The Physiological Effects of Agonistic Behaviour in the Shore Crab, *Carcinus maenas* (L.)

by

Lynne Ure Sneddon B.Sc. (Liverpool)

Division of Environmental and Evolutionary Biology

Institute of Biomedical and Life Sciences

University of Glasgow

A thesis submitted for the degree of Doctor of Philosophy at the Faculty of Science at the University of Glasgow

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

For my parents,

William and Katherine Sneddon

ACKNOWLEDGEMENTS

Many thanks to my supervisors, Felicity Huntingford and Alan Taylor, who have been outstanding in their advice and encouragement during the course of this work.

Thanks to Dr James Currall of Computer Services for help with the statistics and to Dr John P. Swaddle for help with the surface area measurements described in Chapter 3. The strain gauge used in Chapter 4 was developed in collaboration with Dr J.F. Orr of the School of Engineering, The Queen's University of Belfast. Thanks to Dr Dai Roberts and Prof. R.W. Elwood, who enabled me to make a preliminary visit to Queen's University of Belfast and to Mr Alex Torrey who calibrated the strain gauge in Glasgow. Many thanks to Dr Jim Atkinson and the staff and students at the Universities Marine Biological Station, Millport for the use of their facilities during the course of my field work. I am grateful to Dr Tony Clare of the Marine Biological Association for enabling me to obtain a Spooner Bursary to go and work at Plymouth Marine Laboratory. Thanks to Dr Dave Watson at the University of Strathclyde for teaching me biogenic amine analysis. Thanks to Dr Dave Thompson who taught me fat analysis using the soxhelet. Thanks to Prof. Marco Vannini for interesting discussions and the opportunity to visit Florence.

Thanks to all the people who have helped me in anyway, especially June Freel, Graham Adam, Alan McGregor (assistance with the treadmill construction), John Lawrie (tended to my crabs), Willie Orr, Catherine McLagan, Liz Denton, Kate Orr, Dr Ian Morgan, Brenda McPherson, Davie Watson and James Carroll with his quivering tash. Thanks must also go to the academic staff and students who provided useful comments on my work especially Dr Ian Barber, Dr Katie Gilmour, Dr Neil Metcalfe, Dr Ian Morgan and Dr John Swaddle.

I must thank past and current office mates for keeping me entertained:- Kath Sloman, Jason Hall-Spencer, Paul Parslow-Williams, Murray

Roberts, Francesca Marubini and Tony Johns. I also would like to thank all the postgrads who went on big nights out with me and who shared my many flats especially:- Mat Cottam, Scot Ramsay (fellow Falkirkian), Dave Fraser, Grant Stentiford, Mike Kennedy, Dave Stevens, Kirstine O'Conner, Alexandra Hay, Kenny Leung and many others who shall remain anonymous. Special thanks to Scot Ramsay and Kenny Leung who helped me move into my third floor flat when no one else would.

I would like to say a huge thank you to Stuart Fleming for his love and support over what have been, a trying last six months. I would also like to thank Amy Spiller, my twin, for being a true friend and for sharing hilarity and drinking binges. Thanks also to Claire Waluda and John Swaddle for their friendship and innumerable e-mails.

Finally, I would like to express my love and gratitude to my parents for their support, both emotional and financial, and for always being there for me.

The work described in this thesis was funded by a studenship from the Natural Environmental Research Council (GT4/95/110), to who I am grateful.

DECLARATION

I declare that this thesis is my own composition and that the research described herein was performed entirely by myself except where expressly stated.

Lynne U. Sneddon

June 1998

Table of Contents

AGONISTIC BEHAVIOUR IN SHORE CRABS, *CARCINUS MAENAS* (L.): METABOLIC CONSEQUENCES OF VARIABLE OXYGEN TENSIONS.

SUMMARY

The effects of altering resource value on agonistic behaviour were investigated by staging fights between pairs of male shore crabs, *Carcinus maenas* (L.) in the presence and absence of food. The nature of the agonistic behaviour of *Carcinus maenas* is described. The behaviour of winners and losers appears to be different from an early stage in the fights. Contests do not begin with initial display followed by a progressive increase in intensity. The fights become very intense almost immediately at the start of a bout and display acts are performed throughout the fights. As expected, there appears to be an increase in intensity in the contests with food. The duration of fights is highly variable but is significantly lower for contests when food is present than in contests without food (mean fight length 559 ± S.E. 53 and 1143 ± S.E. 135 seconds respectively). The results are discussed in relation to current predictions from game theory models.

Relative body size (carapace width) and weapon size (chela length) were used as indicators of resource holding potential (RHP) in the agonistic behaviour of male shore crabs, *Carcinus maenas* (L.). Weapon size was found to be a more reliable predictor of the outcome of pairwise fights than body size. Crabs with longer chelae than their opponents were more likely to win fights than crabs with relatively larger bodies. Body size had less influence on the outcome of fights. Relative body and weapon size did not influence initiation of contests but did have an

advantage with respect to winning however, this was significant only for weapon size. Winning crabs had heavier claws with greater surface area than losing crabs. There was no relationship between relative size and fight duration. The frequency of cheliped display increased with chela length and winners performed significantly more displays than losers.

Weapon size has been shown to be a better predictor of fight outcome than body size in the shore crab, *Carcinus maenas*. However, when the weapon size disparity is small between two opponents, it is still difficult to predict the victor. The role of weapon strength in pairwise fights between male shore crabs was investigated, to determine if relative force influences contest content, duration and outcome. Weapon strength was ascertained using a force transducer on live crabs, then fights between crabs were staged between size matched males. Winning crabs had major (crusher) claws and minor (cutter) claws which exerted a significantly greater force than losing crabs even when claw length was the same. Winners and losers were matched for body size and claw length but not claw height, or claw length to the dactyl, or dactyl length. Winners had greater claw height and claw length to the dactyl in the major claw giving them a mechanical advantage when closing the claw and thus exerting a greater force. The forces exerted by the major and minor claws were analysed for any relationship between force and morphological measurements. Winning crabs appear to be fitter in having a better claw structure which exerts a greater force and are more successful in agonistic interactions.

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 study, we also examine mechanisms which underlie state-dependent strategies using shore crabs, *Carcinus maenas* and how, by altering the environment, behaviour and physiology is affected. 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 Po_2. After fighting, blood and tissue samples from each crab were taken and analysed for metabolites associated with anaerobiosis (L-lactate, glucose and glycogen). The spectrum of behavioural acts performed during contests was unaffected by hypoxic conditions. However, fight duration was significantly shorter in the hypoxic treatment. 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. 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. 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.

The energetic consequences of fighting, which can be an important factor shaping contest strategy and duration, are not fixed and may depend on environmental conditions. Energy expenditure is costly to fitness because it depletes reserves which otherwise could have been allocated to reproduction, and metabolites are produced that may constrain subsequent activities. The variation in metabolic consequences of fighting was examined in relation to an ecological variable, namely hypoxia. Contests were staged between pairs of size matched male shore crabs, *Carcinus maenas* (L.) under a range of oxygen tensions (between 10 and 100 % oxygen saturation) which crabs experience in their natural habitat. Behaviour was recorded and concentrations of key haemolymph (L-lactate and glucose) and tissue (L-lactate, glucose and glycogen) metabolites associated with anaerobiosis were determined. Concentrations of the key metabolites in fought crabs were compared with those from two extremes of activity i.e. crabs at rest and after exercising to exhaustion, under normoxic (Po_2 = 154 Torr) and severely hypoxic (Po_2 = 15 Torr) conditions. Fighting under both conditions resulted in significantly elevated concentrations of haemolymph metabolites compared to crabs at rest. However, these metabolites were much lower than values obtained from exercised crabs. Significant differences in glycogen concentrations were only shown under hypoxic conditions where glycogen stores were reduced in crabs after fighting and this reduction was similar to values

obtained after exercise. Duration of contests declined significantly when they were staged below 50 Torr (~30% normoxia). As water oxygen tensions were reduced, concentrations of L-lactate and glucose increased in the blood and tissues of fought crabs whilst glycogen stores were significantly reduced. Fight duration declined when crabs were exposed to severe hypoxia (15 Torr) for increasing lengths of time and there was also an increase in blood L-lactate concentrations. The results suggest that as fights progress, crabs experience an increasing metabolic debt, in the form of accumulation of L-lactate and a reduction in energy stores, that is amplified by hypoxic conditions.

To bridge the gap between field and laboratory studies in the agonistic behaviour of the shore crab, *Carcinus maenas* (L.) the physiological status of crabs after fighting in the field and the laboratory was determined. The behavioural aspects of fights and the physiological consequences were examined using established laboratory techniques and a specially-developed field system in which controlled experimentation was possible. The metabolic consequences of aggressive behaviour were assessed by analysis of key metabolites (L-lactate, glucose and glycogen) associated with anaerobiosis in pairs of fought male crabs in the laboratory and in the field. Contests were staged in differing levels of water Po_2 since shore crabs regularly experience hypoxia in the field. These experiments demonstrated that the behavioural acts performed by fighting shore crabs were the same in the field as those seen in the laboratory. There was also a similar influence of being a larger relative size and winning in the field

and the laboratory. Hypoxic conditions in the field resulted in shorter contest durations and increased concentrations of L-lactate and glucose and a reduction in glycogen in the blood and tissues of fought crabs, when compared with contests staged under normoxic conditions in the field. However, contest duration in the field was much shorter than under laboratory conditions and, unlike the laboratory results, there were no differences in metabolite concentrations between fought crabs and crabs which had not fought.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Why fight ?

Aggression is necessary in most species for the acquisition of resources such as food, shelter and mates and is, therefore, an important component of an animal's behavioural repertoire. Competitive ability will have a great effect on an animal's fitness since, if it can outcompete conspecifics for essential resources, then it will be a more successful, fitter individual (Davies & Krebs 1978). When resources are of limited availability, theory predicts that they should be contested intraspecifically by means of agonistic behaviour or fighting (Huntingford & Turner 1987). Agonistic behaviour between individuals will result in one gaining immediate or future access to a resource at the expense of others. Therefore, agonistic interactions may result in a potential increase in fitness through gaining the resource or "benefits" of fighting, but there may also be an associated decrease in fitness or "costs" of fighting. The net change in an individual's fitness is, therefore, the sum of these costs and benefits, and this idea is central to current ecological theory (Davies & Krebs 1978; Maynard Smith 1982).

1.2 Agonistic behaviour and game theory

Considerations of the costs and benefits of agonistic behaviour has led to the use of game theory to develop an explanation for the non-injurious resolution of agonistic interactions in species that are capable of injuring each other (Maynard Smith & Price 1973). Game theory, originally

developed by economists to analyse human decisions in situations involving a conflict of interests, has become a major theoretical framework on which many studies concerning contest behaviour are tested (Maynard Smith 1982). When applied to animal behaviour, an analogy is drawn between human choice of optimum strategy and natural selection acting on genetically determined behavioural phenotypes. Competition for resources is viewed as a game, in which individual animals are players adopting particular strategies. A strategy is a specification of what an animal will do in particular circumstances and results in a pay-off in terms of fitness, that is related to the probability of acquiring the resource and the costs of competing for it (Maynard Smith 1982). The concept of an evolutionary stable strategy or ESS is central to the application of game theory to animal behaviour (Maynard Smith & Price 1973). An ESS is a strategy which, when adopted by the majority of a population, has a higher average pay-off when played against itself and other strategies than the pay-off to any other strategy played against it.

Maynard Smith & Price (1973) used simple models of animal contests to show how individual selection could result in evolutionary stable strategies specifying that, under certain circumstances, individuals should withdraw from their opponents without risking injury. They also predicted that, where the value of the resource was less than the cost of injury, "pure" strategies that specified only one type of contest behaviour would not be evolutionary stable, whereas "mixed" strategies could be. A "mixed" strategy specifies the probabilities with which two or more pure

strategies are played in a population. Subsequent theoretical studies have developed game theory models of agonistic behaviour to take account of asymmetries between contestants (Parker 1974; Maynard Smith & Parker 1976; Hammerstein & Parker 1982; Leimar & Enquist 1984; Enquist & Leimar 1987), contests over resources of different value (Bishop *et al.* 1978; Hammerstein & Parker 1982; Enquist & Leimar 1987), contests between relatives (Maynard Smith 1982), and agonistic behaviour in sexual populations (Maynard Smith 1982). Asymmetries between opponents may be related to differences in fighting ability e.g., size, strength or differences in resource assessment (Parker 1974). Differences between opponents that are unrelated to fighting ability or resource assessment may also be used to settle contests without recourse to escalation i.e. uncorrelated asymmetries such as prior ownership (Maynard Smith & Parker 1976; Hammerstein & Parker 1982).

1.3 Resource value

A clear prediction from game theory models is that behaviour during fights, in particular intensity and duration, should be related to the value of the disputed resource (Bishop *et al.* 1978; Hammerstein & Parker 1982; Enquist & Leimar 1987). An animal should adjust its behaviour during agonistic interactions according to the value it places on a contested resource (Archer 1987). If an animal values a resource highly, then it should fight more intensely than it would over a resource it values less. One of the aims of the study described in this thesis was to determine

whether agonistic interactions of shore crabs, *Carcinus maenas* are influenced by an extreme difference in resource value. This question and that of whether the behaviour of eventual winners and losers should differ from an early stage in contests is addressed in Chapter 2.

1.4 Relative size of opponents

Game theory also predicts that, if fighting is costly, encounters are likely to be resolved at an early stage on the basis of relative fighting ability or resource holding potential (RHP) of the opponents when resource value and ownership are symmetrical (Parker 1974; Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Hammerstein & Parker 1982). RHP can be thought of as number of factors such as morphology (body size, weight), physiology (energy reserves) and previous agonistic experience. Animals should base their strategic decisions during fights on their opponent's RHP relative to their own and where contestants are evenly matched, long intense fights are likely to occur (Maynard Smith 1982; Enquist & Leimar 1983; Archer 1988). Many empirical studies have used overall body size as an index of RHP and have found that when there are large size differences between opponents, the larger contestant usually wins. However, a few studies have shown that weapon size can be important in agonistic interactions (review in Andersson 1994). Perhaps, the ability to inflict injury or weapon strength or size could be a better indicator of RHP. The question of whether relative body size or relative

weapon size or strength is a better indicator of RHP in shore crab fights is examined in Chapters 3 and 4.

1.5 Costs of agonistic behaviour

The costs of agonistic behaviour can be injury or death, and expenditure of time and energy. Injury or death, which are obvious risks of fighting and enhanced predation on animals engaged in fights or displays, are easily quantifiable consequences. Time spent engaged in particular activities is also easily measured and may be related to fitness, especially when the activity is mutually exclusive with feeding and reproduction. The costs of an activity can also be considered as the resultant net reduction in energy stores. Energy spent on agonistic behaviour is not available for feeding and reproduction and, therefore, represents a reduction in fitness. Additionally, depletion of energy reserves and accumulation of certain metabolites, such as L-lactate, may impede an animal's subsequent activities (Eckert *et al.* 1988). This may result in a reduced ability to engage in routine activities and in a quicker onset of exhaustion if maximal exertion, such as escape from a predator is required (Ellington 1983). Therefore, there are two potentially costly consequences of vigorous activity:- (1) the depletion of reserves that need to be replenished and (2) the accumulation of by-products of the respiratory process that need to be removed.

1.6 Metabolic consequences of agonistic behaviour

The physiological effects of agonistic behaviour are an important factor which must be considered if we wish to understand the mechanisms which underlie this behaviour. Recent theories have incorporated assumptions about the mechanisms that underlie behavioural changes during fights (Enquist & Leimar 1987). Behavioural endocrinologists and biochemists have demonstrated rapid changes in physiology in response to behavioural experience including encounters with an aggressive rival (Huntingford & Turner 1987). Many studies have quantified behaviour and physiology to examine the processes that are involved in the participation, escalation and resolution of fights. Some studies on the physiological effects of fighting have shown that animals can incur significant metabolic costs and these increase with fight duration and differ between winners and losers (e.g. fish such as *Tilapia zillii* (Neat *et al.* 1998), *Betta splendens* (Haller 1995), *Gasterosteous aculeatus* (Chellappa & Huntingford 1989); crickets, *Achetus domesticus* (Hack 1997); rats (Haller *et al.* 1990); and birds (Caraco 1979, Røskaft *et al.* 1986, Hogstad 1987). The metabolic variables examined in these studies were respiration rate, concentrations of L-lactate, the by-product of anaerobic respiration, depletion of glycogen and the resultant increase in glucose. Fighting resulted in an increase in respiration rate (e.g. *Necora puber*, Smith & Taylor 1993; *Achetus domesticus*, Hack 1997), increased L-lactate production (*T. zillii*, Neat *et al.* 1998) and reduced energy stores (rats, Haller *et al.* 1990; *G. aculeatus*, Chellappa & Huntingford 1989). There can also be differences in energy reserves between winners and losers and some studies have demonstrated

that relative energy reserves can decide the outcome of fights in damselflies, *Calopteryx* spp. (Marden & Rollins 1990; Plaistow & Siva-Jothy 1996), and starlings, *Sturnus vulgaris* (Witter & Swaddle 1995). All of these metabolic consequences of agonistic behaviour could potentially impede an animal's subsequent activity. For example it has been shown that depleted energy reserves impaired the ability of damselflies, *C. maculata* to secure a mating territory (Marden & Waage 1990) and the attraction of mates through signalling in the bush cricket, *Requena verticalis* (Simmons *et al.* 1992). In contrast to all of these findings, studies on the pumpkinseed sunfish, *Lepomis gibbosus* (Blanckenhorn 1992); spiders (Riechert 1988); the salamander, *Desmognathus ochrophaeus* (Bennett & Houck 1983); and the velvet swimming crab, *Necora puber* (Thorpe *et al.* 1995) have suggested that the energetic costs of agonistic behaviour are negligible. Therefore the objective of this study was to characterise the metabolic effects of agonistic behaviour using male shore crabs, *Carcinus maenas* (L.) and compare the metabolite profiles obtained from fought crabs to quiescent crabs and exercised crabs. This comparison would allow an assessment of the extent of the effects of fighting on the metabolic physiology of the shore crab since, if the metabolite concentrations are similar to quiescent crabs, then fighting has little effect on metabolites. If the metabolic effects from fought crabs are similar to those obtained from crabs subjected to sustained exercise, then fighting may incur a significant metabolic cost. One of the aims of the present study was to characterise the metabolic effects of fighting and

compare these with the effects of sustained exercise and this is described in Chapter 6.

1.7 Impact of environmental variables on the metabolic consequences of fighting

Environmental conditions can affect the costs of a behaviour. In brook charr, *Salvelinus fontinalis* the energy costs of agonistic behaviour increased as water velocity increased (McNicol & Noakes 1984). Few studies have addressed the effects of ecological variability on behaviour but it is likely that the environment in which fights take place will modify the costs of fighting. Where respiratory physiology is concerned, in intertidal organisms oxygen tension is likely to be a key environmental variable influencing costs. Therefore, the main aim of this study was to determine what influence variable oxygen tension had on the agonistic behaviour and metabolic physiology of shore crabs. The effects of varying oxygen tensions on contest behavioural content and metabolite concentrations are described in Chapters 5 and 6.

1.8 Agonistic behaviour in crustaceans

Crustaceans make ideal subjects for studies of agonistic behaviour since they are easily maintained under laboratory conditions, fight readily and are capable of a number of visual displays and postures (Dingle 1983). Agonistic interactions occur in the context of competition for resources such as food, mates or shelter, as well as resulting from chance encounters

(e.g. Hazlett 1968, 1974; Jachowski 1974; Hyatt 1983; Reid *et al.* 1994). In crustaceans, the cost of agonistic behaviour has been analysed in terms of the duration of contests and risk of injury (Berzins & Caldwell 1983; Dingle 1983; Shuster & Caldwell 1989; Glass & Huntingford 1988; Adams & Caldwell 1990) but there is a lack of documented information on the metabolic consequences of fighting. Studies on the velvet swimming crab, *Necora puber*, have shown that fights were associated with a marked increase in respiration rate, which was maintained for several hours after fighting (Smith & Taylor 1993). The increased respiration rate after fighting was related to the duration and intensity of the contests. Thorpe *et al.* (1995) examined changes in the concentrations of L-lactate in the blood and tissues of *N. puber* after fights and found that fighting did not result in a significant elevation of L-lactate or depletion of energy reserves.

1.8 The shore crab *Carcinus maenas*

The shore crab, *C. maenas*, is an abundant species found on North Atlantic shores. It performs daily and tidal migrations with large crabs moving onshore with the tide at night (Kitching *et al.* 1959; Crothers 1964; Reid & Naylor 1993). Such migrations can involve large numbers of shore crabs and fights have been frequently observed in the field (Sekkelsten 1988; Abello *et al.* 1994; Reid *et al.* 1994). This crab is known to inhabit rock pools that become hypoxic at night (lower than normal water oxygen conditions) or even anoxic (no oxygen) due to

respiration of the pool biota and this species is very tolerant of these conditions (Hill *et al.* 1991). Shore crabs can withstand up to 12 hours of anoxia (Robertson 1989). Extensive field and laboratory studies have shown that when the crabs are under hypoxic conditions, there is a greater reliance on anaerobic respiration with a reduction in glycogen stores and L-lactate accumulates in the blood and tissues (Hill 1989; Hill *et al.* 1991). Therefore the effects of this ecological variable on the agonistic behaviour and metabolic physiology were also examined in the present study.

1.9 Objectives of this study

The main aims were to examine:-

- the nature of agonistic behaviour of the male shore crab, *Carcinus maenas* and the influence of resource value.
- the influence of relative size and relative claw strength on the agonistic behaviour of the shore crab.
- the effects of hypoxia on the contest content, duration and outcome of shore crab fights and the effects of low oxygen tensions on the metabolic consequences of fighting in the shore crab.
- a comparison of field and laboratory studies of the agonistic behaviour and metabolic physiology of the shore crab.

CHAPTER TWO

THE NATURE AND INFLUENCE OF RESOURCE VALUE ON THE AGONISTIC BEHAVIOUR OF THE SHORE CRAB, *CARCINUS MAENAS* (L.).

2.1 INTRODUCTION

Crustaceans are very suitable subjects for the experimental study of aggression since they are easily maintained in the laboratory where they fight readily, are capable of a number of postures and visual displays and have obvious weapons, the chelae or claws (Dingle 1983; Hyatt 1983). Studies of the aggressive behaviour of Crustacea have shown that fights between conspecifics commonly involve an exchange of agonistic displays in which the body is raised and the ambulatory legs spread, thus increasing apparent size, and in which the weapons (usually the chelipeds) are presented (for review see Dingle 1983). This exchange of agonistic signals can be sufficient to resolve encounters, suggesting assessment of a potential opponent's fighting ability. However, in other situations fights escalate through increasingly intense displays to physical violence. Grasping, striking and pushing occur during fights in a number of species of crab, for example the swimming crab, *Liocarcinus depurator* (Glass & Huntingford 1988), the velvet swimmer, *Necora puber* (Smith 1990; Smith *et al.* 1994; Thorpe 1994; Thorpe *et al.* 1994) and the hermit crab, *Pagurus bernhardus* (Elwood & Glass 1981).

In recent years studies of this group have been extensively used to provide empirical tests of the predictions of game theory (Maynard Smith 1982). An important insight gained from game theory is that, if fighting is costly, encounters are likely to be resolved at an early stage on the basis of the relative fighting ability or resource holding potential (RHP) of the opponents (Parker 1974; Maynard Smith & Parker 1976; Parker &

Rubenstein 1981; Hammerstein & Parker 1982). Body size may be an indicator of strength, experience and ultimately the ability to inflict injury and is therefore likely to be closely related to fighting ability (Archer 1987). In a wide range of species, including a number of crustaceans such as *Procambarus alleni* (Bovbjerg 1956), *Uca pugilator* (Hyatt & Salmon 1978), *Alpheus heterochaelis* (Schein 1975), *Necora puber* (Smith *et al.* 1994; Thorpe *et al.* 1994), the probability of an individual winning increases as the size of its opponent decreases (Archer 1987).

Another clear prediction of several game theory models is that as resource value increases, behaviour during fights, in particular intensity and duration, should be related to the value of the disputed resource (Bishop *et al.* 1978; Hammerstein & Parker 1982; Enquist & Leimar 1987). Because it is easier to manipulate relative size than resource value, the effects of this variable have been less thoroughly documented. One of the aims of the study described here was to determine whether agonistic interactions of shore crabs, *Carcinus maenas,* are influenced by an extreme difference in resource value. Since there has been extensive discussion in the game theory literature about whether the behaviour of eventual winners and losers should differ early in a fight (Archer 1987), this question was also addressed.

The shore crab, *Carcinus maenas,* is common on the shores of the British Isles and the North Atlantic. It exhibits a diel and tidal migration pattern, with mature crabs moving onshore at night with the tide searching for food (Kitching *et al.* 1959; Crothers 1964; Reid & Naylor 1993). As a

consequence, large numbers of active crabs may congregate and therefore intraspecific competition is likely to occur. Dare and Edwards (1981) recorded a population density of between 1.8 and 2.5 crabs per square metre dispersed over the upper shore of a tidal flat. Although many aspects of the biology of this species have been investigated, the nature of aggressive behaviour in *Carcinus maenas* has been largely ignored. Such information as we have, is limited to general observations that agonistic interactions occur (Jensen 1972; Eales 1974; Berril & Arsenault 1982; Smith 1990). Studies on competition for mates by males have shown that larger males with both chelipeds intact are more likely to mate successfully (Christy 1987; Reid *et al.* 1994). Therefore, this study has the following objectives:- to describe movements and postures used during pairwise fights between male *Carcinus maenas*, differentiating between winners and losers, and to determine the effects of experimentally adding a resource, i.e. food extract, to the arena at the beginning of the interactions.

2.2 METHODS

Male *Carcinus maenas* (carapace width range 55-80 mm) were obtained from the University Marine Biological Station, Millport, Isle of Cumbrae. On arrival at Glasgow the crabs were transferred to individual holding tanks (18 x 21 x 23cm). The tanks were supplied by circulating seawater (salinity 32-34‰) maintained at 12 ± 2°C. The aquaria had a 12 : 12 light-dark cycle and experiments were carried out during the light period. The crabs were kept in these isolated conditions for at least seven days prior to any behavioural observations and were fed every seven days with whitebait. This period of captivity ensured that any tidal rhythms were lost.

Crabs were only used in behavioural observations if the exoskeleton was hard, there was no excessive epifaunal growth and there were no missing or recently regenerated limbs. After completion of experiments, the crabs were held for a further two weeks to make sure they were not in proecdysis, since moulting is known to affect behaviour (Jachowski 1974; Tamm & Cobb 1978; Adams & Caldwell 1990).

Approximately fifty crabs were used in this study. Crabs had to be used more than once due to the limited space available to house them. They were allocated into pairs with the requirements that they had not fought each other previously and had been kept in separate sets of holding tanks. Crabs were used no more than twice and the second fight was staged using pairs of winners or losers of previous fights, to eliminate the prior experience effect. After fighting a rest period of at least seven days

was allowed and a uniform number of days elasped between last feeding and testing. Similar sized crabs were used in the two types of treatment.

Fights were staged by placing two crabs in a glass observation tank (48 x 43 x 34cm) with a gravel substratum. They were separated by a vertically sliding opaque partition to allow them a settling time of fifteen minutes in continued isolation. The tank was screened from visual disturbance and was filled with aerated seawater. There was a possibility of chemical communication during the settling period in the observation tank and to reduce the probability of this occurring, the air and water pumps were switched off. The tank was illuminated from above by lamps containing 18 watt bulbs, the intensity of which was reduced by a tracing paper shade. The light intensity was 1.82 - 2.66 $\mu E. m^{-2} sec^{-1}$ at the top and 0.89 - 1.40 $\mu E. m^{-2} sec^{-1}$ at the bottom of the tank. Observations were made through a small opening in the screen. After the initial settling period and when both crabs were stationary the partition was raised from outside the screen via a pulley mechanism. The actions of both crabs were recorded using a lap top computer as an event recorder. Thirty fights were staged in tanks with no tangible resource and thirty were staged with a resource present, by adding ten ml of seawater and ten ml of food extract (whitebait homogenised in seawater) respectively, via a burette, to the arena as the partition was raised.

2.3 RESULTS

2.3.1 The nature of *Carcinus maenas* fights.

Content : Most pairs of crabs fought readily in the experimental aquaria with only seven pairs in contests without food which did not fight. Fights consisted of a series of discrete action patterns, defined in Table 1 and illustrated in Figure 1. The initiator of a fight was defined as the first crab to move towards its opponent and make physical contact. The winner of a fight was the crab that elicited repeated retreats from its opponent or successfully climbed on top of the other contestant. The end of a contest was defined as the point when the two crabs separated and did not interact for five minutes. The time taken from first lifting the partition to the first crab moving to its opponent was the initiation; and the duration was the time taken for all aggressive behaviour to stop. For both categories of fight (with/without food), there was a tendency for the crab that initiated to be more likely to win but in neither case was this significant (Table 3, X^2 = 1.10, $P > 0.05$, n = 46 in contests with food, n = 23 in contests without food).

Duration : Fights without food ranged in duration from 182 to 2403 seconds whereas fights with food ranged in duration from 154 to 983 seconds. Mean fight length was significantly lower (559 ± S.E. 53 secs) than in those fights without food (1143 ± S.E. 135 secs; T-Test = -4.01, $p<0.01$).

Intensity : Seven categories of interactions were identified on the basis of behavioural content and intensity (Table 2). The distribution of these fight

categories differed significantly in the fights with and without food (X^2 = 13.29, p<0.01). Most contests with food were of the most intense kind (type 7), whereas the highest proportion of contests without food consisted of low intensity interactions of types 1 and 3 (Fig. 2). For encounters with food, there was no relationship between fight category and fight duration (Rs = -0.040, N = 30, P> 0.05). However for fights without food, fight length and fight intensity increased in parallel (Rs = 0.639, N = 22, P< 0.01).

2.3.2 The dynamics of *Carcinus maenas* fights

Fights were divided into four sections, on the basis of behavioural "landmarks":-

the initiation period = time from partition rise until first physical contact of crabs;

the first half = approximately half the duration;

the second half = from the end of the first half until aggressive behaviour was no longer performed by the crabs;

the resolution period = the period after the fight had been settled.

Figure 3 shows the duration of these four phases in fights with and without food. The initiation and resolution phases lasted similar lengths of time, but the 1st and 2nd halves of the interaction were significantly longer in fights without food (1st half, T-Test = - 4.23, p< 0.01, 2nd half, T-Test = - 2.55, p= 0.02).

To examine patterns of behavioural change within fights, the frequency of key acts in each phase, performed by winners and losers was calculated

and analysed separately for fights occurring in the presence or absence of food. These acts were chosen since they represent a difference in level of escalation. The data are presented in Figure 4 and the results of statistical analyses are given in Table 4. The mean number per minute of "move to" acts performed by winners peaked sharply after the initiation period and remained static through the fight before declining rapidly at the end. Losers performed fewer "move to" acts. The frequency of "move away" acts performed by losers also peaked sharply after the initiation period, then increased as the contest progressed and decreased dramatically at the end. Winners performed fewer "move away" acts. In the initiation period, very little cheliped display occurred. As the fight proceeded there was a rapid increase in this behaviour by both winners and losers, which remained stable at a high level throughout the fight before decreasing sharply as the fight ended. Contact acts performed by winners increased dramatically in the first half of fights. Losers generally performed far fewer contact acts than winners and these decreased sharply after the first half of fights (Fig. 4). Table 4 shows the results of Friedman tests which compare the behaviour of winners and losers of each bout separately for contests where food is present or absent during the four time periods. Winners performed more aggressive acts than losers, who performed more "move away" acts (Table 4). The major difference between contests with and without food was in striking which was mainly performed by winners in fights in the presence of food ($F = 20.920$; $P < 0.01$; d.f. = 52). Table 5 shows results of Kruskal Wallis tests which compare the behaviour of

winners and losers when food is present or absent. When food is present winners perform more "move to "; "cheliped display" and contact acts whereas losers perform more "move away" and "cheliped display" acts when food is present.

Table 1. Action patterns used during *Carcinus maenas* fights.

ACT	DESCRIPTION
CROUCH	The crab is stationary with the chelipeds folded in front of the cephalothorax. The body rests on the substrate with all the walking legs completely flexed.
STAND	The crab is stationary with the chelipeds folded in front of the cephalothorax. The legs are in contact with the substrate and support the body which is slightly raised over the ground.
MOVE TO	One crab approaches the other crab.
MOVE AWAY	The crab moves away directly from the crab in an aggressive interaction.
CHELIPEDS IN, BODY RAISED	The body of the crab is raised as high as possible over the substrate by the fully extended legs. The chelipeds are folded in front of the cephalothorax or occasionally pointed downwards.
CHELIPED DISPLAY	The body of the crab is raised as high as possible over the substrate using the fully extended legs. Chelipeds are held out in front with the chelae open or closed.
LATERAL MERUS DISPLAY	As cheliped display but the chelipeds are held out at 180 degrees to the body with the chelae open.
STRIKE	One crab suddenly hits out at the other with one or both chelipeds.
GRASP	When one crab using its chelae to pinch the carapace, chelipeds or legs of the other crab.
CLIMB ON	One crab attempts to or actually climbs on top of the other crab. When on top of the opponent the chelae are usually directed in front of the cephalothorax of its opponent and grasping may occur.
CLIMB OFF	After successfully climbing on, the crab climbs off the other crab.
PUSH	The crab pushes its opponent using its chelipeds, pushing forward with the walking legs and rubs the body of its opponent or grasps using the chelae; or pushes the other crab away using the chelipeds only.

