

RESEARCH ARTICLE

Thermal games in crayfish depend on establishment of social hierarchies

Glenn J. Tattersall*, Joshua P. Luebbert, Olivia K. LePine, Kiel G. Ormerod and A. Joffre Mercier

Department of Biological Sciences, Brock University, 500 Glendridge Avenue, St Catharines, ON, Canada, L2S 3A1

*Author for correspondence (gtatters@brocku.ca)

Accepted 8 February 2012

SUMMARY

An unequal resource distribution is commonly seen in dominance hierarchies, in which the individual with the higher status is more successful in obtaining the resource. One possible resource is preferred temperature. When situations allow, ectotherms regulate their body temperature by behaviourally selecting different environmental conditions, achieving, when possible, a preferred temperature. Using a shuttlebox, the preferred temperature for *Procambarus clarkii* was determined to be 23.9°C with upper and lower voluntary escape temperatures of 25.9 and 21.8°C, respectively. If this preferred temperature zone (21.8–25.9°C) was valued as a resource, given the choice between a preferred temperature and a non-preferred temperature, crayfish should compete over the preferred temperature, with the dominant individual of dyadic pairs achieving the preferred temperature more often than the subordinate. Using a dual-choice experimental tank, competition over a binary temperature choice between rank-established paired crayfish was determined under both warm and cold challenge conditions (warm vs preferred temperature and cold vs preferred temperature, respectively). In naive pairings, similar levels of competition over the preferred temperature occurred in both warm and cold challenge trials, as predicted by game theory. In established pairings, however, dominant crayfish gained significantly greater access to preferred temperature in both warm and cold challenge conditions. These results demonstrate that crayfish engage in a cost–benefit assessment during their initial agonistic contests over temperature, but as hierarchies mature, these thermal games are decided by the dominant animal gaining primary access to the temperature resource.

Key words: crayfish, dominance, agonistic behaviour, thermoregulation, resource, game theory, thermal game.

INTRODUCTION

Aggression and conflict occur ubiquitously throughout the animal kingdom (van der Dennen and Falger, 1990). Aggressive behavior often leads to the formation of stable dominance hierarchies, which form as a result of competition over a resource and can provide a peaceful resolution whereby the resource is often unequally divided (Barnard and Burk, 1979; Chase et al., 2002; Pusey and Packer, 1997). Generally, social animals fight when resources, such as food, shelter and space, are limited. Dominance hierarchies define an individual's status and access to certain resources, including food and shelter. The higher the social status, the more advantaged the individual. This reinforces the fitness of the dominant individual, which wins the majority of the encounters, over the subordinate individual, which loses the majority of the encounters and retreats. Winning an agonistic encounter increases the likelihood of winning the next encounter, and the opposite is also true (Chase et al., 1994; Dugatkin, 1997; Hsu and Wolf, 1999; McDonald et al., 1968). These are known as the winner and loser effects, which help to both separate and stabilise dominance hierarchies.

Aggressive behavior has been well studied in crayfish, which readily fight over shelters (Martin and Moore, 2008; Ranta and Lindstrom, 1992) or space when no shelters are provided (Bovbjerg, 1956). Under such conditions, aggressive encounters include several well-characterised behaviours, such as approaching, pushing, grasping, striking with the claws and the eventual retreat of one opponent (Bruski and Dunham, 1987; Herberholz et al., 2003; Issa et al., 1999; Tierney et al., 2000). Dyadic interactions between crayfish ultimately distinguish dominant and subordinate members of the pair, and interactions within larger groups result in linear

dominance hierarchies (Bovbjerg, 1956; Copp, 1986; Issa et al., 1999) (Martin and Moore, 2008; Ranta and Lindstrom, 1992). Size difference is an important factor in determining the outcome of such encounters, with larger crayfish generally having an advantage, but other factors such as claw size, previous fighting experience and prior residence also affect the outcome (Bergman et al., 2003; Daws et al., 2002; Issa et al., 1999; Peeke et al., 1995; Ranta and Lindstrom, 1992; Rutherford et al., 1995). The frequency and intensity of agonistic encounters increase sharply within the first few minutes and subsequently decrease, rapidly during the first hour, and slowly over days to weeks (Huber et al., 2000; Issa et al., 1999). In at least some cases, stable dominance hierarchies can take hours or days to be established (Fero et al., 2007; Huber et al., 2000) and can reverse following the introduction of a large intruder (Graham and Herberholz, 2009). There is evidence that dominant crayfish have preferred access to shelter, food and mates (Herberholz et al., 2007; Herberholz et al., 2003; Ranta and Lindstrom, 1992; Villanelli and Gherardi, 1998).

Agonistic behaviours, however, use physiological resources that could be available for other behaviours (Briffa and Sneddon, 2007), and are considered costly (Castro et al., 2006; Hack, 1997). As a result, they occur at frequencies inversely related to their cost (Hack, 1997), although game theory can be used to assess whether the benefits of aggression outweigh the costs of contest escalation (Sneddon et al., 2000), and would predict that traits that enhance an animal's resource holding potential are the result of selection (Angilletta, 2009; Briffa and Sneddon, 2007). Whole-organism physiological performance predicts success in agonistic encounters (Mowles et al., 2010) and may underlie the dominance hierarchies

(Briffa and Sneddon, 2007; Seebacher and Wilson, 2006; Seebacher and Wilson, 2007). Acclimation to different temperatures will also affect the outcome of agonistic encounters in crayfish, because of changes in intrinsic biochemical properties of the muscles rather than chelae force generation (Seebacher and Wilson, 2006). Indeed, Wilson and colleagues (Wilson et al., 2007; Wilson et al., 2009) have shown that in adult crayfish, although chela size is a dishonest signal of strength, it is a reliable predictor of resource holding potential and contest outcome. As swimming speed is inversely related to chela size, chela size may represent a trade-off (*via* the handicap principle), signaling greater resource holding potential based not on force production, but on other parameters that cannot be readily detected, such as thermoregulation (Darnell and Munguia, 2011). Because of the risks of injury, however, accurate signaling between conspecifics may still be important in minimising the escalation of contests.

The costs of interspecific or intraspecific interactions as they offset the benefits of resource acquisition have been explored using game theory (Angilletta, 2009; Briffa and Sneddon, 2007; Crowley, 2001; Dugatkin, 1997; Dugatkin et al., 2000; Mitchell and Angilletta, 2009). The general premise is that there are evolutionarily stable strategies (the Nash equilibria) that describe the behavioural phenotypes reflecting the best strategy for a 'rational' individual (i.e. one seeking to maximise its payoff) to 'play' against another individual (Crowley, 2001). These strategies are argued to be 'resistant to invasion by other behavioural strategies', and thus maintained by natural selection (Cronin, 1992). Although detractors of game theory's broader applicability exist (see Siegfried, 2006), evolutionary biologists argue for the utility of this approach in predicting optimal behavioural strategies (Dugatkin et al., 2000). This approach has recently demonstrated that predator habitat selection is based on prey thermoregulatory behaviour (Angilletta, 2009), with the surprising prediction that predators choose temperatures for which they derive no thermal benefit, whereas prey distribute more evenly among available thermal patches (Mitchell and Angilletta, 2009).

To the best of our knowledge, game theory has not been applied in the context of a preferred temperature serving as a resource over which animals compete, particularly in animals that form dominance hierarchies. This question is important when considering ectothermic species, many of which regulate body temperature (T_b) behaviourally (Myhre and Hammel, 1969). In groups of bluegill fish (*Lepomis macrochirus*), dominant members set up territories within their preferred temperature (PT), and subordinates are displaced to areas of lower temperature (Beitinger and Magnuson, 1975; Medvick et al., 1981). The notion that crayfish have a specific PT is not firmly established. Crawshaw (Crawshaw, 1974) observed that crayfish (*Orconectes immunis*) avoid extreme temperatures while moving freely in a wide temperature zone, in what is thought to best represent the optimal thermal range for physiological processes (Fraenkel and Gunn, 1961). Peck (Peck, 1985), however, reported that *Orconectes virilis* select nearly the same temperature when tested and re-tested in a thermal gradient. Interestingly, Peck (Peck, 1985) also observed that although dominant and subordinate crayfish select the same temperature when placed individually in a thermal gradient, dominants and subordinates segregated when placed in the gradient together, with dominants obtaining the PT (in 40% of trials) and subordinates obtaining lower temperatures (Peck, 1985). These observations strongly suggest that crayfish can exhibit a specific PT and that they will compete for it within a thermal gradient.

One disadvantage with using thermal gradients to measure PT is that the animal may simply be exploring the chamber and

experiencing a wide range of temperatures, but not specifically selecting those temperatures. Mean thermal preference and avoided temperatures are more accurately determined using a 'shuttlebox', with two chambers whose temperatures are controlled independently. Movement into one chamber causes temperature to increase, and movement into the other chamber causes temperature to decrease (Cadena and Tattersall, 2009b). The animal shuttles from one chamber to the other; this approach estimates the PT as that chosen behaviourally through operant conditioning, as well as defining the upper and lower avoided temperatures (Barber and Crawford, 1979; Dreisig, 1984; Garrick, 1979).

The aims of the present work were to demonstrate that juvenile crayfish *Procambarus clarkii* (Girard 1852) have a specific PT and to determine whether dominant and subordinate members of crayfish pairs compete for the PT. Our approach was to estimate PT and upper and lower voluntary escape temperatures precisely by placing crayfish individually in a dual chamber shuttlebox and then to allow crayfish to compete for this PT after placing them together in a dual chamber box, with one side held at the PT and the other side either 4°C warmer or 4°C cooler. This approach allowed us to determine whether subordinate crayfish prefer cooler temperatures in the presence of a dominant crayfish, or if they are simply excluded from accessing the PT entirely. We performed these experiments on naive pairs of animals and on pairs whose dominance ranks had been established over a 3-day period. The results indicate that dominant members of established pairs select the PT and displace subordinates to either warmer or cooler 'non-preferred' temperatures (NPTs), but that such differences are not apparent within the first 4 h of pairing.

MATERIALS AND METHODS

Animals and husbandry

Juvenile crayfish (*P. clarkii*) 2.5 to 5.0 cm in length were purchased from Atchafalaya Biological Supply Co. (Raceland, LA, USA). Except for the naive pairs, which were held in solitary containers for at least 2 weeks prior to experimentation, all crayfish were kept in a communal tank. The water was held at 22–23°C, a 12 h:12 h light:dark cycle was maintained, and several plastic (PVC/ABS) pipes (10 to 15 cm in length) were given as shelters. To maintain the water in the tank, an aquarium filter was used in addition to weekly water replacement. Crayfish were fed artificial crabmeat (Imitation crab meat, Aquamore Inc., Rancho Cucamonga, CA, USA) three times per week and were allowed to acclimate for 2 weeks prior to beginning any experiments. No crayfish was used in more than one experiment.

