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Impact of an ecological factor on the costs of resource acquisition: fighting and metabolic physiology of crabs

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Summary

1. Current game theory models and recent experimental evidence suggests that the strategy an animal adopts in agonistic encounters is determined by individual state. Therefore manipulation of an individual's state should elicit different behavioural responses. In this paper, mechanisms are examined that underlie state-dependent strategies using Shore Crabs, *Carcinus maenas*, and how, by altering the environment, behaviour and physiology are affected.

2. Fights were staged between pairs of male crabs under normoxic and severely hypoxic (< 15 torr) conditions to determine if the metabolic costs of fighting and resource acquisition are affected by water P_{O_2}. After fighting, blood and tissue samples from each crab were taken and analysed for metabolites associated with anaerobiosis (L-lactate, glucose and glycogen).

3. The spectrum of behavioural acts performed during contests was unaffected by hypoxic conditions. However, fight duration was significantly shorter in the hypoxic treatment.

4. The phenomenon of being of a larger relative size and winning had a greater influence in the contests staged under hypoxia with 93% of the victors being of a larger size compared to 78% in normoxic conditions. Fight duration and intensity had no relationship with relative size in either treatments.

5. The accumulation of L-lactate was significantly greater in the blood and tissues of crabs after fighting under hypoxia than in normoxic conditions. In addition, there was greater glycolytic activity in the tissues of these crabs, shown by elevated concentrations of glucose in the blood and increased breakdown of glycogen.

6. This study demonstrates that the internal state of the crabs altered the length of time they were willing to engage in fighting and that fighting was energetically more expensive under hypoxic conditions.

Key-words: Agonistic behaviour, *Carcinus maenas*, hypoxia, L-lactate, relative size

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Introduction

Predictions from game theory models about agonistic behaviour rest on the assumption that behavioural strategies evolve through selection that maximizes the fitness of an individual. To assess any net change in fitness due to a particular behaviour pattern there must be an understanding of the benefits and costs associated with it (Maynard Smith 1982). However, these costs and benefits will be determined by individual state and new developments have identified state-dependent strategies as important aspects of fights (Cristol 1992; Rodríguez-Gironés, Drummond & Kacelnik 1996). While these state-dependent strategies have been demonstrated, the mechanisms underlying them are not well known. We describe a study, using Shore Crabs as subjects, that identifies such mechanisms.

Crustaceans fight readily in laboratory conditions and have been the subject of a number of studies in aggression. The rules of crustacean contests obey the predictions of game theory for the most part. Clear physiological consequences of fighting behaviour have been identified, such as increased respiration rate, in the Velvet Swimming Crab, *Necora puber* (Smith & Taylor 1993; Thorpe, Taylor & Huntingford 1995).

The Shore Crab, *Carcinus maenas* (L.), the subject of this study is one of the most common decapods found in northwest European rock pools, and exhibits both circatidal and circadian rhythms in locomotion which have been well studied (Edwards 1958; Kitching, Sloane & Ebling 1959; Crothers 1964; Muntz, Ebling