Table 2. Types of contest shown by male *Carcinus maenas* in fights with and without food.

TYPE	DESCRIPTION	NUMBER OF FIGHTS	
		WITH	WITHOUT
1	The crabs did not behave aggressively and no contest occurred.	0	7
2	Crab 1 attempted to or climbed onto its opponent, crab 2 which behaved submissively throughout the interaction.	2	5
3	Crab 1 attempted to or climbed onto crab 2 at which crab 2 acted aggressively and grasping occurred. The contest continued until one crab, the winner, successfully climbed on or the other repeatedly retreated from the winner.	6	7
4	Crab 1 and crab 2 entered into a pushing contest (Fig.1f) where grasping may occur. The first crab to retreat from this was the loser and subsequently continued retreating acting submissively.	6	5
5	Crab 1 and crab 2 entered into a pushing contest and the first crab to retreat from this was the loser, which kept on retreating but held the chelipeds out as if ready to re-engage.	4	2
6	Crab 1 and crab 2 entered into a pushing contest which developed into blows or strikes by crab 1, at which crab 2 retreated and continued to retreat at subsequent advances from crab 1.	4	3
7	Crab 1 and crab 2 entered into a pushing contest which developed into blows by one or both crabs. Either one crab retreated and is the loser or both crabs re-engaged in the pushing contest.	8	1

Table 3. Number of crabs initiating and losing, and initiating and winning in contests with and without food.

	INIT/LOSE	INIT/ WIN
WITH	20	26
WITHOUT	7	16

Table 4. The behaviour of winners and losers compared within each of the four time periods, with the data being paired for each contest and the results shown separately for contests with (+) and without (-) food. Only significant results are shown. There were no differences in behaviour in the initiation and resolution periods. Friedman tests were performed on the six acts tested in table 1 and the p values adjusted for testing the same data set six times (To = "move to"; Aw = "move away"; Con = "strike", "grasp", "push" and "climb on").

FOOD +/-	ACT	TIME	MEDIAN		S	P VALUE
			W	L		
+	To	1st half	0.95	0.15	13.37	<0.00001
+	To	2nd half	1.1	0.1	28.0	<0.00001
+	Aw	1st half	0.15	0.85	19.59	<0.00001
+	Aw	2nd half	0.1	1.1	17.64	<0.00001
+	Con	1st half	2.4	0.9	9.97	0.012
+	Con	2nd half	1.6	0.4	25.0	<0.00001
-	To	1st half	0.7	0.2	7.12	0.048
-	To	2nd half	0.7	0.0	15.06	<0.00001
-	Aw	2nd half	0.15	0.55	7.12	0.048
-	Con	1st half	1.35	0.35	9.0	0.018
-	Con	2nd half	0.95	0.25	14.0	<0.00001

Table 5 A comparison of the behaviour in contests with and without food analysed separately for winners (W) and losers (L). The data were analysed by Kruskal Wallis tests and the resultant P values adjusted for analysing the same data set six times (Stationary = "crouch" and "stand"; Display = "cheliped display" and "LMD"; Chelae in = "chelipeds in , body raised"; Contact = "strike" "grasp" "push" and "climb on") .

ACT	W/L	MEDIAN		H	P VALUE
		+ FOOD\\	-FOOD		
Stationary	**W**	**0.1**	**0.1**	**0**	**>0.05**
Stationary	**L**	**0.05**	**0.1**	**1.31**	**>0.05**
Move To	**W**	**1.08**	**0.65**	**10.0**	**0.012**
Move To	**L**	**0.15**	**0.1**	**1.39**	**>0.05**
Move Away	**W**	**0. 1**	**0.15**	**3.41**	**>0.05**
Move Away	**L**	**1.0**	**0.6**	**8.58**	**0.018**
Display	**W**	**1.25**	**0.8**	**8.43**	**0.024**
Display	**L**	**1.22**	**0.7**	**7.02**	**0.048**
Chelae In	**W**	**0.5**	**0.5**	**0.35**	**>0.05**
Chelae In	**L**	**0.73**	**0.3**	**6.39**	**>0.05**
Contact	**W**	**2.2**	**1.1**	**8.71**	**0.01**
Contact	**L**	**0.47**	**0.25**	**3.08**	**>0.05**

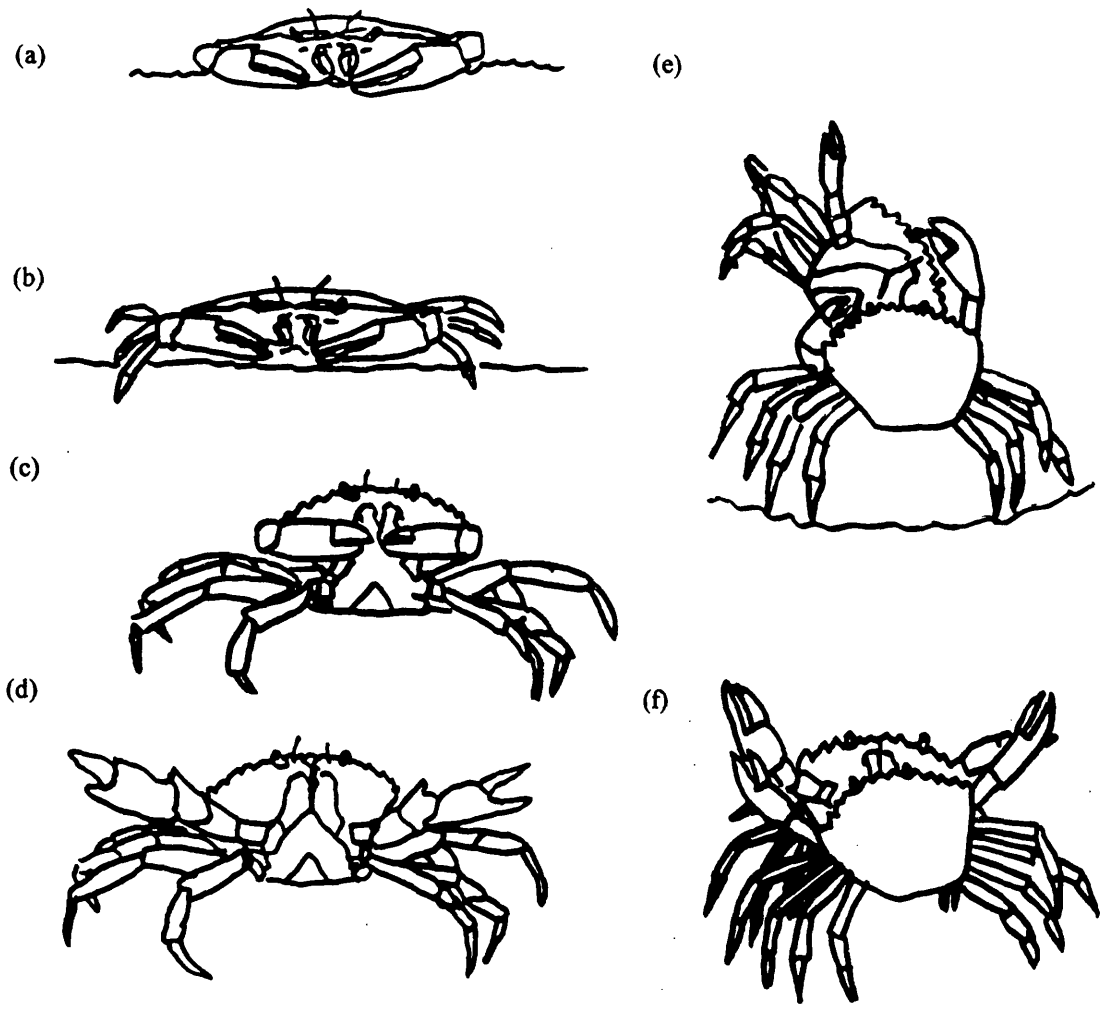


Figure 1. Illustrations depicting the actions of fighting shore crabs:- (a) "crouch", (b) "stand", (c) "chelipeds in, body raised", (d) "LMD", (e) "strike", (f) wrestle.

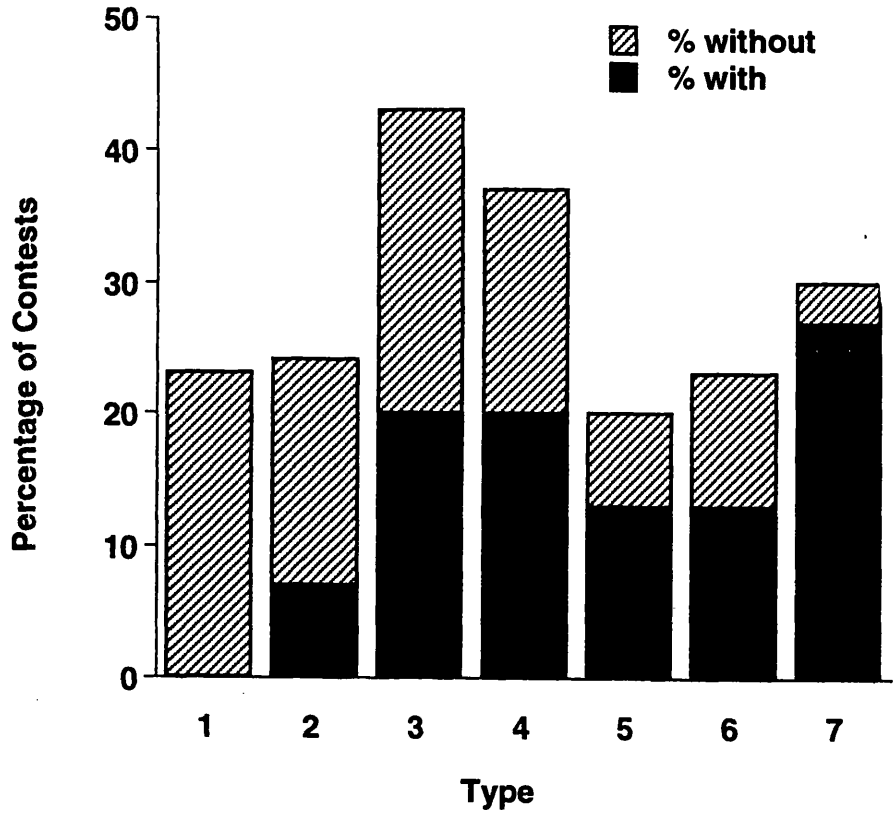


Figure 2. Percentage of contests staged in the presence (solid areas) or absence (hatched areas) of food of each type with type 1 being the least intense increasing to type 7, the most intense.

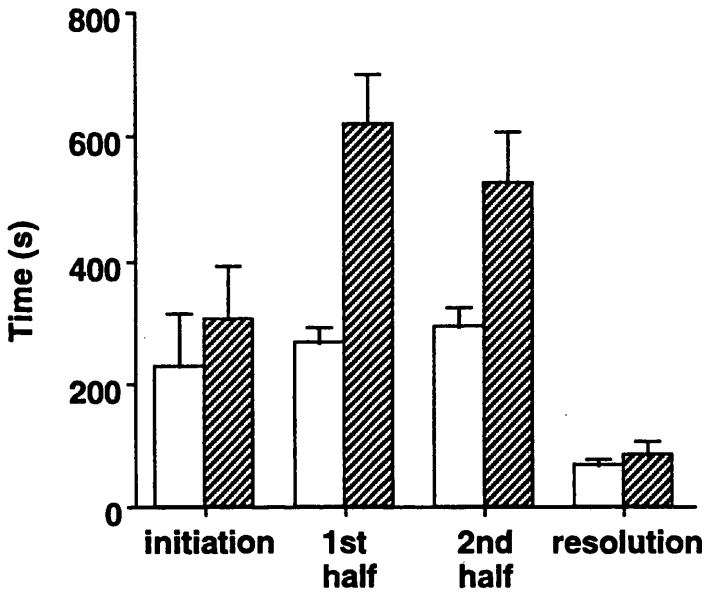


Figure 3. Mean duration (seconds) of the four phases of contests with and without (shaded) food (n = 30 for contests with food).

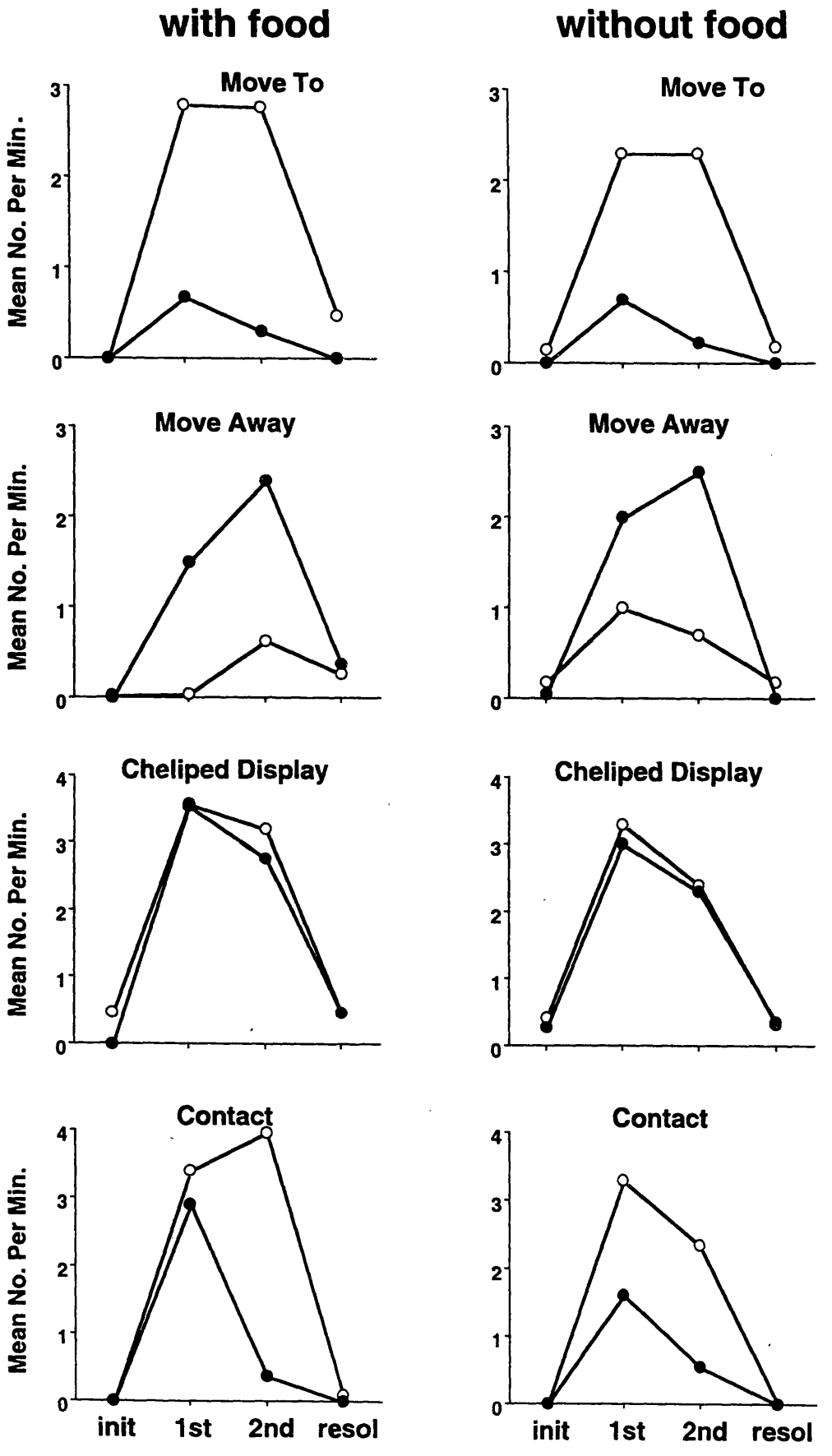


Figure 4. Mean frequency of key acts performed by winners (empty circle) and losers (solid circle) over the four phases in contests with and without food (n=30 for contests with food; n=23 for contests without food). Key acts include "move to", "move away", "cheliped display", and contact acts (consists of "strike", "grasp", and "climb on").

2.4 DISCUSSION

Fights between male *Carcinus maenas* are complex and vary in both content and duration. Although contests can be categorised into types on the basis of level of intensity, fights of *Carcinus maenas* do not fall into a number of clearly defined phases, as they do in fiddler crabs (Crane 1975; Christy 1988). According to game theory, contests should begin with energetically inexpensive movements, such as display acts, followed by more energetically demanding physical contact acts, and then onto injurious behaviour (Maynard Smith 1976, 1982; Krebs & Davies 1987). Therefore there should be a progressive increase in intensity of fighting or escalation (Huntingford & Turner 1987). In the fights between male *Carcinus maenas* there is no initial display and the fights quickly become very intense at the beginning of the fight. The results suggest that the crabs do not assess each other visually, but must engage in physical contact to decide the outcome of contests.

This is contrary to the findings of studies carried out on other crustaceans. Hazlett (1968) observed hermit crabs, *Clibanarius vittatus*, settling contests by display only and this has been shown in other portunid crabs e.g. *Liocarcinus depurator* (Glass & Huntingford 1988) and *Necora puber* (Smith 1990; Smith *et al.* 1994; Thorpe 1994; Thorpe *et al.* 1995). The pushing contest could be a direct assessment of strength and this information could be decisive on the subsequent content and outcome of the fight. Therefore weight or height above the substratum may be important, as it is in spiders (Riechert 1984). The effect of size differential

on the contests of *Carcinus maenas* is described elsewhere and larger crabs have an advantage in winning fights (see Chapter 3; Sneddon *et al.* 1997). The act of climbing on the opponent has been suggested to be an indicator of dominance in the establishment of hierarchies in groups of crabs (Abele *et al.* 1986). Therefore this type of behaviour may be a low intensity strategy which could pay off without costly escalated fighting if the other crab is submissive.

From early on in the majority of these agonistic interactions the behaviour of winners and losers is different. This would agree with more recent game theory models which predict that contestants should adjust their behaviour according to the value they place on the disputed resource (Enquist 1985). It also agrees with other studies on species such as *Pagurus bernhardus* (Hazlett 1982) and *Betta splendens* (Bronstein 1985). Such effects of resource value must be mediated via an effect on the internal state possibly via changes in the neuroendocrine system. Past experience of the crabs was controlled during the period of the present study but not prior to capture, and this will influence the strategy the animal adopts.

In many crustaceans, agonistic interactions occur in the context of competition for resources such as food, mates or shelter, as well as resulting from chance encounters (Hazlett 1968,1974; Jachowski 1974; Rubenstein & Hazlett 1974; Abele *et al.* 1986; Glass & Huntingford 1988; Smith 1990; Reid *et al.* 1994). To determine whether food is a critical resource over which fights occur, fights were staged both in the absence of

any tangible resource and in the presence of food extract. In 23% of the staged fights without food, no contest occurred and striking was rare, whereas in fights in the presence of food, which are mostly type 6 and 7, striking was performed significantly more by winners. This agrees with game theory predictions that contest content should be related to resource value (Maynard Smith 1982). It is possible that, when there was no obvious resource to contest, the costs of fighting such as vulnerability to predation (Crowley *et al.* 1988; Caine 1989; Green 1990); and seriously depleted energy reserves (Marler & Moore 1991) outweighed the benefits so crabs did not engage in aggressive behaviour or pursue escalated fighting.

The presence of food in these staged fights significantly decreased the duration of the bout and is thus contrary to game theory predictions regarding war of attrition models, which suggest that contestants should engage an opponent for longer as resource value increases (Maynard Smith 1982). This finding agrees with recent theory that contestants should decide early on which strategy to adopt and contests should be settled more quickly as resource value increases (Enquist 1983). One interpretation of this is that, since it is only food odour that is present in the fights, then the lack of a "real" food item was quickly ascertained by the crabs and thus they de-escalated. However from observing a large number of these fights, we believe that the increase in intensity and striking, which is potentially injurious, settled the contests quickly and the winner was decided early on. Perhaps the losing crab does not value the

resource as highly as the winner and is less motivated to participate in an escalated fight. Further work, perhaps using different concentrations of food extract, is necessary to determine the precise relationship between resource value and the agonistic behaviour of shore crabs.

CHAPTER THREE

THE INFLUENCE OF RELATIVE SIZE ON THE AGONISTIC BEHAVIOUR OF THE SHORE CRAB, *CARCINUS MAENAS* (L.).

3.1 INTRODUCTION

When resource value and ownership are symmetrical, the probability of an individual winning an agonistic interaction is closely related to its absolute fighting ability or resource holding potential (RHP) relative to that of its opponent (Parker 1974). The RHP of an animal is influenced by a number of interacting factors; for example, morphology (body size in stomatopods, Caldwell & Dingle 1979; weight in spiders, Riechert 1984), physiology (energy reserves in crayfish, Hazlett *et al.* 1975), and previous agonistic experience (Thorpe 1994). Game theory predictions suggest that during aggressive encounters contestants compare their own RHP with that of opponents before deciding to escalate and that where contestants are evenly matched, long, intense fights are likely to occur (Maynard Smith 1982; Enquist & Leimar 1983; Archer 1988). This has been demonstrated in animals as diverse as bowl and doily spiders, *Frontinella pyramitela* (Austad 1983; Suter & Keiley 1984), red deer, *Cervus elaphus* (Clutton-Brock *et al.* 1982), the portunid crab, *Necora puber* (Smith *et al.* 1994) and the Mozambique mouthbrooder, *Oreochromis mossambicus* (Turner & Huntingford 1986).

Many of these empirical studies used overall body size as an index of RHP. However, RHP is complex, and may actually depend on something that correlates with body size (e.g. weapon size) and perhaps the ability to inflict injury, rather than on body size *per se* (Maynard Smith & Brown 1986). Weapons such as horns in ungulates, claws and mandibles in arthropods, and spurs in birds have been shown to be important in

agonistic behaviour (review in Andersson 1994). Removal of antlers in male reindeer, *Rangifer tarandus*, and red deer reduces their fighting ability and dominance status, although age, body size and condition also play a role (e.g. Espmark 1964; Lincoln 1972; Prowse *et al.* 1980; Clutton-Brock *et al.* 1982). In the study described here, in an attempt to try to disentangle the relative effects of body size and weapon size on the outcome of agonistic behaviour in shore crabs, *Carcinus maenas* (L.), contests between pairs of males are staged.

Crustaceans are ideal subjects for laboratory studies of aggression since they fight readily in laboratory conditions, have formidable weapons (the chelae or claws), and are relatively easy to manipulate and measure. The general nature of agonistic behaviour in crustaceans involves an exchange of agonistic signals where the body is raised high above the substratum by the ambulatory legs and the weapons are presented to the opponent (Dingle 1983). Agonistic interactions may be resolved at this point or may escalate to fighting behaviour. In studies of hermit crabs, *Pagurus bernhardus* (Neil 1985), crayfish, *Cherax cuspidatus* (Pavey & Fielder 1996) and portunid crabs (e.g. Thorpe *et al.* 1995) larger individuals were found to have a high probability of winning against a smaller opponent. These investigations interpreted body size (carapace width or length) as the empirical determinant of RHP.

After maturation, the chelae of decapod crustaceans grow allometrically to the carapace size and hence a linear relationship exists between these two variables, with the chelae length increasing with

carapace width (Hartnoll 1982). It is therefore possible that the effect of size on fight outcome is actually predicted by chela size rather than body size. In addition, due to inherited individual differences and loss of chelipeds through damage or escape from predators, for any given carapace width there can be a range of chela sizes (Fig. 1 shows data for the crabs used in this study). Such differences in weapon size may be particularly important in settling fights between individuals with evenly matched body size. Therefore the relative effects of weapon size and body size on the content, duration and outcome of pairwise fights between male shore crabs are examined.

3.2 METHODS

3.2.1 Maintenance of Study Animals

Male *Carcinus maenas* (carapace width range 55-80 mm, 64 crabs) from the University Marine Biological Station, Millport, Isle of Cumbrae where they had been freshly caught by creeling from the Clyde Sea area. On their arrival the crabs were transferred to individual holding tanks (18 x 21 x 23cm), supplied with circulating seawater (salinity 32-34$^{o}/_{oo}$) maintained at 12 +/- 2°C and on a 12 : 12 h light-dark cycle, with experiments being carried out in the light period. The crabs were kept in these isolated conditions for at least seven days prior to any behavioural observations and during this time they were provided with whitebait to excess once every five days. Crustaceans can survive for up to three months without food so this regime kept the crabs healthy but motivated to fight over food.

3.2.2 Assessing Size

Crabs were used in behavioural observations only if they were in pristine condition i.e. the exoskeleton was hard, there was no excessive epifaunal growth and there were no missing or recently regenerated limbs. A few days prior to each fight the carapace width of the crabs were measured to the nearest 0.1mm between the fourth and fifth lateral spines, using vernier callipers. From these measurements the body size ratio of the interactants was calculated by dividing the carapace width of the

smaller opponent by that of the larger. This was repeated for the major chela length (the total propodus length). After completion of experiments the crabs were held for a further two weeks to make sure they were not in proecdysis, which none were.

3.2.3 Experimental Protocol

Crabs had to be used more than once due to the financial constraints of obtaining animals and the limited space available in which to house them. Crabs were fought no more than twice and in the second interaction, fights were staged between a pair of winners or a pair of losers from previous fights. At least seven days were allowed between fights and crabs were never fought against opponents they had previously encountered. Therefore each pairing was treated as an independent event.

Fights (46) were staged by placing two crabs in a glass observation tank (48 x 43 x 34cm), with a gravel substratum, filled with aerated seawater which was screened from visual disturbance. Crabs were separated by a vertically sliding opaque partition to allow them a settling time of fifteen minutes in continued isolation. To reduce the possibility of chemical communication, the air and water pumps were switched off to minimise mixing during the settling period. The tank was illuminated from above at a light intensity of 1.82 - 2.66 μ E. m^{-2} sec^{-1} at the top and 0.89 - 1.40 μ E. m^{-2} sec^{-1} at the bottom of the tank. Observations were made through a small opening in the screen. After the initial settling period and when both crabs were stationary, the partition was raised from

outside the screen by a pulley. Ten ml of food extract (whitebait homogenised in seawater) was added, via a burette, into the centre of the arena at the start of the experiment. We recorded the actions of both crabs using a lap top computer as an event recorder. These behavioural data are presented elsewhere (see Chapter 2; Sneddon *et al.*, 1997). The frequency of cheliped display was recorded and analysed for this study and also the intensity of the contest. The end of a contest was judged to have occurred when the two crabs separated and did not interact for five minutes.

The crab that first moved to its opponent and engaged in physical contact was defined as the initiator. The winner was the crab that successfully climbed on top of its opponent or elicited repeated retreats from the other crab, (the loser). The duration of the first bout of fights was measured by analysis of the event recorder data. The duration of the first bout was the time from initiation to when the two crabs disengaged for the first time or one crab retreated from the other. The overall duration was the time from initiation to when no further aggressive acts were performed by either crab. Wrestle duration was the time taken from the crabs first entering into a pushing contest until the first crab disengaged the other.

Fourteen pairs of crabs, taken from a separate group of crabs with each pair being matched for chela length, were humanely killed by immersion in liquid nitrogen after fighting and the major chela taken off by careful dissection using a scalpel. The sample size of fourteen was chosen for ethical reasons. The area of the outer surface of the major chela of each crab was calculated by video image analysis. After removal from the crab, each

chela was held in a standardised orientation at a specified distance from a video camera (Sony CCD-FX700E) on a set magnification. A scale bar was also filmed so that the videos could be calibrated accurately. The video tapes were subsequently digitised and analysed using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health) on a Power Macintosh 7500/100AV to render estimates of chela surface area. Each measurement was performed twice and surface area measures were shown to be highly repeatable (intraclass correlation coefficient (Zar 1984), $r_I = 0.9973$, $F_{14,1} = 742.85$, $P < 0.00001$.).

The major chelae used in the surface area analyses were placed in a preweighed vial and freeze dried (Edwards Modulyo) then weighed accurately.

3.2.4 Statistical Analysis

A chi square test was performed on the number of absolute smaller or larger crabs (chela and body size) initiating fights and winning fights. A binary logistic regression was used to determine the importance of the proportional difference in chela length (relative chela length) and carapace width (relative carapace width) in predicting the outcome of fights. The event probability (likelihood of larger or smaller crab winning) was also calculated.

The surface area and weight of the major chela of winners and losers were compared using a paired T-Test.

To determine if fight duration, length of first bout or wrestle duration was related to relative size of body and weapons of fighting pairs, a regression analysis was carried out. Spearman Rank tests were performed on size ratio and intensity of contests to determine if intensity increased as crabs became evenly matched.

The frequency of cheliped display was calculated for winners and losers and a paired T-Test performed on the data to compare the behaviour of winners and losers. Regression analysis of frequency of cheliped display against chela size was carried out and ANOVA used to compare the regression lines of winners and losers. Two tailed tests of significance were used throughout.

3.3 RESULTS

The relations between absolute size difference in carapace and chela size and the probability of initiation and winning are shown in Table 1. Size was not related to initiation but there was a significant relationship between chela length and the likelihood of winning ($X^2 = 4.08$, $P < 0.01$, $n = 46$). A similar but not significant effect was found for carapace width ($X^2 = 1.6$, $P > 0.05$, $n = 46$). In both cases there is a range of size differences where the winner may be smaller (chela difference 0-6mm; carapace difference 0-9mm). The surface area of the major chela of winning crabs (mean 1.717 cm^2, SE 0.123) was significantly higher than that of losers (mean 1.485 cm^2, SE 0.104, $T = 3.38$, $P = 0.0049$, $n = 14$) and the dry weight of chela was also found to be significantly higher in winners (mean 3.728 g, SE 0.481) than in losers (mean 3.022g, SE 0.403, $T = 8.44$, $P < 0.00001$, $n = 14$) even when the chela of both crabs were matched for length ($T = -0.96$, $P = 0.37$).

Figure 2 shows the results of a logistic regression showing the importance of relative carapace width and chela length (Fig. 2). When the probability (solid line, Fig. 2) of the larger crab winning is equal to 0.5 there is an equal chance of either opponent winning; i.e. the two crabs are evenly matched. As the line moves towards 1.0, the probability of the larger opponent winning increases. Relative carapace width does not reliably predict the outcome of fights ($z = 0.50$, $P = 0.618$, odds ratio = 9.35, Fig. 2b) and the slope of the line is not significantly different from

zero (G = 0.259, d.f. = 1, P = 0.611, Fig. 2b). In contrast, relative chela length is a reliable predictor of the larger crab winning (z = 2.227, P = 0.027, odds ratio = 4.67E06, Fig. 2a), with the slope of the line (before reaching 1.0) being significantly different from zero (G = 6.853, d.f. = 1, P = 0.009, Fig. 2). Therefore chela length is a more reliable predictor of the winner of pairwise fights between shore crabs than carapace width.

Overall fight duration is not related to either carapace width or chela length differential (Table 2), and thus duration cannot be predicted by the relative size of opponents. Similarly the length of the first bout of these fights and wrestle duration is unrelated to either relative carapace width or chela length (Table 2). Relative size (Fig. 3), both carapace width and chela length, and overall duration is also unrelated to contest intensity (Table 3).

The frequency of cheliped display per minute was calculated for winners (mean 1.51, SE 0.09, n = 32) and losers (mean 0.963, SE 0.105, n = 32). A regression analysis showed that, using data for both winners and losers, cheliped display frequency increased significantly with chela size (regression equation $y = 0.11 + 0.0331x$, $r^2 = 0.188$; $F_{62} = 6.94$, P = 0.013). Figure 4 shows the frequency of cheliped display for winners and losers separately. When comparing the slope of both lines using ANOVA, it was found that they were not significantly different ($F_{30} = 0.04$, P = 0.85) but winners performed significantly more cheliped displays than losing crabs ($F_{30} = 9.64$, $P < 0.01$) and a paired T-Test confirmed this (T = 5.28, $P < 0.01$, n=32).

Table 1. The number of larger and smaller crabs initiating and winning fights in staged contests with percentage shown in brackets. Results for carapace width and chela length ratio are shown separately. The significance of X^2 test is shown by the resulting P value (n = 46).

	CARAPACE WIDTH			CHELA LENGTH		
	Smaller	**Larger**	**p**	**Smaller**	**Larger**	**p**
INITIATE	21 (45%)	26 (55%)	n.s.	19 (41%)	27 (59%)	n.s
WIN	15 (32%)	32 (68%)	n.s.	10 (22%)	36 (78%)	0.01

Table 2. Regression analyses of relative size, both carapace width and chela length, and total duration, duration of first bout and wrestle duration.

	Carapace width	**Chela length**
Total duration	**F = 0.16, p =0.694**	**F = 0.02, p = 0.881**
First bout	**F= 0.24, p = 0.624**	**F = 0.07, p = 0.792**
Wrestle	**F = 2.38, p = 0.135**	**F = 2.12, p = 0.158**

Table 3. The results of Spearman rank tests performed on contest type and carapace width, chela length, and duration.