Experimental apparatus

The experimental apparatus used throughout allowed for a dual-choice behavioural design for solitary or paired animals (Table 1). The experimental tank was constructed of Plexiglas® (26.8×10.7×9.0 cm) lined with white contact paper with a height-adjustable central divider to partition the tank into two approximately rectangular chambers (Fig. 1A). To allow access of the crayfish from one chamber to the other, the centre contained a 4.9×4.7 cm opening with a slightly inclined ramp to help commit the crayfish to a given side and to minimise idling between the chambers. At either end of the tank, a smaller compartment (6.4×3.7×14.6 cm) connected *via* numerous perforations to the larger compartments was used to aerate the water (Fig. 1A). Air was vigorously bubbled into the smaller chambers, to act as a mixing compartment, allowing the water in the larger chambers to be constantly aerated without bubble agitation interfering with the behaviour or visual detection of the crayfish.

Table 1. Dual-choice behavioural design, indicating how the shuttlebox chamber was used in the various experimental series

	'Left' side (T_L)	'Right' side (T_R)
Series I: habituation	23°C	23°C
Series II: thermoregulation ^a	$T_R - 4^\circ\text{C}$, ramping at $\pm 0.5^\circ\text{C min}^{-1}$	T_R , ramping at $\pm 0.5^\circ\text{C min}^{-1}$
Series IIIa: naive pairings		
Cold challenge ^b	PT - 4°C	PT
Warm challenge ^b	PT	PT + 4°C
Series IIIb: established pairings		
Constant temperature	23.9°C	23.9°C
Cold challenge ^b	PT - 4°C	PT
Warm challenge ^b	PT	PT + 4°C

^aNote: one temperature (T_L) is always 4°C cooler than the other side (T_R). Direction (+ vs -) of temperature ramp is dictated by the location of the crayfish (+ when on the right side, - when on the left side).

^bSide of PT (preferred temperature from Series II) was randomised with each trial ('left' and 'right' orientation is arbitrary for illustration purposes).

To control the temperature of the chambers independently, a temperature probe was attached at the bottom outer corner of both chambers, opposite from the inflow of water (Fig. 1A). These probes relayed information to a computer. Using custom-built software (ICFish v.2.0, Brock University Electronics Shop, St Catharines, ON, Canada), the set-point temperature for both chambers was changed independently and precisely ($\pm 0.1^\circ\text{C}$). The dual chamber temperature box regulated the water temperature continuously to its set point, which depended on the experimental condition. Constant flow of water was achieved through an inflow of water towards the surface and an outflow of water towards the base of each chamber. Water was pumped from each chamber using a magnetic impeller pump (Eheim model 1046A) through a cooling element consisting of copper coils placed in a water bath containing antifreeze (Lauda Brinkmann RE 106), allowing the water in the system to be cooled. The water then passed through separate 600 W heating elements (modified Black and Decker 5-cup coffee makers), rheostatically powered by the dual chamber temperature box, thereby initiating precise heating when needed. Before returning to the tank, the water passed through flow valves allowing the flow rates to be set accordingly.

Crayfish pairings and agonistic assessments

Prior to assessing competition between two crayfish over temperature, crayfish had to be paired to determine dominance status. Two juvenile crayfish were selected randomly from the communal tank and placed into separate TupperwareTM containers. Body length and mass were measured, after which the crayfish were given unique identifiers using white nail polish. They were placed back into their respective TupperwareTM containers and set aside for 30 min without any distractions. The crayfish then were transferred together into another container where they were allowed to interact while being recorded to video for 30 min. In the case of the naive pairings, this assessment occurred within the dual-choice chamber, immediately preceding the temperature-choice trials (see Series IIIa, below), whereas in the established pairings, the behavioural assessments were followed by 3 days of continuous pairing under laboratory conditions, with one plastic pipe placed in the TupperwareTM container to act as a shelter.

To identify the winner and loser in each pairing, the 30-min video was scored for withdrawals and avoidances, as described previously (May and Mercier, 2006; May and Mercier, 2007). A withdrawal was defined as one crayfish retreating after an encounter involving physical contact, whereas an avoidance was defined as a direction change after a head-on approach without making contact. The crayfish with the lowest number of withdrawals and retreats was

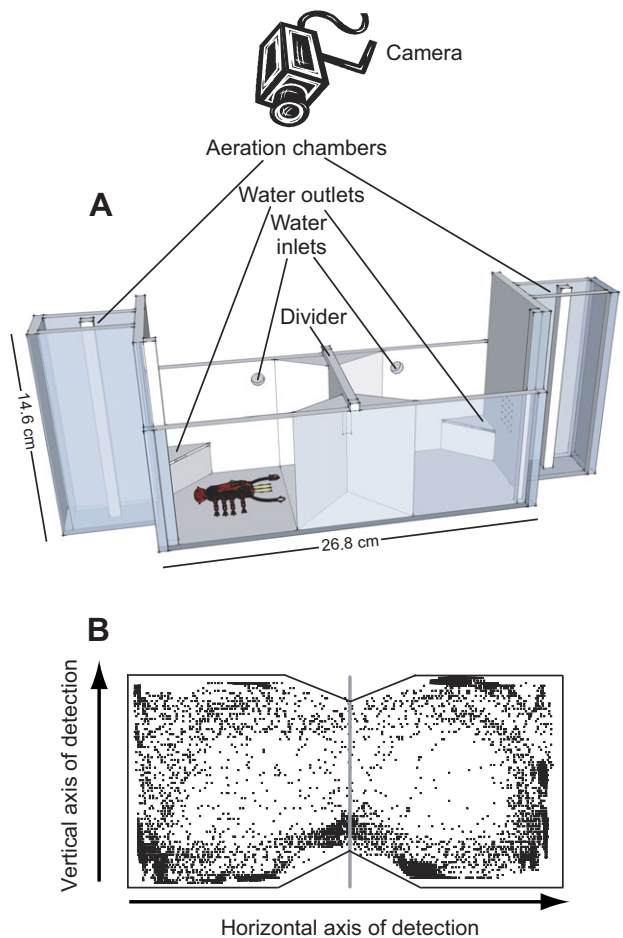


Fig. 1. (A) Schematic drawing of the experimental tank (drawn using Google SketchUp[®] software). The left and right chambers are divided with a partial or full divider depending on the experiment. The tank is lined with white adhesive tape to establish a contrast between the crayfish and the tank. Each end of the tank contains an aeration chamber to separate the continual water bubbling from the crayfish. Water is drawn out of the tank at the bottom sides and circulates back in towards the top of the tank, after passing first through computer-controlled temperature controllers. Each compartment contains a temperature probe to allow for independent, continuous regulation of the temperature of each chamber. When a partial divider is in place, the crayfish is free to move from one side of the chamber to the other (i.e. perform a shuttle). (B) A depiction of the second-by-second detection of crayfish movement within the experimental tank. Individual crayfish were tracked using motion detection software.

termed the winner whereas the other crayfish was termed the loser; a dominance index was calculated as in May and Mercier (May and Mercier, 2006) as the proportion of encounters won divided by the sum of all withdrawals and avoidances. Within the established pairs, to verify that the winner could be equated with dominant status, the crayfish were viewed once daily after being paired to determine which crayfish occupied the shelter. These daily viewings lasted a minimum of 5 min, and in all instances, the winner had gained exclusive access to the shelter, suggesting no rank reversals. Shelter occupancy has been a useful indicator of dominance status in crayfish (Capelli and Hamilton, 1984; Fero and Moore, 2008; Fero et al., 2007; Herberholz et al., 2007; Loring and Hill, 1976; Martin and Moore, 2008; May and Mercier, 2007; Peeke et al., 1995; Ranta and Lindstrom, 1992). In the present study, it was only used as further evidence that we correctly understood the hierarchy. The paired crayfish remained together in the Tupperware™ container for 3 days prior to conducting any experiments on them, and no reversals in dominance status were observed, which is consistent with previous research (May and Mercier, 2006; May and Mercier, 2007).

Experimental procedures

Series I: habituation and side bias trials

To establish the duration of time required for crayfish to become accustomed to the experimental chamber and reduce its exploratory activity to a consistent level (Cadena and Tattersall, 2009a; Cadena and Tattersall, 2009b), a 'habituation' experiment was performed ($N=10$ individuals). This experiment was also used to determine whether a side preference or bias existed in the test tank. Both sides of the experimental tank were set at a constant temperature of 23°C, after which a crayfish was randomly selected from the communal tank and placed into the experimental tank. The initial side placement of the crayfish was counter-balanced, with five of the trials having the crayfish placed in the left side initially and five of the trials having the crayfish placed in the right side initially. Each crayfish was allowed to explore freely both sides of the tank for a total of 4 h.

Series II: PT assessment

To determine the PT for *P. clarkii*, a set of thermoregulation experiments was performed ($N=10$ individuals). The resulting temperature was used as the 'preferred temperature' in the pairing trials (Series III). To determine the PT, the experimental tank was converted into a ramping temperature, dual-choice shuttlebox (see Cadena and Tattersall, 2009b) as follows: first, the crayfish location was detected continuously using custom-built software (Fig. 1B); second, this positional information was translated by the software to allow continuous determination of which side the crayfish occupied; and third, this location information was utilised to initiate the appropriate temperature change in both chambers (positive or negative). When the crayfish occupied the left (arbitrary) side of the tank, the entire system would cool, whereas when the crayfish occupied the right (arbitrary) side, the system would warm. The two sides were always held 4°C apart from one another, thereby continually providing the crayfish the opportunity to select its PT behaviourally. To ensure that lethal temperatures would not be reached, upper and lower temperature maxima/minima were set at 35 and 12°C, respectively. The body temperature was assumed to be the instantaneous temperature of the chamber that the crayfish occupied because of the small size of the crayfish, the high heat capacity of water, and the low temperature ramp speed used (0.5°C min⁻¹).

The initial condition for the PT experiments consisted of a constant temperature of 23°C for both the right and left chambers. A juvenile crayfish was placed randomly into either the left or right side of the choice tank. During an initial 1 h period, the crayfish was allowed to habituate to the tank, moving freely from side to side, after which a 4°C temperature difference was initiated with a temperature ramp speed of 0.5°C min⁻¹ for a subsequent 4 h (experimental duration). Each time the crayfish moved from one side to the other, a shuttle was deemed to have occurred, and the temperature ramping slope was modified. By shuttling from one side to the other, the crayfish was always able to select a temperature that differed by $\pm 4^\circ\text{C}$ from the one currently occupied and/or choose to remain in one chamber while the water temperature slowly rose or fell to the voluntary escape temperatures.