& Kitching 1965; Allen 1966; Dare & Edwards 1981; Reid & Naylor 1993; Aagard *et al*. 1995). Adult crabs are known to inhabit rock pools at night and are regularly exposed to hypoxia or even anoxia during the summer months because of oxygen depletion resulting from the respiration of the pool biota (Edwards 1958; Dare & Edwards 1981; Robertson 1989; Hill, Taylor & Strang 1991). When in these hypoxic pools at night, the crabs have the problem of maintaining oxygen uptake at a rate sufficient to meet their metabolic demands. If the P_{O_2} of the water in the pool falls below the 'critical' P_{O_2} or P_C for this species (≈ 60–80 torr; Taylor 1976) the crabs have to resort to anaerobic metabolism to meet their energy requirements (Burke 1979; Hill *et al*. 1991). In decapod crustaceans such as *C. maenas* it is now well established that the concentration of the end product of anaerobic metabolism, L-lactate, increases within the tissues and haemolymph of crabs in a hypoxic pool environment (Hill *et al*. 1991). Many studies have shown that, after periods of exercise or exposure to hypoxia, the main end product of anaerobic respiration, L-lactate, accumulates in the tissues and haemolymph of crustaceans (Booth, McMahon & Pinder 1982; Zebe 1982; Gäde 1984; Morris & Greenaway 1989; Hill *et al*. 1991). In *C. maenas*, during exposure to periods of severe hypoxia, L-lactate concentrations increase in the haemolymph and tissues due to a total reliance on anaerobic metabolism when the water P_{O_2} is below 20 torr (Burke 1979; Hill *et al*. 1991). However, this crab is able to sustain vigorous activity in hypoxic conditions and can survive up to 12 h in anoxia (Robertson 1989). Other investigations have shown that exercise or exposure to declining oxygen tensions results in the breakdown of glycogen giving elevated concentrations of glucose (Lynch & Webb 1973; van Aardt 1988; Weinstein *et al*. 1988).

Field observations have indicated that *C. maenas* may still engage in agonistic interactions in rock pools at night when conditions have become severely hypoxic (L. U. Sneddon, unpublished observations). Few studies have examined the metabolic costs of agonism in aquatic organisms, but Thorpe *et al*. (1995) investigated the metabolic costs of fighting in the Velvet Swimming Crab, *Necora puber*, and found that fighting under normoxic conditions resulted in an accumulation of L-lactate, but that this was not statistically different from resting values and there were no significant differences between winners and losers in concentrations of key metabolites such as glucose and L-lactate.

The aim of this investigation was to determine the effect of hypoxic conditions on the intensity, duration and outcome of Shore Crab contests and to characterize the physiological consequences of fights in this species. Therefore the implications of the metabolic cost of resource acquisition under extreme environmental conditions will be examined. Fights were therefore staged between pairs of male Shore Crabs under both normoxic and hypoxic conditions; and haemolymph and tissue samples from fought crabs were analysed for L-lactate, glucose and tissue glycogen to determine the metabolic effects of fighting at these two oxygen levels.

Methods

EXPERIMENTAL PROTOCOL

Male Shore Crabs (n = 128) were obtained by creeling in the vicinity of the University Marine Biological Station, Isle of Cumbrae, between the months of April and June. Following transportation to Glasgow, the crabs were housed in individual holding tanks ($18 \times 21 \times 23$ cm^3) supplied with circulating sea water (32–34‰) maintained at 10 ± 1 °C and on a 12:12 h light:dark cycle, with experiments being carried out in the light period. The crabs were not fed for 7 days prior to any experimentation, to reduce the variation in the concentrations of metabolites such as glucose and glycogen that was found among freshly collected crabs in a previous study (Hill 1989). This is a relatively short period of food deprivation since the Shore Crab can withstand 3 months of starvation (Wallace 1973). Crabs were used only if they possessed a full complement of limbs (which had not been recently regenerated), were not covered with excessive epibiotic growth and had no obvious signs of parasitism. The crabs were used within 10 days of capture since muscle condition and metabolic capacity are known to deteriorate in *C. maenas* after 3 weeks of captivity (Houlihan & Mathers 1985). Crabs were kept for 2 weeks after experimentation to ensure that they were not in proecdysis, and none was.

Fights between pairs of crabs were staged in a small tank ($55 \times 28 \times 30$ cm^3) so that the P_{O_2} of the water could be reduced to the required level in a relatively short time. A gas mixture of oxygen, nitrogen and carbon dioxide supplied by a precision gas-mixing system was bubbled through the water via an air stone to reduce the P_{O_2} to the required level. The small percentage of carbon dioxide (0·3%) in the gas mixture was included to maintain a constant water pH of 8·1. Careful adjustment of the flow rate ensured that the P_{O_2} of the water remained relatively constant (15 ± 2 torr) throughout the contests. Bubbling the gas mixture through the water via air stones at opposite ends of the tank caused thorough mixing of the water. To ensure that fights staged under normoxia took place under similar flow conditions, air was bubbled continuously through the water.