	Rs	n	p value
Carapace width	**-0.063**	**27**	**0.745**
Chela length	**-0.270**	**27**	**0.157**
Duration	**-0.040**	**17**	**0.871**

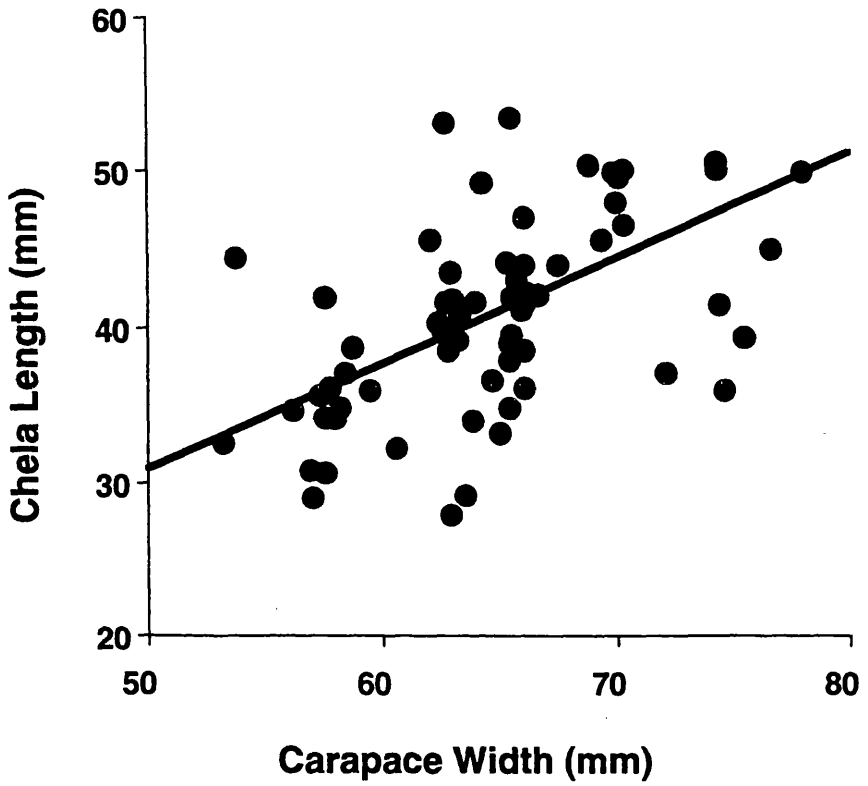
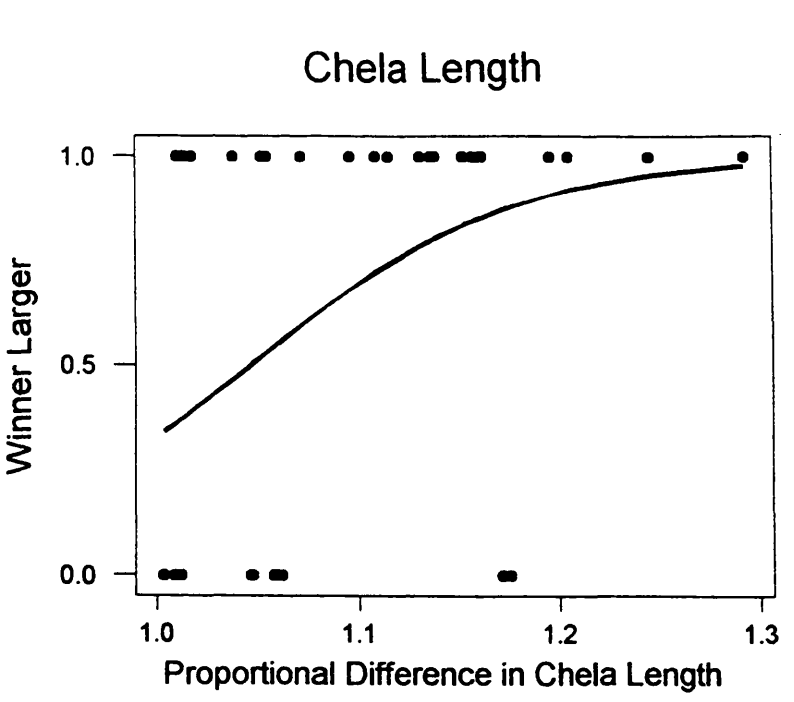


Figure 1. The relationship between chela length and carapace width showing the amount of variation in chela size for a given carapace width. Data points refer to crabs used in this study ($y = -2.9272 + 0.6757x$, $r^2 = 0.38$; $n = 94$)



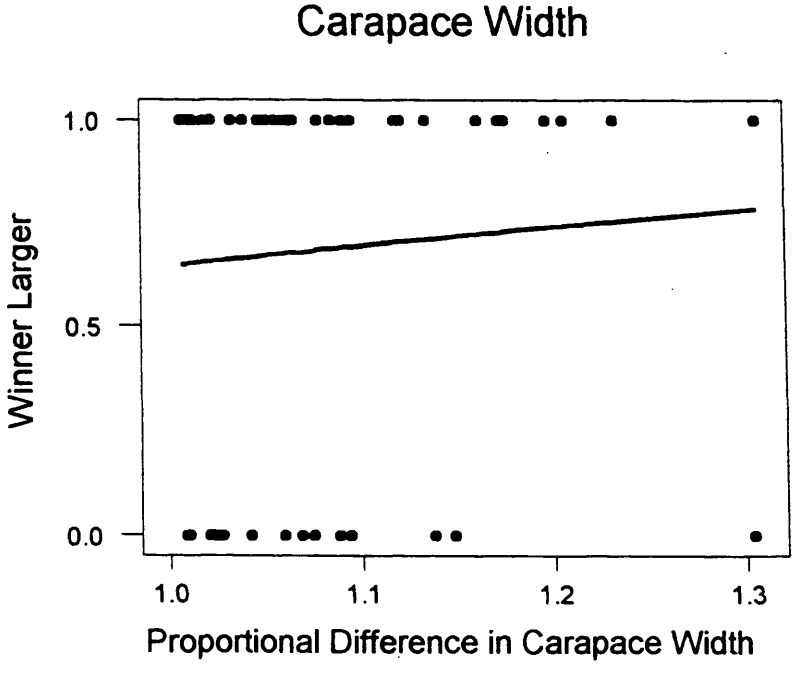


Figure 2. These graphs illustrate the results of a logistic regression performed on the proportional size difference in chela length (a) and carapace width (b) and on whether the winner was larger or smaller. The solid lines are the probabilities of a smaller or larger crab winning (points at 0.0 and 1.0 are smaller and larger winners respectively).

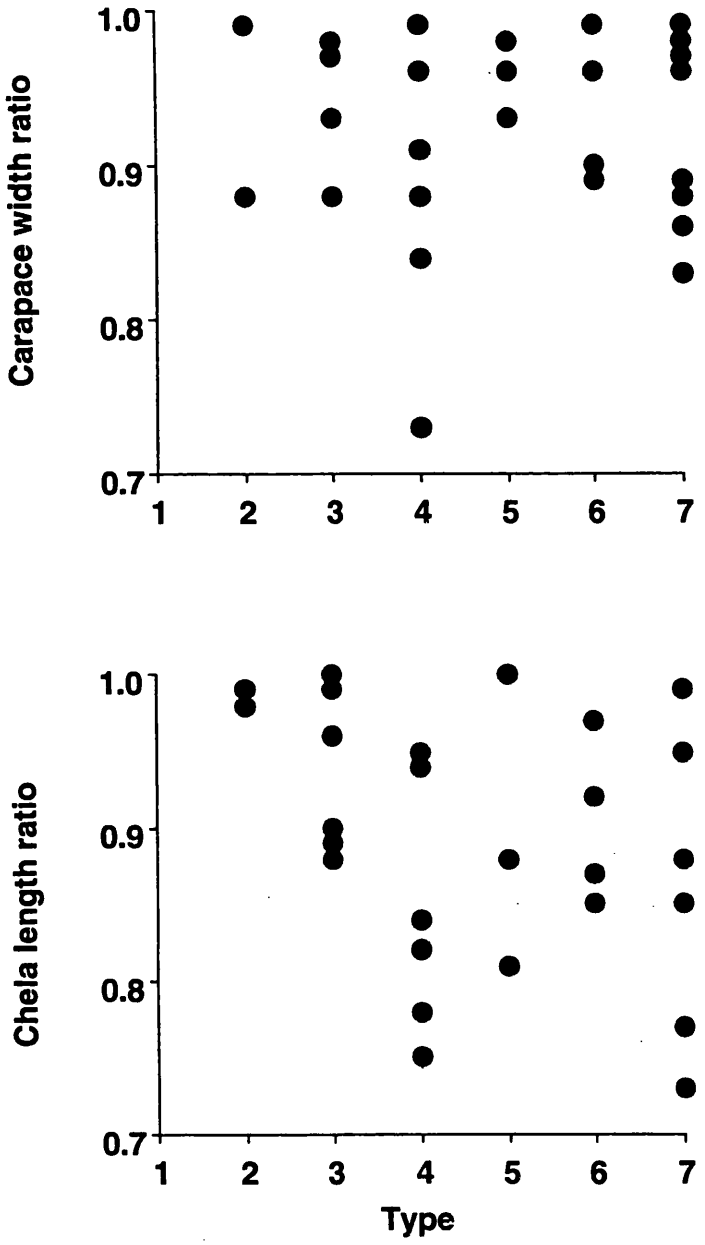


Figure 3. The distribution of contests type (Type 1 least intense, Type 7 most intense) with interactant size ratio for both chela length and carapace width.

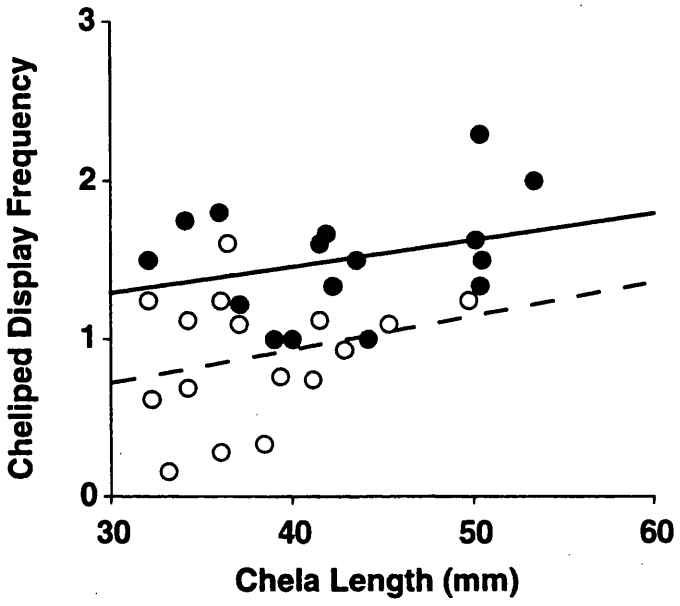


Figure 4. Mean cheliped display with chela length is shown for winners (solid circle, $y = 0.78 + 0.017x$, $r^2 = 0.093$; $n = 32$, solid line) and losers (empty circle, $y = 0.13 + 0.022x$, $r^2 = 0.068$; $n = 32$, dashed line) of pairwise fights.

3.4 DISCUSSION

Relative chela size appears to be more important than relative body size in influencing the outcome of agonistic contests between male *C. maenas*. This result indicates that weapon size may be a more reliable indicator of RHP than body size in these crabs. However, neither relative weapon nor relative body size are good indicators of overall fight duration or intensity. The absence of a relationship between either index of relative size and duration of first bout suggests that the lack of a relationship where overall duration is concerned, is not due to the crabs being confined in an experimental tank where they are forced to stay in close proximity to one another. From field observations, shore crabs fights involve one bout and no re-engagement of an opponent occurs since the loser can retreat far distances (Chapter 7; L.U. Sneddon, pers. ob.).

The chelipeds comprise a substantial proportion of total body weight (20% in *Carcinus maenas*, Lee & Seed 1992) and chela size is critically important in mating success, foraging, mate acquisition and defence, and to reduce inter- and intraspecific attack (Lawton 1989; Bildsten *et al.* 1989; Snedden 1990; Davenport *et al.* 1992; Garvey & Stein 1992; Claxton *et al.* 1994; Juanes & Smith 1995). In the shore crab *C. maenas*, it has been shown that paired, mating males have larger chelae compared to the average of the entire male population of that site (Lee & Seed 1992) however no data were collected with respect to body size. In addition, it is known that limb damage has a negative effect on male

Several studies have shown the importance of weapon size in male agonistic behaviour (e.g. the horned beetle, *Bolithotherus cornutus*, Brown & Bartalon 1986; red deer, Clutton-Brock *et al.* 1982; pseudoscorpions, *Dinocheirus arizonensis*, Zeh 1987a,b). It has been suggested that males with large weapons may be in "good" condition and that this trait may be an indicator of male phenotypic and genotypic quality (Kodric-Brown & Brown 1984; Andersson 1986). This may be important in the mating success of individual male *C. maenas* since studies have shown larger clawed crabs are more successful in obtaining mates (Lee & Seed 1992).

Winners perform significantly more cheliped displays than losers. This may indicate a difference in aggressiveness, with crabs with larger claws displaying them more. Alternatively, if claw size is the decisive factor, then the increased presentation of the claws by winners is a strategy they adopt to establish their dominance over an opponent. Therefore it may pay for a small crab to avoid aggressive interactions, to ensure chelipeds are not lost and hence impair chela growth, which may mean the chela would remain relatively large with body size which might enable the larger clawed crab to outcompete conspecifics. Another explanation may be that crabs in "good" condition may be able to grow bigger claws and that they are also more active and are victorious over smaller clawed crabs. The results of the present study support this hypothesis since crabs with larger surface area and heavier chelae won most fights.

Relative size, for both carapace width and chela length, does not influence the duration of bouts and hence does not agree with the

prediction of Game Theory that as opponents become more evenly matched the duration of fights increases (Maynard Smith 1982). Other studies have also failed to find a relationship between fight duration and size e.g. in dungflies, *Scatophaga stercoraria* (Sigurjonsdottir & Parker 1981) and scorpion flies, *Harpobatacus* spp. (Thornhill 1984). Fight duration is highly variable and unpredictable in the contests of *C. maenas* In contrast, in fights between male *N. puber*, the duration of interactions decreased as crabs became more disparate in size (Smith *et al.* 1994).

In other species of Crustacea (Hyatt 1983) and in other taxa (Archer 1988), relative size of combatants influences the duration and outcome of agonistic interactions between them. Contests were usually won by the larger crab but smaller crabs were successful only when the size difference was relatively small. Size is very likely to be related to strength and age in the decapods and thus to reflect RHP (Hartnoll 1982). This effect of size, both of the body and the chela (the more important factor), as a measure of relative RHP and as a predictor of the outcome in the fights of *C. maenas* agrees with Game Theory predictions (Parker 1974; Maynard Smith & Parker 1976) and is found in many other species such as the crayfish, *Orconectes rusticus* (Rubenstein and Hazlett 1974), *Cherax cuspidatus* (Pavey & Fielder 1996), the fiddler crabs, *Uca* spp. (Hyatt & Salmon 1978), snapping shrimps, *Alpheus heterochaelis* Say (Schein 1975) and other portunid crabs, e.g. *Callinectes sapidus* (Jachowski 1974), *Liocarcinus depurator* and *N. puber* (Huntingford *et al.* 1995). Since the difference in size between two opponents appears to influence winning but

not initiation, this would suggest that assessment may be occurring during rather than prior to the interaction (Enquist & Leimar 1983).

This study has shown that weapon size is a more reliable predictor of the outcome of these fights than carapace size and hence is a better indicator of RHP. The majority of other studies have used overall body size as a measure of RHP however weapon size may explain why, when there is little distinction in body size, the smaller bodied opponent wins. In stomatopod species, those species with heavier raptorial appendages are more aggressive (Dingle 1983) and this dissimilarity in weapon size may explain why smaller opponents won, when body length was less than 30% larger (Caldwell & Dingle 1979). Cheliped display frequency was higher for winners than in losers which may indicate that this behavioural trait, together with chela length, might also affect the outcome of fights.

Since chela size is more important than body size and there are equal numbers of smaller and larger crabs initiating fights, perhaps assessment of an opponent is occurring during rather than prior to an interaction. Assessment therefore could be tactile in grasping by the weapons and not visual and so future research on claw strength is essential to fully answer this question.

CHAPTER FOUR

WEAPON STRENGTH AS AN INDICATOR OF RESOURCE HOLDING POTENTIAL (RHP) IN FIGHTS BETWEEN SHORE CRABS

4.1 INTRODUCTION

The likelihood of an animal winning an agonistic interaction depends upon its fighting ability or RHP relative to that of its opponent, when factors such as resource value and ownership are symmetrical (Parker 1974). Opponents may base their strategic decisions during fights on comparing their RHP with that of their opponent's. Game theory models suggest that when there are large differences in RHP, then fights should be short and of low intensity, whereas fights should be of long duration and high intensity when RHP is similar between opponents (Maynard Smith 1982; Enquist & Leimar 1983; Archer 1988). This prediction has been supported by many studies where body size was measured and used as an indicator of RHP (review in Archer 1988). A few studies have used weapon size as an indicator of RHP and this has demonstrated that it is a much better index of RHP than body size (review in Andersson 1994) and a more reliable predictor of fight outcome (e.g. shore crabs, *Carcinus maenas*, see chapter 3; Sneddon *et al.* 1997a). Behavioural decisions of an animal can be strongly influenced by its perceived risk of danger (Lima & Dill 1990) and so the potential ability to inflict injury using the weapons may be more decisive in animal conflicts. Weapons are known to be important in agonistic behaviour (Andersson 1994) but few studies have measured actual weapon strength directly.

The present study examines the influence of relative weapon strength in the agonistic interactions of the shore crab, *Carcinus maenas*, a species in which relative weapon size has been demonstrated to be a more reliable

predictor of the victor of a fight than body size (Sneddon *et al.* 1997a). Contests between male shore crabs do not begin with initial display and crabs must engage one another in direct physical contact to resolve a contest which suggests that assessment is occurring during, rather than prior to a physical interaction (see chapter 2; Sneddon *et al.* 1997b). However, the visual cue, surface area of the claw, was found to be as important as the tactile cue, weight of the claw, in deciding the outcome of a fight (see chapter 3; Sneddon *et al.* 1997a). The aim of the present study, therefore, is to determine if weapon strength decides fight outcome and behavioural content.

The claws or chelae of decapod crustaceans are part of the specialised pereiopods, the chelipeds. They are used in many different contexts, among which are their nearly universal use in feeding, and their common use in social interactions as display and fighting organs (Dunham 1981). Chelipeds are used to threaten and attack opponents during conflicts as well as to secure, defend and position females prior to and during copulation (Snedden 1990). *C. maenas* has dimorphic chelipeds, having one claw larger (the major or crusher claw), than the other (the minor or cutter claw, Norman & Jones 1991). The claws are employed in aggressive encounters in a wide range of decapod species and it has been suggested that bigger claws confer selective advantages by increasing success in mate competition and intraspecific agonistic interactions (review in Lee & Seed 1992). In *C. maenas*, loss of the chelipeds was detrimental in their ability to defend a mate (Sekkelsten 1988; Abello *et*

al. 1994) and in the natural habitat, the majority of crabs which obtained females had larger sized claws than the average of the whole male population (Reid *et al.* 1994). During pairwise fights, male shore crabs grasp or pinch their opponents' body and limbs using the claws but injuries are not sustained when fighting over food (see Chapter 3; Sneddon *et al.* 1997b). It is conceivable that this behaviour provides a means of assessing an opponent's strength and so RHP may be assessed and strategic decisions can be made as to whether or not to escalate.

Therefore the objectives of this study were to determine if relative weapon strength exerted an influence on the outcome, behavioural content and duration of fights and if any morphological factors (i.e. carapace width and claw size) correlated with the forces exerted by the claws.

4.2 METHODS

4.2.1 Maintenance of the study animals

Male *Carcinus maenas* (carapace width range 55-80 mm, n = 54 crabs) were obtained from the University Marine Biological Station, Millport, Isle of Cumbrae where they had been freshly caught by creeling from the Clyde Sea area. On their arrival in Glasgow the crabs were transferred to individual holding tanks (18 x 21 x 23cm), supplied with circulating seawater (salinity 32-34°/$_{oo}$) maintained at 10 ± 2°C and on a 12 : 12 h light-dark cycle, with experiments being carried out in the light period. The crabs were kept in these isolated conditions for seven days prior to any behavioural observations and were deprived of food during this period. Crustaceans can survive for up to three months without food so this regime kept the crabs healthy but motivated to fight over food.

Crabs were only used in behavioural observations if they were in pristine condition i.e. the exoskeleton was hard, there was no excessive epifaunal growth and there were no missing or recently regenerated limbs. The carapace width of each crab was measured to the nearest 0.1mm between the fourth and fifth lateral spines, using vernier callipers 24 hours prior to each fight. The major chela length or the total propodus length (CL); length to the start of the dactyls (L-D), dactyl length(DL) and chela height (CH) were also measured as shown in figure 1. After completion of the experiments the crabs were held for a further two weeks to make sure they were not in proecdysis, and none were. During this time the crabs were fed *ad libitum* every 3 days.

4.2.2 Determination of claw force

The strength of both claws was tested using a customized strain gauge load transducer (similar to the gauge used by Preston *et al.* 1996). The transducer was fitted with precision constantan, fully encapsulated strain gauges (CEA-06-125UN-120, Measurements Group, Basingstoke, UK). Each claw was tested separately and the crab was allowed to exert pressure on the endplates. It was found that when any attempt was made to withdraw the transducer from the crab claw, the crab would respond by attempting to retain its grip on the endplates; the forces exerted were recorded as maximal chelar forces under these experimental conditions. A computer link allowed a plot of force exerted against time (s) to be produced from which maximum force exerted could be noted. Owing to difficulties experienced when attempting to measure crab cheliped strength in seawater, each crab was tested out of water and held in position by hand, as done in previous studies (Preston *et al.* 1996; Blundon & Kennedy 1982). The transducer was calibrated on a JJ tensile testing machine to a maximum value of 300 Newtons (N), loading from 0N to 300N and back again to 0N. The relationship between microstrain (y) and force or load (x) exerted on the endplates of the transducer was found to be approximately a 1:1 relationship where dy/dx had an average value of 1.015 (hysteresis = 0.005). Thus values of microstrain could be used to determine the force (N) applied to the transducer. This allowed the estimation of crab claw strength from the microstrain values recorded

when the crab gripped the 2 endplates of the transducer. The crabs were tested twice, 24 hours and 6 days after their arrival in Glasgow. The time of testing was chosen to give the crabs 24 hours to recover from creeling and to test the strength of claws 24 hours prior to fights to obtain an accurate measure of claw force that can be potentially exerted during the fights. The claw force measurements were shown to be highly repeatable between the two days of testing (intraclass correlation coefficient (Zar 1984), $r_I = 0.9973$, $F_{54,1} = 742.85$, $P < 0.00001$).

4.2.3 Behavioural Observations

Fights (n = 27) were staged by placing two size-matched crabs in a glass observation tank (48 x 43 x 34cm), containing a gravel substratum, and filled with aerated seawater. The tank was screened from visual disturbance (see Chapter 3). Crabs were separated by a vertically sliding opaque partition to allow them a settling time of fifteen minutes in continued isolation. To reduce the possibility of chemical communication, the air and water pumps were switched off to minimise mixing during the settling period. The tank was illuminated from above at a light intensity of 1.82 - 2.66 μ E. m^{-2} sec^{-1} at the top and 0.89 - 1.40 μ E. m^{-2} sec^{-1} at the bottom of the tank. Observations were made through a small opening in the screen. After the initial settling period and when both crabs were stationary, the partition was raised from outside the screen by a pulley. Ten ml of food extract (whitebait homogenised in seawater) was added, from a syringe via plastic tubing, into the centre of the arena at the start of

the experiment. The actions of both crabs were recorded using a lap top computer as an event recorder. The intensity of the contests was then determined from the behavioural content (see chapter 2 and Sneddon *et al.* 1997b for details). The end of a contest was judged to have occurred when there was a clear winner which elicited two repeated retreats from its opponent or successfully climbed on top of the other contestant, the loser.

The crab that first moved to its opponent and engaged in physical contact was defined as the initiator. The winner was the crab that successfully climbed on top of its opponent or elicited repeated retreats from the other crab, (the loser). The duration of the fights was defined as the time from initiation to when the fight was resolved.

4.2.4 Weight of claws

Ten pairs of crabs, chosen at random from the 27 staged contests, were humanely killed by immersion in liquid nitrogen after fighting and the major chela taken off by careful dissection using a scalpel. The sample size of no more than ten was chosen for ethical reasons. The major chelae used in the surface area analyses were placed in a preweighed vial and freeze dried (Edwards Modulyo) then weighed accurately (to 0.001g).

4.2.5 Statistical Analyses

To determine if the potential of the chelae to exert a greater force influences initiation or winning of contests, a chi square test was performed on the numbers of weaker or stronger clawed crabs which initiated or won fights. Paired T-Test analyses were used to compare the forces in both claws exerted by winners and losers. Regression was performed on each of the morphological measurements separately to determine if claw force was correlated with size for both claws and multiple regression was used to determine if any of these morphological attributes were correlated with one another. Paired T-tests were used to compare each of the morphological measurements of both winners and losers. The ratio of weapon strength in each pair was calculated by dividing the smaller force exerted by a contestant in a fighting pair by the greater force of their opponent for both the crusher and cutter claw. This ratio was used in Spearman rank correlation analyses to determine if relative weapon strength was related to the intensity or duration of fights.

4.3 RESULTS

4.3.1 The influence of relative force on initiating and winning fights

Crusher claw force ranged from 34.90N to 148.47N and cutter claw force ranged from 17.64N to 86.24N. The number of crabs with claws capable of exerting more or less force than their opponent who either initiated or won fights is shown in table 1. Approximately equal numbers of crabs with greater or less relative force initiated fights but more crabs with stronger claws won fights (89%). A paired T-Test confirms that winners had both crusher and cutter claws capable of exerting a greater force than either claw in losers (Table 2; Fig. 2).

4.3.2 The relationship between force and morphological measurements

Crusher claw force in winners was related separately to carapace width and CL but not to L-D, DL or CH (Table 3). In losers, crusher force was related to carapace width, CL and DL but not to L-D and CH (Table 3). The cutter claw of winners exerted a force which correlated with carapace width, CL, L-D and CH but not to DL (Table 3). In losers, the force exerted by the cutter claw was similarly related to these morphological traits except CH (Table 3).

Multiple regression showed that DL was highly correlated with all the other morphological variables and was not entered into the regression analyses for either the crusher or cutter claws for both winners and losers.

The results of this analysis are shown in Table 4, where force is related to the other morphological measurements taken.

Winners had claws which were heavier than losers (crusher $T = 4.15$, $p = 0.004$; cutter $T = 3.68$, $p = 0.008$, $n = 10$; Fig. 3) and claw force was positively related to dry weight (crusher $F_{1,9} = 5.01$, $p = 0.004$; cutter $F_{1,9} = 11.83$, $p = 0.002$).

4.3.3 Relative size and contest content

Winners and losers were matched for body size ($T = 1.39$; $p = 0.21$) and crusher CL ($T = 1.39$; $p = 0.18$) but winners tended to have crushers with greater height or CH ($T = 2.34$; $p = 0.006$) and with greater L-D ($T = 2.34$; $p = 0.027$). Cutter claws were matched in winners and losers for all of the measurements (CL - $T = -0.30$, $p = 0.77$; L-D - $T = -0.28$, $p = 0.78$; DL - $T = -0.53$, $p = 0.60$; CH - $T = 0.25$, $p = 0.81$).

The ratio of the claw force of both claws for each fighting pair was not correlated with either intensity (crusher $Rs = 0.366$, $p = 0.856$; cutter $Rs = 0.210$, $p = 0.294$) or duration of fights (crusher $Rs = -0.196$, $p = 0.326$; cutter $Rs = -0.096$, $p = 0.633$).

Table 1. The number (and percentage) of crabs who initiated or won fights and who exerted a lesser or greater force with the crusher claw ($X^2 = 6.18$, $p < 0.05$, $n = 27$).

	FORCE	
	Lesser	**Greater**
Initiate	11 (41%)	16 (59%)
Win	3 (11%)	24 (89%)

Table 2. A comparison of the force exerted by winners (W) and losers (L) by both the crusher and cutter claws using paired T-Tests (n = 27).

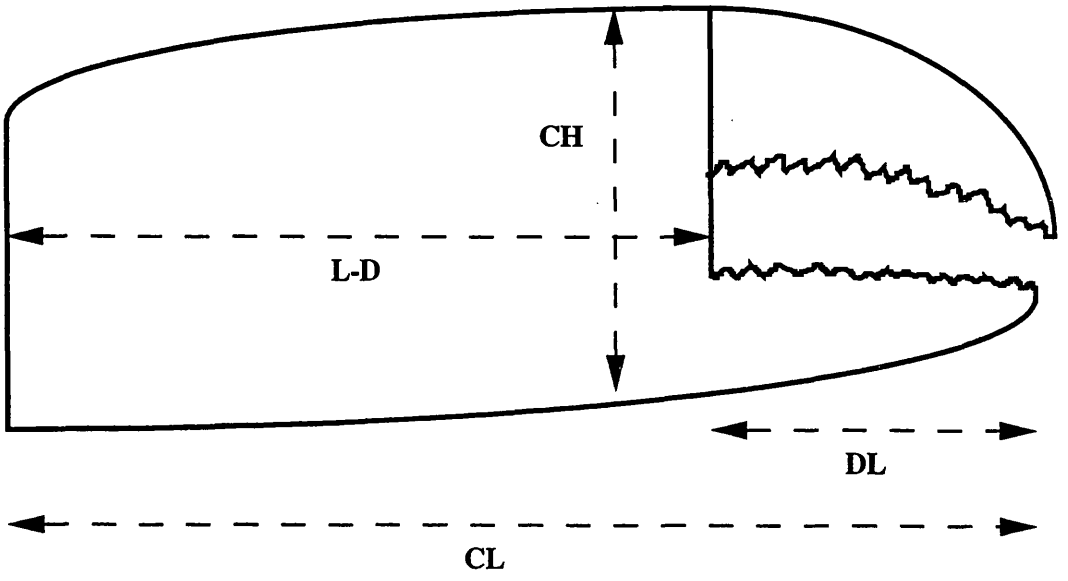
	Mean Force (±SE)		**T**	**p**
	W	**L**		
Crusher	95.45 (±6.04)	75.80 (±6.45)	6.15	<0.0001
Cutter	54.76 (±3.38)	40.87 (±2.55)	5.49	<0.0001

Table 3. Regression analyses of each of the morphological measurements and the forces exerted by both the crusher and cutter claws of winners and losers (n = 27 winning and losing crabs).

	Crusher		Cutter	
	$F_{1,26}$	p	$F_{1,26}$	p
Winners Force				
Carapace width	5.89	0.023	13.68	0.001
CL	5.95	0.023	12.98	0.001
L-D	2.13	0.157	10.72	0.003
DL	2.55	0.123	1.50	0.231
CH	3.40	0.077	12.35	0.002
Losers Force				
Carapace width	4.46	0.050	8.36	0.009
CL	4.44	0.050	4.70	0.042
L-D	0.51	0.480	7.05	0.015
DL	9.08	0.006	0.48	0.495
CH	1.63	0.214	1.47	0.237

Table 4. Results of multiple regression analyses performed on crusher and cutter claw force and all morphological measurements for both winners (W) and losers (L). The dactyl (DL) was removed from all the regressions as it was highly correlated with all the other measurements.