Series IIIa: naive crayfish competition over PT

To evaluate whether dominance status influences competition over temperature, warm and cold temperature challenge experiments were conducted, where the experimental tank was organised with a simple binary choice: PT *versus* warm NPT (warm challenge) or PT *versus* cold NPT (cold challenge). Initially, this was conducted using naive pairs of crayfish, whose winner/loser status had only just been determined. Crayfish were placed randomly into a particular side of the experimental tank at a constant temperature of 23.9°C (the PT determined in Series II). A complete divider separated the crayfish. After a 30-min period to adjust to the initial period of agonistic assessment, a temperature difference was initiated (15 min), such that one side of the tank was held at the PT whereas the other side was held 4°C higher (warm challenge=27.9°C; $N=10$ pairs) or 4°C lower (cold challenge=19.9°C; $N=10$ pairs). Following this 15-min equilibrium period, the divider was removed and the crayfish were allowed to interact for a subsequent 4 h period. They were videotaped at constant temperature for only the first 30 min of this time, for subsequent scoring of winner/loser status, but their presence in either side of the tank was recorded throughout. These experiments were deemed thermal 'challenge' experiments, as the binary choice in temperature was PT *versus* an alternative temperature that was above or below the voluntary escape temperatures.

Series IIIb: established pairs and competition over PT

Crayfish that were assessed for dominance status and subsequently paired for 3 days to reinforce the hierarchy were examined in an identical matter as in Series IIIa, with the following exceptions. First, interactions between crayfish were videotaped 3 days prior to placement in the temperature-choice tank and were not videotaped thereafter. Second, in addition to similar warm ($N=12$ pairs) and cold challenge ($N=12$ pairs) experiments, a constant temperature control experiment was added to assess the interactions between dyadic crayfish behaving at their PT. This control experiment ($N=8$ pairs) consisted of both sides of the choice chamber being held at the PT (23.9°C). In all cases, crayfish were placed into the experimental tank, randomly with respect to side, prior to assessing the time spent in each side of the choice chamber for a similar, 4 h period of interaction.

Image capture and crayfish detection

For experiments involving solitary crayfish (Series I and II), custom-designed motion tracking software (ICFish v.2.0) was used to follow the crayfish (1 frame s⁻¹; Fig. 1B). The white adhesive tape lining the tank allowed for a contrast-based software recognition algorithm to detect the crayfish. The location of the crayfish in Series

II was used by the temperature controller to modify the direction of thermal ramping (positive vs negative). For experiments involving crayfish pairs, focal individual motion tracking was not feasible; therefore, time-lapsed webcam-based image capture (Flix v.2.0, <http://www.nimisis.com/projects/flix.php>) was used throughout the 4 h duration. Images were analysed manually by determining, for each time point, the position of the dominant and subordinate crayfish within their respectively occupied chambers.

Determining crayfish location for every second of the experimental duration allowed us to assess events such as when crayfish began to share a compartment and when crayfish departed from a compartment. As a result, we were able to characterise interactions between crayfish based on which animal initiated sharing chambers and which animal called off this interaction (i.e. retreated); we referred to these as induced retreats. D\S retreats were interactions where the dominant induced the subordinate to retreat to the opposing chamber, and S\D retreats were interactions when the subordinate induced the dominant to retreat to the opposing chamber. The mean number and duration of these induced retreats were assessed for all agonistic trials.

Data analysis

Data (crayfish location, temperature of both chambers) from all experiments were binned at 1-s intervals, allowing the timing that each animal initiated a shuttle from one side of the choice chamber to the other, as well as the proportion of time spent on each side, to be assessed. To determine whether there was a side bias in Series I, a paired *t*-test was performed comparing the total time the crayfish spent in the left *versus* the right chamber. To determine thermoregulatory parameters (Series II), the mean selected temperature was used, obtained from the instantaneous temperature of the occupied chamber. Knowledge of every shuttle event (defined as the departure from one chamber to the adjacent chamber) also allowed for the upper and lower extreme temperatures (UETs and LETs) to be determined. The PT was determined using the mean selected temperatures over the final 3 h, to account for habituation to the available temperature choices. In the Series III experiments, images from every second were observed to determine the side that the dominant and subordinate crayfish occupied throughout the experiment.

Because each animal was not free to behave independently of the other, we elected to minimise multiple statistical analyses by testing only the percent time that each animal occupied the 'preferred temperature' side of the test tank in the final hour. These data were compared across experimental series using a Kruskal–Wallis one-way ANOVA on ranks, and with a Wilcoxon signed rank test to test against a random value of 0. The percent time the animals occupy their PT was compared between social status within an experiment using a Wilcoxon signed rank test. The effective selected temperature (T_{sel}) in the pairing trials was also calculated for the

dominant and subordinate crayfish by using a time-weighted equation as follows:

$$T_{sel} = \frac{\sum_i^n T_i}{n}, \quad (1)$$

where n corresponds to the number of observations and T_i corresponds to the temperature of the occupied compartment at the i th observation, where i is measured in seconds. T_{sel} was compared using a two-way repeated-measures (RM) ANOVA with treatment and social status as factors, and crayfish pair as the subject. Throughout all experimental series, the number of shuttles per hour and/or instantaneous velocity were used to determine the amount of time required for the crayfish to habituate to the experimental conditions (the dominant individual was used as the focal individual when necessary). A two-way RM ANOVA was used with experimental series and time as treatments to test for differences in shuttle frequency. Where appropriate, Holm–Sidak *post hoc* tests (or Dunn's test for ranked data) were used to compare multiple groups following ANOVA tests. An α of 0.05 was used to assess significance. Where normality was violated, the data were ranked and equivalent non-parametric analysis was performed. All data are reported as means \pm s.d. (unless otherwise indicated).

Game theory assessment

We utilised a modified hawk–dove game to describe the equilibrium game (Crowley, 2001), where the cost of fighting is incurred when sharing chambers, and the benefits of temperature are realised based on which chamber is occupied (see Table 2 for parameters). For the purposes of this study, it was not necessary to derive empirical values for all of the parameters; however, the values used for costs of fighting and benefits of temperature, as well as probability of winning contests, are based on previously used values (Crowley, 2001). Two Nash equilibria result from this type of situation (each with one animal achieving PT and the other achieving NPT; Fig. 2). Because there are two equilibria, a mixed strategy outcome can be assessed from this kind of analysis, given the relative costs of fighting and benefits of temperature. The probability that a focal crayfish will choose PT can be calculated from the payoff matrix by optimising the payoff for one individual given the other individual's behaviour (Siegfried, 2006); when there is no temperature difference, the probability of choosing left or right is 0.5, whereas when the temperature difference is 4°C, the probability is at least 0.6 (note: this probability will change, depending on what benefits are used).

RESULTS

Habituation, side bias and overall shuttling behaviour

Animals exhibited no preference for either side of the shuttlebox (Series I); there was no significant difference in the mean time spent

Table 2. Parameters used for estimating a payoff matrix (see Fig. 2) describing the trade-off between conflict resolution and temperature preference for two equivalently sized, non-hierarchical crayfish

Parameter	Value	Constraint
V , thermal gain associated with PT (fitness)	5	$V > W$; the gain from fighting should exceed the risk of injury
T , thermal gain associated with NPT (fitness) ^a	4	$T \leq 0.8V$; gain from NPT is estimated to be 80% of PT gain (Angilletta, 2009)
L , cost of losing a fight (fitness)	2	See Crowley, 2001
W , cost/risk of injury when winning a fight (fitness)	1	Risk of injury is assumed to be similar to losing a fight
p , probability of winning fight	0.5	50% chance of winning a fight with equal-sized opponent

NPT, non-preferred temperature; PT, preferred temperature.

^aIn the case of a constant temperature trial, $T=V$. We assumed that the probability of winning a contest was that of random chance for the simple case.

		Other	
		PT	NPT
Focal	PT	$p(V-W)-(1-p)L$	V
	NPT	T	$p(T-W)-(1-p)L$

		L	R
Focal	L	1	5
	R	5	1

		PT	NPT
Focal	PT	1	5
	NPT	4	0.5

Fig. 2. Proposed payoff matrix (modified from Crowley, 2001) describing the trade-off between the benefits and costs of fighting over a resource (shared space or preferred temperature). (A) Equations for the generalised payoff to a focal animal when played against another animal. See Table 2 for parameter definitions. (B) Estimated payoffs for the symmetrical condition of two animals competing only over space (arbitrarily left vs right) held at the animal's preferred temperature (PT). (C) Estimated payoffs for two animals competing over PT and non-preferred temperature (NPT) based on values from Table 2. In both cases, there is no single predicted outcome; grey shading indicates the predicted Nash equilibria. Based on the estimated fitness parameters (Table 2), the probability of either animal choosing the left or right chamber in B is 50%, whereas the probability of choosing PT is 60%.

on the left side ($55.2 \pm 20.1\%$) compared with the right side ($t_9 = 0.86$; $P = 0.4125$). There was an overall effect of time ($F_{3,195} = 56.1$, $P < 10^{-5}$) and treatment ($F_{6,195} = 4.93$, $P = 0.0001$) on the number of shuttles, but no significant interaction effect ($F_{18,195} = 1.48$, $P = 0.1$) for the Series I experiments. Upon initial placement in the experimental tank, crayfish exhibit a higher activity in the first hour, followed

by an overall decline (Table 3, Fig. 3). Under all experimental series, the number of shuttles was significantly greater during the first hour than in the second, third or fourth hours (Table 3). Significantly more shuttling occurred in the thermoregulation trials than in the constant temperature pairing trials ($P < 0.001$). In addition, the cold challenge in the established pairings also exhibited significantly more shuttling ($P < 0.001$) than the constant temperature pairing experiments (Table 3).

Preferred temperatures

After habituating to the shuttle box at a constant temperature, and following the initiation of the ramping temperature protocol, variability in selected temperature was initially elevated for 1 h (Fig. 3), although PT itself was not significantly influenced by time ($F_{3,27} = 0.831$, $P = 0.49$). There was also a significant effect of time on instantaneous velocity ($F_{3,27} = 11.8$, $P < 0.001$; Fig. 3), with the first hour demonstrating a higher rate of movement. To account for the habituation required to react to the ramping shuttle box, thermal preference was expressed as the average temperature over the final 3 h period (Fig. 4). The mean PT for over this final 3 h period was $23.9 \pm 1.0^\circ\text{C}$ (range 22.2 – 25.8°C). The mean UET and LET over this same time course were 25.9 ± 0.9 and $21.8 \pm 1.0^\circ\text{C}$, respectively.