Approximately 100 crabs (range 55–80 mm carapace width) were used in this study. Pairs of size matched (carapace width and claw length to ±1%) crabs were transferred separately to a partitioned tank (see Sneddon, Huntingford & Taylor 1997a) in which the water was fully aerated with air and kept at a constant temperature (10 ± 1 °C) for both hypoxic (n = 30)

and normoxic ($n = 20$) contests. Calibrated oxygen electrodes were positioned on the opposite sides from the air stones to measure P_{O_2} in either side of the tank which was always within ± 2% agreement. The crabs were left for a settling period of 1 h during which time the P_{O_2} of the water was reduced to 20 torr. The partition was then carefully raised to minimize disturbance to the crabs. For contests staged under normoxia crabs were left for the same settling period before raising the partition. To promote fights, food extract (Whitebait homogenized in sea water) was slowly injected from a syringe into the middle of the arena via plastic tubing as the partition was raised. Behavioural data were collected by observations made through a small opening in the screening which surrounded the tank and were logged using a laptop computer as an event recorder.

In 14 contests staged in severe hypoxia, crabs were chosen at random from a separate group of crabs and were therefore size mismatched.

BIOCHEMICAL ANALYSES

In 15 of the size-matched contests staged under hypoxic conditions, both crabs were removed immediately with minimal disturbance at the end of a contest and a haemolymph sample taken by piercing the arthrodial membrane at the base of the third pereiopod with a hypodermic needle (21 g) attached to a syringe (1 ml). This was repeated for 15 contests staged under normoxic conditions.

In another five of the size-matched contests that took place under hypoxic conditions, crabs were removed immediately at the end of a contest and immediately immersed in liquid nitrogen to freeze the tissues so that muscle tissue could be removed from the merus of one of the walking legs (the 4th pereiopod). This muscle was chosen as an index of metabolic effects in the tissues since when the crabs fight they stand up on these legs and push against their opponent. Three different tissue types were analysed as part of another study and there are no significant differences between metabolite concentrations in the three tissues (L. U. Sneddon, unpublished observations). This procedure was repeated for five contests staged under normoxic conditions.

Samples of haemolymph and muscle tissue were treated with perchloric acid as outlined in Thorpe *et al*. (1995). The concentration of L-lactate in both tissues was determined using the method of Gutmann & Wahlefeld (1974) with the modification suggested by Engel & Jones (1978). The method used for glucose determination was based on that of Kunst, Draeger & Ziegerhorn (1981). The concentration of glycogen in the muscle tissue was determined using the method of Keppler & Decker (1974) which involved the hydrolysis of the glycosidic bonds of glycogen by 1–4,1–6-amyloglucosidase to release D-glucose which was then assayed using the method of Kunst *et al*. (1981). Full details of these methods can be found in Hill *et al*. (1991). All reagents used to perform metabolite assays were supplied by the Sigma Chemical Co. Ltd (Poole, UK).

Table 1. A comparison of the behaviour of size matched winners (W) and losers (L) to determine the influence of normoxia and hypoxia using Kruskal–Wallis tests

		Median			
Act	W/L	Hypoxia	Normoxia	*H*	*P*-value
To	w	1·0	1·075	1·67	> 0·05
To	l	0·5	0·15	5·84	> 0·05
Away	w	0·0	0·1	0·38	> 0·05
Away	l	1·0	1·0	0·19	> 0·05
Display	w	1·15	1·25	0·94	> 0·05
Display	l	0·93	1·225	4·18	> 0·05
Chelae in	w	0·0	0·5	4·72	> 0·05
Chelae in	l	0·3	0·725	2·77	> 0·05
Contact	w	2·0	2·2	0·08	> 0·05
Contact	l	1·0	0·475	0·97	> 0·05

To = ‘move to’; Away = ‘move away’; Display = ‘Cheliped display’; Chelae in = ‘chelipeds in, body raised’ and Contact = ‘strike’, ‘grasp’, ‘push’ and ‘climb on’; see Sneddon *et al*. 1997a for a detailed description of acts.