	CRUSHER FORCE		CUTTER FORCE	
	W	**L**	**W**	**L**
$F_{4,21}$	4.70	3.05	3.70	4.60
p	0.042	0.038	0.019	0.009



CL = claw length
DL = dactyl length
L-D = length minus the dactyl
CH = claw height

Figure 1. Diagram of a claw indicating what measurements were taken from the claws of each crab used in this study

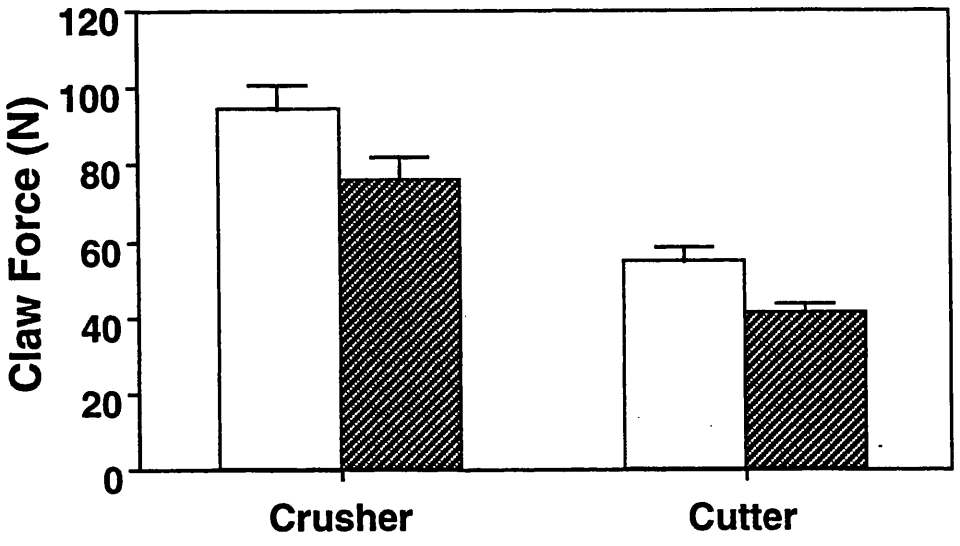


Figure 2. Mean (±SE) claw force for the crusher and cutter claws for both winners (empty bars) and losers (hatched bars) of fights between pairs of shore crabs, *Carcinus maenas* (n = 54 crabs)

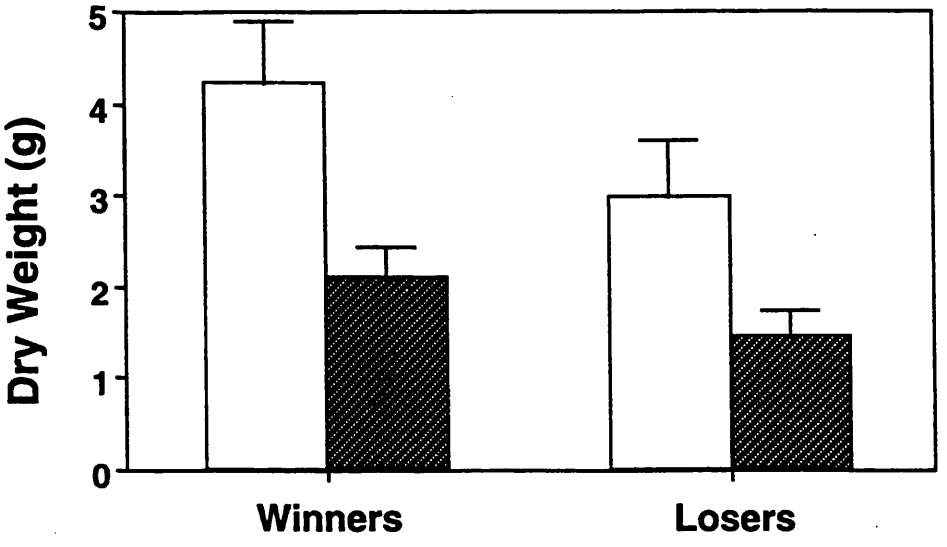


Figure 3. Mean (±SE) dry weight of crusher (empty bars) and cutter (hatched bars) claws for both winners and losers of fights between pairs of shore crabs, *Carcinus maenas* (n = 54 crabs)

4.4 DISCUSSION

Having stronger claws than an opponent increases the likelihood of winning fights but does not influence initiating them, which agrees with the results of a previous study on body size and claw length (see chapter 3; Sneddon *et al.* 1997a). This suggests that shore crabs must engage one another during agonistic interactions to assess RHP and this probably does not occur in display before physical contact is made. Relative weapon strength is a more reliable predictor of fight outcome than weapon size, since a greater percentage of fights were won by crabs which had stronger claws than their opponent (89%) than having longer claws or CL (78%, Sneddon *et al.* 1997a). Hence weapon strength is a better indicator of RHP than body or claw size. Only 3 of these contests were won by the weaker clawed crab, and in these contests the maximum force difference between the two opponents was only 5%, whereas in a previous study, the maximum size difference between crabs in contests in which the smaller crab won, was 18% and 30%, for claw length and body size, respectively (Sneddon *et al.* 1997a). Therefore the assessment of an opponent's strength may be crucial in making strategic decisions in this species and may enable crabs to decide whether or not to escalate since the behaviour of winners and losers is very different from an early stage in shore crab fights (Sneddon *et al.* 1997b). This agrees with research carried out on the freshwater crab, *Potamon fluviatile*, in which claw strength was important in the establishment of dominance hierarchies but only in female crabs and not males (Gabbanini *et al.* 1995).

Although the claw length was matched for each fighting pair in this study, winners had crusher claws with greater height (CH) and length to the dactyl (L-D) and this may give winners a mechanical advantage which allows them to exert greater force when pinching. These differences also mean that winners have claws with greater surface area which, when presenting them to competing conspecifics or to attacking predators, may aid deterrence of the attacker, and winners claws may also have a greater muscle mass which is confirmed by the dry weight results. However, the cutter claws are matched in all the size measurements yet winners have cutters which exert a greater force than losers. This may be explained by the fact that winners have cutters with a greater dry weight than losers and so have a greater muscle mass within the claw which allows them to exert a greater force. This may be due to the crabs being in intermoult for a longer period than their opponent. After moulting, the muscle mass within the exoskeleton is greatly reduced and the muscle tissue increases during intermoult (Hartnoll 1982). Another explanation could be that winning crabs are in better condition than losing crabs and are able to invest more energy into growing larger, heavier claws with greater musculature. They are, therefore, able to potentially inflict greater injury on their opponent and this may be decisive on the outcome of fights since animals should base their behavioural decisions on their perceived risk of danger (Lima & Dill 1990). Other studies have shown that larger clawed crabs are more successful in obtaining mates and so having larger claws is a desirable trait (Sekkelsten 1988; Abello *et al.* 1994; Reid *et al.* 1994). Many studies

have suggested that having larger armaments or weapons indicates better male quality (Kodric-Brown & Brown 1984; Andersson 1986) and they have a dual function as ornaments which may be important in female choice (Berglund *et al.* 1996). This hypothesis is supported by the results of a study on salmon, *Salmo salar*, in which dominant males have larger kype size and are also more successful in securing matings than subordinates (Järvi 1990). Amongst shore crabs where males establish dominance hierarchies, a female may gain fitness by choosing among the most dominant males in the short term since she will be protected during the vulnerable post moult period and will mate without being interrupted. In the long term, the advantages of choosing dominant mates are that the offspring will be of better genetic quality, provided fighting ability and weapon strength has a genetic basis.

The larger crusher claws were able to exert greater forces than the smaller cutter claws and the forces generated were similar to those found in a previous study on *C. maenas* (Preston *et al.* 1996). It has been shown that there is a positive correlation between force and body size (Preston *et al.* 1996) and in the present study, when analysing each of the morphological measurements separately, force was related to carapace width in both claws in winners and losers. Force is also related to claw length, but this is to be expected since claw length grows relatively to carapace width in these crabs (Hartnoll 1982). The length of the claw excluding the dactyl (L-D) is related to force only for the cutter claws and claw height does not appear to be a good indicator of claw force. This is

contrary to a previous study which suggested that claw height was an acceptable indicator of claw strength (Lee 1993). However, Lee (1993) examined sarcomere length in the muscle of the claw and used a wider size range of crabs (30 - 70mm carapace width; Lee 1993) than the present study. Perhaps sarcomere length would have been a more reliable indicator of claw force and should be examined in future experiments, since studies have shown that claws with a high mechanical advantage have longer sarcomeres than those with a low mechanical advantage (Abby Kalio & Warner 1984).

The dry weight of the claws was greater in winners than losers for both claws and force was related to dry weight. Therefore, winners have heavier, stronger weapons than losers, even though they are matched in claw length. It appears as if this gives winners a competitive advantage over losers and it would pay crabs to invest energy into growing claws with greater height (CH), and longer L-D giving an overall greater surface area, and greater muscle tissue.

Relative force influences the outcome of fights but not the intensity or duration of contests for both the crusher and cutter claws. This agrees with a previous study on *C. maenas* which showed that neither body size or weapon size is related to the length or intensity of contests (see chapter 3; Sneddon *et al* 1997a). Therefore relative weapon strength can only be used as a predictor of fight outcome but not as an indicator of behavioural content. Weapon or claw size has been shown to be important in a number of studies on agonistic behaviour in crustaceans (e.g. crayfish, *Orconectes*

propinquus (Smith & Dunham 1990); *O. rusticus* (Snedden 1990; Garvey & Stein 1993; Rutherford *et al.* 1994); fiddler crabs, *Uca burgersi* (Jones 1980); stomatopods, *Gonodactylus viridis* (Caldwell & Dingle 1979)). However, none of these studies have examined the influence of relative weapon strength and perhaps using this as an RHP index may explain why there has to be large size differences between opponents before the larger contestant will definitely win (e.g. 30% difference in body size of *G. viridis*, Caldwell & Dingle 1979; 18% difference in weapon size of *C. maenas*, Sneddon *et al.* 1997a). It would be also interesting to determine the effects of weapon strength on agonistic interactions in other taxa since, weapon size is important in animals such as red deer, *Rangifer tarandus* (Clutton-Brock *et al.* 1982), horned beetles, *Bolithotherus cornutus* (Brown & Bartalon 1986), and pseudoscorpions, *Dinocheirus arizonensis* (Zeh 1987a,b). However, to do this, the mechanical difficulties, for example, of measuring a red deer pushing against its opponent using the antlers, would have to be overcome.

CHAPTER FIVE

IMPACT OF AN ECOLOGICAL FACTOR ON THE COSTS OF RESOURCE ACQUISITION: FIGHTING AND METABOLIC PHYSIOLOGY OF CRABS.

5.1 INTRODUCTION

Predictions from game theory models about agonistic behaviour rest on the assumption that behavioural strategies evolve through selection that maximises 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 *et al.* 1996). While these state dependent strategies have been demonstrated, the mechanisms underlying them are not well known. Here a study is described, 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 *et al.* 1995).

The shore crab, *Carcinus maenas* (L.), the subject of this study is one of the most common decapods found in north-west European rock pools, and exhibits both circatidal and circadian rhythms in locomotion which have been well studied (Edwards 1958; Kitching *et al.* 1959; Crothers 1964; Muntz *et al.* 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 due to oxygen depletion resulting from the respiration of the pool biota (Edwards 1958; Dare & Edwards 1981; Robertson 1989; Hill *et al.* 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 Po_2 of the water in the pool falls below the 'critical' Po_2 or Pc for this species (approximately 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 (Zebe 1982; Booth et al. 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_{O2} 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 twelve hours 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 (Chapter 7). 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 this was not statistically different from resting values and that 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 characterise 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.

5.2 METHODS

5.2.1 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 x 21 x 23cm) 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 recorded 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 three weeks of captivity (Houlihan & Mathers 1985). Crabs were kept for two weeks after experimentation to ensure that they were not in proecdysis, and none were.

Fights between pairs of crabs were staged in a small tank (55 x 28 x 30 cm) so that the P_{O2} 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 Po_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 Po_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 - 80mm 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 Chapter 2; Sneddon *et al.* 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 Po_2 in either side of the tank which was always within ± 2% agreement. The crabs were left for a settling period of 1 hour during which time the Po_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 homogenised 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 lap top 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 mis-matched.

5.2.2. 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 (21g) attached to a syringe (1ml). This was repeated for 15 contests staged under normoxic conditions.

In another 5 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 tissues types were analysed as part of another study and there are no significant differences between metabolite concentrations in the three tissues (Chapter 6). This procedure was repeated for 5 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 et al. (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, Dorset U.K.).

5.3 RESULTS

5.3.1. 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" ($S = 6.4$, $P = 0.012$), "cheliped display" ($S = 8.0$, $P = 0.005$) and contact acts ($S = 9.3$, $P = 0.002$) whereas losers perform more "move away" ($S = 13.0$, $P < 0.0001$).

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; $T = -5.75$, $P = < 0.00001$; 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; $T = 0.14$, $P = 0.89$).

5.3.2. The influence of relative size

The effects of relative size, both carapace width and chela length, on contest initiation and outcome were tested by Chi Square. 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, $X^2 = 4.78$, $P < 0.05$; chela length, $X^2 = 8.05$, $P < 0.01$) whereas in contests staged under normoxia the results are only significant for chela length ($X^2 = 4.08$, $P< 0.01$) and not carapace width ($X^2 = 1.6$, $P> 0.05$) with a smaller proportion of larger individuals winning (see Chapter 3; Sneddon *et al.* 1997b).

Paired T-Tests showed that in fights staged under hypoxic conditions, winning crabs have wider carapaces ($T = 4.33$, $P = 0.0007$), and greater claw length ($T= 3.36$, $P = 0.0046$) and claw height ($T= 4.51$, $P = 0.0005$) as was also the case in contests staged under normoxia (Chapter 3; Sneddon *et al.* 1997b).

Regression analysis was used to determine if there was any relationship between relative size and 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 ($F_{1,12} = 0.05$, $P = 0.829$; $F_{1,29} = 0.24$, $P = 0.624$ respectively) or chela length ($F_{1,12} = 0.35$, $P = 0.563$; $F_{1,29} = 0.07$, $P = 0.792$ respectively). The duration of bouts of wrestling was also unrelated to relative size (carapace width $F_{1,6}$

= 0.5, P = 0.510; $F_{1,29}$ = 2.38, P = 0.135; chela length $F_{1,6}$ = 0.09, P = 0.819; $F_{1,17}$ = 2.12, P = 0.158) in contests staged under hypoxia and normoxia, respectively.

Spearman Rank coefficients were calculated to the examine effects of intensity and water PO_2 levels. Contest intensity in hypoxia was unrelated to relative size (carapace width Rs = 0.22, P = 0.40; chela length Rs = 0.39, P = 0.12; n = 14) and to contest duration (Rs = 0.12, P = 0.66, n = 14) which agrees with the results from normoxic contests obtained in a previous study (carapace width Rs = -0.06 P = 0.75; chela length Rs = -0.27, P = 0.16, n = 27; duration Rs = -0.04, P = 0.87, n = 17; Sneddon *et al.* 1997b).

5.3.3. Physiological consequences

To compare levels of haemolymph metabolites in winners and losers the values 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 = 0.18, P = 0.674; 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 $F_{1,29}$ = 1044.5, P <0.0001; glucose

$F_{1,29}$ = 1499.3, P <0.0001; 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 Chapter 2 (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 $F_{1,29}$ = 0.32; P = 0.57; glucose $F_{1,29}$ = 0.00, P = 0.96).

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 $F_{1,14}$ = 1.56, P = 0.23; loser L-lactate $F_{1,14}$ = 0.00, P = 0.98; winner glucose $F_{1,14}$ = 0.27, P = 0.61; loser glucose $F_{1,14}$ = 0.31, P = 0.55; Hypoxia - winner L-lactate $F_{1,14}$ = 1.66, P = 0.22; loser L-lactate $F_{1,14}$ = 0.06, P = 0.80; winner glucose $F_{1,14}$ = 1.84, P = 0.20; loser glucose $F_{1,14}$ = 0.55, P = 0.473).

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 between winners and losers of each contest (L-lactate $F_{1,9}$ = 0.04, P = 0.84; glucose $F_{1,9}$ = 0.44; P = 0.52; glycogen $F_{1,9}$ = 0.10, P = 0.75). L-lactate ($F_{1,9}$ = 158.7, P <0.0001) and glucose ($F_{1,9}$ = 38.42, P <0.001) 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 ($F_{1,9}$ = 576.5, $P < 0.0001$, Fig. 3).

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

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 (To = "move to"; Away = "move away"; Display = "Cheliped display"; Chelae in = "chelipeds in, body raised" and Contact = "strike", "grasp", "push" and "climb on"; see Chapter 2; (Sneddon *et al.* 1997a) for a detailed description of acts).

Act	W/L	Median		H	P value
		hypoxia	normoxia		
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

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 ($X^2 = 4.78$, $P < 0.05$) and chela length ($X^2 = 8.02$, $P < 0.01$).

	Carapace width		Chela length	
		Hypoxia		
	Smaller	**Larger**	**Smaller**	**Larger**
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%)**

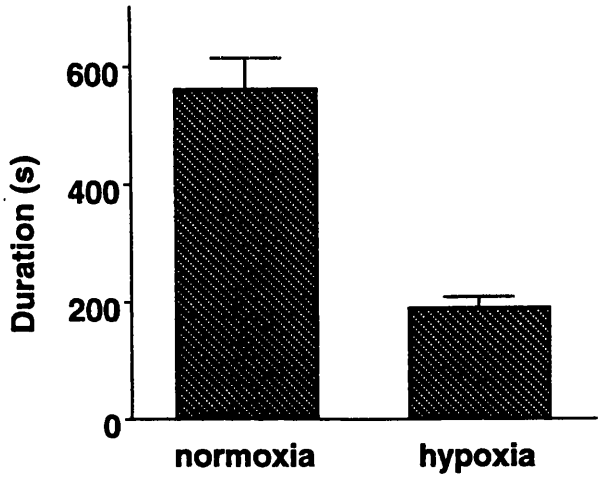


Figure 1. Mean duration of contests staged between size matched male *Carcinus maenas* under normoxic and severely hypoxic conditions. Error bars are SE.

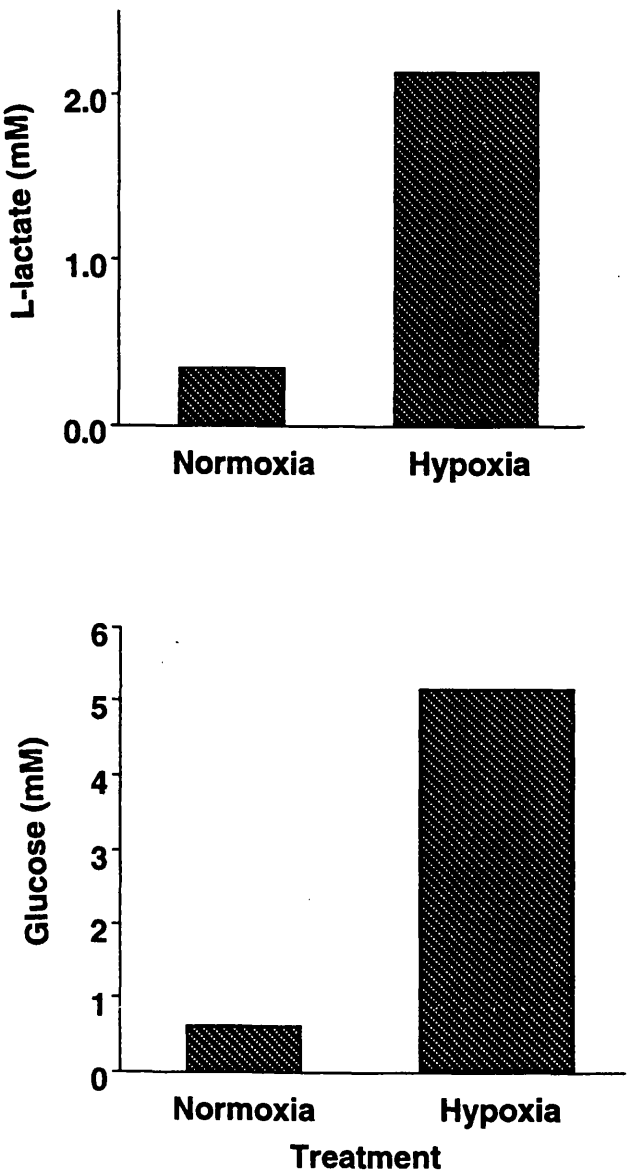


Figure 2. The concentrations (mM) of L-lactate and glucose in the haemolymph of size matched *Carcinus maenas* after contests staged under normoxic and severely hypoxic conditions. Values are medians.

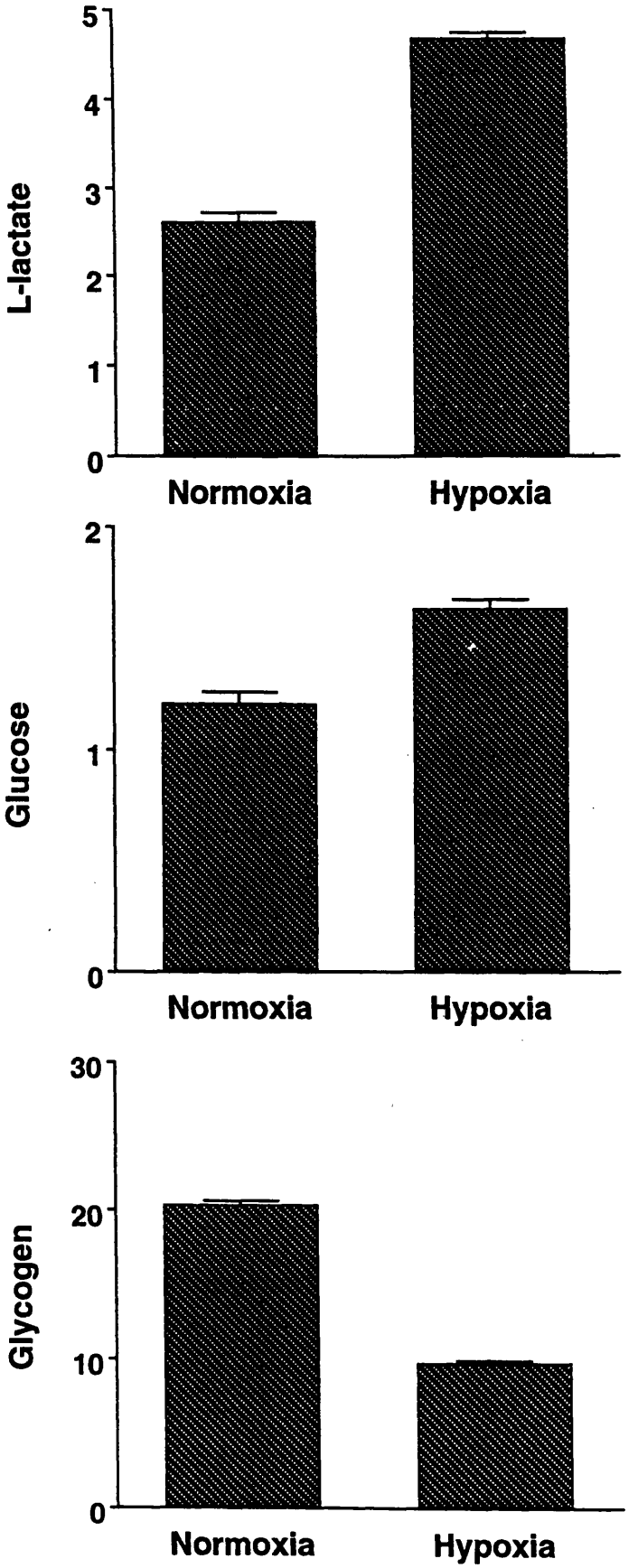


Figure 3. The mean concentrations ($\mu mol.g^{-1}$) of L-lactate and glucose in the leg muscle tissue (4th pereiopod) of size matched *Carcinus maenas* after contests staged under normoxic and severely hypoxic conditions. Error bars are SE.

5.4 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 (Po_2 < 20 Torr) which regularly occur in the field, resulted in *C. maenas* of 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 to 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 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 concetrations 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 (Chapter 2; 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 et al. (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. brook charr, *Salvelinus fontinalis,* Kieffer *et al.* 1996; and rainbow trout, *Oncorhynchus mykiss,* Ferguson et al. 1993).

Fighting in normal oxygen conditions for *Carcinus maenas* is sufficiently energetically demanding to force the crabs to resort to anaerobic respiration, but not as demanding as exhaustive exercise on a treadmill (Chapter 6). 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 *et al.* 1994) whereas in *C. maenas*, the difference in behaviour of winners and losers is evident from an early stage (Chapter 2; 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 to 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 Po_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 hours 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.

CHAPTER SIX

METABOLIC CONSEQUENCES OF AGONISTIC BEHAVIOUR: CRAB FIGHTS IN DECLINING OXYGEN TENSIONS

6.1 INTRODUCTION

The concept of the "cost" of a given behaviour pattern, in terms of its negative effects on fitness, is critical to current ecological theory (Krebs & Davies 1978). In the case of agonistic behaviour various negative consequences have been considered including injury (Shuster & Caldwell 1983), increased predation risk (Ellington 1983), reduction of time spent on other activities (Glass & Huntingford 1988), and increased energy expenditure (Hack 1997). Two potentially detrimental consequences of fights, or any vigorous activity, are depletion of energy reserves (Plaistow & Siva-Jothy 1996; Neat *et al.* 1998) and accumulation of potentially harmful respiratory by-products (Thorpe *et al.* 1995; Neat *et al.* 1998). Both of these may place constraints on the performance of subsequent activity (Eckert *et al.* 1988; Ellington 1983). In the cichlid fish, *Tilapia zillii*, fighting resulted in depletion of energy reserves and accumulation of L-lactate in the muscle tissues (Neat *et al.* 1998). The metabolic consequences of agonistic interactions are not necessarily fixed, but may depend on ecological conditions (Sneddon *et al.* 1998). For example, population density increased the costs of fighting in demoiselles, *Chromis dispilus* (Barnett & Pankhurst 1996), and red backed salamanders, *Plethodon cinereus* (Jaeger *et al.* 1983), and water velocity increased the energy costs of territorial defence in brook charr, *Salvelinus fontinalis* (McNicol & Noakes 1984). The present study attempts to quantify the extent to which energy stores are depleted and respiratory by-products accumulated in male shore crabs (*Carcinus maenas*) after fighting.

The shore crab, *C. maenas*, is known to inhabit rock pools which regularly become hypoxic (lower than normal oxygen conditions) or even anoxic (no oxygen) due to respiration of the pool biota (Hill *et al.* 1991) and this species is very tolerant of these conditions, being capable of withstanding 12 hours of anoxia (Robertson 1989). Extensive field and laboratory studies have shown that when the crabs are under hypoxic conditions, there is a greater reliance on anaerobic respiration with increased concentrations of L-lactate and glucose in the blood and tissues, and a reduction in glycogen stores (Hill *et al.* 1991). Male shore crabs have been observed fighting under hypoxic conditions in rock pools (Chapter 7) and since this inter-tidal species fights under a wide range of oxygen tensions in its natural habitat, the metabolic consequences of agonistic behaviour in relation to water oxygen tension were also examined. An earlier study has shown that, while the intensity of fights between crabs is unchanged by hypoxia, the time spent engaged in fights is reduced from an average of 559 seconds under normal oxygen conditions to 193 seconds under severely hypoxic conditions (Chapter 5; Sneddon *et al.* 1998). Accumulation of L-lactate was much more marked in the low oxygen environment suggesting that glycogen depletion and/or L-lactate accumulation may be part of a mechanism by which fights are resolved (Chapter 5; Sneddon *et al.* 1988). To test this, the relationship needs to be examined in more detail to determine if these metabolic effects are a means by which the resolution of contests are determined as in the cichlid (Neat *et al.* 1998). In addition, to assess the extent to which

these metabolic consequences may represent energetic costs, it is necessary to investigate how they compare with the metabolic consequences of activity unrelated to fights. The purpose of the present study was, therefore, to:- (1) compare the metabolic consequences of fights at different oxygen levels with those generated by two extremes of activity unrelated to fighting; (2) examine in more detail how the duration of fights and their metabolic consequences vary in relation to declining oxygen tensions; and (3) see whether pre-exposure to hypoxic conditions affects the duration of conflicts and its metabolic consequences.

Therefore, it was decided to stage contests under normoxic conditions (100% saturation of O_2 in water or when the partial pressure of water (Po_2) is 154 Torr) and under hypoxic conditions (down to 15 Torr or ~10% normoxia) to examine the metabolic effects of fighting under different environmental conditions which the crabs experience in their natural habitat. We measured changes in energy stores by glycogen depletion and resultant glucose release (Lynch & Webb 1973; van Aardt 1988; Weinstein *et al.* 1988). By-products of respiration where measured in terms of accumulation of L-lactate, the end-product of anaerobic respiration that is known to increase in the tissues and crabs after activity (Houlihan *et al.* 1985; Hill *et al.* 1991; Hamilton & Houlihan 1992). In an attempt to gauge the metabolic effects of fighting, the metabolites were also measured in crabs at rest and after exhaustive activity, so that a comparison could be made between fighting and these two extremes of activity to see if fighting actually does have an effect on metabolite

concentrations and if this is anywhere near maximal rates of anaerobic respiration.

6.2 METHODS

6.2.1. Maintenance of Study Animals

90 male shore crabs (carapace width range 55 - 80mm) were obtained, with no missing or regenerated limbs, no excessive epibiont coverage and no obvious signs of parasitism, by creeling in the Clyde Sea area between March and May in the vicinity of the University Marine Biological Station and they were transported to Glasgow where they were housed in individual holding tanks (18 x 21 x 23cm). The tanks were supplied by circulating sea water (32 - 34$^{o}/_{oo}$) at 10 ± 1°C and were maintained under a 12:12 light dark cycle with all experiments being carried out during the light period. The crabs were deprived of food for 7 days prior to any experiments since this is known to reduce intra-individual variation in metabolite concentrations (Hill 1989). This species can withstand three months of food deprivation (Wallace 1973), so this starvation period is relatively short. Concentrations of L-lactate, glucose and glycogen before fighting were not measured since this would involve either removing a blood sample, which would have a direct effect on metabolite concentrations itself, or obtaining tissue samples, which would mean sacrificing the crabs. There were no significant differences between winners and losers (see below) in each metabolite after fighting so it is assumed that each pair had similar metabolite profiles prior to fighting. Since captivity is known to have a detrimental effect on metabolic capacity (Houlihan *et al.* 1985), crabs were used within 10 days of capture to minimise this effect and to make the tests consistent. Crabs were used

only once for experimentation and then were retained for two weeks to ensure they were not in proecdysis (none were).

6.2.2. Experiment 1 - Metabolic consequences of agonistic behaviour and exercise under normoxic and severely hypoxic conditions

Crabs were allocated to one of three groups as follows:-

1. REST (n = 20). Each crab was transferred separately to a partitioned arena (55 x 25 x 30cm) which was screened from visual disturbance and left them for one hour. During this hour under normoxia, air was bubbled through air stones which were positioned at opposite ends of the tank. Oxygen electrodes were positioned on opposite sides from the air stones to closely monitor oxygen tensions and these were always within 2% agreement. In the severely hypoxic treatment, the Po_2 of the water was decreased gradually over the hour to the required level by altering the percentage composition of a gas mixture (N_2, CO_2, O_2) through a precision gas mixing system. The partition was raised and then lowered after the mean duration of contests staged in normoxia (559 secs) and in hypoxia (193 secs; see Chapter 5; Sneddon *et al.* 1998). The Po_2 of the water under normoxia was 154 Torr and under severe hypoxia, 15 Torr, at 10°C. The crabs were immediately removed with minimal disturbance and a haemolymph sample (250 µl) was taken by piercing the arthrodial membrane at the base of the third pereiopod with a hypodermic needle (21g) and syringe (1ml) and treated as described below. A further 5 crabs

were treated in this manner, but instead of taking a haemolymph sample we immediately placed them into liquid N_2 to freeze the tissues.

2. EXERCISE (n = 10 both under normoxia and hypoxia). The crabs were removed from their holding tanks and were placed onto a treadmill submerged in a tank of seawater and kept in position by a clear perspex box which was positioned just above the treadmill (Fig.1). The crab was placed onto the middle of the treadmill, left for one hour and then exercised at a constant speed ($2.5 m.min^{-1}$) until the crab appeared fatigued i.e. it dropped its chelipeds and stopped walking. During the hour before the treadmill was switched on, under normoxia, air was bubbled through air stones which were positioned at opposite ends of the tank. In the severely hypoxic treatment, the Po_2 of the water was decreased gradually over the hour as described before. The crab was then removed from the treadmill when it ceased walking and a haemolymph sample taken. A further 5 crabs were exercised thus under both oxygen tensions in order to obtain tissue samples and when the crabs stopped walking, they were placed into liquid N_2. The time to reach exhaustion was between 40 and 45 minutes under normoxia and 22 and 25 minutes under severe hypoxia.

3. FIGHTING. Fights were staged between pairs of size matched (by carapace width and claw length to 1%) male crabs under normoxia (n = 15 pairs) and severe hypoxia (n = 14 pairs). Each pair was transferred of crabs separately to a partitioned arena (55 x 25 x 30cm) which was screened from visual disturbance and left them for one hour where air or a gas mixture was used to obtain the desired Po_2. The partition was raised

from outside the screening, food extract (whitebait homogenised in seawater which was a chemical stimulus and provided a perceived resource) was injected into the middle of the arena via tubing and the interaction viewed through a hole in the screen. After the resolution of a fight, when a clear winner and loser was apparent (the winner of a fight was the crab that elicited 2 successive retreats from its opponent or successfully climbed on top of the other contestant, the loser, see Chapter 2; Sneddon *et al.* 1997), each crab was removed immediately and a haemolymph sample taken. We staged a further 5 fights with crabs being removed after the resolution of a contest and each placed immediately into a Dewar of liquid N_2. Tissue samples were taken from claw, the merus of the 3rd walking leg (4th pereiopod) and the merus of the swimming paddle (5th pereiopod) from the fought crabs to examine the metabolites in a number of muscle tissues to determine if there are variations amongst different sets of muscles.

6.2.3. Enzymatic analyses

The haemolymph and tissue samples were treated with perchloric acid to denature the enzymes and halt all metabolic processes as outlined in Thorpe *et al.* (1995). The method used for L-lactate determination was based on that of Gutmann and Wahlefeld (1974) where the L-lactate is oxidised to pyruvate in a reaction catalysed by L-lactate dehydrogenase with the modification of adding EDTA to the buffer as suggested by Engel and Jones (1978) to eliminate the interference of copper ions on the

absorption spectrum of pyruvate. The method used for glucose determination was based on that of Kunst *et al.* (1981) which involves a two step reaction using hexokinase and glucose-6-phosphate dehydrogenase (G-6-PDH). The hexokinase catalyses the phosphorylation of glucose whilst G-6-PDH catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate. Glycogen determination was carried out using the method of Keppler and Decker (1974) and involved hydrolysis of 1-4 and 1-6 glycosidic bonds of glycogen by 1-4. 1-6 amyloglucosidase to release D-glucose which was assayed using the method of Kunst *et al.* (1981). All reagents were supplied by the SIGMA Chemical Co. LTD (Poole, Dorset U.K.).