Agonistic interactions

Establishment of winning and dominance status as determined by the initial 30-min video analysis, yielded dominance indices (pooled across all agonistic trials) of the winning crayfish of 0.935 ± 0.084 and the losing crayfish of 0.065 ± 0.084 , which were significantly different (paired t -test, $t_{51} = 37.5$, $P < 0.001$). Winner status was not influenced by body mass, as the winning crayfish had body masses similar to those of the losing crayfish (pooled across all agonist trials: 3.34 ± 1.22 g vs 3.27 ± 1.28 g; $F_{1,51} = 1.226$, $P = 0.276$). In the established hierarchies, none of these dominance associations reversed (based on which crayfish was observed occupying a shelter in the home cage) during the 3-day socialisation period.

The duration of agonistic behaviours was assessed by examining the duration of bouts when crayfish occupied the same chambers in the agonistic trials (Series III). These contests were classified based on which crayfish entered and which crayfish subsequently exited a particular chamber (Table 4). In the case of the established control trials, these contests all lasted for a similar length of time (21.8 to 28.7 s; Friedman RM ANOVA, $\chi^2_3 = 0.384$, $P = 0.943$). During thermal agonistic challenges, there were distinct differences in the duration of these bouts (interaction between experimental group and direction of retreat, $F_{3,123} = 16.49$, $P < 0.001$; Table 4). Bouts involving contests over PT were significantly longer if the dominant

Table 3. Number of shuttles over time in the habituation, thermoregulation and agonistic pairing trials

	1st hour	2nd hour	3rd hour	4th hour
Series I: habituation	47 ± 19^c	10 ± 8.0	9.8 ± 8.2	6.6 ± 4.9
Series II: thermoregulation ^a	41 ± 32^c	29 ± 28	22 ± 18	31 ± 25
Series IIIa: naive pairing ^b				
Cold challenge	28 ± 18^c	12 ± 13	10 ± 7	8 ± 4
Warm challenge	31 ± 16^c	10 ± 10	12 ± 10	11 ± 11
Series IIIb: established pairing ^b				
Constant temperature	27 ± 19^c	4.0 ± 4.0	4.1 ± 5.1	3.1 ± 3.3
Cold challenge ^a	45 ± 21^c	15 ± 9.8	13 ± 8.1	9.5 ± 6.0
Warm challenge	29 ± 23^c	17 ± 30	14 ± 18	9.0 ± 10

Data are means \pm s.d.

^aSignificant overall difference from the constant temperature trials ($P < 0.001$).

^bFocal crayfish was the dominant.

^cSignificant difference between the first hour and the remaining hours ($P < 0.05$).

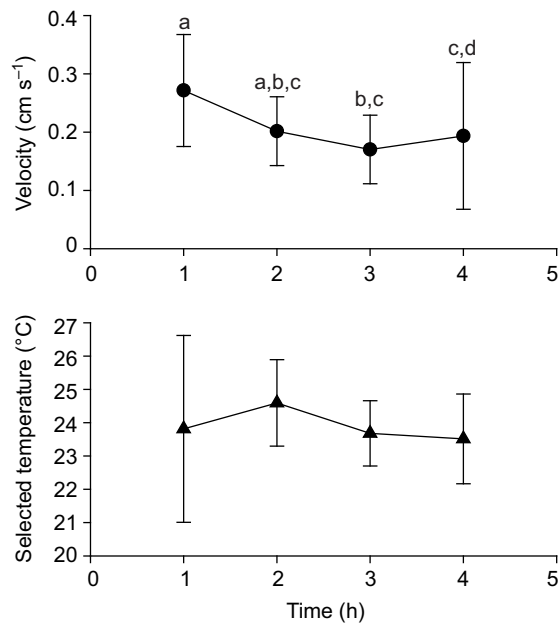


Fig. 3. Mean (\pm s.d.) hourly time course of the (A) instantaneous movement velocity and (B) selected temperature in *Procamburus clarkii* from the thermoregulation trials. Letters depict statistical similarity using Holm–Sidak’s method for *post hoc* testing. Selected temperature was not significantly different across the 4 h time period.

induced retreat of the subordinate (D \ S), whereas disputes where the subordinate induced retreat of the dominant (S \ D) were shorter in duration, and virtually non-existent at the NPT (although dominants did induce retreats from NPT). The actual number of induced retreats was not dependent on social status in the control, constant temperature trials (Fig. 5), whereas dominance status was a significant factor in the thermal agonism experiments (Fig. 6), with the dominant inducing far more retreats than the subordinate (interaction between experimental group and direction of retreat, $F_{1,40}=36.2$, $P<0.001$). There was no influence of experimental treatment (naive *vs* established, cold or warm challenge) on the overall number of induced retreats.

Over the 4 h of examination, crayfish pairs altered their time spent together and apart during their contests over temperature (Fig. 6). In all cases, the percent time spent at NPT was either low (<20%) throughout, or declined throughout the 4 h experiment (to as low as 2%), whereas the percent time spent together at PT was 21–35%. Naive dominant crayfish were unable to gain solitary access to PT, whereas established dominant crayfish achieved almost 50% of their time at PT on their own.

Consequences of thermal choice in the context of agonistic pairings

Providing crayfish with a binary choice in temperature (PT *vs* warm and PT *vs* cold) in the context of competition still resulted in an evident preference for 23.9°C: in trials where a thermal choice existed, crayfish spent a majority of time at the PT (Fig. 7), although this was influenced by rank in established pairs. In the established hierarchies, the percentage time spent at the PT for the dominant crayfish was significantly different from that of the subordinate (established warm, Wilcoxon $T_{0.05(2),12}=6$, $P<0.01$; established cold, Wilcoxon $T_{0.05(2),12}=3$, $P<0.005$), but was only significantly different from the game theory expectation of 60% (see Materials and

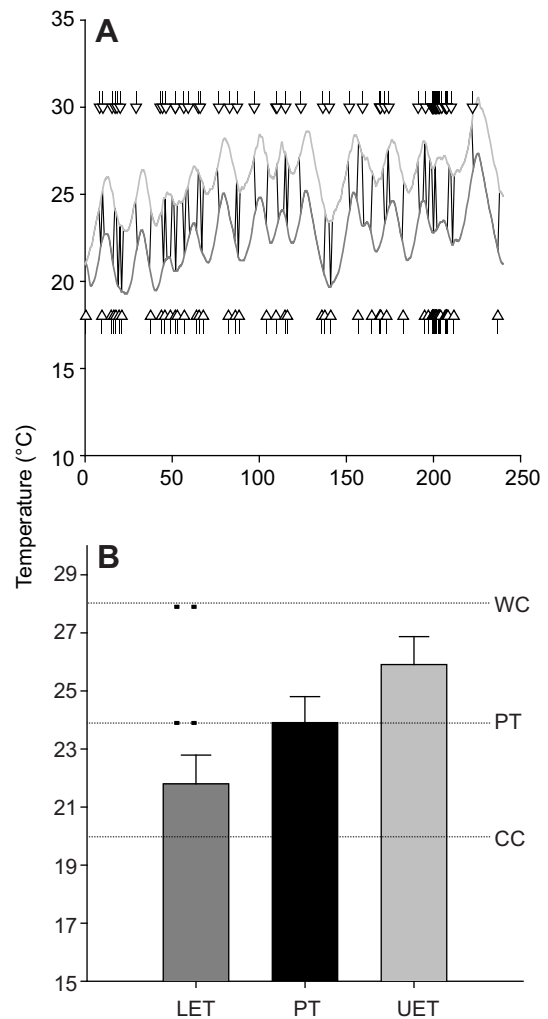


Fig. 4. Behavioural thermoregulation in *P. clarkii*. (A) Sample trace from one individual within a dual-choice, ramping temperature (0.5°C min⁻¹) shuttlebox. The dark grey and light grey lines represent the temperature of the cold and warm chambers, respectively, and the solid line represents the estimated temperature of the crayfish. The upward pointing arrows indicate times when the crayfish shuttled from the lower temperature chamber to the higher temperature chamber, and the downward pointing arrows represent the opposite; these escape times allowed for upper and lower escape temperatures to be determined. (B) Summary of thermoregulatory parameters from all crayfish ($N=10$). Data are mean \pm s.d. values of lower extreme temperature (LET), preferred temperature (PT) and upper extreme temperature (UET) for *P. clarkii*. WC and CC refer to the warm challenge and cold challenge temperatures used in the agonistic encounter trials, which were chosen to be greater than and less than the UET and LET, respectively.

methods) for the established warm crayfish ($t_{11}=2.95$, $P=0.013$ and $t_{11}=1.37$, $P=0.19$). In the control, constant temperature trials, the mean percentage of time the dominant crayfish spent on the left (arbitrary) side was not significantly different from the subordinate (established control: Wilcoxon $T_{0.05(2),8}=9$, $P>0.2$; Fig. 5), nor from game theory predictions ($t_7=0.25$, $P=0.81$). In the naive pairs, however, there was no clear effect of winner/loser status on the percentage time spent at the PT; both winner and loser spent 60–65% of their time at PT (Figs 6, 7). In none of the naive trials was there a difference in the total time spent at PT between winner and loser crayfish (naive warm, Wilcoxon $T_{0.05(2),12}=21$, $P=0.2$; naive cold,

Table 4. Median duration (s) of the bouts of time spent together by dominant (D) and subordinate (S) crayfish under constant temperature conditions (where retreats occur within two chambers, left and right) and under thermal choice situations (preferred vs non-preferred temperature)

	D\S left	D\S right	S\D left	S\D right
Constant temperature	21.8	22.2	24.6	28.7
	D\S PT	D\S NPT	S\D PT	S\D NPT
Overall	47.1	<u>25.0</u>	<u>20.0</u>	0

NPT, non-preferred temperature; PT, preferred temperature. Bouts of time were assessed based on which animal (dominant vs subordinate) initiated contact and which exhibited a retreat (denoted by the arrow). D is used to describe both the dominant (established) and winner (naive) crayfish, and S is used to describe the subordinate (established) and loser (naive) crayfish. Overall refers to the pooled values from the naive and established trials (cold and warm challenge trials combined). Underlined values denote statistical similarity (Holm-Sidak *post hoc* test, $P < 0.05$) within a row.

Wilcoxon $T_{0.05(2),12}=15, P=0.08$), nor from a game theory expectation of 60% ($t_9=0.50, P=0.81$ and $t_9=0.094, P=0.98$).