Results

BEHAVIOURAL CONTENT AND DURATION

The behavioural data were not normally distributed; therefore non-parametric tests were applied. Kruskal–Wallis tests were used to compare the behaviour of winners and losers separately in normoxic and hypoxic contests. A comparison of the behaviour of winners and losers of contests staged under normoxic and hypoxic conditions is shown in Table 1. Hypoxic conditions do not alter the spectrum of behaviour shown during fights.

Friedman tests were used to compare the behaviour of each pair of winners and losers in hypoxic fights. Comparisons of the behaviour of winners and losers of fights staged under hypoxic conditions showed that there are the same differences in behaviour that were observed during normoxic contests, i.e. winners perform more ‘move to’ ($S = 6{\cdot}4$, $P = 0{\cdot}012$), ‘cheliped display’ ($S = 8{\cdot}0$, $P = 0{\cdot}005$) and contact acts ($S = 9{\cdot}3$, $P = 0{\cdot}002$) whereas losers perform more ‘move away’ ($S = 13{\cdot}0$, $P < 0{\cdot}0001$).

Total duration of fights in normoxia and hypoxia were compared using a *t*-test. The duration of hypoxic contests (mean 193 ± 24 s) was significantly shorter than those staged under normoxia (559 ± 53 s; $t = -5{\cdot}75$, $P \leq 0{\cdot}0001$; Fig. 1). The duration of bouts of wrestling during fights held under hypoxic and normoxic conditions did not differ significantly (60 ± 2·4 s and 63 ± 9·4 s, respectively; $t = 0{\cdot}14$, $P = 0{\cdot}89$).

THE INFLUENCE OF RELATIVE SIZE

The effects of relative size, both carapace width and chela length, on contest initiation and outcome were

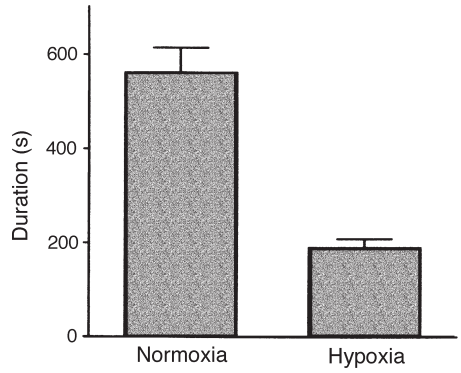

Fig. 1. Mean duration of contests staged between size matched male *Carcinus maenas* under normoxic and severely hypoxic conditions. Error bars are SE.

tested by χ^2. Equal numbers of smaller and larger crabs initiated fights but under hypoxic conditions, the majority of contests were won by larger individuals (Table 2; carapace width, $\chi^2 = 4{\cdot}78, P < 0{\cdot}05$; chela length, $\chi^2 = 8{\cdot}05, P < 0{\cdot}01$) whereas in contests staged under normoxia the results are only significant for chela length ($\chi^2 = 4{\cdot}08$, $P < 0{\cdot}01$) and not carapace width ($\chi^2 = 1{\cdot}6, P > 0{\cdot}05$) with a smaller proportion of larger individuals winning (see Sneddon, Huntingford & Taylor 1997b).

Paired *t*-tests showed that in fights staged under hypoxic conditions, winning crabs have wider carapaces ($t = 4{\cdot}33, P = 0{\cdot}0007$), and greater claw length ($t = 3{\cdot}36$, $P = 0{\cdot}0046$) and claw height ($t = 4{\cdot}51$, $P = 0{\cdot}0005$) as was also the case in contests staged under normoxia (Sneddon *et al*. 1997b).