6.3.4. Statistical analyses

Values obtained for haemolymph and tissue samples for each metabolite in the three treatment groups were normally distributed and so measures were compared using ANOVA. Data for winners and losers were pooled since they were not significantly different. Tissue metabolites were analysed using ANOVA to determine if there were any differences in metabolite concentrations in the different tissue types.

6.3.5. Experiment 2 - Fight duration and metabolic consequences in relation to declining oxygen tensions

Contests were staged as described before at a range of water oxygen tensions (Po_2 = 123; 91; 61; 46; 30 ± 2 Torr; n = 8 for each Po_2). Each pair of crabs was placed in the experimental tank in fully aerated seawater and left for 1 hour and the Po_2 reduced gradually to the required level as described before. Contest duration was logged onto a lap top computer used as an event recorder and after the resolution of fights crabs were removed immediately and a haemolymph sample taken for metabolite analyses.

Further contests were staged at each water Po_2 (n = 5 at each Po_2). After the resolution of fights, the crabs were immediately removed and placed them into a Dewar of liquid N_2 to freeze the muscle tissues.

6.3.6. Statistical analyses

The duration of fights was determined for each Po_2 treatment and compared with the duration of normoxic contests using T-Tests. Mean haemolymph L-lactate and glucose concentrations at each Po_2 treatment were compared with the values obtained under normoxia using T-Tests. Mean tissue values for glycogen, glucose and L-lactate were obtained for fought crabs and compared with those crabs at rest using T-Tests.

6.3.7. Experiment 3 - Effects of pre-exposure to hypoxia on contests duration and blood metabolites

Contests were staged under severe hypoxia as described before, however, once the Po_2 was reduced to 15 Torr, the crabs were left for additional time periods before they were allowed to fight. Contests (n = 3 at each hour) were staged with increased settling periods at hourly intervals to a maximum of 7 hours. Each pair of crabs fought and were immediately removed once the fight was resolved and a blood sample taken. Blood was also taken from crabs at rest left for the same time periods at 15 Torr (n = 3 at each hour). The samples were analysed for L-lactate and glucose. Tissue samples were not taken after these experiments. Contest duration was logged onto an event recorder.

6.3.8. Statistical Analysis

The metabolite data were not normally distributed so Kruskal Wallis tests were used to compare metabolite concentrations in the blood of fought crabs to resting crabs, and the metabolite concentrations at each hourly interval to the concentrations obtained from crabs left for the initial one hour. The duration of contests at each hourly interval were compared to the duration of contests staged after one hour using T-tests.

6.3.9. Ethical considerations

1. Crabs can quickly make up the relatively small volume of blood, which was taken for metabolite determination, from the surrounding sea water and can replace lost constituents within 48 hours (J.D. Robertson, pers. comm.). All animals from which blood was removed survived for the two

week holding period after experimentation and appeared to behave normally and were subsequently released into the Clyde Sea area.

2. The crabs were removed immediately from the treadmill once signs of fatigue (reduced ability to maintain position on the treadmill) were visible.

3. Immersion in liquid N_2, necessary to instantly halt all metabolic reactions, is the least distressing and rapid way of killing crabs.

4. This species regularly experiences hypoxic and even anoxic conditions in nature and is physiologically tolerant of these. Therefore, the conditions they were subjected to in this study were similar to those that they would encounter in their natural habitat and shore crabs have been observed fighting under severely hypoxic conditions in the field (Sneddon, unpub. data). After exposure to hypoxic conditions, living crabs were subsequently placed into individual holding tanks with fully aerated seawater to allow them to recover. The experimental design did not, therefore, expose crabs to conditions that were outwith those they would experience in nature and to which they have effective physiological adaptations.

5. The resolution of fights was readily apparent when an individual switched from actively engaging in fights to withdrawal. At this point they were immediately removed from the experimental tank, and no injuries were sustained as a result of fighting. Since there was no risk of injury and the crabs were only allowed to fight until one crab withdrew, and because all crabs recovered quickly, I feel that the welfare of the crabs was not at risk.

6.4 RESULTS

Each pair of crabs fought readily in the experimental arena. Shore crab fights consisted of a series of discrete action patterns where the contestants did not initially perform display but engaged each other in a pushing contest where both crabs stand high on the tips of the ambulatory legs, facing one another with chelipeds open wide, in what appears to be a trial of strength (Sneddon *et al.* 1997). The contests quickly became very intense with most physical contact acts being performed most frequently in the first half of fights. No injuries were sustained by the crabs. Maximum fight duration was 983 seconds and 390 seconds under normoxic and hypoxic conditions, respectively.

6.4.1 Experiment 1 - Metabolic consequences of agonistic behaviour and exercise under normoxic and severely hypoxic conditions

By-product accumulation

Values for the concentrations of L-lactate in the haemolymph of crabs after fighting were significantly higher than resting values (N - $F_{1,20}$ = 13.3, $p = 0.001$; H - $F_{1,20} = 68.3$, $p < 0.001$; Fig. 1), but significantly lower than concentrations observed after exercise (N - $F_{1,27} = 36.6$, $p < 0.001$; H - $F_{1,27} = 192.6$, $p < 0.001$; Fig. 1) under both normoxia (N) and hypoxia (H). Values in crabs that had fought under hypoxia were similar to those of crabs exercised under normoxia (Fig. 1). The concentrations of L-lactate in the muscle tissue were higher in crabs fought under normoxic

conditions than in crabs at rest ($F_{1,13} = 45.7$, $p < 0.001$), but not as high as values obtained after exercise on a treadmill ($F_{1,13} = 607.2$, $p < 0.001$; Fig.2). A similar effect can be seen under hypoxic conditions, but mean concentrations of L-lactate were much higher than those found under normoxia (Comparison of rest and fought crabs, $F_{1,13} = 21.9$, $p < 0.001$; fought and exercised crabs, $F_{1,13} = 22.0$, $p < 0.001$; Fig. 2)

Energy reserves

Glucose concentrations in the blood after fighting were significantly higher than values obtained at rest ($F_{1,20} = 39.7$, $p < 0.001$) under normoxia but not as high as values obtained from exercised crabs ($F_{1,27} = 173.3$, $p < 0.001$). Mean glucose concentrations in the blood of fought crabs were also higher than resting values under severe hypoxia ($F_{1,20} = 26.2$, $p < 0.001$). However, values obtained after exercise under hypoxia were lower than those found after fighting ($F_{1,27} = 26.6$, $p < 0.001$). Glucose concentrations in the muscle tissue of fought crabs were greater than in crabs at rest but lower than in exercised crabs (Comparison of rest and fought crabs, $F_{1,13} = 18.1$, $p = 0.001$; $F_{1,13} = 80.2$, $p < 0.001$; fought and exercised crabs, $F_{1,13} = 275.1$, $p < 0.001$; $F_{1,13} = 513.0$, $p < 0.001$ under normoxia and hypoxia respectively; Fig. 2).

Fighting under normoxia did not result in a significant breakdown of leg muscle glycogen (4th pereiopod) compared with resting crabs ($F_{1,18} = 27.8$, $p < 0.001$; Fig. 2). Exercise under normoxia resulted in a dramatic

reduction in the concentration of glycogen ($F_{1,18}$ = 187.0, $p < 0.001$) and thus fighting under normoxia does not appear to be as metabolically demanding as exercise since there is less glycolytic activity. Under hypoxic conditions, the breakdown of glycogen is significantly greater in fought crabs than crabs at rest ($F_{1,18}$ = 21.0, $p < 0.001$) and this is similar to the concentrations of glycogen found in crabs that had been exercised ($F_{1,18}$ = 2.77, p = 0.113; Fig. 2).

There were no significant differences in metabolite composition between chela, leg (4th pereiopod) and swimming paddle (5th pereiopod) tissue of fought crabs (Table I). Therefore we are justified in using the results from one muscle set in the rest of the statistical analysis. L-lactate concentrations under hypoxic conditions were 40% greater those under normoxic conditions ($F_{1,28}$ = 248.64, $p<0.001$). There was an increase of approximately 30% in glucose concentrations in the muscle tissues of crabs fighting under hypoxia compared with those fought under normoxia ($F_{1,28}$ = 290.68, $p<0.001$). Glycolytic activity was greater in the muscle tissues of crabs fought under hypoxia with a reduction of glycogen stores of around 50 % compared with the values obtained from crabs fought under normoxia ($F_{1,28}$ = 648.87, $p<0.001$).

6.4.2. Experiment 2. Fight duration and metabolic consequences in relation to declining oxygen tensions

Duration

The duration of fights under hypoxia did not differ significantly from those staged in normoxia until the fights were staged at P_{O2} values below 46 ± 2 Torr ($T = -3.24$, $p = 0.007$; Fig. 3), at which point the duration of contests became significantly shorter.

By-product accumulation

The concentrations of L-lactate in the haemolymph of crabs fought under hypoxic conditions did not differ significantly from those in normoxia until the P_{O2} that the contests were staged under was below 30 Torr ($T = 2.04$, $p = 0.05$, $df = 19$; Fig. 4a - the results are shown for both winners and losers which are not significantly different). Analyses of leg (4th pereiopod) muscle tissue (Fig. 5) demonstrated that fighting under increasingly hypoxic conditions appears to involve an increase in anaerobic metabolism as reflected in an increased concentration of L-lactate (below 40 Torr, $T = -12.9$, $p < 0.0001$, $df = 15$).

Energy reserves

There was a significant increase in blood glucose ($T = 26.8$, $p < 0.001$, $df = 18$; Fig. 4b) and tissue glucose ($T = 6.42$, $p < 0.0001$, $df = 17$); and also a reduction in glycogen stores ($T = -11.6$, $p < 0.0001$, $df = 16$; Fig. 5) when contests were staged at oxygen tensions below 40 Torr.

6.4.3. Experiment 3 - Effects of pre-exposure to hypoxia on contests duration and blood metabolites

Metabolites

The median concentrations of L-lactate and glucose obtained from crabs at rest and from fought crabs during exposure to different periods of severe hypoxia are shown in figure 6a. L-lactate concentrations increase in the blood of crabs at rest and after fights with increasing length of settling periods under 15 Torr, whereas glucose decreases. Fought crabs have significantly higher concentrations of L-lactate ($H = 21.6$, $p < 0.001$, $df = 1$) and glucose ($h = 41.3$, $p < 0.001$, $df = 1$) than resting crabs. When fights are staged after 4 hours under severe hypoxia, the blood L-lactate concentrations are significantly higher in fought crabs than in the blood of crabs after only one hour ($H = 38.3$, $p < 0.001$, $df = 6$), and glucose concentrations in the blood are significantly different from values obtained from crabs fought after only a one hour settling period ($H = 33.7$, $p < 0.001$, $df = 6$).

Duration

The duration of contests declines with increasing length of time spent under hypoxia and the contests are significantly shorter than those staged after a one hour settling period, when the contests are staged after 3 hours or longer periods of exposure to hypoxia ($T = 3.14$, $p = 0.007$).

Table I. Comparison of metabolite concentrations (values shown are means from claw tissue, 4th pereiopod and 5th pereiopod; $\mu mol.g^{-1}$) and percentage difference (%) in the three tissue types from crabs (n = 10 crabs for both N and H) which had fought under normoxia (N) and hypoxia (H)

	N	H		N	H		N	H	
	L-lactate		%	Glucose		%	Glycogen		%
Claw	3.398	5.841	+42	1.271	1.813	+30	19.74	9.650	-49
4th	3.037	5.323	+43	1.278	1.898	+33	19.24	9.336	-49
5th	3.323	5.671	+41	1.254	1.929	+35	19.68	9.026	-46
$F_{2,12}$	0.28	1.67		0.11	0.283		0.22	0.50	
p	0.703	0.230		0.897	0.283		0.809	0.618	

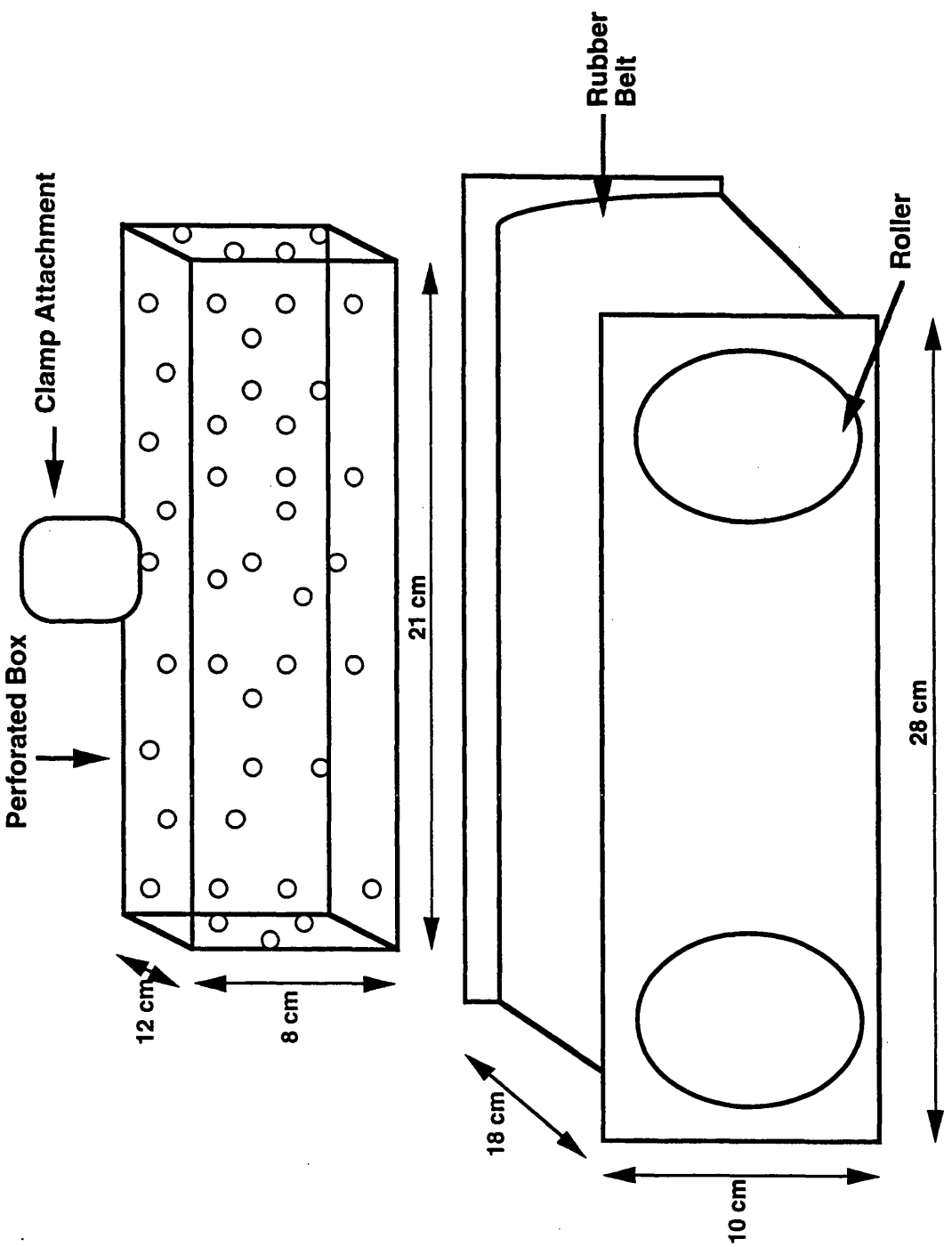


Figure 1. Diagram of the treadmill and the perforated box which kept the crab in position once it was placed on to the rubber belt.

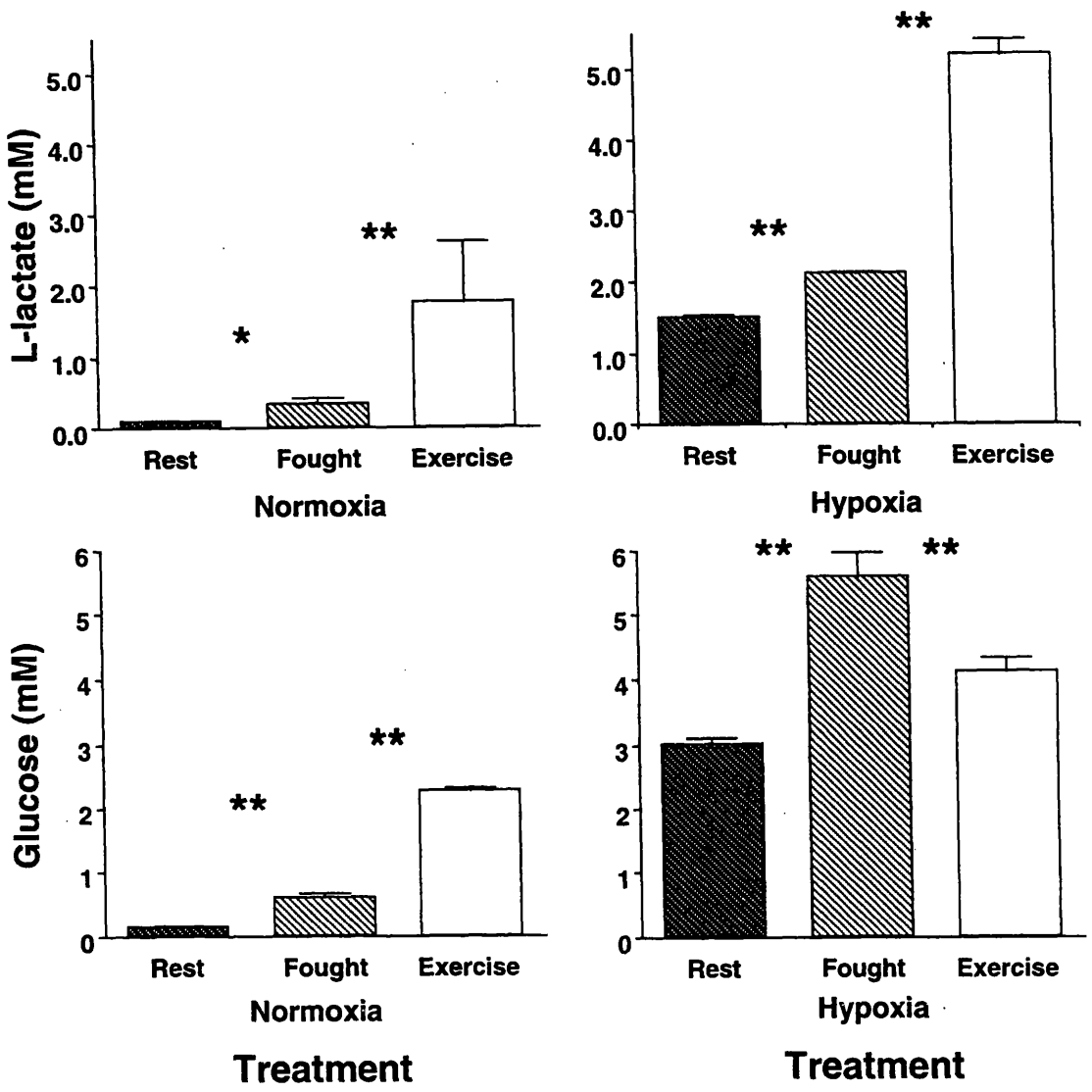


Figure 2. Mean (+SE) concentrations of L-lactate and glucose obtained from the haemolymph of crabs at rest (n = 10 under both N and H), after fighting (n = 30 under N; n = 28 under H) and after exercise (n = 10 both under N and H) on a treadmill under normoxic (N) and hypoxic (H; 15 Torr) conditions (* indicates p = 0.001; ** indicates p < 0.001)

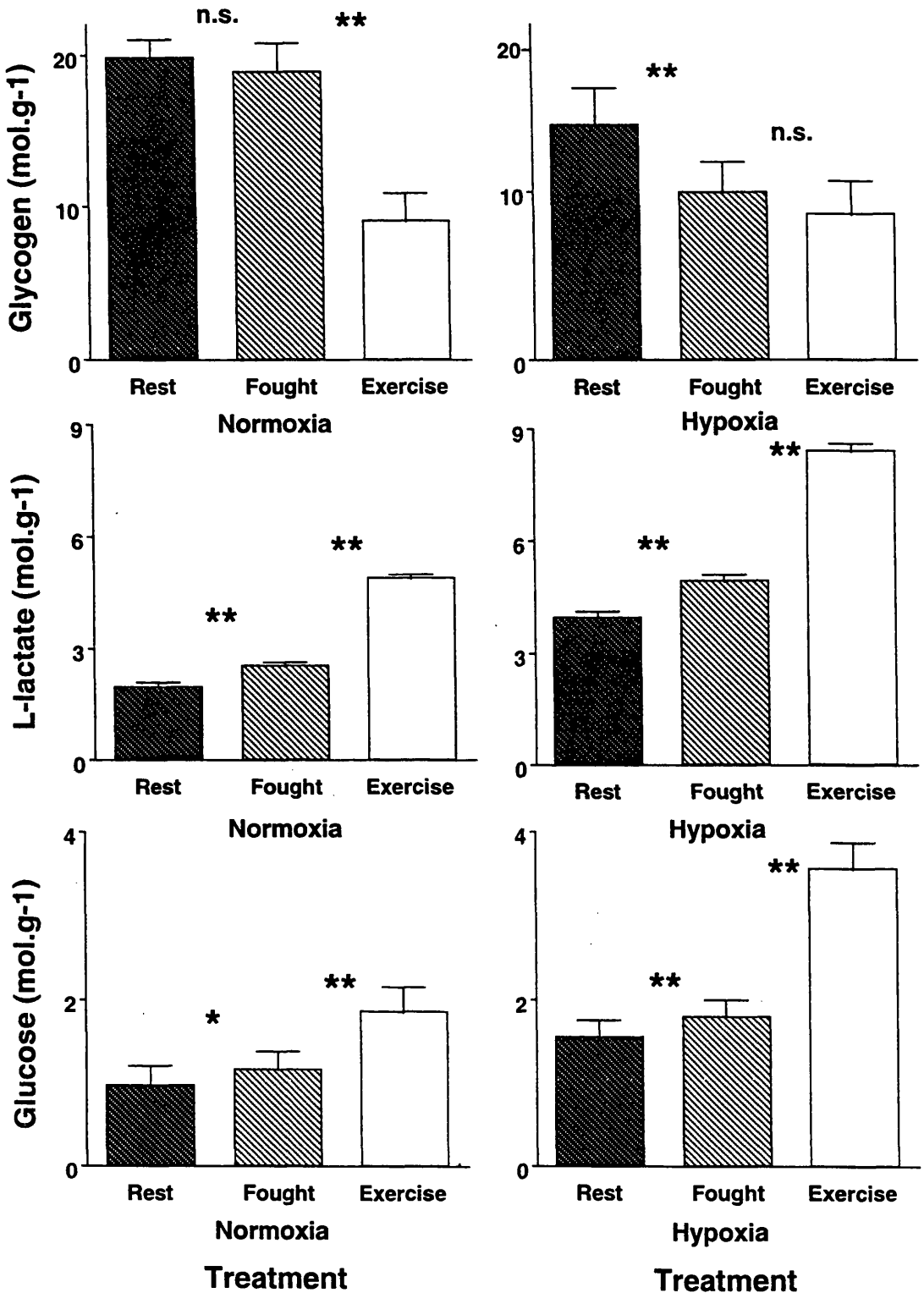


Figure 3. Mean (+SE) concentrations of L-lactate, glucose and glycogen from the leg tissue (4th pereiopod) of crabs at rest (n = 5 under both N and H), after fighting (n = 10 under both N and H) and after exercise on a treadmill (n = 5 under both N and H) under normoxic (N) and hypoxic (H; 15 Torr) conditions (n.s. indicates $p > 0.05$; * indicates $p = 0.001$; ** indicates $p < 0.001$)

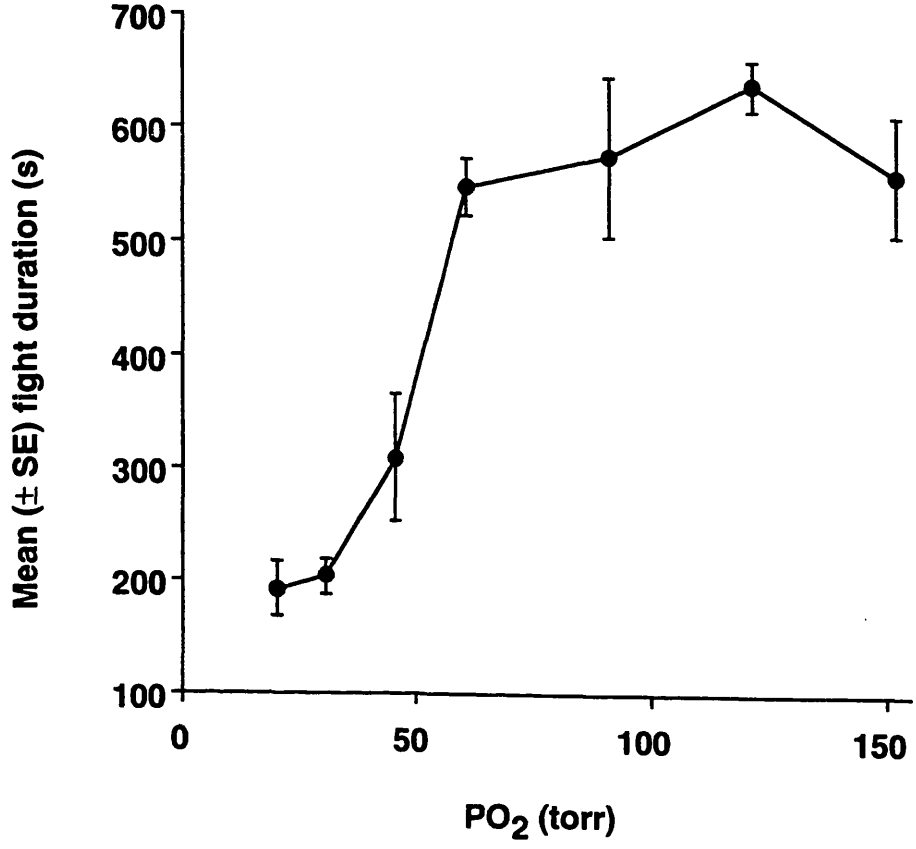


Figure 4. Mean (±SE) duration of contests staged at different oxygen tensions (n = 8 at each P_{O2})

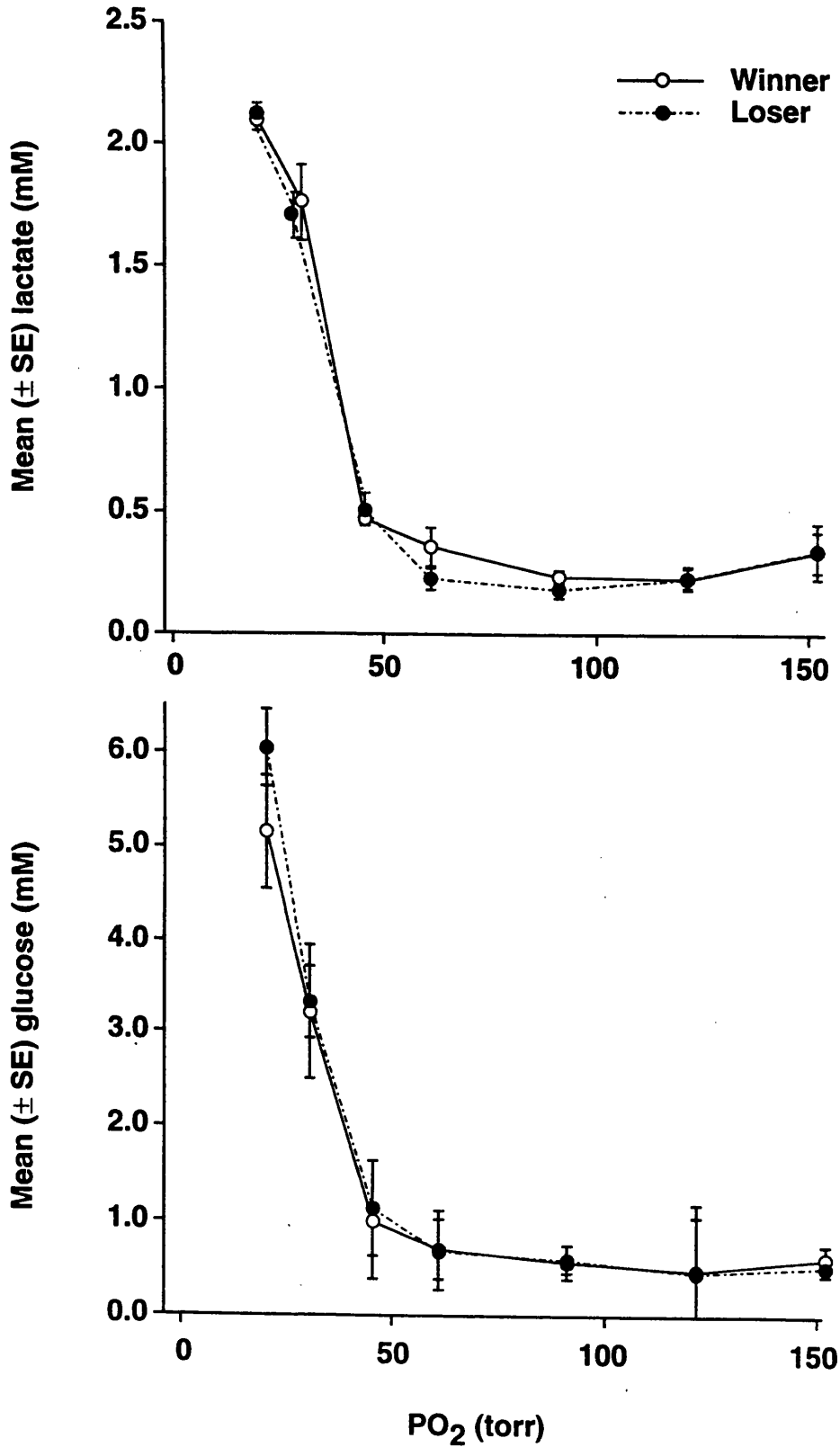


Figure5. Mean (±SE) concentrations of L-lactate (a) and glucose(b) obtained from the haemolymph of crabs from contests at different oxygen tensions (n = 16 crabs at each P_{O2})

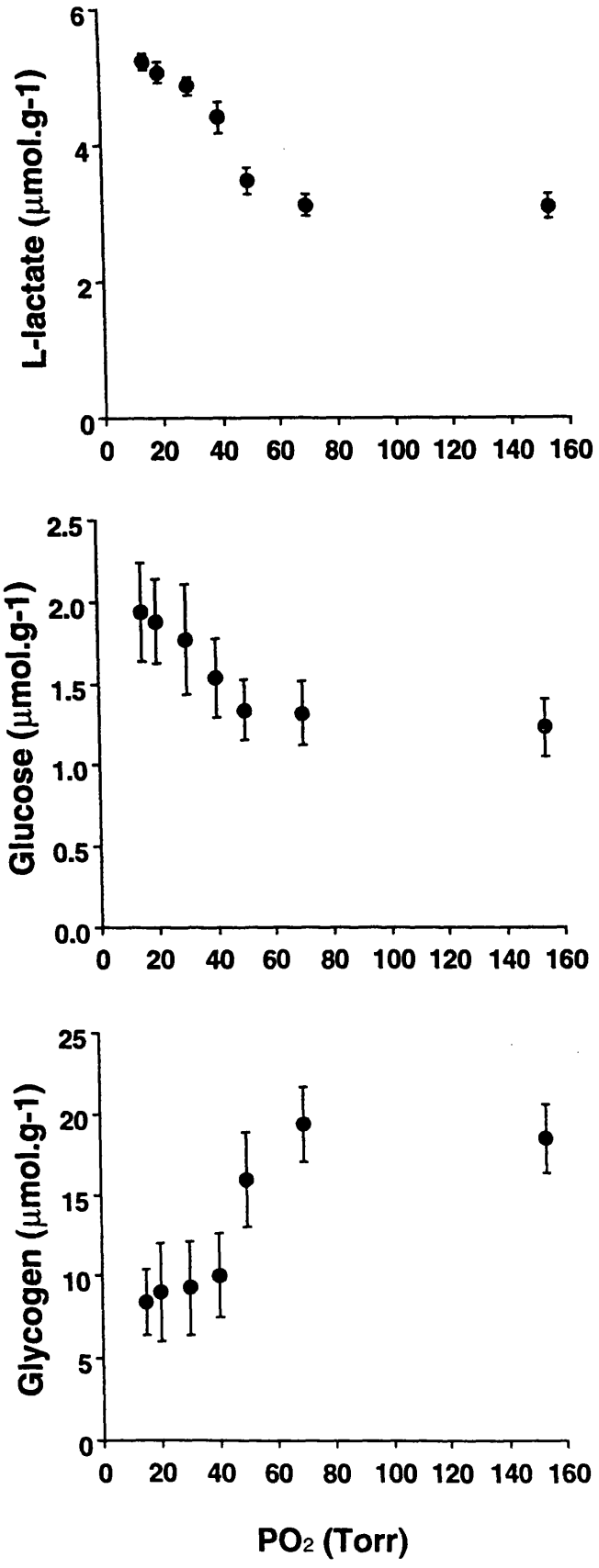


Figure 6. Mean concentrations (±SE) of L-lactate, glucose and glycogen obtained from the leg tissue (4th pereiopod) of crabs which had fought under a range of water P_{O2} (n = 10 crabs at each P_{O2})

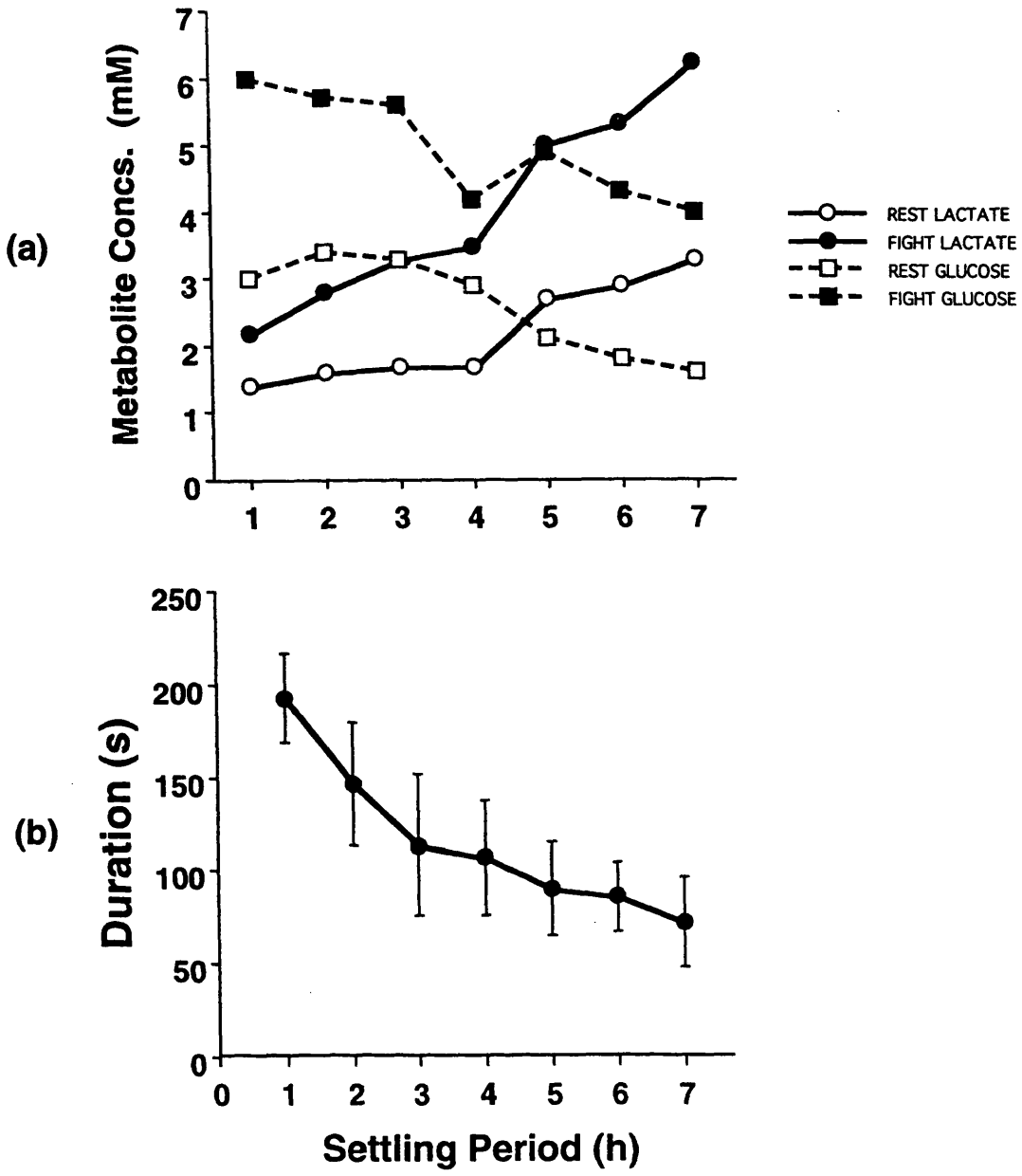


Figure 7. (a) Median concentrations of L-lactate (circles) and glucose (squares) from the haemolymph of crabs at rest (empty circles and squares; n = 3 at each interval) and after fighting (solid circles and squares; n = 6 at each interval) left for increasing lengths of settling periods under severe hypoxia.