The effective selected temperature (T_{sel}) within the pairing trials resulted in different thermal outcomes depending on social status (two-way ANOVA interaction effect, $F_{3,40}=3.49, P < 0.024$; Fig. 7). Although treatment had an overall significant effect as well ($F_{3,40}=64.6, P < 0.001$), this was driven by how the design of the warm *versus* cold challenges constrained available temperatures. In terms of social status, however, winner crayfish from the naive pairs did not exhibit statistically distinguishable differences in the resultant temperatures from loser crayfish. Dominant crayfish from the established pairs exhibited T_{sel} values that were significantly

different than those of subordinate crayfish under both warm and cold challenges (Fig. 7).

DISCUSSION

In species where aggressive and/or social interactions occur, the benefits of a higher social rank are clearly seen through agonistic interactions; dominant crayfish have more access to resources such as food and shelter (Herberholz et al., 2007). In the present study, temperature was determined to be a resource over which crayfish will compete, which has not been explicitly demonstrated previously. Under both warm and cold challenge conditions, where crayfish have a choice between a preferred and a non-preferred temperature, the subordinate crayfish occupied the non-preferred temperature for the majority of the time. Interestingly, however, such an outcome of thermal agonism depends on prior establishment of dominance ranks, suggesting that competition for thermal resources depends on the social context. Naively paired crayfish continue to compete for temperature over the 4 h time course of these experiments. Hierarchies established over a 3-day period, however, resolved thermal competition within a 4 h time period, and the dominant crayfish obtained predominant access to the resource.

Because winners and losers are clearly distinguishable as early as 30 min after adult crayfish are paired (May and Mercier, 2006; May and Mercier, 2007), we might have predicted that naive juvenile crayfish pairs would resolve conflicts over temperature and dissociate between the preferred and non-preferred temperatures within the subsequent 4 h period. Our data, however, indicate that even in the fourth hour of pairing, losing crayfish from naive pairs do not withdraw to the chamber with the non-preferred temperature, whereas losing crayfish of established pairs do (Fig. 6). Thus, behaviours of losers must change between the first and third days of continuous pairing. Winners also occupy the preferred temperature for longer times following 3 days of

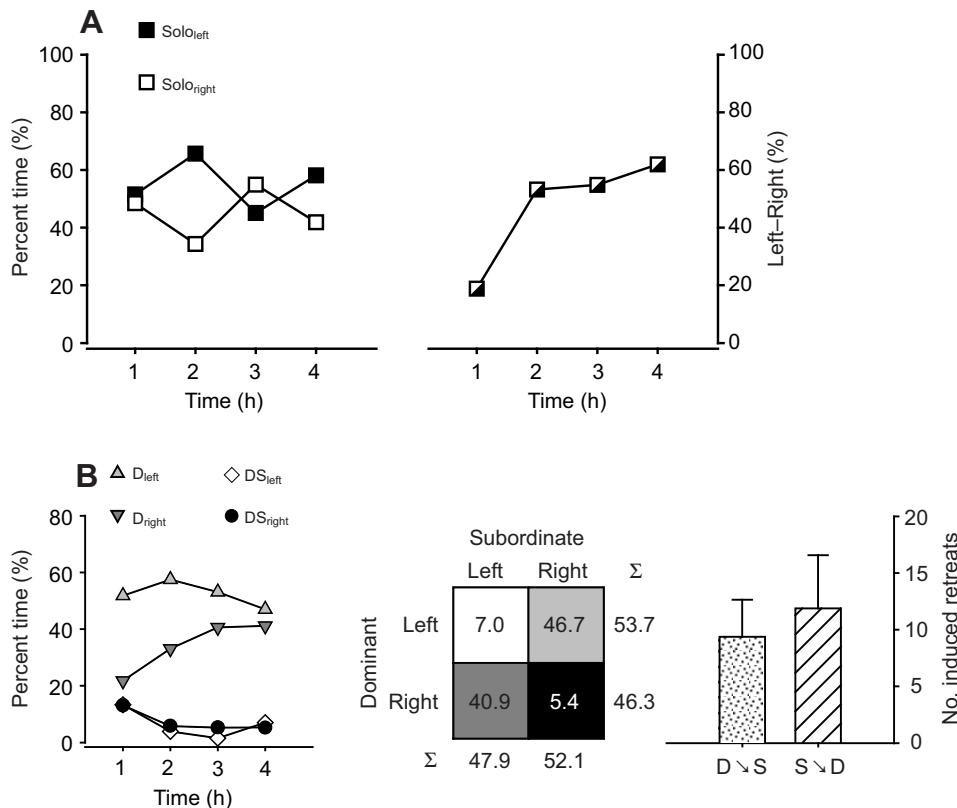


Fig. 5. Outcomes of habituation experiments (A) and constant temperature agonistic trials (B) over the 4 h of observation. The top left plot depicts the results from the habituation experiments where solitary crayfish were studied at constant temperature (23°C), showing the percent time spent on either the left or the right side (note the values are ~50% and sum to 100%). The top right plot depicts the absolute difference (or bias) of individual crayfish during the same 4 h time course (a large difference demonstrates that an individual animal spends more time on an arbitrary side). The bottom three plots depict results from the established control agonistic trials. The lower left plot depicts the changes in percent time over the 4 h experiments, showing the four possible outcomes of either sharing 'left' or 'right', or gaining exclusive access to 'left' or 'right'. The middle plot depicts the outcome matrix (in percent time) for the fourth hour (fill reflects the same points in the time course plots to the left). The lower right plot depicts the number of induced retreats over the entire 4 h experiments. There was no significant difference between the number of retreats induced by the dominant (D\S) compared with those induced by the subordinate (S\D).

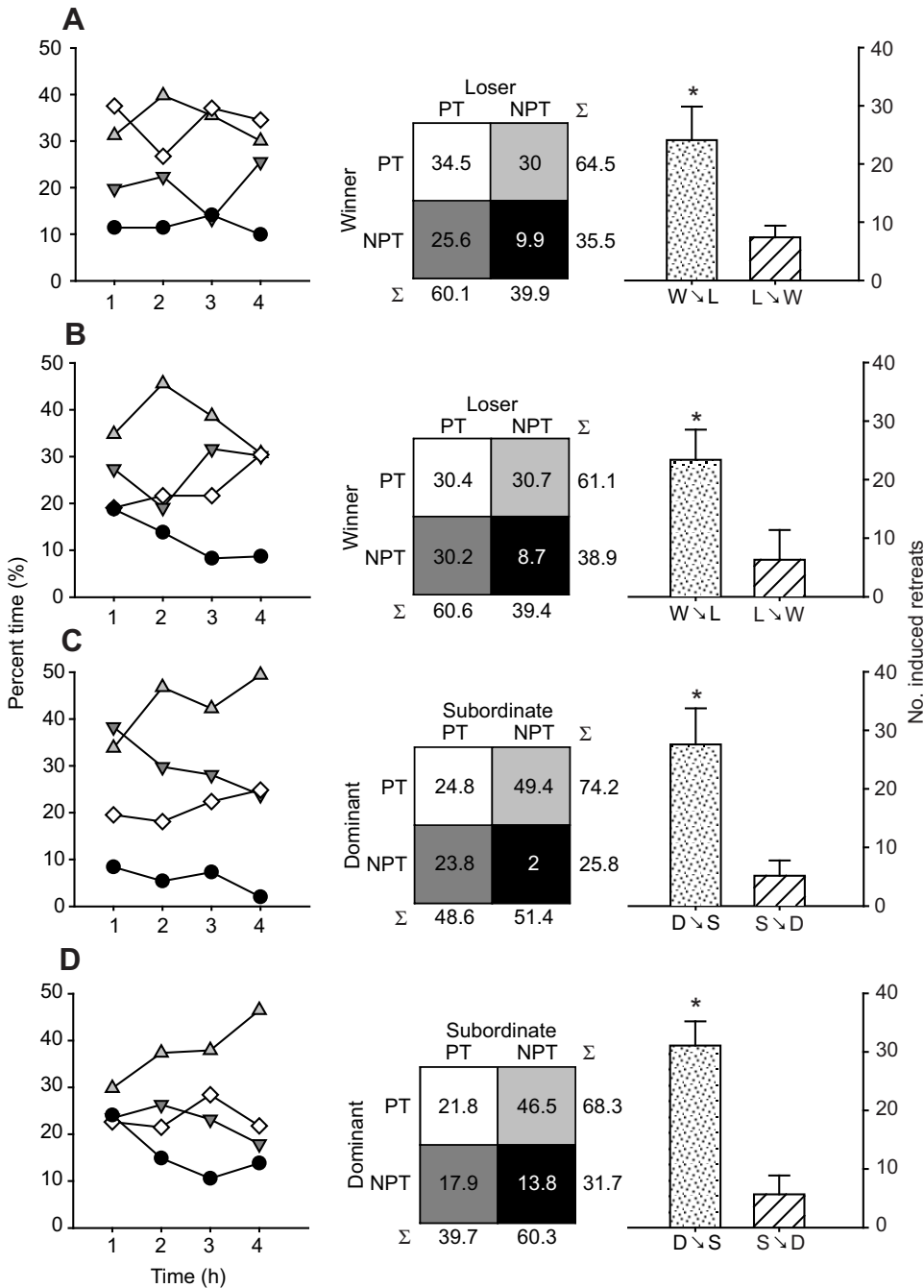


Fig. 6. Outcomes of the agonistic interactions between newly paired (naive) crayfish and established pairs tested under conditions where only binary choices in temperatures (PT vs warm NPT and PT vs cold NPT) were available. Each set of three plots corresponds to a given suite of experiments (Series III): (A) naive warm, (B) naive cold, (C) established warm and (D) established cold. In all cases, the left-hand plots depict the changes in percent time over the 4 h experiments, showing the four possible outcomes of either sharing PT or NPT, or gaining exclusive access to PT or NPT (open diamonds: both animals at PT; light gray triangles: dominant at PT, subordinate at NPT; dark gray triangles: dominant at NPT, subordinate at NPT; filled circles: both animals at NPT). The middle plots depict the outcome matrix (in percent time) for the fourth hour (fill reflects the same points in the time course plots to the left). The right-hand plots depict the number of induced retreats over the entire 4 h experiment. All induced retreats were significantly ($P < 0.001$ as indicated by the asterisk) different from one another, where the dominant/winner crayfish induced more retreats ($D \setminus S$ and $W \setminus L$) than the subordinate/loser crayfish ($S \setminus D$ and $L \setminus W$).

pairing (Fig. 6), which implies that their behaviour may change as well. Thus, our results indicate that behaviours of juvenile crayfish continue to change after ‘winner’ and ‘loser’ status has been determined. This is not surprising, as dominance status is associated with successful resource acquisition (Crowley, 2001). Furthermore, after dominance ranks are established in crayfish, the frequency of agonistic encounters declines rapidly for a few hours but continues to decline slowly for a few days (Huber et al., 2000; Issa et al., 1999). Finally, responses to a reflective environment are remarkably similar between winners and losers after 30 min of pairing, with both members approaching the reflection (a dominant-like behaviour), whereas responses of the losers change over 3 days of continuous pairing to indicate an increase in avoidances rather than approaches (May and Mercier, 2007). Because memory plays an important role in conspecific

recognition (Crowley, 2001), the differences between naive and established pairs may be based on slowly developing neurophysiological differences (Fujimoto et al., 2011; Sneddon et al., 2000).