Regression analysis was used to determine if there was any relationship between relative size, both carapace width and chela length, the duration of hypoxic contests and also wrestle duration (where the two opponents stand on their 4th and 5th pereiopods and push against one another). The relative size of interactants did not influence the duration of hypoxic contests or normoxic contests for either carapace width ($F_{1,12} = 0{\cdot}05$, $P = 0{\cdot}829$; $F_{1,29} = 0{\cdot}24$, $P = 0{\cdot}624$, respectively) or chela length ($F_{1,12} = 0{\cdot}35, P = 0{\cdot}563$; $F_{1,29} = 0{\cdot}07, P = 0{\cdot}792$, respectively). The duration of bouts of wrestling was also unrelated to relative size (carapace width $F_{1,6} = 0{\cdot}5$, $P = 0{\cdot}510$; $F_{1,29} = 2{\cdot}38$, $P = 0{\cdot}135$; chela length $F_{1,6} = 0{\cdot}09$, $P = 0{\cdot}819$; $F_{1,17} = 2{\cdot}12$, $P = 0{\cdot}158$) in contests staged under hypoxia and normoxia, respectively.

Spearman rank coefficients were calculated to examine effects of intensity and water P_{O_2} levels. Contest intensity in hypoxia was unrelated to relative size (carapace width $R_s = 0{\cdot}22$, $P = 0{\cdot}40$; chela length $R_s = 0{\cdot}39$, $P = 0{\cdot}12$; $n = 14$) and to contest duration ($R_s = 0{\cdot}12$, $P = 0{\cdot}66$, $n = 14$) which agrees with the results from normoxic contests obtained in a previous study (carapace width $R_s = -0{\cdot}06$, $P = 0{\cdot}75$; chela length $R_s = -0{\cdot}27, P = 0{\cdot}16, n = 27$; duration $R_s = -0{\cdot}04$, $P = 0{\cdot}87, n = 17$; Sneddon *et al*. 1997b).

PHYSIOLOGICAL CONSEQUENCES

To compare levels of haemolymph metabolites in winners and losers, the value obtained for losers was subtracted from that of winners and since the residuals were not normally distributed, a Kruskal–Wallis test was applied. The concentrations of glucose and L-lactate in the haemolymph were not significantly different between each pair of winners and losers (L-lactate $H = 0{\cdot}18, P = 0{\cdot}674$; glucose $H = 0, P = 1$, $n = 30$ crabs for each treatment; Fig. 2).

The data were transformed to make the residuals normal (ln + 1). ANOVA analysis confirmed that fighting under hypoxic conditions resulted in significantly higher concentrations of L-lactate and glucose in the haemolymph of crabs (L-lactate $F_{1,29} = 1044{\cdot}5$, $P < 0{\cdot}0001$; glucose $F_{1,29} = 1499{\cdot}3, P < 0{\cdot}0001$; Fig. 2). To determine if levels of haemolymph metabolites were affected by contest intensity, the fights were divided into two categories of low intensity (types 2–4) and high intensity (types 5–7) based on their behavioural content (see Sneddon *et al*. 1997a for detailed explanation of contest types). The intensity of contests did not influence metabolite concentrations under either normoxic or hypoxic conditions (L-lactate $F_{1,29} = 0{\cdot}32; P = 0{\cdot}57$; glucose $F_{1,29} = 0{\cdot}00, P = 0{\cdot}96$).

