concentration for both lactate dehydrogenase and the citric acid cycle (Muñoz & Ponce 2003). Pyruvate kinase activity varies with acute temperature but it does not change with acclimation, which indicates that the activity of this enzyme is not limiting ATP supply to the tissues. The sex-specific response of lactate dehydrogenase activity in tail muscle to thermal acclimation is not easily explained in terms of gender-specific metabolic demands, unless glycolytically produced ATP contributes to the growth of reproductive tissues and eggs in females. Otherwise, the dominance behaviour of males and females is indistinguishable.

The importance of acclimation of aerobic metabolic capacity of chelae muscle may lie in supplying ATP for sustained activity and in providing energy for gluconeogenesis. If the latter pathway is important, it may explain the lack of response of pyruvate kinase to thermal acclimation. Despite changes in metabolic capacity with acclimation and/or acute temperature, the force produced by the chelae is insensitive to acute temperature changes within acclimation treatments, although there are differences between treatments. The thermal insensitivity of chelae force indicates that ATP supply does not limit muscle performance, and that muscle performance is up-regulated at low temperatures.

Thermal acclimation is a phylogenetically widespread response of organisms to temperature fluctuations (Scheiner 1993; Piersma & Drent 2003; Seebacher 2005), and we confirm that a flexible phenotype would manifest in obvious fitness benefits for acclimated individuals relative to nonacclimated conspecifics (Wilson & Franklin 2002). Acclimation to the thermal environment clearly benefits dominance behaviour in *C. destructor*. Social position is an important component of individual fitness, so that acclimation will have positive fitness consequences. Capacity changes in oxidative metabolism are one mechanism that facilitates the positive effects of acclimation. None the less, acclimation only partially explains fighting success, and the importance of claw size indicates that patterns of growth and development interact with acclimation.

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