Thermoregulation in crayfish

In the wild, crayfish are primarily nocturnal but have been found to be active both at night and during the day (Crawshaw, 1974). Nevertheless, during the day, *P. clarkii* move into deeper, cooler water, with mean temperatures of 24.0°C, whereas at night they select water temperatures of up to 26.7°C (Bückle Ramirez et al., 1994). By selecting cooler waters during the day, crayfish lower their metabolic energy expenditure during their normally inactive periods, which presumably are associated with increased predator avoidance in deeper waters (Crawshaw, 1974). In contrast, during

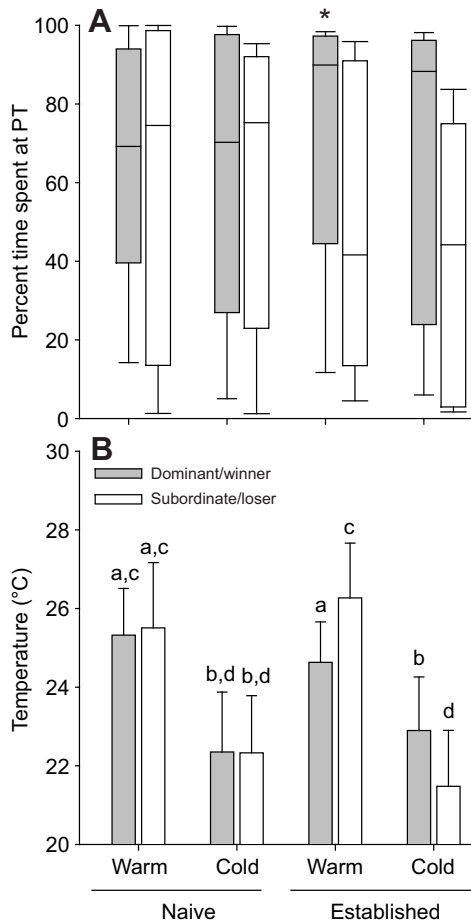


Fig. 7. Summary outcomes from the experiments testing the competition over temperature between dominant/winner and subordinate/loser crayfish. (A) Percentage of time (as box plots showing 5th, 25th, 75th and 95th percentiles, mean and median) the dominant/winner (grey fill) and subordinate/loser (open fill) crayfish spent at the preferred temperature within the agonistic trials (Series III). The asterisk denotes a significantly greater amount of time than game theory expectation (60%; Wilcoxon signed rank test, $P < 0.05$). In the established hierarchies, dominant crayfish spent a significantly greater time at PT compared with subordinate crayfish (see Results for statistical results). (B) Effective selected temperatures (T_{sel} ; mean \pm s.d.) resulting from the pairing trials (naive vs established). Letters denote statistically similar values based on *post hoc* multiple comparisons.

active periods, crayfish select warmer temperatures, increasing metabolic energy expenditure, with a trade-off being an increased predation risk as the crayfish that have burrowed congregate with other crayfish in shallower waters. Thus, it appears that the detection and selection of temperature is an important eco-physiological trait in *P. clarkii* and, presumably, other crayfish (Lagerspetz and Vainio, 2006).

In the present study, we demonstrated that *P. clarkii* learn to thermoregulate behaviourally within a dual-choice shuttlebox. The resulting PT of 23.9°C is very similar to previously published values [23.4°C (Espina et al., 1993)], whereas the symmetric upper and lower escape temperatures (i.e. $\pm 2^\circ\text{C}$ from the PT) support the notion that crayfish actively select and defend a temperature (Bückle Ramirez et al., 1994; Casterlin and Reynolds, 1977; Casterlin and Reynolds, 1980; Crawshaw, 1974; Payette and McGaw, 2003; Taylor, 1984; Taylor, 1990). Furthermore, crayfish required to thermoregulate are far more active than those that are held at a

constant temperature, indicating that temperature selection is a strong motivating factor (Cadena and Tattersall, 2009a; Cadena and Tattersall, 2009b). Indeed, previous research has demonstrated that the crayfish *Orconectes immunis* will also raise their thermal preference upon infection, by as little as 1°C (Casterlin and Reynolds, 1977). This alteration in thermal preference is driven by humoral substrates (prostaglandin E1), because upon injection with the antipyretic, paracetamol, the behavioural rise in thermal preference disappears (Casterlin and Reynolds, 1980). Thus, even subtle changes in temperature are readily detected and carefully regulated in crayfish.

Behavioural response to experimental conditions and binary choices

Based on the rapid decline in the number of shuttles, an initial 1 h period was required to habituate crayfish to changes in the experimental tank, after which they behaved consistently, as Payette and McGaw (Payette and McGaw, 2003) have observed. Because the activity level of the crayfish continually decreased from the first to the fourth hour, but did not cease entirely, the last hour was most representative of conflict resolution in the agonistic trials. In the case of the naive hierarchies, the level of shuttling was similar to that observed in the established hierarchies (Table 3), suggesting that overall movement does not drive the agonistic outcome. During all experiments, the crayfish were initially very active in the experimental tank, and while they explored, they initiated encounters upon coming close to each other. The subsequent reduction in activity may have indicated some conflict resolution, but this is unlikely, because solitary crayfish reduced shuttling to a steady-state level similar to that seen in the agonistic trials. Indeed, only when both crayfish were provided with no conflicting resource (PT in both chambers) did shuttling rate decline to very low levels (Table 3). When faced with a thermal choice, it may be more efficient for the subordinate to tolerate the NPT instead of competing with the dominant crayfish and risking injury.

Interestingly, in the absence of a temperature difference, established crayfish spent little time together, presumably shuttling past one another on those few occasions (Table 4, Fig. 5). Some salient results occurred when a temperature choice was provided to crayfish pairs. For example, there are more interactive bouts resolved by dominant-induced retreat of the subordinate throughout all thermal agonism trials (Fig. 6), suggesting that the dominant drives the social dynamics. One consequence of this is that the dominant will still leave the PT to pursue the subordinate within the opposite chamber (see Table 4), suggesting that territoriality is an important factor in their assessment of temperature as a resource. Because these NPT interactions were relatively brief and similar in duration to those observed in the constant temperature trials, they may simply represent non-aggressive searching behaviour. Interestingly, game theory would predict that two Nash equilibria exist for these types of interactions (where individuals achieve the highest fitness by segregating between the two available choices); the outcome from the constant temperature agonistic trials confirmed this expectation, with nearly complete segregation occurring (Fig. 5). When temperature is a limited resource over which to compete, there is still evidence that segregation occurs, but a substantial (~25–30%) percentage of time with both crayfish occupying the PT chamber occurs. In the naive trials, this outcome was consistent with the game theory predictions, where we estimated that the overall probability of occupying PT would be 0.6. However, established crayfish in the warm and cold challenge trials spent less time together, with the dominant predominantly occupying the PT. On the whole, this

suggests that the crayfish simply alternate the chambers they occupy, driven by the dominant's activity. In the cold challenge conditions, however, both crayfish still spent some time together at the NPT (Fig. 6), which is in sharp contrast to the warm challenge condition where they spend only 2% of time together in the warm, NPT. This result supports the contention of Vickers et al. (Vickers et al., 2011) that the higher fitness costs of warmer temperatures will drive more precise thermoregulatory behaviour, although only the dominant is able to realise this potential.

The naive crayfish, in contrast, appear to show no particular resolution over temperature. We surmise that immediately following our initial scoring of the dominance index, the introduction of new benefits and costs (by adding a temperature choice) alters the context of competition, which may alter the rate of conflict resolution and increase the tendency for dominance reversal over the course of the thermal agonism experiments. Given the close size and novel establishment of the hierarchy in the naive pairs, it is reasonable to expect that a change in resource may alter the outcome. The fact that the 4 h time course responses in the established group follows a resolution-based pattern (Fig. 6), whereas the time course within the naive crayfish demonstrates fluctuations over time, highlights the very different nature of hierarchy establishment. Indeed, the payoff of each behavioural strategy (dominant vs subordinate) must change within the 3 days of hierarchy formation. For example, the dominant crayfish would be more assured of winning agonistic encounters, whereas in the naive pairs, there would still be little physiological difference between the winner and loser crayfish (Fujimoto et al., 2011; May and Mercier, 2007). According to game theory, increasing the probability (or cost) of winning a fight would increase the overall probability of the focal animal choosing the PT, which is consistent with how the established dominant crayfish performs. Overall, this suggests that the payoff of the dominant choosing the PT is higher than that for the subordinate, consistent with the evidence that contests over PT last longer, and that dominant crayfish induced subordinate crayfish to retreat more often. It is unlikely that other components (i.e. V , T and L in Table 2) of the payoff matrix (Fig. 2) change within the 3 days of hierarchy reinforcement, although we need to confirm this with empirical data.

Temperature selection during agonistic interactions

The method of assessing thermal preference in ectotherms can affect the conclusions drawn regarding thermoregulation and behavior (Anderson et al., 2007; Cadena and Tattersall, 2009b). Peck (Peck, 1985) reported that when two crayfish from an established hierarchy were placed in a temperature gradient, the dominant crayfish displaced the subordinate, and rarely did the two crayfish occupy the same section of the gradient. The average temperature difference between the dominant and the subordinate crayfish was up to 6.6°C, showing that the dominant significantly displaced the subordinate (Peck, 1985). There was, however, no constant temperature control trial to verify that space was not driving the behavioural displacement. Whereas the mean temperature for both the dominant and subordinate crayfish when they were alone was approximately 24°C, when placed together, the subordinate was usually displaced to lower temperatures with a mode of approximately 16°C (Peck, 1985). If the PT was the only driving force for agonistic behaviour, then it is expected that the subordinate crayfish would be displaced equally to higher and lower temperatures. Instead, the subordinate was displaced to colder temperatures, which suggests that the subordinate crayfish was competing to select cooler temperatures rather than warmer temperatures, but not necessarily competing over

its PT. It also indicates, however, that the dominant crayfish was competing/defending the higher temperature conditions. In addition, only 41% of the dominant crayfish retained the same temperature preference in pairs compared with when they were tested alone, and in a few trials, the dominant crayfish actually selected higher temperatures (Peck, 1985). Thus, the results from thermal gradient experiments suggest that thermal preferences have a strong social context, and that the presence of conspecifics can profoundly alter attempts to select optimal temperatures behaviourally, even in the absence of overt agonistic behaviours (see Khan et al., 2010).