Regression analysis was used to determine any effects of contest duration on levels of both metabolites. It was also found that fight duration was unrelated to the accumulation of L-lactate or to the concentration of glucose (normoxia – winner L-lactate $F_{1,14} = 1{\cdot}56$, $P = 0{\cdot}23$; loser L-lactate $F_{1,14} = 0{\cdot}00$, $P = 0{\cdot}98$; winner glucose $F_{1,14} = 0{\cdot}27$, $P = 0{\cdot}61$; loser glucose $F_{1,14} = 0{\cdot}31, P = 0{\cdot}55$; hypoxia – winner L-lactate $F_{1,14} = 1{\cdot}66, P = 0{\cdot}22$; loser L-lactate $F_{1,14} = 0{\cdot}06$, $P = 0{\cdot}80$; winner glucose $F_{1,14} = 1{\cdot}84$, $P = 0{\cdot}20$; loser glucose $F_{1,14} = 0{\cdot}55, P = 0{\cdot}473$).

The concentrations of glycogen, glucose and L-lactate in leg muscle tissue (merus of the 4th pereiopod) were found to be normally distributed and so ANOVA analysis was used to compare levels of metabolites in winning and losing crabs, and crabs in hypoxic and normoxic conditions. For all three metabolites there was no significant difference

Table 2. The number of smaller and larger crabs initiating size mismatched contests and winning contests under hypoxic conditions. Results are shown for both carapace width ($\chi^2 = 4{\cdot}78, P < 0{\cdot}05$) and chela length ($\chi^2 = 8{\cdot}02, P < 0{\cdot}01$)

	Carapace width		Chela length	
	Smaller	Larger	Smaller	Larger
Hypoxia				
Initiate	6 (43%)	8 (57%)	8 (57%)	6 (43%)
Win	1 (8%)	13 (92%)	1 (8%)	13 (92%)
Normoxia				
Initiate	21 (45%)	26 (55%)	19 (41%)	27 (59%)
Win	15 (32%)	32 (68%)	10 (22%)	36 (78%)

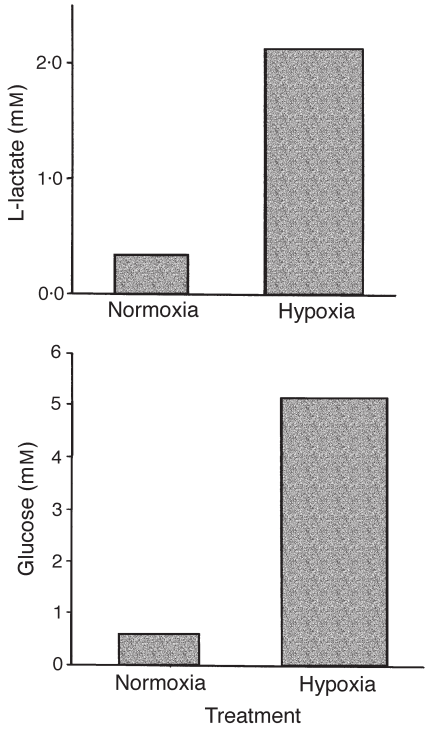

Fig. 2. The concentrations (mM) of L-lactate and glucose in the haemolymph of size matched *Carcinus maenas* after contests staged under normoxic and severely hypoxic conditions. Values are medians.

between winners and losers of each contest (L-lactate $F_{1,9} = 0{\cdot}04$, $P = 0{\cdot}84$; glucose $F_{1,9} = 0{\cdot}44$; $P = 0{\cdot}52$; glycogen $F_{1,9} = 0{\cdot}10$, $P = 0{\cdot}75$). L-lactate ($F_{1,9} = 158{\cdot}7$, $P < 0{\cdot}0001$) and glucose ($F_{1,9} = 38{\cdot}42$, $P < 0{\cdot}001$) concentrations were significantly higher in crabs after fighting in hypoxia (Fig. 3; $n = 10$ animals for each treatment). Glycogen levels were significantly lower in the crabs after fighting in the hypoxic conditions than in normoxia ($F_{1,9} = 576{\cdot}5$, $P < 0{\cdot}0001$, Fig. 3).

P-values were adjusted throughout for multiple testing as appropriate (Zar 1984).