(b) Mean duration (±SE) of contests staged after increased lengths of settling period under severe hypoxia (n = 3 contests at each interval)

6.4 DISCUSSION

One aim of this study was to compare the metabolic consequences of fighting with those of the metabolite profiles of two extremes of activity (no activity and sustained strenuous exercise) under both normoxic and hypoxic conditions. This study has shown that fighting constitutes a metabolically costly activity for male shore crabs and is reflected in in enhanced glycolytic activity and increased concentrations of L-lactate in the haemolymph and in the muscle tissues. Fighting under normoxic conditions is sufficiently metabolically demanding to force the crabs into anaerobic respiration, but not as anaerobically demanding as sustained exercise on a treadmill. Under hypoxic conditions all categories of crabs had greater accumulations of L-lactate and greater depletion of glycogen compared with crabs under normoxic conditions. In fought crabs under severe hypoxia, the glycogen depletion is comparable to that of sustained exercise. It would be expected that energy demands would be higher for exercise since, in this study this lasts around four times longer than fights under both conditions and crabs are forced to walk whereas fighting is a voluntary activity based on a set of strategic decisions. Fights staged under severe hypoxia are significantly shorter in duration and the crabs show increased levels of L-lactate in haemolymph and muscle tissue (Sneddon *et al.*, 1998). This implies that L-lactate accumulation may be acting as a physiological constraint that limits the crabs to a few minutes of activity. However, the concentrations of L-lactate in undisturbed animals under

severe hypoxia are quite high indicating that the crabs are already metabolically compromised and are having to resort to anaerobic respiration to supply their normal energy requirements. However, crabs exercised under hypoxic conditions show a much greater accumulation of L-lactate than fought crabs and thus, fought crabs have not reached their maximum levels of L-lactate production.

One hypothesis for the decrease in duration of contests under hypoxia is that, if concentrations of L-lactate after fighting are lower than after exercise, it may be that the crabs decide to de-escalate since the metabolic cost of fighting for long periods in severe hypoxia may exceed the benefits. Subsequent behaviour may be constrained by energetic status and since fighting is sufficiently demanding to result in increased anaerobic respiration, then perhaps the crabs are conserving energy for other activities, such as predator escape, by limiting the time they engage an opponent. If an animal's energy demand under hypoxia remains at the pre-hypoxic rate, the rate of glycolysis must be enhanced which would rapidly deplete the carbohydrate pool and animals should overcome this possibly by reducing their energy demand. In brook charr and demoiselles, the increased energy costs of defending territories when population density increases, are reduced by decreasing the frequency of agonistic acts (McNicol & Noakes 1984; Barnett & Pankhurst 1996).

Under hypoxic conditions, blood glucose concentrations are higher in fought crabs than exercised crabs and this may be due to the available glucose being used up in anaerobic respiration to meet the extra energy

demands of exercised crabs. Under normoxia, this effect is not seen, possibly because energy is being produced via aerobic respiration and anaerobic respiration is not relied upon heavily. When the crabs are subject to increasing periods of exposure to hypoxia in experiment 3, glucose concentrations decline and again, this is probably because available glucose is being used to provide energy over the longer period of hypoxia. Alternatively, this effect could be due to a time effect where glucose is released and increases in the blood sampled after a short time in fought crabs but, decreases in the blood of crabs left for a longer time before sampling since, it is being used in respiration i.e. exercised crabs and crabs in experiment 3. The reduction of glycogen stores was much greater in exercised crabs under normoxia than in resting concentrations. The reduction, however, was similar to crabs fought and crabs exercised under severe hypoxia. It appears as if, when the crabs are "under pressure" metabolically, they release a large amount of glucose from the breakdown of glycogen so that plentiful glucose is available for respiratory pathways if it is needed (Lynch & Webb 1973; van Aardt 1988; Weinstein *et al.* 1988). Any glucose not used in respiration will eventually be returned to glycogen through gluconeogenesis during the recovery period (Lynch & Webb 1973; van Aardt 1988; Weinstein *et al.* 1988).

A second aim of this study was to examine fight duration and levels of target metabolites across a range of oxygen tensions. Experiment 2 showed that staging fights under increasing degrees of hypoxia resulted in a decrease in the duration of contests only below 50 Torr (~ 30%

normoxia). Haemolymph metabolites were affected below 30 Torr (~ 20% normoxia) where an increase in L-lactate and glucose concentrations were shown. These results agree with previous findings that shore crabs respond to hypoxic conditions when Po_2 falls below 50 and have to physiologically compensate for the reduction in oxygen in the environment (Burke 1979; Taylor 1982). This involves hyperventilation, bradycardia and an increase in anaerobic respiration. *C. maenas* abandons aerobic respiration below 20 Torr and utilises anaerobic pathways for energy production which result in L-lactate accumulation (Burke 1979). However, glycolytic activity in these crabs is only greater when water Po_2 is below 50 Torr where the crabs are metabolically compensating for the declining oxygen tensions. This would suggest that the metabolic consequences of fighting are much greater in an hypoxic environment, which these crabs frequently encounter (Hill 1989), since there is both enhanced glycolytic activity and a greater metabolic debt created that has to be repaid. The results of experiment 2 support those of experiment 1 suggesting that L-lactate production may constrain activity in crabs, but only when oxygen is in limited supply and energy demands have to be supplemented by anaerobic respiration. The concentrations of L-lactate recorded in this study are not as high as those found in a previous investigation, although the crabs were subjected to complete anoxia (tissue L-lactate 8$\mu mol.g^{-1}$, blood L-lactate 2mM; glycogen reduction from 18$\mu mol.g^{-1}$ to 5$\mu mol.g^{-1}$; Hill 1989). Other studies have shown that energy demands are higher when crabs recover metabolically from anaerobiosis

(Hill *et al.* 1991). There is an increase in respiration rate and concentrations of L-lactate initially increase during the recovery period and it takes 8 hours for shore crabs to re-pay the metabolic debt (Hill *et al.* 1991). Therefore, fighting under hypoxia is more energetically costly due to this "metabolic recovery" and depletion of glycogen stores. This suggestion is supported by the results of experiment 3 which showed that leaving the crabs for longer under severely hypoxic conditions prior to fights resulted in a decrease in contest duration and this was correlated with an increase in L-lactate. Because the time spent fighting declined, it is conceivable that any subsequent activities will also be impaired.

There are no metabolite differences between winners and losers even though there are behavioural differences. This suggests that the metabolic effects of fighting are similar for winners and losers, unlike animals such as the cichlid, *T. zillii* (Neat *et al.* 1998) and the cricket, *Achetus domesticus*, (Hack 1997). However, we do not know the values for each metabolite in individual crabs prior to fighting. Perhaps winners have relatively greater energy reserves than losers before fighting as in the damselfly, *Calopteryx splendens xanthostoma*, (Plaistow & Siva-Jothy 1996), or the increase in L-lactate from rest to fought values is less in winners compared with losers as demonstrated in studies on the cichlid, *T. zillii* (Neat *et al.* 1998).

In this study the environmental conditions may have raised the energy costs of fighting per unit time and so constrained fighting behaviour by forcing crabs to readjust their strategic decisions about how long to fight.

Therefore the metabolic consequences of a given behaviour can be variable and this may be dependent on the physiological status of the animal. In brook charr, agonistic behaviour was effected by environmental conditions such that, as water current velocity increased, there was an associated increase in energy costs of territorial defence and the animal responded by decreasing the frequency of chasing intruders to more display acts, to resolve the interactions. Further experiments are required to determine if it is a physiological constraint or a motivational difference or both that determines the duration of contests. The energy stores that are depleted as a result of enhanced energy demand during a fight under hypoxia are quite substantial and this may ultimately have an effect on fitness and so may represent a real cost of fighting. Energy expended during fighting must either be replenished through foraging or it will cause a permanent reduction in reserves. The acquisition of food may possibly involve increased predation risk and a loss in time spent mating. For example, depleted energy reserves reduce an individual's ability to secure a mating territory in the damselfly species *Calopteryx maculata* (Marden & Waage 1990) and *C. s. xanthostoma* (Plaistow & Siva-Jothy 1996); and to attract mates through signalling in the bush cricket *Requena_verticalis* (Simmons *et al.* 1992).

It would be interesting to investigate what effect the metabolic debt of fighting has on subsequent activity by re-staging fights between crabs which have recently fought under severe hypoxia, since if their ability to engage in activity is impaired then so should their fighting ability, the length of time they spend fighting and their energetic status.

CHAPTER 7

FIELD AND LABORATORY STUDIES ON AGONISTIC BEHAVIOUR IN SHORE CRABS, *CARCINUS MAENAS* (L.): METABOLIC CONSEQUENCES OF VARIABLE OXYGEN TENSIONS.

CHAPTER 7

FIELD AND LABORATORY STUDIES ON AGONISTIC BEHAVIOUR IN SHORE CRABS, *CARCINUS MAENAS* (L.): METABOLIC CONSEQUENCES OF VARIABLE OXYGEN TENSIONS.

7.1 INTRODUCTION

Most studies on individual behaviour in aquatic crustaceans have been made under tightly controlled laboratory conditions which are necessary when looking into behavioural mechanisms (review in Dingle 1983). However, such experiments cannot demonstrate how relevant this behaviour is in the ecology of an individual *in situ*. Without further knowledge of context, the results from controlled experiments cannot be interpreted in functional terms. There are many difficulties in observing aquatic organisms in their natural environment, since there are problems such as limited observation time under water using SCUBA (e.g. velvet swimming crab, *Necora puber*; Smith 1990; demoiselle, *Chromis dispilus*, Barnett & Pankhurst 1996) and many species are nocturnal, which makes locating them difficult. Many studies have used bait in order to attract animals and observe intraspecific agonistic interactions (e.g. *Nephrops norvegicus*, Bjordal 1986; *N. puber*, Smith 1990).

Non-manipulative studies in semi-natural environments in large aquaria circumvent some of the disadvantages of both highly controlled environments and manipulative field studies. They have provided detailed knowledge about aggressive behaviour, courtship, pair formation, feeding and timing of moult and mating in crustaceans (Stein *et al.* 1975; Atema *et al.* 1979; Karnofsky & Price 1989) and aggression in fish (McNicol & Noakes 1984). However, even these conditions remain artificial, since it is impossible to imitate realistic emigration and immigration, the threat of

predation and fluctuation in environmental factors. Many questions about the incidence and nature of agonistic interactions cannot be addressed without knowledge of behaviour in the natural context.

Therefore, the purpose of this study was to obtain observations on the intraspecific agonistic behaviour between shore crabs, *Carcinus maenas,* in the field to determine if the aggressive behaviour the crabs display in the laboratory is also shown in the natural environment. Smith (1990) observed *C. maenas* fighting in the subtidal zone but made no quantitative measurements of this behaviour. Ideally these should be non-manipulative observations, but this was not feasible during the course of the present study. Under laboratory conditions, fighting resulted in an elevation of L-lactate and glucose in the blood and tissues of *C. maenas* which was amplified under low oxygen tensions and was accompanied by a significant reduction in glycogen stores (see chapters 5 & 6; Sneddon *et al.* 1998). Also the duration of contests between shore crabs were much shorter under very low oxygen tensions. Therefore, the behavioural aspects of fights and the physiological consequences were examined using established laboratory techniques and a specially-developed field system in which controlled experimentation was possible. The present work was carried out on the upper shore in a large rock pool. This allowed easy access to the study site and avoided the problems of night time SCUBA diving. The metabolic consequences of agonistic behaviour were assessed by analysis of key metabolites (L-lactate, glucose, glycogen) associated with anaerobiosis in fought crabs. Also the rock pool became hypoxic so observations on the crabs experiencing these conditions in the field were

made and the effects of hypoxia on behaviour and metabolic physiology of agonistic interactions in the field will be compared with laboratory results.

7.2 METHODS

7.2.1. Field Study Site

Field observations were made on the rocky shore in the vicinity of the University Marine Biological Station, Millport, Isle of Cumbrae during the months of June, July and August in 1996 and 1997. The pool was chosen since *C. maenas* were present (2-4 crabs were present on each sampling occasion), the steep sides would prevent crabs from leaving the pool once released into it and the pool was located high on shore which allowed a longer access time when the tide was out. Plastic mesh (0.5 x 0.5cm) barriers were placed at either end of the pool as indicated in figure 1, to prevent crabs leaving the observation area.

7.2.2. Experiments Under Ambient Oxygen Tensions

Crabs used in field observations were freshly creeled from the Clyde Sea area and males were marked numerically with nail varnish on the back of the carapace, measured (carapace width and propodus length to 0.1mm) and placed in separate holding tanks for 24 hours prior to experimentation. The crabs were placed into ten individual plastic mesh cages (cylindrical shape, 8cm diameter x 15cm length) and taken to the rock pool at 10 pm. Observations were made between 2.30 am and 4 am when oxygen tensions in the pool water would be lowest (Hill 1989). The Po_2 of the pool was measured using a calibrated, hand-held oxygen meter and was found to lie between 30 and 42 Torr. The cages were split into two groups of five and tied to rocks and placed in the pool at either end (Fig. 1).

Crabs were chosen at random and 2 individuals released into the pool. Bait was thrown into the pool to encourage the crabs to fight. Baiting individuals to attract them and encourage fights has been used in a variety of animals such as *Necora puber* (Smith 1990); *Nephrops norvegicus* (Bjordal 1986) and fulmars, *Fulmaris glacialis* (Enquist *et al.* 1991).

The acts of the fighting crabs were observed with the aid of a diving lamp, UK 400R with a red filter, and were recorded on audio tape by speaking into a Sony Dictaphone. Aquatic crustaceans are not able to detect light of wavelength of 500nm (Loew 1976) and light of this colour has no effects on behaviour (Chapman & Howard 1979). A total of 22 fights were taped and the information transferred onto the event recorder by playing back the audio tapes. This provided information on behavioural content and duration of fights (see chapter 2). The influence of relative size was determined since the crabs had been marked and measured previously.

Five pairs of crabs which had fought at ambient oxygen conditions, were chosen at random and immediately removed from the pool after fighting and a blood sample (0.1ml) taken by piercing the arthrodial membrane at the base of the third pereiopod using a needle (19g) and syringe (1ml). The sample was immediately added to 0.1ml of 0.6M Perchloric acid (PCA) in an 1.5ml Eppendorf tube, shaken and then placed into a box containing ice to keep the sample chilled whilst on the shore. The PCA deproteinises the blood and halts all metabolic processes. Another three pairs of crabs, chosen at random, were immediately

removed from the pool after fighting and each crab was placed into a Dewar of liquid N_2 to instantly freeze the tissues and halt all metabolic processes. Five crabs, which were treated as described above but were released singly into the pool, were removed after three minutes and a blood sample taken to obtain values of metabolites from crabs who had not engaged in fighting. Three crabs were also treated in this manner but were placed into liquid N_2 after the three minutes. The crabs were relatively inactive after their release into the pool. These samples will be referred to as samples from crabs at rest.

7.2.3. Experimental manipulation of oxygen conditions in the field

The conditions in the rock pool were made normoxic by aerating the pool using a cylinder of compressed air and a regulator to which airline tubing and three airstones were attached. The airstones were positioned at either end of the pool and one in the middle and air was gently bubbled through the water one hour prior to behavioural observations. The P_{O2} was measured using a hand held oxygen meter and was found to lie between 145 and 156 Torr. Experimental conditions were as described before and 12 fights were recorded. Of these 12 fights, 5 were chosen at random and blood samples obtained from the crabs after fighting. Another 3 were chosen at random and each crab was placed in liquid N_2 after fighting. Blood ($n = 5$) and tissue ($n = 3$) samples were also obtained from crabs which had not fought as described before.

7.2.4. Laboratory Experiments

Male shore crabs were obtained (carapace width range 55 - 80mm), with no missing or regenerated limbs, no excessive epibiont coverage and no obvious signs of parasitism, by creeling in the Clyde Sea area between June and August in the vicinity of the University Marine Biological Station and transported them to Glasgow where they were housed in individual holding tanks (18 x 21 x 23cm). The tanks were supplied with circulating sea water (32 - 34‰) at 10 ± 1°C and were maintained under a 12:12 light dark cycle with all experiments being carried out during the light period. The crabs were deprived of food for 7 days prior to any experiments since this is known to reduce intra-individual variation in metabolite concentrations (Hill 1989). This species can withstand three months of food deprivation (Wallace 1973), so this starvation period is relatively short. Since captivity is known to have a detrimental effect on metabolic capacity (Houlihan *et al.* 1985), crabs were used within 10 days of capture to minimise this effect and to make the results comparable. Crabs were used only once for experimentation and after experimentation the crabs were retained for two weeks to ensure they were not in proecdysis (none were).

The crabs were transferred separately to a partitioned arena (55 x 25 x 30cm) which was screened from visual disturbance and left there for four hours. During the first hour under normoxia, air was bubbled through air stones which were positioned at opposite ends of the tank. Oxygen electrodes were positioned on opposite sides from the air stones to closely

monitor oxygen tensions and these were always within 2% agreement. In the severely hypoxic treatment, the Po_2 of the water was decreased gradually over the hour to 35 Torr (mean oxygen tension in the field) by altering the percentage composition of a gas mixture (N_2, CO_2, O_2) through a precision gas mixing system. The partition was raised from outside the screening, food extract (whitebait homogenised in seawater which was a chemical stimulus and provided a perceived resource) was injected into the middle of the arena via tubing and the interaction viewed through a hole in the screen. After the resolution of a fight, when a clear winner and loser was apparent (the winner of a fight was the crab that elicited 2 successive retreats from its opponent or successfully climbed on top of the other contestant, the loser, chapter 2; Sneddon *et al.* 1997a), each crab was removed immediately and a haemolymph sample taken. A further 5 fights were staged with crabs being removed after the resolution of a contest and each placed immediately into a Dewar of liquid N_2.

To obtain blood and tissue samples from crabs which had not fought, crabs were treated as described above and each crab was transferred singly to the arena. The partition was raised and then lowered after the mean duration of contests staged in normoxia (559 secs) and in hypoxia (193 secs; see Sneddon *et al.* 1998) and then crabs were removed and had a blood sample taken (n = 5) or placed into liquid N_2 (n = 5).

7.2.5. Enzymatic analyses

The haemolymph and tissue samples were treated with perchloric acid to denature the enzymes and halt all metabolic processes as outlined in Thorpe *et al.* (1995). The method used for L-lactate determination was based on that of Gutmann and Wahlefeld (1974) where the L-lactate is oxidised to pyruvate in a reaction catalysed by L-lactate dehydrogenase with the modification of adding EDTA to the buffer suggested by Engel and Jones (1978) to eliminate the interference that copper ions have on the absorption spectrum of pyruvate. The method used for glucose determination was based on that of Kunst *et al.* (1981) which involves a two step reaction using hexokinase and glucose-6-phosphate dehydrogenase (G-6-PDH). The hexokinase catalyses the phosphorylation of glucose whilst G-6-PDH catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate. Glycogen determination was carried out using the method of Keppler and Decker (1974) and involved hydrolysis of 1-4 and 1-6 glycosidic bonds of glycogen by 1-4. 1-6 amyloglucosidase to release D-glucose which was assayed using the method of Kunst *et al.* (1981). All reagents were supplied by the SIGMA Chemical Co. LTD (Poole, Dorset U.K.).

7.2.5. Statistical Analyses

The behaviour of winners in the pool fights was compared with that of winners in the contests staged under hypoxia in the laboratory by Kruskal

Wallis Tests. This was also repeated for losers. Mean duration of contests in the field was calculated and compared with laboratory contest duration under normoxia and hypoxia using Kruskal Wallis analysis. The influence of relative size on initiation and winning contests in the field was tested using Chi Square under normoxia and hypoxia.

Metabolites obtained in the field from crabs at rest were compared with those obtained from crabs at rest in the laboratory using Kruskal Wallis tests under both normoxia and hypoxia. The metabolites found in fought crabs under field conditions were compared with those from crabs fought in the laboratory using Kruskal Wallis tests and this analysis was also used to compare the metabolites of crabs at rest to fought crabs in the field under both normoxia and hypoxia.

7.3 RESULTS

7.3.1. Behavioural content

The behavioural acts of fighting *C. maenas* in the field are similar to these observed during fights in the laboratory (Table 1; see Chapter 2 for details of laboratory behaviour; Sneddon *et al.* 1997a). However, winners perform fewer "move to" acts and losers perform fewer "move away" acts, since in the pool contests, fights consisted of one interaction with no re-engagement of an opponent.

7.3.2. Duration

The durations of contests under laboratory conditions were much longer than in the field under hypoxia ($H = 9.06$, $df = 1$, $p = 0.003$) and under normoxia ($H = 15.14$, $df = 1$, $p < 0.001$, Fig. 2). However, fights under normoxic conditions in the field are of similar duration to laboratory contests staged under hypoxia ($H = 1.57$, $df = 1$, $p = 0.210$).

7.3.3. Relative size of winners and losers

Under normoxic conditions in the field, equal numbers of crabs initiated fights but larger individuals tended to win, for both body ($X^2 = 2.8$, $p = 0.1$, $n = 12$) and claw size ($X^2 = 5.6$, $p < 0.05$, $n = 12$; Table 2). However this is significant for claw size only. This is similar to the results of laboratory studies in which crabs with larger claws ($X^2 = 4.08$; $p < 0.01$; $n = 46$; see Chapter 3; Sneddon *et al.* 1997b) tended to win more fights than crabs with larger bodies ($X^2 = 1.6$, $p > 0.05$, $n = 46$). When

conditions were hypoxic in the pool, equal numbers of crabs initiated fights but the majority were won by the larger individual for both body ($X^2 = 4.7$, $p < 0.05$, $n = 22$) and claw size ($X^2 = 12.78$, $p < 0.01$, $n = 22$). This effect is stronger for weapon size, where 86% of fights were won by crabs with larger claws compared with 77% of fights won by crabs with a larger body size.

7.3.4. Metabolic physiology of crabs at rest in the field and laboratory

Concentrations of L-lactate in the blood of crabs at rest under normoxia were higher in the field than in laboratory conditions (Fig. 3, Table 3). Under hypoxic conditions, concentrations of L-lactate in the blood of crabs at rest from the field were similar to those obtained in the laboratory (Fig. 3, Table 3). Under both normoxic and hypoxic conditions in the field, concentrations of L-lactate in the tissues of resting crabs, were similar to those found under laboratory conditions (Fig. 3, Table 3). Under both normoxic and hypoxic conditions in the field, glucose concentrations in the blood of crabs at rest were higher than concentrations obtained from crabs at rest in the laboratory (Fig. 4, Table 4). Tissue glucose concentrations in the field were similar to those found in the laboratory for crabs at rest under both normoxia and hypoxia (Fig. 4, Table 4). Under both oxygen tensions, crabs at rest in the field had similar glycogen concentrations to those in the laboratory (Fig. 5, Table 5).

7.3.5. Metabolic physiology of fought crabs

Concentration of L-lactate in the blood of crabs after fighting under normoxia were higher in the field than in laboratory conditions (Fig. 3, Table 3). However, under hypoxia, blood L-lactate concentrations from fought crabs were similar to those obtained in the laboratory (Fig. 3, Table 3). Under both normoxic and hypoxic conditions in the field, concentrations of L-lactate in the tissues of fought crabs, were similar to those found under laboratory conditions (Fig. 3, Table 3). Under both oxygen tensions in the field, glucose concentrations in the blood of crabs after fighting were higher than concentrations obtained from crabs in the laboratory (Fig. 4, Table 4). Tissue glucose concentrations in the field were similar to those found in the laboratory for crabs after fighting under both normoxia and hypoxia (Fig. 4, Table 4). Under normoxia, crabs after fighting in the field had similar glycogen concentrations to those in the laboratory (Fig. 5, Table 5). However, under hypoxic conditions in the field, glycogen concentrations were higher in the tissues of fought crabs compared to those fought in the laboratory.

7.3.6. Comparison of crabs at rest with fought crabs

There was no significant difference in L-lactate concentrations between crabs at rest and after fighting in the field but, under laboratory conditions L-lactate concentrations were higher in fought crabs than those obtained from crabs at rest (Table 3). There was no significant difference between L-lactate concentrations in the tissues of crabs at rest and after fighting in

the field but, in the laboratory L-lactate concentrations were higher in the tissues of fought crabs compared with crabs at rest (Table 3). In the field, under both oxygen tensions, fought crabs had similar blood glucose concentrations when compared to crabs at rest but in the laboratory, fought crabs had higher concentrations of glucose than resting crabs. In the field, there was no significant difference in tissue glucose concentrations between crabs at rest and fought crabs under both oxygen tensions, however, in the laboratory, fought crabs had higher concentrations of tissue glucose than crabs at rest (Table 4). Under laboratory conditions, glycogen concentrations were lower in fought crabs compared with crabs at rest under hypoxia but were similar under normoxia (Table 5). Under both oxygen tensions in the field, crabs at rest had similar glycogen concentrations to fought crabs

Table 1. A comparison of the behavioural content of contests observed in the rock pool and the behaviour of crabs in laboratory using Kruskal Wallis tests (To = "move to"; Away = "move away"; Display = "cheliped display" and Contact = "strike", "grasp", and "push").

Act		W/L Pool	Median Lab	H	p value
To	W	0.65	1.0	10.0	0.002
To	L	0.15	0.5	5.84	0.016
Away	W	0.0	0.1	0.38	0.536
Away	L	0.6	1.0	8.58	0.003
Display	W	1.2	1.1	0.94	0.332
Display	L	0.4	0.4	2.57	0.109
Contact	W	1.8	2.0	0.09	0.770
Contact	L	1.0	1.0	0.35	0.555

Table 2. Numbers of smaller and larger crabs initiating fights and winning fights in the rock pool under normoxic (n = 12) or hypoxic conditions (n = 22). Results are shown for both carapace width and chela length under normoxia and under hypoxia.

	NORMOXIA			
	Carapace width		**Chela length**	
	Smaller	**Larger**	**Smaller**	**Larger**
Initiate	**7**	**5**	**6**	**6**
Win	**3**	**9**	**1**	**11**
	HYPOXIA			
Initiate	**12**	**10**	**11**	**11**
Win	**5**	**17**	**3**	**19**

Table 3. Comparisons of L-lactate concentrations obtained from the blood and tissues of crabs at rest and from fought crabs under field and laboratory conditions for both hypoxic and normoxic conditions using Kruskal Wallis analyses (R_F = crabs at rest in the field; F_F = crabs fought in the field; R_L = crabs at rest in the laboratory; and F_L = crabs fought in the laboratory).

	NORMOXIA			
	BLOOD		TISSUE	
	H	p	H	p
R_F & R_L	6.82	0.009	1.09	0.297
F_F & F_L	14.30	<0.001	1.43	0.232
R_F & F_F	2.94	0.086	0.07	0.796
R_L & F_L	5.19	0.023	6.01	0.014
	HYPOXIA			
R_F & R_L	1.87	0.172	1.80	0.180
F_F & F_L	0.76	0.384	0.11	0.745
R_F & F_F	0.01	0.903	0.20	0.606
R_L & F_L	6.23	0.013	8.48	0.004

Table 4. Comparisons of glucose concentrations obtained from the blood and tissues of crabs at rest and from fought crabs under field and laboratory conditions for both hypoxic and normoxic conditions using Kruskal Wallis analyses (R_F = crabs at rest in the field; F_F = crabs fought in the field; R_L = crabs at rest in the laboratory; and F_L = crabs fought in the laboratory).

	NORMOXIA			
	BLOOD		TISSUE	
	H	p	H	p
R_F & R_L	6.82	0.009	1.84	0.175
F_F & F_L	8.48	0.004	0.42	0.515
R_F & F_F	0.14	0.713	0.08	0.784
R_L & F_L	6.82	0.009	5.08	0.024
	HYPOXIA			
R_F & R_L	5.77	0.016	0.56	0.456
F_F & F_L	6.23	0.013	1.06	0.302
R_F & F_F	0.24	0.624	0.30	0.584
R_L & F_L	14.30	<0.001	7.65	0.007

Table 5. Comparisons of glycogen concentrations obtained from the tissues of crabs at rest and from fought crabs under field and laboratory conditions for both hypoxic and normoxic conditions using Kruskal Wallis analyses (R_F = crabs at rest in the field; F_F = crabs fought in the field; R_L = crabs at rest in the laboratory; and F_L = crabs fought in the laboratory).