In the present study, a choice chamber was used containing two compartments, one with the PT and one with an NPT. The aggressive interactions resulted in much simpler spatial segregation. Nevertheless, with binary choices in temperature, dominant crayfish of established pairs are able to achieve effective selected temperatures (24.6 and 22.9°C in the warm and cold challenge trials, respectively) much closer to the PT value (23.9°C) than those achieved by subordinates (26.3 and 21.5°C, respectively). Interestingly, these resultant temperatures demonstrate that subordinate crayfish are relegated to selecting temperatures that are both above and below their UET (25.9°C) and LET (21.8°C), respectively. This suggests that the displacement of the subordinate crayfish is effectively to just beyond the mean voluntary escape temperature, whereas the dominant crayfish remains within the refractory zone for behavioural thermoregulation (Bückle Ramirez et al., 1994; Crawshaw, 1974; Fraenkel and Gunn, 1961). In short, both crayfish are able to regulate temperature, but the subordinate is forced to inhabit NPTs for longer periods of time.

These results have important implications. For many biological processes including enzyme function, metabolic rate and organism development, there is an optimal temperature range (Angilletta, 2009); temperatures above this can have detrimental effects (Taylor, 1990), particularly if metabolic demand outstrips the respiratory capacity, or if animals approach the upper lethal temperatures that are associated with cellular thermal stress. By comparing the thermal optimum for *Procambarus spiculifer* to the optimal growth rate, Taylor (Taylor, 1990) found that in the spring and fall, when temperatures were below the thermal optimum, the growth rate was the highest, whereas in the summer, when the environmental temperatures were above the thermal optimum, the growth rate was at a minimum. Therefore, at higher temperatures, an increased metabolic rate and a higher rate of catabolism results in less energy being available for growth and development. Even though the summer temperatures exceeded the thermal optimum of 23.4°C by only 6°C, the resulting growth rate was minimal, showing that higher temperatures may be more detrimental than lower temperatures.

It would be beneficial to test the present paradigm of PT versus NPT, but at increasingly further differences in temperature. Game theory would predict increased levels of conflict between the two crayfish as the benefit of achieving PT exceeds that of the non-optimal temperature. Although the voluntary UET and LET were within the range of the temperatures of the chambers, by using temperatures that are closer to the upper and lower limits of *P. clarkii* (Payette and McGaw, 2003), it would be possible to explore questions of whether warm temperatures, by virtue of being closer to lethal values, are less 'desirable' than cold temperatures (see Vickers et al., 2011).

Context of thermal agonism

Social compromises related to or constrained by temperature have been studied in numerous other contexts in the past. From trade-offs between oxygen and temperature in amphibians (Noland and Ultsch,

1981; Ultsch et al., 1999), thermoregulation and social aggregation or predator avoidance in reptiles (Khan et al., 2010; Lelievre et al., 2011; Vickers et al., 2011), food and temperature in daphnia (Lampert, 2005; Lampert et al., 2003), to social trade-offs with habitat selection (Hughes and Grand, 2000; Hugie and Dill, 1994; Nocera and Forbes, 2010), animals must make decisions based on minimising the costs of conflict and maximising access to the desired resource. Indeed, Hugie and Dill (Hugie and Dill, 1994) argue that game theory can explain why prey will weigh the risk of predation when making foraging choices, demonstrating that these trade-offs can be used to predict behaviours within the context of conflicting choices.

As a result of the high growth rates, dense populations and the agonistic nature of *P. clarkii* (Barbaresi and Gherardi, 2000), competition for resources in their natural environment is quite fierce. Furthermore, because of the temperature dependency of growth, metabolism and locomotory processes (Croll and Watts, 2004), and the fact that crayfish prefer certain temperatures, competition over specific thermal environments is expected. Indeed, thermal niches in the crayfish's environment might be exploited in nature, or may simply augment agonistic interactions over mutually occurring resources because of the thermal resource's attractiveness. In their natural environment, crayfish compete over resources such as food and shelter, but the relative importance of these resources may change depending on the situation. For example, when shelters are placed within a thermal gradient at temperatures above and below the favourable thermal zone (for the species *Orconectes causeyi*, 14–29°C), the crayfish will not occupy the shelter (Loring and Hill, 1976). However, when the shelter is placed within this zone, the mean selected temperature of the crayfish tracks the location of the shelter. This shows that to a certain extent, crayfish will exhibit a shift in their body temperature in order to obtain the benefits of obtaining a shelter. However, above and below a certain point, the importance of maintaining a favorable temperature is greater than occupying a shelter. Therefore, depending on habitat conditions, certain resources will be favoured, which will help in reinforcing the status of the dominant crayfish.

Conclusions and perspectives

Our results indicate that temperature is a resource over which crayfish will compete for occupancy when exposed to temperatures $\geq 4^\circ\text{C}$ from the preferred condition. In other words, competition for a thermal resource involves holding and securing the resource, much like inhabiting a shelter or holding a food item. It is important to consider temperature as a possible resource (in addition to food and shelter), particularly because of the temperature dependency of ectothermic physiology. Nevertheless, agonistic pair history also determines how temperature is valued as a resource, as naive pairs continue to compete over temperature for 4h whereas established hierarchies exhibit conflict resolution. Our experimental design is well suited for theoretical modeling, and we anticipate that more accurate mathematical models of agonistic interactions can be placed into the context of trade-offs with relevant abiotic constraints for subsequent empirical testing. To help understand the driving forces that influence the competitive context of crayfish in the wild, future research may consider whether other naturally varying environmental parameters, such as dissolved oxygen, carbon dioxide (see Bierbower and Cooper, 2010) or water flow, are also resources over which crayfish will compete.

ACKNOWLEDGEMENTS

We would like to acknowledge Viviana Cadena, Jacob Berman and Qian Long for assistance with experiments, Brock University's Technical Services team for

building the electronic shuttle box, Miriam Richards for guidance with the behavioural assessment, and Mat Vickers for consultation with parts of the analysis.

FUNDING

This research was funded by Discovery Grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to G.J.T. and A.J.M.

REFERENCES

- Anderson, J. L., Albergotti, L., Proulx, S., Peden, C., Huey, R. B. and Phillips, P. C. (2007). Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. *J. Exp. Biol.* **210**, 3107-3116.
- Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.
- Barbaresi, S. and Gherardi, F. (2000). The invasion of the alien crayfish *Procambarus clarkii* in Europe, with particular reference to Italy. *J. Biol. Invas.* **2**, 259-264.
- Barber, B. J. and Crawford, E. C. (1979). Dual threshold control of peripheral temperature in the lizard *Dipsosaurus dorsalis*. *Physiol. Zool.* **52**, 250-263.
- Barnard, C. J. and Burk, T. (1979). Dominance hierarchies and the evolution of "individual recognition". *J. Theor. Biol.* **81**, 65-73.
- Beitinger, T. L. and Magnuson, J. J. (1975). Influence of social rank and size on thermoselection behavior of bluegill (*Lepomis macrochirus*). *J. Fish. Res. Board Can.* **32**, 2133-2136.
- Bergman, D. A., Kozlowski, C. P., McIntyre, J. C., Huber, R., Daws, A. G. and Moore, P. A. (2003). Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. *Behaviour* **140**, 805-825.
- Bierbower, S. M. and Cooper, R. L. (2010). The effects of acute carbon dioxide on behavior and physiology in *Procambarus clarkii*. *J. Exp. Zool. A* **313**, 484-497.
- Bovbjerg, R. V. (1956). Some factors affecting aggressive behavior in crayfish. *Physiol. Zool.* **29**, 127-136.
- Briffa, M. and Sneddon, L. U. (2007). Physiological constraints on contest behaviour. *Funct. Ecol.* **21**, 627-637.
- Bruski, C. A. and Dunham, D. W. (1987). The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I: An analysis of bout dynamics. *Behaviour* **103**, 83-107.
- Bückle Ramirez, L. F., Herrera, F. D., Sandoval, F. S., Sevilla, B. B. and Rodriguez, M. H. (1994). Diel thermoregulation of the crayfish *Procambarus clarkii* (Crustacea, Cambaridae). *J. Therm. Biol.* **19**, 419-422.
- Cadena, V. and Tattersall, G. J. (2009a). Decreased precision contributes to the hypoxic thermoregulatory response in lizards. *J. Exp. Biol.* **212**, 137-144.
- Cadena, V. and Tattersall, G. J. (2009b). The effect of thermal quality on the thermoregulatory behavior of the bearded dragon *Pogona vitticeps*: influences of methodological assessment. *Physiol. Biochem. Zool.* **82**, 203-217.
- Capelli, G. M. and Hamilton, P. A. (1984). Effects of food and shelter on aggressive activity in the crayfish *Orconectes rusticus* (Girard). *J. Crust. Biol.* **4**, 252-260.
- Casterlin, M. E. and Reynolds, W. W. (1977). Behavioral fever in crayfish. *Hydrobiologia* **56**, 99-101.
- Casterlin, M. E. and Reynolds, W. W. (1980). Fever and antipyresis in the crayfish *Cambarus bartoni*. *J. Physiol.* **303**, 417-421.
- Castro, N., Ros, A. F. H., Becker, K. and Oliveira, R. F. (2006). Metabolic costs of aggressive behaviour in the Siamese fighting fish, *Betta splendens*. *Aggressive Behav.* **32**, 474-480.
- Chase, I. D., Bartolomeo, C. and Dugatkin, L. A. (1994). Aggressive interactions and inter-contest interval: how long do winners keep winning? *Anim. Behav.* **48**, 393-400.
- Chase, I. D., Tovey, C., Spangler-Martin, D. and Manfredonia, M. (2002). Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proc. Natl. Acad. Sci. USA* **99**, 5744-5749.
- Copp, N. H. (1986). Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda, Astacidea). *Crustaceana* **51**, 9-24.
- Crawshaw, L. I. (1974). Temperature selection and activity in the crayfish, *Orconectes immunis*. *J. Comp. Physiol.* **95**, 315-322.
- Croll, S. L. and Watts, S. A. (2004). The effect of temperature on feed consumption and nutrient absorption in *Procambarus clarkii* and *Procambarus zonangulus*. *J. World Aquacult. Soc.* **35**, 478-488.
- Cronin, H. (1992). *The Ant and the Peacock: Altruism and Sexual Selection from Darwin to Today*. Cambridge: Press Syndicate of the University of Cambridge.
- Crowley, P. H. (2001). Dangerous games and the emergence of social structure: evolving memory-based strategies for the generalized hawk-dove game. *Behav. Ecol.* **12**, 753-760.
- Darnell, M. Z. and Munguia, P. (2011). Thermoregulation as an alternate function of the sexually dimorphic fiddler crab claw. *Am. Nat.* **178**, 419-428.
- Daws, A. G., Grillis, J., Konzen, K. and Moore, P. A. (2002). Previous experiences alter the outcome of aggressive interactions between males in the crayfish, *Procambarus clarkii*. *Mar. Freshw. Behav. Physiol.* **35**, 139-148.
- Dreisig, H. (1984). Control of body temperature in shuttling ectotherms. *J. Therm. Biol.* **9**, 229-233.
- Dugatkin, L. A. (1997). Winner and loser effects and the structure of dominance hierarchies. *Behav. Ecol.* **8**, 583-587.
- Dugatkin, L. A., Reeve, H. K. and Ebrary Inc. (2000). *Game Theory and Animal Behavior*. Oxford: Oxford University Press.
- Espina, S., Herrera, F. D. and Bückle, L. F. (1993). Preferred and avoided temperatures in the crayfish *Procambarus clarkii* (Decapoda, Cambaridae). *J. Therm. Biol.* **18**, 35-39.
- Fero, K. and Moore, P. A. (2008). Social spacing of crayfish in natural habitats: what role does dominance play? *Behav. Ecol. Sociobiol.* **62**, 1119-1125.
- Fero, K., Simon, J. L., Jourdie, V. and Moore, P. A. (2007). Consequences of social dominance on crayfish resource use. *Behaviour* **144**, 61-82.