Discussion

The behavioural content of fights between pairs of male Shore Crabs was unaffected by severe hypoxia. Fight duration was significantly shorter in hypoxic contests and it appeared as if the effect of having a larger relative size and being victorious was amplified in hypoxic contests. As in contests staged in normoxia, contest duration and intensity were unrelated to relative size (Sneddon *et al.* 1997b).

Hypoxic conditions such as those used in this study ($P_{O_2} < 20$ torr), which regularly occur in the field, resulted in *C. maenas* having to resort to anaerobic respiration to meet energy demands. Anaerobiosis is a very inefficient way of producing energy with more glycogen molecules being broken down for each molecule of energy produced compared with aerobic respiration. Crabs are normally quiescent under hypoxic conditions in order to conserve energy (Taylor & Spicer 1988). Therefore a low oxygen environment amplifies the costs of fighting as is shown by increased accumulation of L-lactate in the crab blood and tissues which may represent a constraint on contest duration. Thus, the agonistic interactions in severe hypoxia were of much shorter duration than those staged under normoxia, where oxygen was readily available for aerobic energy production. These results show that the internal state of the animal has altered the length of time the crabs were willing to engage one another. However, fighting over the perceived resource (food extract) in these experiments is clearly a high priority and hypoxic conditions did not stop the animals from behaving agonistically. Further investigation

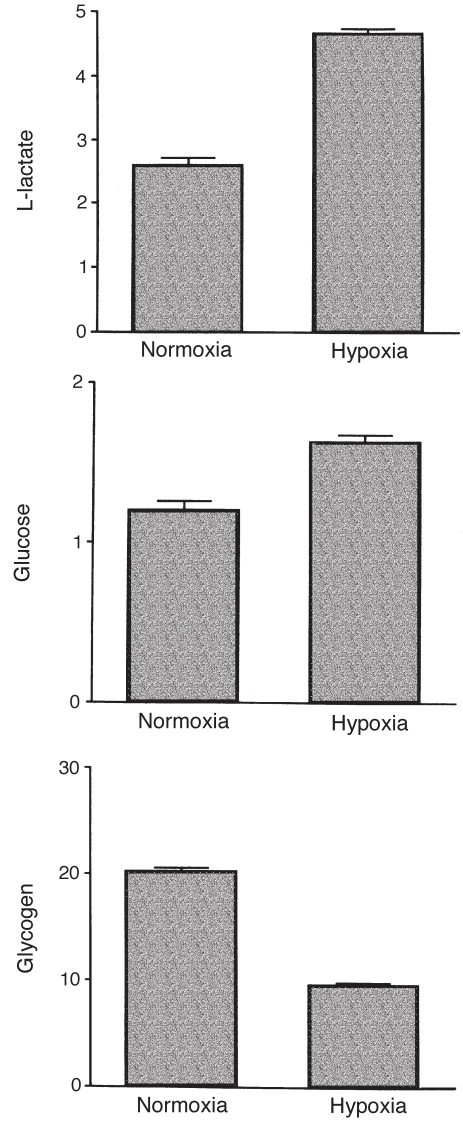

Fig. 3. The mean concentrations (μmol g^{-1}) of L-lactate and glucose in the leg muscle tissue (4th pereiopod) of size matched *Carcinus maenas* after contests staged under normoxic and severely hypoxic conditions. Error bars are SE.

of the metabolic effects of fighting is required to determine if L-lactate is a constraining factor in these contests. The tissue data show that in fights staged under hypoxia, fighting was sufficiently energetically demanding to cause more glycogen to be converted into L-lactate during glycolysis than during fights under normoxic conditions. This would suggest that the costs of fighting are greater when the environment is hypoxic and thus an animal may be metabolically limited in the time that it can perform aggressive behaviour, with only the fittest individuals able to win fights. This is perhaps why there was only one case during this study in which a smaller crab won a contest. The tissue results are taken from one set of muscles but for future analysis, to obtain a true picture of energetic costs of fighting, the measurement of oxygen consumption and whole body metabolite concentrations would be highly desirable.