	NORMOXIA		HYPOXIA	
	H	**p**	**H**	**p**
R_F & R_L	0.56	0.456	0.56	0.456
F_F & F_L	0.95	0.329	5.19	0.023
R_F & F_F	0.60	0.439	1.07	0.302
R_L & F_L	1.57	0.210	5.19	0.023

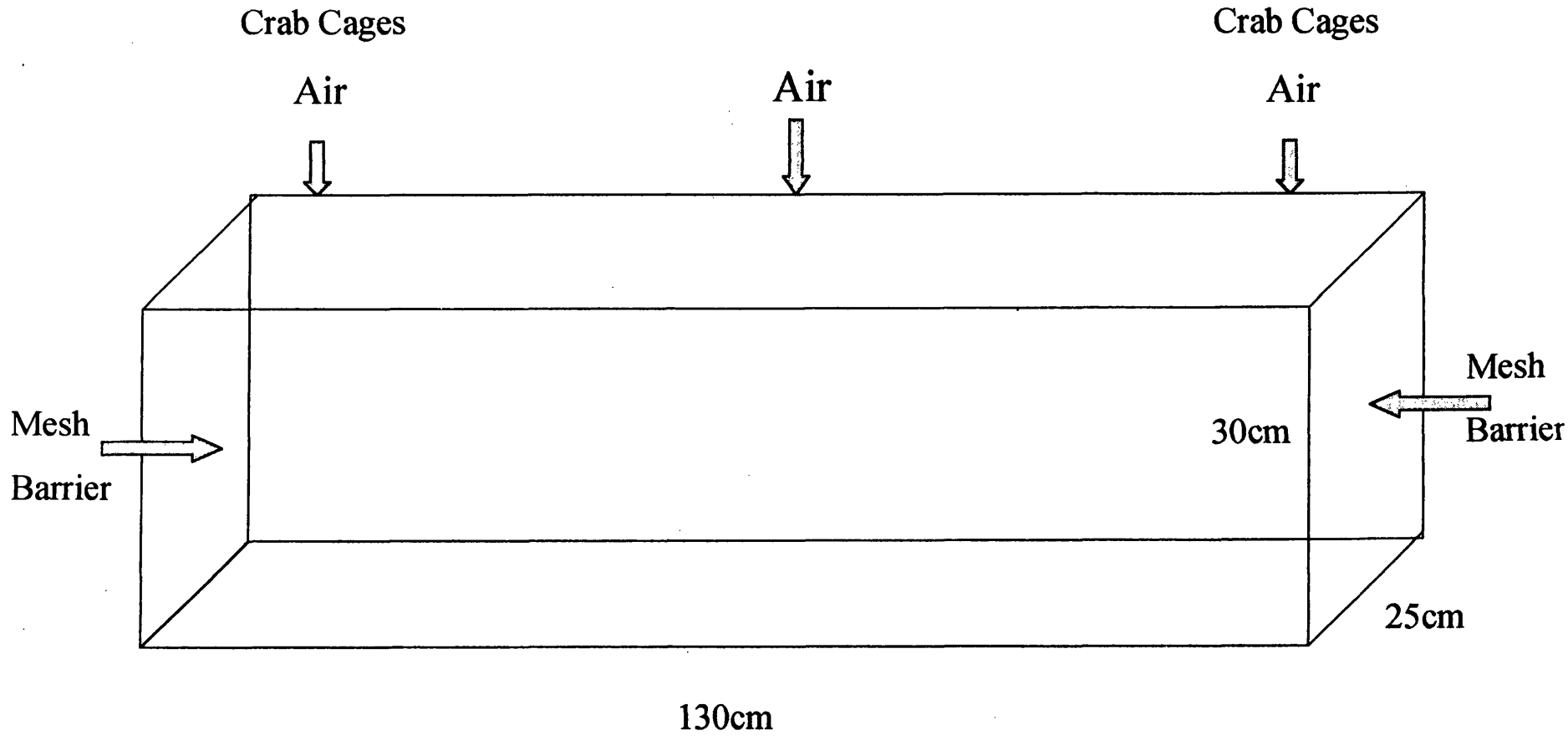


Figure 1. Diagrammatic representation of the rock pool used in this study. The position of netlon barriers, where the netlon cages where placed and the position of the airstones under normoxic conditions are shown. This diagram is based upon the maximum measurements obtained for length (130cm), breadth (25cm) and depth (30cm).

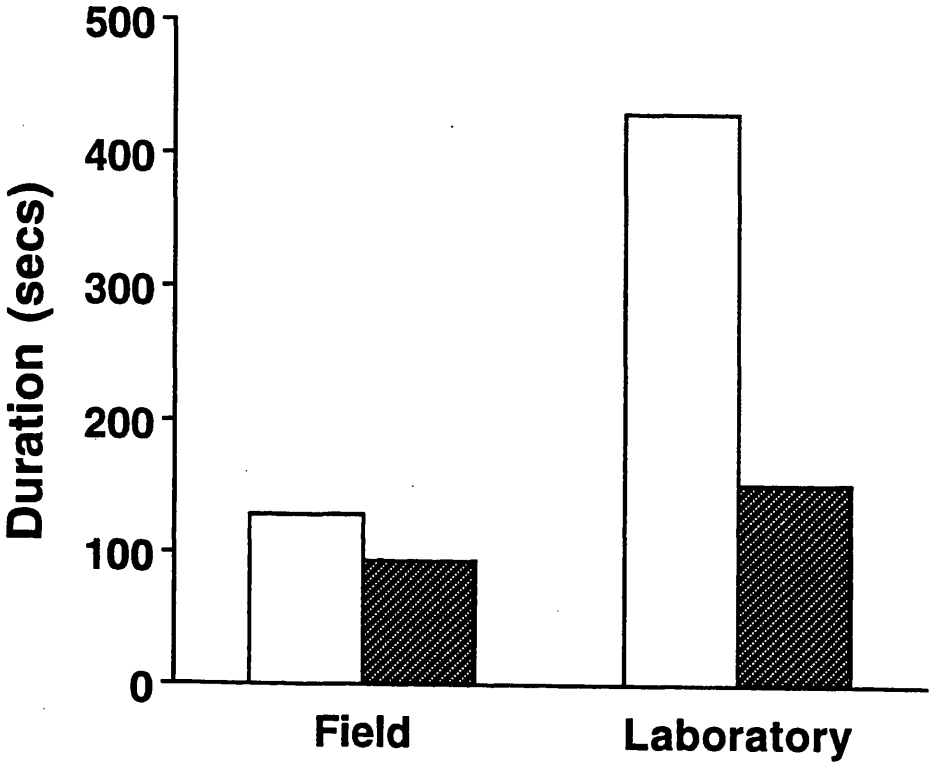


Figure 2. The median duration of fights under normoxia (empty bars) and hypoxia (shaded bars) in both field and laboratory observations.

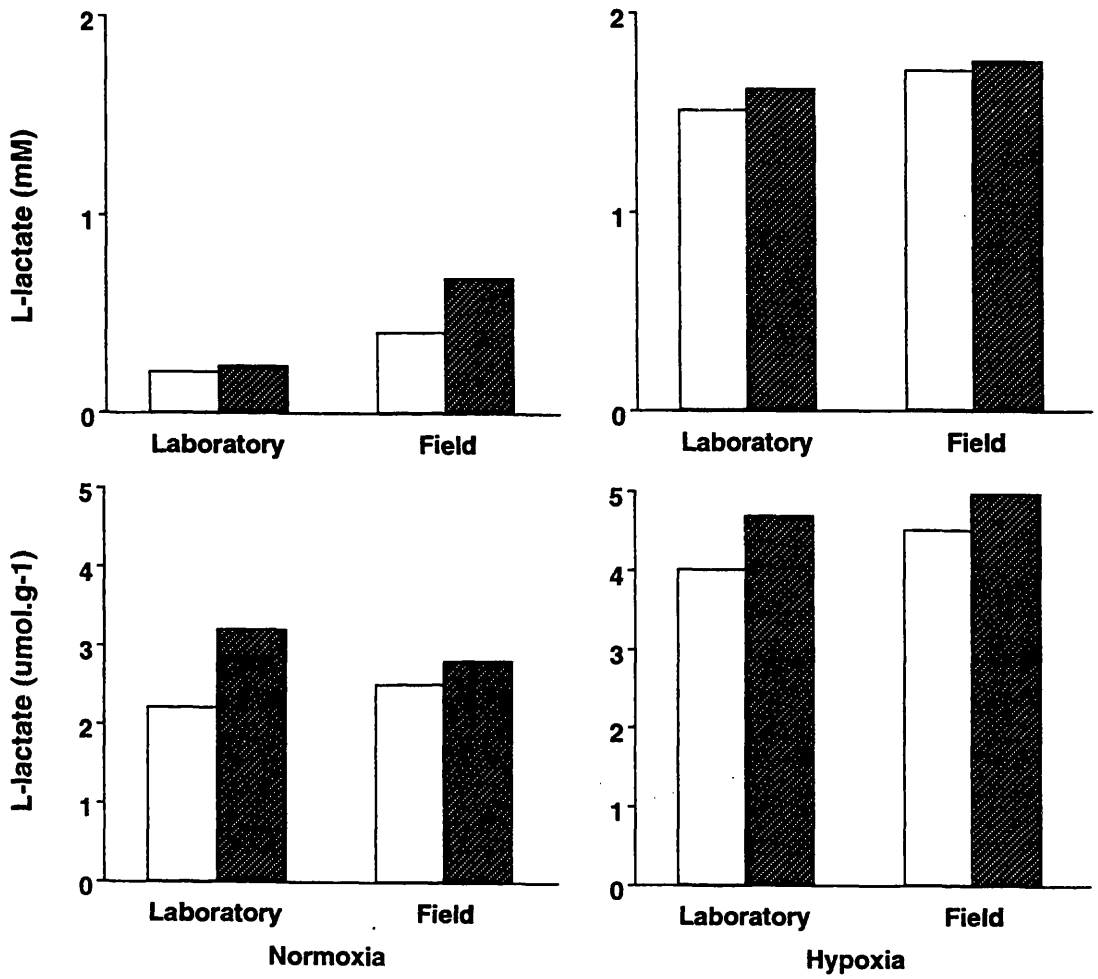


Figure 3. The median concentrations of L-lactate in the blood (mM) and tissues ($\mu mol.g^{-1}$) of crabs at rest (empty bars) and crabs fought (shaded bars) in the field and in the laboratory under both normoxia and hypoxia.

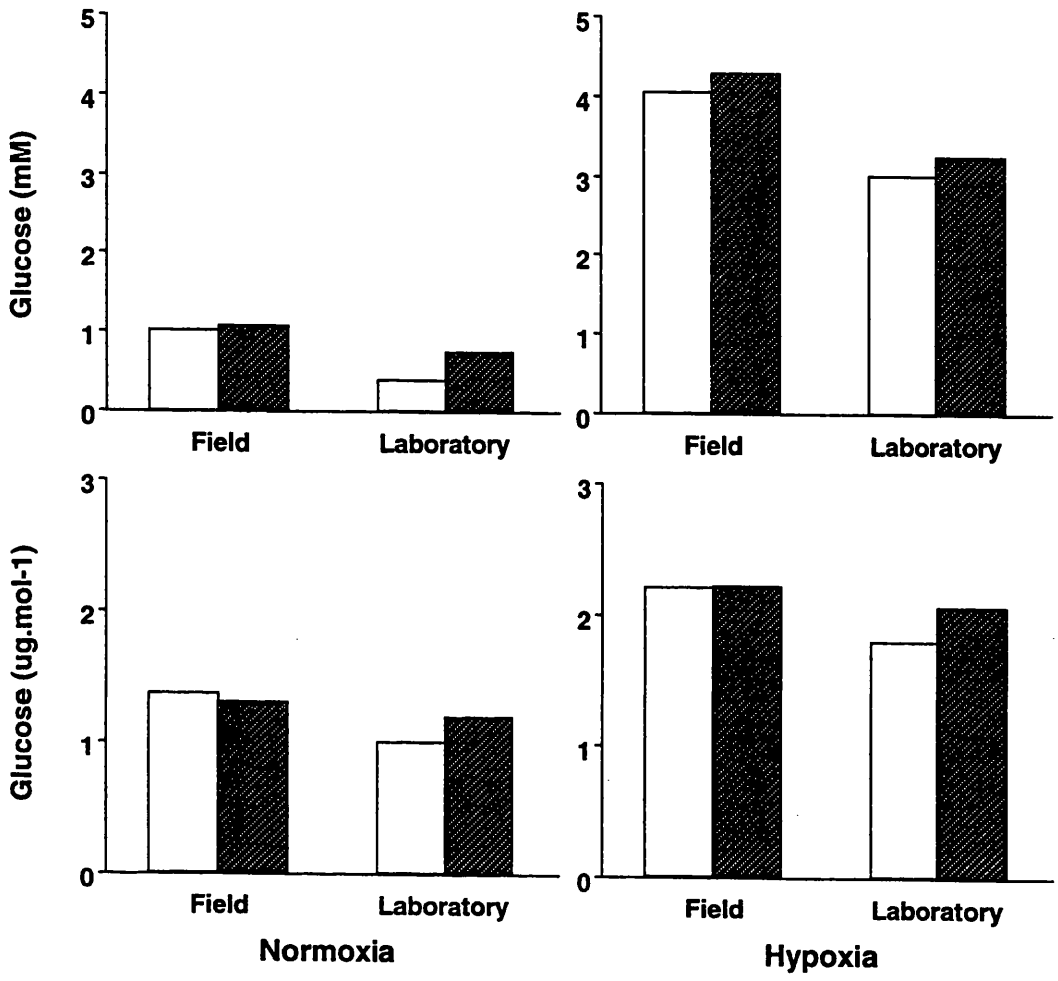


Figure 4. The median concentrations of glucose in the blood (mM) and tissues ($\mu mol.g^{-1}$) of crabs at rest (empty bars) and crabs fought (shaded bars) in the field and in the laboratory under both normoxia and hypoxia.

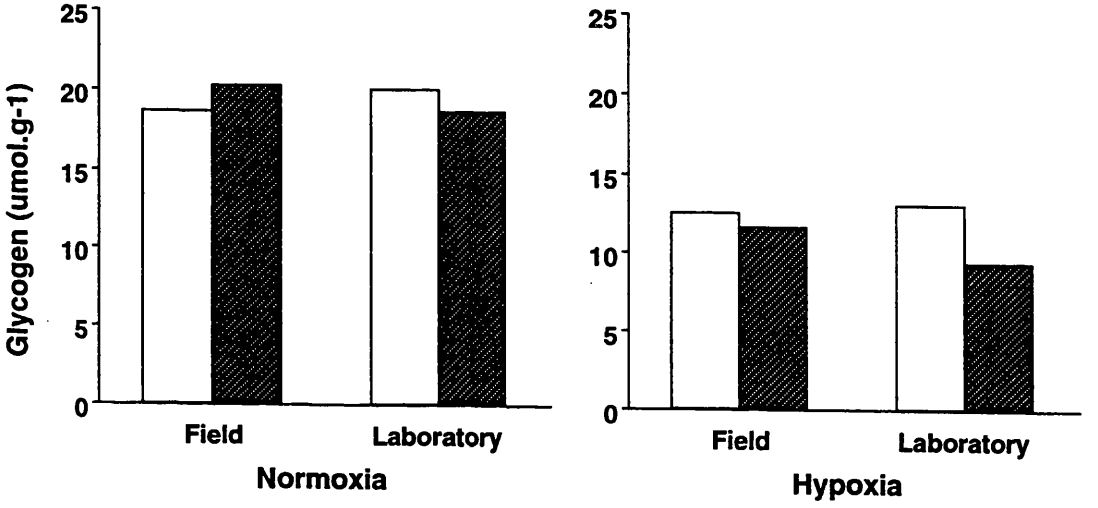


Figure 5. The median concentrations glycogen in the tissues ($\mu mol.g^{-1}$) of crabs at rest (empty bars) and crabs fought (shaded bars) in the field and in the laboratory under both normoxia and hypoxia.

7.4 DISCUSSION

The agonistic behaviour of the shore crab in the natural environment is similar to the behaviour observed in the laboratory. However, the fights in the rock pool consisted of only one aggressive interaction and no re-engagement of an opponent occurred. This agrees with field observations on *N. puber* where similar action patterns were performed by crabs but the fights were shorter in duration and consisted of only one bout (Smith 1990). In the laboratory, the fighting pairs of shore crabs are confined to a tank where they have to remain in close proximity to one another whereas, in a rock pool, crabs were able to retreat long distances from an opponent. The behaviour of fighting *C. maenas* in the pool agrees with theories that agonistic behaviour should not involve lengthy interactions since this may increase attraction of predators and competing conspecifics (Crowley *et al.* 1988; Caine 1989; Green 1990; Smith 1990). Duration may also be influenced by the relative motivation of the crabs to fight. Crabs used in laboratory experiments were starved 7 days prior to contests in order to increase motivation but crabs used in field observations were only starved for 24 hours. Therefore, the crabs may have been less motivated to fight for long periods over food in the field since they may have been less hungry than those fought in the laboratory. It was decided to hold them for a short time to keep conditions as natural as possible. However, it would be interesting to hold crabs 7 days prior to the field experimentation to determine if they fought for longer. Another possibility is that the value

the crabs in the field place on the food items is low, since there is abundant molluscan prey available to them on the shores of Cumbrae, and so they may not be so highly motivated to fight for long over food. It is unlikely that food will be a scarce commodity for the crabs inhabiting this area and, therefore, fights probably occur when chance items of food such as carcasses of dead fauna are available, or in the breeding season, when competition occurs over receptive females (Sekkelsten 1988; Smith 1990; Abello *et al.* 1994; Reid *et al.* 1994). In future field observations, it would be interesting to place a caged female into the pool to determine if fights become longer and more intense due to the presence of this highly valued resource.

The effects of relative size in the rock pool fights under both normoxia and hypoxia, were similar to the results of laboratory studies, with approximately equal numbers of smaller and larger crabs initiating fights but more larger crabs winning (see Chapters 3 & 5; Sneddon *et al.* 1997b, 1998). This suggests that assessment is occurring during rather than prior to interactions and that assessment can only be made when individuals are close to or engage one another. Perhaps visual perception in *C. maenas* is limited since they are mostly nocturnal and can only assess visually when opponents are in very close proximity, or that assessment is tactile when the strength of an opponent is assessed in the pushing contest or during grasping. Perhaps the costs of fighting are greater for smaller crabs and so they decide to retreat from larger opponents. When comparing body and weapon size, relative weapon size exerts a stronger influence on the

outcome of fights in the field which confirms the laboratory studies (Chapter 3; Sneddon *et al.* 1997b). Animals should base their strategic decisions on their perceived risk of danger (Lima & Dill 1990), and so smaller clawed crabs may detect that their opponent has larger weapons and, therefore, a greater ability to inflict injury and this may influence their decision to de-escalate. It can be concluded that claw size may be a major indicator of status and competitive ability in this species as it is in the grapsid crabs, *Aratus pisoni* and *Goniopsis cruentata* (Warner 1970).

In the laboratory, fighting resulted in greater concentrations of L-lactate and glucose in the blood and tissues of shore crabs under normoxia and hypoxia (see Chapters 5 & 6; Sneddon *et al.* 1998). Glycogen concentrations were only affected under hypoxic conditions, where there was a reduction in the tissues of fought crabs. There were no such metabolic effects shown by crabs which fought in the rock pool. This may be due to the fights being shorter in the field and so, there is not a sufficiently increased energy demand caused by fighting unlike those staged under laboratory conditions which were of much longer duration. Fighting in the laboratory resulted in enhanced anaerobic respiration and increased accumulation of L-lactate; and breakdown of glycogen into glucose causing hyperglycaemia in the blood and tissues. Another possibility why there appear to be no effects of fighting on metabolite levels in the field is that the sample sizes in the field were low and these metabolites are highly variable in freshly caught crabs (Hill 1989). In the laboratory, the crabs are starved for 7 days which reduces the variability

in these metabolites between individuals (Hill 1989) and so the crabs may have similar metabolite profiles before fighting. This variability may be confounding the field data and thus, there was no difference between crabs at rest and after fighting in the pool.

Blood L-lactate and glucose concentrations were higher in resting and in fought crabs in the field than in the laboratory. This could be due to the field crabs having recently fed before capture which is known to elevate glucose and L-lactate concentrations (Wallace 1973). Some studies have shown that hyperglycaemia and production of L-lactate is a response to stress in the shore crab (Hill 1989) and in other crustaceans (e.g. *Nephrops norvegicus*, Spicer *et al.* 1990) and the greater concentrations of these metabolites in the blood of field crabs may be due to the crabs not having recovered from the stress of creeling.

Better experimental protocol and increased sample sizes are needed to determine the metabolic effects of fighting in this species under natural conditions. New technology, such as biosensors which can detect metabolite levels instantly from a small amount of human blood, should be investigated and utilised for animal experimentation in the field. These biosensors would enable us to know an individual's blood L-lactate and glucose concentrations, prior to and after fighting and so, the metabolic effects of agonistic behaviour within individuals can be thoroughly examined.

The field observations confirmed that the behavioural acts performed during the agonistic interactions of male *C. maenas* in the laboratory are

the same as would be seen in the natural environment. However, fights are of much shorter duration in the field than those in the laboratory and the crabs do not seem to show the same metabolic consequences of fighting as they do in the laboratory. With increased sample sizes, a similar experimental protocol as used in the laboratory studies, and lower water oxygen tensions, perhaps the metabolic effects of fighting would be more apparent in the field manipulations. However, it could be that *C. maenas* fights in the field are never as long as fights under laboratory conditions and therefore, the crabs do not incur a metabolic cost as a result of fighting.

CHAPTER EIGHT

GENERAL DISCUSSION

8.1 Resource value and agonistic behaviour

Increasing the value of the resource that the shore crabs, *Carcinus maenas* contest, resulted in an increase in the intensity of fights and a decrease in duration. Game theory models predict that, as resource value increases, so should intensity of contests and this was confirmed by the results of Chapter 2 (Bishop *et al.* 1978; Hammerstein & Parker 1982; Enquist & Leimar 1987). Other studies have demonstrated a similar effect of resource value in contests between other species e.g. the hermit crab, *Pagurus bernhardus*, (Hazlett 1982); the fish, *Betta splendens*, (Bronstein 1982). In *C. maenas*, the behaviour of winners and losers was very different from an early stage in the fights. This agrees with current theory that contestants should adjust their behaviour according to the value that they place on the disputed resource (Enquist & Leimar 1987).

Further work is necessary to determine the precise relationship between resource value and the agonistic behaviour of shore crabs. This could involve using different concentrations of food extract or a higher value resource such as receptive females. In the natural environment of the Clyde Sea area, where the crabs used in this study were obtained, the shore crab's prey are in abundance (L.U. Sneddon pers. obs.). It is unlikely, therefore, that food will be a scarce commodity in this area, whereas receptive females are only available for a short time period once a year (Crothers 1964). It would be expected that contests between male shore crabs over receptive females, would be more intense than contests

over food but perhaps, of much shorter duration. It would be interesting to test this theory using receptive females as the contested resource.

8.2 Resource holding potential (RHP) and agonistic behaviour

The agonistic behaviour of male *Carcinus maenas* is typical of many brachyuran species (Dingle 1983). However, fights do not begin with low intensity acts progressively increasing in escalation to physical violence (Maynard Smith 1982; Huntingford & Turner 1987). Instead, fights can become very intense at the beginning of a bout. In other portunid species, *Liocarcinus depurator* and *Necora puber*, conflicts can be resolved simply by display (Glass & Huntingford 1988; Smith 1990; Smith *et al.* 1994; Thorpe 1994) but it seems that shore crabs must engage one another in physical contact for assessment of an opponent's RHP to occur. In male *C. maenas*, as in several crustacean species (Hyatt 1983) and other taxonomic groups (Huntingford & Turner 1987; Archer 1988), the size difference between opponents greatly influences the outcome of fights but, not the content or duration of contests. A comparison of body size with weapon or claw size in Chapter 3, demonstrated that weapon size was the better predictor of which crab would win, and this agrees with many other studies (Andersson 1994). Equal numbers of smaller and larger males initiated contests, which is similar to the contest behaviour of *N. puber* (Smith *et al.* 1994; Thorpe *et al.* 1995). The predictions of game theory suggest that animals should not engage opponents in fights that they have little chance of winning (Parker 1974; Maynard Smith & Parker 1976;

Hammerstein & Parker 1982). It is probable that shore crabs cannot accurately judge the size of their opponent until they engage in physical contact. Perhaps size is less important in assessing an opponent and strength may be the crucial factor. Weapon strength was shown to exert a greater influence than weapon size on the outcome of shore crab fights in Chapter 4 and thus crabs may assess the ability to potentially inflict injury and base their strategic decisions on this. Weapon strength also influenced the establishment of dominance hierarchies in *Potamon fluviatile* (Gabbanini *et al.* 1995). It would, therefore, pay shore crabs to invest energy into growing larger claws with greater musculature, since this appears to give an advantage in agonistic interactions. This would also apply to the weapons of other animal groups.

8.3 Costs and metabolic consequences of fighting

Comparison of animal contest behaviour with game theory predictions requires a knowledge of the relative costs associated with a given strategy. In addition to the risk of predation, the potential costs of agonistic behaviour are likely to be the risk of injury and expenditure of time and energy (Archer 1988). While injuries do occur in fights between *C. maenas* and may have adverse effects on fitness, they do not appear to be a common result of fighting in portunid crabs since, injuries were not seen in fights between *Liocarcinus depurator* (Glass & Huntingford 1988) or *Necora puber* (Thorpe 1994). The results of Chapters 5 & 6 demonstrate that shore crabs do incur a metabolic cost of fighting reflected in

accumulation of L-lactate in the blood and tissues. Chapter 6 demonstrated that very low oxygen conditions amplify this metabolic cost, creating a greater respiratory debt; and a significant depletion of glycogen reserves which was similar to crabs which were subjected to sustained exercise. The accumulation of L-lactate correlates with a decrease in duration of fights and under severely hypoxic conditions, fights were of much shorter duration than under normal oxygen tension. Therefore, it appears as if the ecological environment has raised the costs of fighting per unit time.

8.4 Impact of an environmental variable

Shore crabs are known to experience hypoxic conditions regularly in the field (Hill *et al.* 1991) and so the metabolic costs of fighting under natural conditions have real implications. It takes shore crabs up to 8 hours to repay the metabolic debt which has been created after being exposed to low oxygen conditions (Hill *et al.* 1991). After fighting in low oxygen tensions, the ability to perform any subsequent activities may be impaired such that, performing high exertion activities is limited. Also the need to replenish the energy reserves which were depleted during fighting, will require increased foraging and will increase an animal's exposure to predation and reduce the time spent on other activities such as mating. Therefore, fighting under severely hypoxic conditions has a real cost which is greater than fighting under normal oxygen conditions. The

metabolic cost of fighting in *C. maenas* is not fixed and appears to vary with environmental oxygen tensions as demonstrated in Chapters 5 and 6.

This work has shown that an ecological variable such as hypoxia has implications for the costs of agonistic behaviour. Few studies have examined this relationship between the costs of fighting and environmental variability. Altering population density increased the costs of fighting in demoiselles, *Chromis dispilus* (Barnett & Pankhurst 1996) and red backed salamanders, *Plethodon cinereus* (Jaeger *et al.* 1983) and varying water velocity increased the energy costs of territorial defence in the brook charr, *Salvelinus fontinalis* (McNicol & Noakes 1984). Future research on the costs of a behaviour should consider ecological variability in an animal's natural environment and assess costs under natural environmental fluctuations that the animal experiences.

8.5 Mechanisms underlying agonistic behaviour

Fighting in low oxygen tensions appeared to increase the metabolic costs of fighting which constrained fighting behaviour by forcing shore crabs to readjust their strategic decisions about how long to fight. L-lactate accumulation may have been a mechanism by which crabs decided how long to fight for. It is interesting to note, however, that exercised crabs had higher concentrations of L-lactate than fought crabs and so fought crabs had not reached a maximum limit of L-lactate production and, therefore, could potentially engage in activity for longer. Perhaps *C. maenas* engaged in fighting detect that they are creating a metabolic debt and thus

the metabolic costs of fighting outweigh the benefits, so motivation to fight for long periods is reduced. The results of Chapter 6 also demonstrate that there are no differences in metabolite concentrations between winners and losers and so these physiological parameters do not appear to have constrained the behaviour of losers as suggested by a study on the cichlid, *Tilapia zillii* where losers had significantly higher L-lactate concentrations than winners (Neat *et al.* 1998). It would be interesting to know the concentrations of each metabolite in crabs prior to fights since this study cannot tell how much L-lactate has been produced during fights. There could be a difference in anaerobic capacity between winning and losing crabs which may play a role in the behaviour of the crabs and may be a decisive factor in contest behaviour.

8.6 Field and laboratory studies

Field observations on the agonistic behaviour of *C. maenas* in Chapter 7 confirm that the behavioural acts performed by fighting shore crabs are similar to those observed in the laboratory in Chapter 2. The results from the field observations also confirm that the effects of relative body and claw size are similar to the results obtained from laboratory studies in Chapter 3. As demonstrated in the laboratory studies described in Chapters 5 and 6, hypoxia has similar effects on the duration of contests and metabolic physiology of shore crabs in the field. Contests under hypoxia in the field were of shorter duration than contests under normoxic conditions; and crabs had higher concentrations of L-lactate and glucose

and lower concentrations of glycogen under hypoxia compared with crabs under normoxia. However, fights in the field were of much shorter duration than those in the laboratory under both hypoxia and normoxia. This agrees with a field study on *N. puber* where the duration of fights were much shorter (Smith 1990). Animal conflicts should be brief since lengthy interactions will attract competing conspecifics and predators. The field studies do not reflect the metabolic effects of fighting that were demonstrated in the laboratory studies in Chapter 7. There were no differences in metabolites between crabs at rest and after fighting. However, the crabs used in the field study were not subjected to exactly the same treatment as the laboratory crabs. They were not starved or kept in isolation for 7 days prior to the contests. Small sample sizes were used in the field studies and perhaps with increased observations and similar experimental protocol as in the laboratory, the same metabolic consequences would occur in field crabs as in crabs fought in the laboratory. Field experiments also need to be conducted in a pool which becomes more hypoxic than the one used in this study (30 - 42 Torr) since laboratory studies show that the metabolic effects are greater in lower oxygen tensions (<30 Torr, Chapter 6). This could also be achieved by bubbling nitrogen gas through the pool water which would displace oxygen and give very low oxygen tensions. Perhaps under severe hypoxia in the field, the metabolic consequences of fighting would be more apparent. However, it may be that the metabolic effects of fighting seen under laboratory conditions are a result of being confined to a relatively

small space where they are forced to engage an opponent in agonistic behaviour for longer than they would in the natural environment. To overcome this problem, the use of a larger arena in the laboratory or perhaps the construction of semi-natural conditions in a very large tank with refugia may enable losers to retreat to a distance where they will stay clear of their opponent and so the duration and metabolic consequences of fights would be similar to those observed in the field.

9. REFERENCES

9.1 Chapter One

Abello P., Warman C.G., Reid D.G. & Naylor E. 1994. Chela loss in the shore crab *Carcinus maenas* and its effect on mating success. *Mar. Biol.* 121, 247-252.

Adams E.S. & Caldwell 1990. Deceptive communication in asymmetric fights of the stomatopod crustacean *Gonodactylus bredini. Anim. Behav.* 39, 706-716.

Andersson M.B. 1994. *Sexual Selection.* Princeton University Press, Princeton.

Archer J. 1988. *The Behavioural Biology of Aggression.* Cambridge, Cambridge University Press.

Bennet A.F. & Houck L.D. 1983. The energetic cost of courtship and aggression in a plethodontid salamander. *Ecol.* 64, 979-983.

Bishop D.T., Cannings C. & Maynard Smith J. 1978. The war of attrition with random rewards. *J. Theoret. Biol.* 74, 377-388.

Blanckenhorn W.U. 1992. Group size and the cost of agonistic behaviour in pumpkinseed sunfish. *Ethol. Ecol. Evol.* 4, 255-271.

Caraco T. 1979. Time budget and group size: a test of theory. *Ecol.* 60, 618-628.

Chellapa S. & Huntingford F.A. 1989. Depletion of energy reserves during reproductive aggression in male 3-spined sticklebacks, *Gasterosteous aculeatus* L. *J. Fish Biol.* 35, 315-316.

Crothers J.H. (1964) The biology of the shore crab *Carcinus maenas* (L.) 2. The biology of the adult crab. *Fld Stud.* 2(5), 579-614.

Davies N.B. & Krebs J.R. 1978. Introduction: ecology, natural selection and social behaviour. In: *Behavioural Ecology: an evolutionary approach.* (Eds J.R. Krebs & N.B. Davies), Blackwell Scientific Publications, p1-18.

Dingle H. 1983. Strategies of agonistic behaviour in Crustacea. In: *Studies in adaptations: the behaviour of the higher Crustacea* (Eds S. Rebach & D.W. Dunham), Wiley Interscience p85-111.

Eckert R., Randall D. & Augustine G. 1988. *Animal Physiology. Mechanisms and Adaptations.* 3rd Edition, New York, Freeman.

Ellington W.R. 1983. The recovery from anaerobic metabolism in invertebrates. *J. exp. Zool.* 228, 431-444.

Enquist M. & Leimar O. 1983. Evolution of fighting behaviour: decision rules and assessment of relative strength. *J. Theoret. Biol.* 102, 387-410.

Enquist M. & Leimar O. 1987. Evolution of fighting behaviour: The effect of variation in resource value. *J. Theoret. Biol.* 127, 187-205.

Glass C.W. & Huntingford F.A. 1988. Initiation and resolution of fights between swimming crabs (Liocarcinus depurator). *Ethology* 77, 237-249.

Hack M.A. 1997. The energetic costs of fighting in the house cricket, *Achetus domesticus* (L.). *Behav. Ecol.* 8(1), 28-36.

Haller J. 1995. Biochemical backround for an analysis of cost-benefit interrelations in aggression. *Neurosci. Biobehav. Rev.* 19, 599-604.

Haller J., Rusu V.M., Giurgia R. & Dat C.P. 1990. Energetical cost of aggression in rats: resident-intruder conflicts. *Rev. roum. Biochim.* 27, 217-224.