- Fraenkel, G. S. and Gunn, D. L. (1961). The orientation of animals: kineses, taxes and compass reactions. *Science* **136**, 707-708.
- Fujimoto, S., Hirata, B. and Nagayama, T. (2011). Dominance hierarchy-dependent behavioural plasticity of crayfish avoidance reactions. *J. Exp. Biol.* **214**, 2718-2723.
- Garrick, L. D. (1979). Lizard thermoregulation: operant responses for heat at different thermal intensities. *Copeia* **1979**, 258-266.
- Graham, M. E. and Herberholz, J. (2009). Stability of dominance relationships in crayfish depends on social context. *Anim. Behav.* **77**, 195-199.
- Hack, M. A. (1997). The energetic costs of fighting in the house cricket, *Acheta domestica* L. *Behav. Ecol.* **8**, 28-36.
- Herberholz, J., Sen, M. M. and Edwards, D. H. (2003). Parallel changes in agonistic and non-agonistic behaviors during dominance hierarchy formation in crayfish. *J. Comp. Physiol. A* **189**, 321-325.
- Herberholz, J., McCurdy, C. and Edwards, D. H. (2007). Direct benefits of social dominance in juvenile crayfish. *Biol. Bull.* **213**, 21-27.
- Hsu, Y. and Wolf, L. L. (1999). The winner and loser effect: integrating multiple experiences. *Anim. Behav.* **57**, 903-910.
- Huber, R., Goessmann, C. and Hemelrijk, C. (2000). The formation and maintenance of crayfish hierarchies: behavioral and self-structuring properties. *Behav. Ecol. Sociobiol.* **48**, 418-428.
- Hughes, N. F. and Grand, T. C. (2000). Physiological ecology meets the ideal-free distribution: predicting the distribution of size-structured fish populations across temperature gradients. *Environ. Biol. Fish.* **59**, 285-298.
- Hugie, D. M. and Dill, L. M. (1994). Fish and game – a game-theoretic approach to habitat selection by predators and prey. *J. Fish Biol.* **45**, 151-169.
- Issa, F. A., Adamson, D. J. and Edwards, D. H. (1999). Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *J. Exp. Biol.* **202**, 3497-3506.
- Khan, J. J., Richardson, J. M. L. and Tattersall, G. J. (2010). Thermoregulation and aggregation in neonatal bearded dragons (*Pogona vitticeps*). *Physiol. Behav.* **100**, 180-186.
- Lagerspetz, K. Y. H. and Vainio, L. A. (2006). Thermal behaviour of crustaceans. *Biol. Rev.* **81**, 237-258.
- Lampert, W. (2005). Vertical distribution of zooplankton: density dependence and evidence for an ideal free distribution with costs. *BMC Biol.* **3**, 10.
- Lampert, W., McCauley, E. and Manly, B. F. J. (2003). Trade-offs in the vertical distribution of zooplankton: ideal free distribution with costs? *Proc. R. Soc. Lond. B* **270**, 765-773.
- Leleuvre, H., Blouin-Demers, G., Pinaud, D., Lisse, H., Bonnet, X. and Lourdaux, O. (2011). Contrasted thermal preferences translate into divergences in habitat use and realized performance in two sympatric snakes. *J. Zool.* **284**, 265-275.
- Loring, M. W. and Hill, L. G. (1976). Temperature selection and shelter utilization of the crayfish *Orconectes causeyi*. *Southwest. Nat.* **21**, 219-226.
- Martin, A. L. and Moore, P. A. (2008). The influence of dominance on shelter preference and eviction rates in the crayfish, *Orconectes rusticus*. *Ethology* **114**, 351-360.
- May, H. Y. and Mercier, A. J. (2006). Responses of crayfish to a reflective environment depend on dominance status. *Can. J. Zool.* **84**, 1104-1111.
- May, H. Y. and Mercier, A. J. (2007). Duration of socialization influences responses to a mirror: responses of dominant and subordinate crayfish diverge with time of pairing. *J. Exp. Biol.* **210**, 4428-4436.
- McDonald, A. L., Heimstra, N. W. and Damkot, D. K. (1968). Social modification of agonistic behaviour in fish. *Anim. Behav.* **16**, 437-441.
- Medvick, P. A., Magnuson, J. J. and Sharr, S. (1981). Behavioral thermoregulation and social interactions of bluegills, *Lepomis macrochirus*. *Copeia* **1981**, 9-13.
- Mitchell, W. A. and Angilletta, M. J. (2009). Thermal games: frequency-dependent models of thermal adaptation. *Funct. Ecol.* **23**, 510-520.
- Mowles, S. L., Cotton, P. A. and Briffa, M. (2010). Whole-organism performance capacity predicts resource-holding potential in the hermit crab *Pagurus bernhardus*. *Anim. Behav.* **80**, 277-282.
- Myhre, K. and Hammel, H. T. (1969). Behavioral regulation of internal temperature in the lizard *Tiliqua scincoides*. *Am. J. Psychol.* **217**, 1490-1495.
- Nocera, J. J. and Forbes, G. J. (2010). Incorporating social information to improve the precision of models of avian habitat use. *Condor* **112**, 235-244.
- Noland, R. and Ullsch, G. R. (1981). The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). *Copeia* **1981**, 645-652.
- Payette, A. L. and McGaw, I. J. (2003). Thermoregulatory behavior of the crayfish *Procambarus clarki* in a burrow environment. *Comp. Biochem. Physiol.* **136A**, 539-556.
- Peck, S. K. (1985). Effects of aggressive interaction on temperature selection by the crayfish *Orconectes virilis*. *Am. Mid. Nat.* **114**, 159-167.
- Peeke, H. V. S., Sippel, J. and Figler, M. H. (1995). Prior residence effects in shelter defense in adult signal crayfish (*Pacifastacus leniusculus* (Dana)) – results in same-sex and mixed-sex dyads. *Crustaceana* **68**, 873-881.
- Pusey, A. E. and Packer, C. (1997). The ecology of relationships. In *Behavioural Ecology: An Evolutionary Approach* (ed. N. B. Davies and J. R. Krebs), pp. 254-283. Oxford: Blackwell Science.
- Ranta, E. and Lindstrom, K. (1992). Power to hold sheltering burrows by juveniles of the signal crayfish, *Pacifastacus leniusculus*. *Ethology* **92**, 217-226.
- Rutherford, P. L., Dunham, D. W. and Allison, V. (1995). Winning agonistic encounters by male crayfish *Orconectes rusticus* (Girard) (Decapoda, Cambaridae) – chela size matters but chela symmetry does not. *Crustaceana* **68**, 526-529.
- Seebacher, F. and Wilson, R. S. (2006). Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*. *Funct. Ecol.* **20**, 1045-1053.
- Seebacher, F. and Wilson, R. S. (2007). Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. *Biol. Lett.* **3**, 471-474.
- Siegfried, T. (2006). *A Beautiful Math: John Nash, Game Theory, and the Modern Quest for a Code of Nature*. Washington, DC: Joseph Henry Press.
- Sneddon, L. U., Taylor, A. C., Huntingford, F. A. and Watson, D. G. (2000). Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenas*. *J. Exp. Biol.* **203**, 537-545.
- Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comp. Biochem. Physiol.* **77A**, 513-517.
- Taylor, R. C. (1990). Crayfish (*Procambarus spiculifer*) growth rate and final thermal preferendum. *J. Therm. Biol.* **15**, 79-81.
- Tierney, A. J., Godleski, M. S. and Massanari, J. R. (2000). Comparative analysis of agonistic behavior in four crayfish species. *J. Crust. Biol.* **20**, 54-66.
- Ullsch, G. R., Reese, S. A., Nie, M., Crim, J. D., Smith, W. H. and LeBerte, C. M. (1999). Influences of temperature and oxygen upon habitat selection by bullfrog tadpoles and three species of freshwater fishes in two Alabama strip mine ponds. *Hydrobiologia* **416**, 149-162.
- van der Dennen, J. and Falger, V. S. E. (1990). *Sociobiology and Conflict: Evolutionary Perspectives on Competition, Cooperation, Violence and Warfare*. London: Chapman and Hall.
- Vickers, M., Manicom, C. and Schwarzkopf, L. (2011). Extending the cost-benefit model of thermoregulation: high-temperature environments. *Am. Nat.* **177**, 452-461.
- Villanelli, F. and Gherardi, F. (1998). Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. *Freshwater Biol.* **40**, 305-315.
- Wilson, R. S., Angilletta, M. J., James, R. S., Navas, C. and Seebacher, F. (2007). Dishonest signals of strength in male slender crayfish (*Cherax dispar*) during agonistic encounters. *Am. Nat.* **170**, 284-291.
- Wilson, R. S., James, R. S., Bywater, C. and Seebacher, F. (2009). Costs and benefits of increased weapon size differ between sexes of the slender crayfish, *Cherax dispar*. *J. Exp. Biol.* **212**, 853-858.