Under hypoxia, the vast majority of contests (93%) were won by the larger opponent with only one instance in which a smaller individual won. In comparison, only 78% of fights were won by larger individuals in contests staged in normoxia (Sneddon *et al*. 1997b). A possible explanation for this is that smaller crabs may have a reduced anaerobic capacity or are less tolerant of anaerobic end products than larger crabs. Houlihan, Mathers & El Haj (1984) suggested that large specimens of *C. maenas* have a greater anaerobic scope than smaller conspecifics based on an investigation of walking performance. Perhaps during severe hypoxia metabolic costs are higher for small crabs and therefore the benefits of fighting are exceeded by the costs and thus they do not pursue escalated fighting against larger opponents. An increase in anaerobic capacity with increasing body size has been demonstrated in other animals (e.g. Brown Trout, *Salvelinus fontinalis*, Kieffer *et al*. 1996, and Rainbow Trout, *Oncorhynchus mykiss*, Ferguson, Kieffer & Tufts 1993).

Fighting in normal oxygen conditions for *C. maenas* is sufficiently energetically demanding to force the crabs to resort to anaerobic respiration, but not as demanding as exhaustive exercise on a treadmill (L. U. Sneddon, unpublished observations). This suggests that, under normoxic conditions, the duration of contests was not constrained by metabolic factors and this is perhaps why there was no relationship between the duration of contests and L-lactate accumulation. Fights staged under severe hypoxia were significantly shorter in duration and the crabs showed increased concentrations of L-lactate but, again there was no relationship between L-lactate concentrations and fight duration. This implies that L-lactate build-up may act as a physiological constraint that limits the crabs to a few minutes of activity. An alternative hypothesis is that if concentrations after fighting are lower than after exercise, it may be that the crabs decide to de-escalate the fights since the cost of fighting for long periods in severe hypoxia may exceed the benefits. Therefore the strategy the crabs adopt is dependent upon internal state.

Concentrations of haemolymph metabolites did not differ between winners and losers so there is no extra energy demand for winning or losing crabs. This agrees with the results of studies on the respiratory and metabolic costs of fighting in *Necora puber* in which respiration rates and L-lactate concentrations did not differ between the two categories of crabs (Smith & Taylor 1993; Thorpe *et al*. 1995). However, in *N. puber* there are no behavioural differences between contestants until the final stages of a fight (Thorpe, Huntingford & Taylor 1994) whereas in *C. maenas*, the difference in behaviour of winners and losers is evident from an early stage (Sneddon *et al*. 1997a). It would be expected therefore that there would be a difference in energy requirements in *C. maenas* between winning and losing crabs similar from those demonstrated in other animals such as the House Cricket, *Achetus domesticus* (Hack 1997a).

It can be inferred from the similar metabolite profiles of winners and losers in normoxia that these variables do not act as a cue causing the eventual loser to behave submissively, as has been described for the Crayfish, *Cherax destructor* (Head & Baldwin 1986), or that energy expenditure is an important cost which influences contest strategies as in the House Cricket (Hack 1997b). Therefore even though winners perform a larger repertoire and more escalated acts than losers, there does not appear to be a greater energy expenditure for winners as reflected by a greater increase in anaerobic respiration.

This study has raised many questions regarding the effects of hypoxia on the agonistic behaviour of the Shore Crab. It would be interesting to see at what point the duration of contests starts to decline with decreasing water P_{O_2} and what role the physiology of the crab plays in the interactions by determining if L-lactate accumulation is a metabolic constraint. This would involve a comparison of the concentrations in crabs at rest and accumulating after fights or after strenuous exercise. There may also be longer term costs since this metabolic debt has to be paid back. It has been shown that it may take between 8 and 24 h for L-lactate concentrations to return to resting levels after exposure to hypoxia (Hill *et al*. 1991) and this may restrict the types of behaviour the crabs can perform if their metabolism is compromised.

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