Hammerstein P. & Parker G.A. 1982. The asymmetric war of attrition. *J. Theoret. Biol.* 96, 647-682.

Hazlett B.A. 1968. Size relationships and aggressive behaviour in the hermit crab *Clibanarus vittatus*. *Z. tierpsychol.* 25, 608-614.

Hazlett B.A. 1982. Resource value and communication strategy in the hermit crab *Pagurus berhardus* (L.). *Anim. Behav.* 30, 135-139.

Hill A.D. 1989. The anaerobic metabolism of the common shore crab, *Carcinus maenas* (L.). Ph.D Thesis, University of Glasgow, 185pp.

Hill A.D., Taylor A.C. & Strang R.H.C. 1991. Physiological and metabolic responses of the shore crab, *Carcinus maenas* (L.) during environmental anoxia and subsequent recovery. *J. Exp. Mar. Biol. Ecol.* 150, 31-50.

Hogstad O. 1987. It is expensive to be dominant. *Auk* 104, 333-336.

Huntingford F.A. & Turner A.K. 1987. *Animal Conflict.* Chapman & Hall, 448pp.

Hyatt G.W. 1983. Qualitative and quantitative dimensions of crustacean aggression. In: *Studies in adaptations: the behaviour of the higher Crustacea* (Eds S. Rebach & D.W. Dunham), Wiley Interscience.

Jachowski R.L. 1974. Agonistic behaviour of the blu crab, *Callinectes sapidus* Rathbun. *Behav.* 50, 232-253.

Kitching J.A., Sloane J.F. & Ebling F.J. 1959. The ecology of Lough Ine VIII. Mussels and their predators. *J. Anim. Ecol.* 28, 331-341.

Leimar O. & Enquist M. 1984. Effects of asymmetries in owner-intruder interactions. *J. Theoret. Biol.* 111, 475-491.

Marden J.H. & Rollins R.A. 1994. Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. *Anim. Behav.* 48, 1023-1030.

Marden J.H. & Waage, J.K. 1990. Escalated damselfly territorial contests are energetic wars of attrition. *Anim. Behav.*, 39, 954-959.

Maynard Smith J. 1982. *Evolution and the theory of games*. Cambridge University Press, 224pp.

Maynard Smith J. & Parker G.A. 1976. The logic of animal conflict. *Anim. Behav.* 24, 154-175.

Maynard Smith J. & Price G.R. 1973. The logic of animal conflict. *Nature* 246, 15-18.

McNicol R.E. & Noakes D.L.G. 1984. Environmental influences on territoriality of juvenile brook charr, *Salvelinus fontinalis*, in a stream environment. *Environ. Biol. Fishes* 10, 29-42.

Neat F.C., Taylor A.C. & Huntingford F.A. 1998. Proximate costs of fighting in male cichlid fish: the role of injury and energy metabolism. *Anim. Behav.* 55, 875-882.

Parker G.A. 1974. Assessment strategy and the evolution of animal conflict. *J. Theoret. Biol.* 47, 223-243.

Parker G.A. & Rubenstein D.I. 1981. Role assessment, reserve strategy and acquisition of information in asymmetric animal conflicts. *Anim. Behav.* 29, 221-240.

Plaistow S. & Siva-Jothy M.T. 1996. Energetic constraints and male mate-securing tactics in the damsel fly *Calopteryx splendens xanthostoma* (Charpentier). *Proc. Roy. Soc. Lond. B* 263, 1233-1238.

Reid D.G., Abello P., Warman C.G. & Naylor E. 1994. Size related mating success in the shore crab *Carcinus maenas. J. Zool. Lond.* 232, 397-407.

Reid D.G. & Naylor E. 1993. Different free running periods in split components of the circatidal rhythm in the shore crab, *Carcinus maenas. Mar. Ecol. Prog. Ser.* 102, 295-302.

Riechert S.E. 1988. The energetic costs of fighting. *Am. Zool.* 28, 877-884.

Robertson J.D. 1989. Physiological constraints upon marine organisms. *Earth Sciences* 80, 225-234.

Røskaft E.T., Järvi T., Bakken M., Beck C. & Reinertsen R.E. 1986. The relationship between social status and resting metabolic rate in great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*). *Anim. Behav.* 34, 838-842.

Sekkelsten G.I. 1988. Effect of handicap on male mating success in shore crabs, *Carcinus maenas*. *Oikos* 51, 131-134.

Shuster S. & Caldwell R.L. 1989. Male defence of the breeding cavity and factors affecting the persistence of breeding pairs in the stomatopod, *Gonodactylus bredini* (Manning). *Ethology* 82, 192-207.

Simmons L.W., Teale R.J., Maier M., Standish R.J. Bailey W.J. & Withers P.C. 1992. Some costs of reproduction for male bush crickets *Requena verticalis*: allocating resources to mate attraction and nuptial feeding. *Behav. Ecol. Sociobiol.* 31, 57-62.

Smith I.P. & Taylor A.C. 1993 The energetic cost of agonistic behaviour in the velvet swimming crab, *Necora* (=*Liocarcinus*) *puber* (L.). *Anim. Behav.* 43, 375-391.

Witter M.S. & Swaddle J.P. 1995. Dominance, competition and energetic reserves in the european starling, *Sturnus vulgaris*. *Behav. Ecol.* 6, 343-348.

9.2 Chapter Two

Abele L.G., Campanella P.J. & Salmon M. 1986. Natural history and social organisation of the semi-terrestrial grapsid crab *Pachygrapsus transversus* (Gibbes). *J. exp. mar. Biol. Ecol.* 104, 153-170.

Adams E.S. & Caldwell R.L. 1990. Deceptive communication in asymmetric fights of the stomatopod crustacean *Gonodactylus bredini*. *Anim. Behav.* 39, 706-716.

Archer J. 1987. *The Behavioural Biology of Aggression.* Cambridge University Press, Cambridge.

Berrill M. & Arsenault M. 1982. Mating behaviour of the green shore crab *Carcinus maenas*. *Bull. Mar. Sci.* 32, 632-638.

Bishop D.T., Cannings C. & Maynard Smith J. 1978. The war of attrition with random rewards. *J. Theoret. Biol.* 74, 377-388.

Bovbjerg R.V. 1956. Some factors affecting aggressive behaviour in crayfish. *Physiol. Zool.* 29, 127-136.

Bronstein P.M. 1985. Predictions of dominance in male *Betta splendens*. *J. Comp. Psychol.* 99, 56-59.

Caine E.A. 1989. Caprellid amphipod behaviour and predatory strikes by fish. *J. exp. mar. Biol. Ecol.* 126, 173-180.

Christy J.H. 1987. Competitive mating, mate choice and mating associations of brachyuran crabs. *Bull. Mar. Sci.* 41(2), 177-191.

Crane J. 1975. *Fiddler crabs of the World.* Princeton University Press, Princeton, New Jersey.

Crothers J.H. 1964. The biology of the shore crab *Carcinus maenas* (L.) 2. The biology of the adult crab. *Fld Stud.* 2(5), 579-614.

Crowley P.H., Gillett S. & Lawton J.H. 1988. Contests between larval damselflies: empirical steps towards a better ESS model. *Anim. Behav.* 36, 1496-1510.

Dare P.J. & Edwards D.B. 1981. Underwater television observations on the intertidal movements of shore crabs, *Carcinus maenas*, across a mudflat. *J. mar. biol. Ass. U.K.* 61, 107-116.

Dingle H. 1983. Strategies of agonistic behaviour in Crustacea. In: *Studies of Adaptation: The Behaviour of the Higher Crustacea*, (Eds Rebach S.& Dunham D.W.), Wiley, New York.

Eales A.J. 1974. Sex pheromone in the shore crab *Carcinus maenas* and the site of its release from females. *Mar. Behav. Physiol.* 2, 345-355.

Elwood R.W. & Glass C.W. 1981. Negotiation or aggression during shell fights of the hermit crab, *Pagurus bernhardus*? *Anim. Behav.* 29, 1239-1244.

Enquist M. 1985. Communication during agonistic interactions with particular reference to variation in choice of behaviour. *Anim. Behav.* 33, 1152-1161.

Enquist M. & Leimar O. 1987. Evolution of fighting behaviour: the effect of variation in resource value. *J. Theoret. Biol.* 127, 187-205.

Glass C.W. & Huntingford F.A. 1988. Initiation and resolution of fights between swimming crabs (*Liocarcinus depurator*). *Ethology.* 77, 237-249.

Green A.J. 1990. Determinants of chorus participation and the effects of size, weight and competition on advertisement calling in the Tungara frog, *Physalaemus pustulosus*. *Anim. Behav.* 39, 620-638.

Hammerstein P. & Parker G.A. 1982. The asymmetric war of attrition. *J. Theoret. Biol.* 96, 647-682.

Hazlett B.A. 1968. Size relationship and aggressive behaviour in the hermit crab, *Clibanarius vittatus*. *Z. tierpsychol.* 27, 369-374.

Hazlett B.A. 1974. Field observations on the interspecific agonistic behaviour in hermit crabs. *Crustaceana* 26, 133-138.

Hazlett B.A. 1982. Resource value and communication strategy in the hermit crab *Pagurus bernhardus* (L.). *Anim. Behav.* 30, 135-139.

Huntingford F.A. & Turner A.K. 1987. *Animal Conflict*. Chapman Hall, London.

Hyatt G.W. 1983. Qualitative and quantitative dimensions of crustacean aggression. In: *Studies of Adaptation: The Behaviour of the Higher Crustacea*, (Eds Rebach S. & Dunham D.W.), Wiley, New York.

Hyatt G.W. & Salmon M. 1978. Combat in fiddler crabs *Uca pugilator* and *Uca pugnax*: A quantitative analysis. *Behav.* 65, 182-211.

Jachowski R.L. 1974. Agonistic behaviour of the blue crab, *Callinectes sapidus* Rathbun. *Behav.* 50, 232-253.

Jensen K. 1972. On the agonistic behaviour in *Carcinus maenas* (L). *Ophelia* 10, 57-61.

Kitching J.A., Sloane J.F. & Ebling F.J. 1959. The ecology of Lough Ine VIII. Mussels and their predators. *J. Anim. Ecol.* 28, 331-341.

Krebs J.R. & Davies N.B. 1987. *An Introduction to Behavioural Ecology.* Blackwell Scientific, Oxford.

Marler C. & Moore M.C. 1991. Supplementary feeding compensates for testosterone induced costs of aggression in male mountain spiny lizards, *Sceloperus jarrovi. Anim. Behav.* 42, 209-219.

Maynard Smith J. 1974. The theory of games and the evolution of animal conflicts. *J. Theoret. Biol.* 47, 209-221.

Maynard Smith J. 1982. *Evolution and the Theory of Games.* Cambridge University Press, Cambridge.

Maynard Smith J. & Parker G.A. 1976. The logic of asymmetric contests. *Anim. Behav.* 24, 159-175.

Parker G.A. 1974. Assessment strategy and the evolution of fighting behaviour. *J. Theoret. Biol.* 47, 224-243.

Parker G.A. & Rubenstein D. I. 1981. Role of assessment, reserve strategy, and acquisition of information in asymmetric animal conflicts. *Anim. Behav.* 29, 221-240.

Reid D.G., Abello P., Warman C.G. & Naylor E. 1994. Size related mating success in the shore crab *Carcinus maenas. J. Zool. Lond.* 232, 397-407.

Reid D.G. & Naylor E. 1993. Different free running periods in split components of the circatidal rhythm in the shore crab, *Carcinus maenas. Mar. Ecol. Prog. Ser.*102, 295-302.

Riechert S.E. 1984. Games spiders play III: cues underlying context-associated changes in agonistic behaviour. *Anim. Behav.* 32, 1-15.

Rubenstein D.I. & Hazlett B.A. 1974. Examination of the agonistic behaviour of the crayfish *Oronectes virilis* by character analysis. *Behav.* 50, 193-216.

Schein H. 1975. Aspects of the aggressive and sexual behaviour of *Alpheus heterochaelis* Say. *Mar. Behav. Physiol.* 3, 83-96.

Smith I.P. 1990. The Agonistic Behaviour of the Velvet Swimming Crab, *Liocarcinus puber* (L.). Ph.D Thesis, University of Glasgow.

Smith I.P., Huntingford F.A., Atkinson R.J.A. & Taylor A.C. 1994. Strategic decisions during agonistic behaviour in the velvet swimming crab, *Necora puber*. *Anim. Behav.* 47, 885-894.

Tamm G.R. & Cobb J.S. 1978. Behaviour and the crustacean moult cycle: changes in the aggression of *Homarus americanus*. *Science* 200, 79-81.

Thorpe K.E. 1994. Behavioural and Physiological Studies of Fighting in the Velvet Swimming Crab, *Necora puber*(L.). Ph.D Thesis, University of Glasgow.

Thorpe K.E., Huntingford F.A. & Taylor A.C. 1994. Relative size and agonistic behaviour in the female velvet swimming crab, *Necora puber* (L.). *Behav. Proc.* 32, 235-246.

9.3 Chapter Three

Abello P., Warman C.G., Reid D.G., & Naylor E. 1994. Chela loss in the shore crab *Carcinus maenas* and its effect on mating success. *Mar Biol* 121, 247-252.

Andersson M.B. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* 40, 804-816.

Andersson M.B. 1994. *Sexual Selection.* Princeton University Press, Princeton.

Archer J. 1988. *The Behavioural Biology of Aggression.* Cambridge, Cambridge University Press.

Austad S.N. 1983. A game theoretical interpretation of male combat in the bowl and doily spider (*Frontinella pyramitela*). *Anim. Behav.* 31, 59-73.

Berrill M. & Arsenault M. 1982. Mating behaviour of the green shore crab *Carcinus maenas*. *Bull. Mar. Sci.* 32, 632-638.

Bildsten K.L., McDowall S.G. & Brisbin I.L. 1989. Consequences of sexual dimorphism in sand fiddler crabs, *Uca pugilator*: differential vulnerability to avian predation. *Anim. Behav.* 37, 133-139.

Brown L. & Bartalon J. 1986. Behavioural correlates of male morphology in a horned beetle. *Am. Nat.* 127, 565-570.

Caldwell R.L. & Dingle J. 1979. The influence of size on agonistic encounters in the mantis shrimp, *Gonodactylus viridus*. *Behav.* 69, 255-264.

Claxton W.T., Govind C.K. & Elner R.W. 1994. Chela function, morphometric maturity, and the mating embrace in the male snow crab, *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* 51, 1110-1118.

Clutton-Brock T.H., Guiness F.E. & Albon S.D. 1982. *Red Deer: Behaviour and Ecology of Two Sexes*. Edinburgh, Edinburgh University Press.

Davenport J., Spikes M., Thornton S.M. & Kelly B.O. 1992. Crab eating in the diamondback terrapin, *Malaclemys terrapin*: dealing with dangerous prey. *J. Ma.r Biol. Ass. U.K.* 72, 835-848.

Dingle H. (1983) Strategies of agonistic behaviour in Crustacea. In: *Studies in Adaptation: The Behaviour of Higher Crustacea* (Eds Rebach S. & Dunham D.W.), New York, Wiley Interscience, pp 85-111.

Enquist M. & Leimar O. 1983. Evolution of fighting behaviour: Decision rule and assessment of relative strength. *J. Theoret. Biol.* 102, 387-341.

Espmark Y. 1964. Studies in dominance - subordination relationship in a group of semi domestic reindeer. *Anim. Behav.* 12, 420-426.

Gabbanini F., Gherardi F., & Vannini M. 1995. Force and dominance in the agonistic behaviour of the freshwater crab *Potamon fluviatile*. *Agg. Behav.* 21, 451-462.

Garvey J.E. & Stein R.A. 1992. Evaluating how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). *Am. Midl. Nat.* 129, 172-181.

Hartnoll R.G. 1982. Growth. In: *The biology of the Crustacea. Vol. 2. Embryology, Morphology, and Genetics* (Ed Abele L.G.), New York, London, Academic Press, pp 111-196.

Hazlett B.A., Rubenstein D.I. & Rittschof D. 1975. Starvation, energy reserves, and aggression in the crayfish *Orconectes virilis*. Crustaceana 28, 11-16.

Huntingford F.A., Taylor A.C., Smith I.P. & Thorpe K.E. 1995 Behavioural and physiological studies of swimming crabs. *J. Exp. Mar. Biol. Ecol.* 193, 21-39.

Hyatt G.W. 1983. Qualitative and quantitative dimensions of crustacean aggression. In: *Studies in Adaptation: The Behaviour of the Higher Crustacea* (Eds Rebach S. & Dunham D.W.), New York, Wiley Interscience pp. 113-139.

Hyatt G.W. & Salmon M. 1978. Combat in the fiddler crabs *Uca pugilator* and *Uca pugnax*: a quantitative analysis. *Behav.* 65, 182-211.

Jachowski RL 1974. Agonistic behaviour of the blue crab, *Callinectes sapidus* Rathbun. *Behav* 50: 232-253

Juanes F. & Smith L.D. 1995. The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. *J. Exp. Mar. Biol. Ecol.* 193, 197-223.

Kodric-Brown A. & Brown J.H. 1984. Truth in advertising: the kinds of traits favoured by sexual selection. *Am. Nat.* 124, 305-322.

Lawton P. 1989. Predatory interaction between the brachyuran crab *Cancer pagurus* and decapod crustacean prey. *Mar. Ecol. Prog. Ser.* 52, 169-179.

Lee S.Y. 1993. Chela height is an acceptable indicator of chela strength in *Carcinus maenas* (L.). *Crustaceana* 65, 115-116.

Lee S.Y. & Seed R. 1992. Ecological implications of cheliped size in crabs: some data from *Carcinus maenas* and *Liocarcinus holsatus*. *Mar. Ecol. Prog. Ser.* 84, 151-160.

Lincoln G.A. 1972. The role of antlers in the behaviour of red deer. *J. exp. Zool.* 182, 233-250.

Maynard Smith J. 1982. *Evolution and the Theory of Games*. Cambridge & New York, Cambridge University Press.

Maynard Smith J. & Brown R.L.W. 1986. Competition and body size. *Theoret. Pop. Biol.* 50, 166-171.

Maynard Smith J. & Parker G.A. 1976. The logic of asymmetric contests. *Anim. Behav.* 24, 159-175.

Neil S.J. 1985. Size assessment and cues: studies of hermit crab contests. *Behav.* 92, 22-38.

Parker G.A. 1974 Assessment strategy and the evolution of fighting behaviour. *J. Theoret. Biol.* 47, 223-243.

Pavey C.R. & Fielder D.R. (1996) The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus*. *J. Zool. Lond.* 238, 445-457.

Prowse D.L., Trilling J.S. & Luich J.R. 1980. Effects of antler removal on mating behaviour of reindeer. In: *Proc of 2nd Internat Reindeer/Caribou Symp.* (Eds Reimers E., Gaare E. & Skjenneberg S.),Direktoratet for Vilt og Ferskvannsfisk, Trondheim, Norway, pp 528-536.

Riechert S.E. 1984. Games spiders play III: cues underlying context-associated changes in agonistic behaviour. *Anim. Behav.* 32, 1-15.

Rubenstein D.I. & Hazlett B.A. 1974. Examination of the agonistic behaviour of the crayfish, *Orconectes virilis* by character analysis. *Behav.* 50, 193-216.

Schein H. 1975. Aspects of the aggressive and sexual behaviour of *Alpheus heterochaelis* Say. *Mar. Behav. Physiol.* 3, 83-96.

Sekkelsten G.I. 1988. Effect of handicap on mating success in male shore crabs *Carcinus maenas*. *Oikos* 51, 131-134.

Sigurjonsdottir H. & Parker G.A. 1981. Dungfly struggles: evidence for assessment strategy. *Behav. Ecol. Sociobiol.* 8, 219-230.

Smith I.P., Huntingford F.A., Atkinson R.J.A. & Taylor A.C. 1994. Strategic decisions during agonistic behaviour in the velvet swimming crab, *Necora puber* (L). *Anim. Behav.* 47, 885-894.

Snedden W.A. 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus*: chela size and sperm competition. *Behav.* 115, 100-113.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997. The influence of resource value on the agonistic behaviour of the shore crab, *Carcinus maenas* (L.). *Mar. Freshw. Behav. Physiol.* 30, 225-237.

Suter R.B. & Keiley M. 1984. Agonistic interactions between male *Frontinella pyramitela*. *Behav. Ecol. Sociobiol.* 15, 1-7.

Thornhill R. 1984. Fighting and assessment in *Harpobatacus* scorpionflies. *Evol.* 38, 204-214.

Thorpe K.E. 1994. Behavioural and Physiological Studies of Fighting in the Velvet Swimming Crab, *Necora puber* (L.). Ph.D. Thesis, University of Glasgow.

Thorpe K.E., Huntingford F.A. & Taylor A.C. 1995. Relative size and agonistic behaviour in the female velvet swimming crab, *Necora puber*(L.). *Behav. Pro.c* 32, 235-246.

Turner G.F. & Huntingford F.A. 1986. A problem for game theory analysis: assessment in male mouthbrooder contests. *Anim. Behav.* 34, 61-70.

Zar J.H. 1984. *Biostatistical Analysis*. New Jersey, Prentice Hall.

Zeh D.W. 1987a. Aggression, density, and sexual dimorphism in chernetid pseudoscorpions. *Evolution* 41, 1072-1087.

Zeh D.W. 1987b. Life history consequences of sexual dimorphism in a chernetid pseudoscorpion. *Ecology* 68, 1495-1501.

9.4 Chapter Four

Abby Kalio N.J. & Warner G.F. 1984. Effects of two different feeding regimes on the chela closer muscles of the shore crab, *Carcinus maenas*. *Mar. Behav. Physiol.* 11, 209-218.

Abello P., Warman C.G., Reid D.G. & Naylor E. (1994) Chela loss in the shore crab *Carcinus maenas* and its effect on mating success. *Mar. Biol.* 121, 247-252.

Andersson M.B. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* 40, 804-816.

Andersson M.B. 1994. *Sexual Selection.* Princeton University Press, Princeton.

Archer J. 1988. *The Behavioural Biology of Aggression.* Cambridge, Cambridge University Press.

Berglund A., Bisazza A. & Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.* 58, 385-399.

Blundon J.A. & Kennedy V.S. 1982. Mechanical and behavioural aspects of the blue crab, *Callinectes sapidus* (Rathbun), predation on Chesapeake Bay bivalves. *J. exp. mar. Biol. Ecol.* 65, 47-65.

Brown L. & Bartalon J. 1986. Behavioural correlates of male morphology in a horned beetle. *Am. Nat.* 127, 565-570.

Caldwell R.L. & Dingle J. 1979. The influence of size on agonistic encounters in the mantis shrimp, *Gonodactylus viridus*. *Behav.* 69, 255-264.

Clutton-Brock T.H., Guiness F.E. & Albon S.D. 1982. *Red Deer: Behaviour and Ecology of Two Sexes*. Edinburgh, Edinburgh University Press.

Dunham D.W. 1981. Chela efficiency in display and feeding by hermit crabs. *Crustaceana* 41(4), 40-45.

Enquist M. & Leimar O. 1983. Evolution of fighting behaviour: Decision rule and assessment of relative strength. *J. theoret. Biol.* 102, 387-341.

Gabbanini F., Gherardi F. & Vannini M. 1995. Force and dominance in the agonistic behaviour of the freshwater crab *Potamon fluviatile*. *Agg. Behav.* 21, 451-462.

Garvey J.E. & Stein R.A. 1992. Evaluating how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). *Am. Midl. Nat.* 129, 172-181.

Hartnoll R.G. 1982. Growth. In: *The biology of the Crustacea. Vol. 2. Embryology, Morphology, and Genetics* (Ed Abele L.G.), New York, London, Academic Press, pp 111-196.

Järvi T. 1990. The effects of male dominance, secondary sexual characteristics and female mate choice on the mating success of male Atlantic salmon, *Salmo salar*. *Ethology* 84, 123-132.

Jones A.R. 1980. Chela injuries in the fiddler crab, *Uca burgersi* Holthius. *Mar. Behav. Physiol.* 7, 47-56.

Kodric-Brown A. & Brown J.H. 1984. Truth in advertising: the kinds of traits favoured by sexual selection. *Am. Nat.* 124, 305-322.

Lee S.Y. 1993. Chela height is an acceptable indicator of chela strength in *Carcinus maenas* (L.). *Crustaceana* 65, 115-116.

Lee S.Y. & Seed R. 1992. Ecological implications of cheliped size in crabs: some data from *Carcinus maenas* and *Liocarcinus holsatus*. *Mar. Ecol. Prog. Ser.* 84, 151-160.

Lima S.L. & Dill L.M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619-640.

Maynard Smith J. 1982. *Evolution and the Theory of Games*. Cambridge & New York, Cambridge University Press.

Norman C.P. & Jones M.B. 1991. Limb loss and its effect on handedness and growth in the velvet swimming crab, *Necora puber*. *J. Nat. Hist.* 25, 639-645.

Parker G.A. 1974. Assessment strategy and the evolution of fighting behaviour. *J. Theoret. Biol.* 47, 223-243.

Preston S.J., Revie I.C., Orr J.F. & Roberts D. 1996. A comparison of the strengths of gastropod shells with forces generated by potential crab predators. *J. Zool., Lond.* 238, 181-193.

Reid D.G., Abello P., Warman C.G. & Naylor E. 1994. Size related mating success in the shore crab *Carcinus maenas*. *J. Zool., Lond.* 232, 397-407.

Rutherford P.L., Dunham D.W. & Allison V. 1994. Winning agonistic encounters by male crayfish *Orconectes rusticus*: chela size matters but chela asymmetry does not. *Crustaceana* 69, 526-529.

Sekkelsten G.I. (1988) Effect of handicap on mating success in male shore crabs *Carcinus maenas*. *Oikos* 51, 131-134.

Smith M.R. & Dunham D.W. 1990. Chela posture and vision compensation for sensory deficit in the crayfish, *Orconectes propinquus*. *Crustaceana* 59, 309-313.

Snedden WA (1990) Determinants of male mating success in the temperate crayfish *Orconectes rusticus*: chela size and sperm competition. *Behav.* 115, 100-113.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997a. Weapon size versus body size as a predictor of winning fights between shore crabs, *Carcinus maenas* (L.). *Behav. Ecol. Sociobiol.* 41(4), 237-242.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997b. The influence of resource value on the agonistic behaviour of the shore crab, *Carcinus maenas* (L.). *Mar. Freshw. Behav. Physiol.* 30, 225 - 237.

Zar J.H. 1984. *Biostatistical Analysis*. New Jersey, Prentice Hall.

Zeh D.W. 1987a. Aggression, density, and sexual dimorphism in chernetid pseudoscorpions. *Evolution* 41, 1072-1087.

Zeh D.W. 1987b. Life history consequences of sexual dimorphism in a chernetid pseudoscorpion. *Ecology* 68, 1495-1501.

9.5 Chapter Five

Aagard A., Warman C.G., Depledge M.H. & Naylor E. 1995. Dissociation of heart rate and locomotor activity during the expression of rhythmic behaviour in the shore crab, *Carcinus maenas*. *Mar. Freshw. Behav. Physiol.* 26, 1-10.

Allen J.A. 1966. The Fauna of the Clyde Sea Area. *Crustacea. Oceanog. Mar. Biol. Ann. Rev.* 4, 247-265.

Booth C.E., McMahon B.R. & Pinder A.W. 1982. Oxygen uptake and the potentiating effects of increased haemolymph L-lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *J. Comp. Physiol.* B 148, 111-121.

Burke E.M. 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. *Biol. Bull. Mar. Biol. Lab., Woodshole* 156, 157-168.

Cristol D. A. 1992. Food deprivation influences dominance status in dark-eyed juncos, *Junco hyemalis*. *Anim. Behav.* 43, 117-124.

Crothers J.H. 1964. The biology of the shore crab, *Carcinus maenas* II. The life of the adult crab. *Fld Stud.* 2(5), 579-614.

Dare P.J. & Edwards D.B. 1981. Underwater television observations on the intertidal movement of shore crabs, *Carcinus maenas* across a mudflat. *J. Mar. Biol. Ass.* UK 61, 107-116.

Edwards R.L. 1958. Movements of individual members in a population of the shore crab, *Carcinus maenas*, in the littoral zone. *J. Anim. Ecol.* 127, 37-45.

Engel P. & Jones J.B. 1978. Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of NAD+ in alkaline hydrazine buffers: improved conditions for the assay of L-glutamate, L-lactate and other metabolites. *Anal. Biochem.* 88, 475-484.

Ferguson R.A., Kieffer J.D. & Tufts B.L. 1993. The effects of body size on the acid-base and metabolite status in the white muscle of rainbow trout before and after exhaustive exercise. *J. Exp. Biol.* 180, 195-207.

Gäde G. 1984. Effects of oxygen deprivation during anoxia and muscular work on the energy metabolism of the crayfish *Oronectes limosus*. *Comp. Biochem. Physiol.* 77A, 495-502.

Gutmann I. & Wahlefeld A.W. 1974. L-lactate determination with lactate dehydrogenase and NAD+. *Methods of Enzymatic Analysis*. 2nd Edition, (Ed Bergmeyer H.U), p464 - 468, New York, Academic Press.

Hack M.A. 1997a. The energetic costs of fighting in the house cricket, *Achetus domesticus* (L.). *Behav. Ecol.* 8(1), 28-36.

Hack M.A. 1997b. Assessment strategies in the contests of male crickets, *Achetus domesticus* (L.). *Anim. Behav.* 53, 753-747.

Head G. & Baldwin S. 1986. Energy metabolism and the fate of L-lactate during recovery from exercise in the australian freshwater crayfish, *Cherax destructor*. *Austral. J. Mar. Freshw. Res.* 37, 641-646.

Hill A.D. 1989. *The anaerobic metabolism of the shore crab, Carcinus maenas*. Ph.D Thesis, University of Glasgow.

Hill A.D., Taylor A.C. & Strang R.H.C. 1991. Physiological and metabolic responses of the shore crab, *Carcinus maenas*, during environmental anoxia and subsequent recovery. *J. exp. Mar. Biol. Ecol.* 150, 31-50.

Houlihan D.F., Mathers E. & El Haj A. 1984. Walking performance and aerobic and anaerobic metabolism of *Carcinus maenas*. *J. Exp. Marine Biol. Ecol*.4, 211-230.

Houlihan D.F. & Mathers E. 1985. Effects of captivity and exercise on the locomotion and muscle of *Carcinus maenas*. *J. Exp. Mar. Biol. Ecol.* 92, 125-142.

Keppler D. & Decker K. 1974. Glycogen determination with amyloglucosidase. *Methods of Enzymatic Analysis*. 2nd Edition (Ed Bergmeyer H.U.), p1129 - 1131, New York, Academic Press.

Kieffer J.D., Ferguson R.A., Tomoa J.E. & Tufts B.L. 1996. Relationship between body size and anaerobic metabolism in Brook trout and large mouth bass. *Trans. Am. Fish. Soc.*125, 760-767.

Kitching J.A., Sloane J.F. & Ebling F.J. 1959. The ecology of Lough Ine VIII. Mussels and their predators. *J. Anim. Ecol.* 34, 315-329.

Kunst A., Draeger B. & Ziegerhorn J. 1981. UV methods with hexokinase and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis*. 2nd Edition (Ed Bergmeyer H.U.), p163-172, New York, Academic Press.

Lynch M.P. & Webb K.L. 1973. Variations in serum constituents of the blue crab, *Callinectes sapidus* : Glucose. *Comp. Biochem. Physiol.* 45A, 127-139.

Maynard Smith J. 1982. *Evolution and Theory of Games.* Cambridge University Press, Cambridge.

Morris S. & Greenaway P. 1989. Adaptations to a terrestrial existence in the robber crab, *Birgus latro* L. - IV. L-lactate dehydrogenase function and L-lactate accumulation during exercise. *Comp. Biochem. Physiol.* 94B, 59-64.

Muntz L., Ebling F.J. & Kitching J.A. 1965. The ecology of Lough Ine VIV. Predatory activity of large crabs. *J. Anim. Ecol.* 34, 315-329.

Reid D.G. & Naylor E. 1993. Different free running periods in split components of the circatidal rhythm in the shore crab, *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* 102, 295-302.

Robertson J.D. 1989. Physiological constraints upon marine organisms. *Earth Sciences* 80, 225-234.

Rodríguez-Gironés M. A., Drummond H. & Kacelnik A. 1996. Effect of food deprivation on dominance status in blue-footed booby (*Sula nebouxii*) broods. *Behav. Ecol. Sociobiol.* 7, 82-88.

Smith I.P. & Taylor A.C. 1993. The energetic cost of agonistic behaviour in the velvet swimming crab, *Necora puber* (L.). *Anim. Behav.* 45, 375-391.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997a. The influence of resource value on the agonistic behaviour of the shore crab, *Carcinus maenas* (L.). *Mar. Freshw. Behav. Physiol.* 30, 225-237.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997b. Weapon size versus body size as a predictor of winning fights between shore crabs, *Carcinus maenas* (L.). *Behav. Ecol. Sociobiol.*41(4), 237-242.

Taylor A.C. 1976. The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. Exp. Biol.* 65(2), 309-322.

Taylor A.C. & Spicer J.I. 1988. Functional significance of a partial emersion response in the intertidal prawn, *Palaemon elegans* during environmental hypoxia. *Mar. Ecol. Prog. Ser.* 44, 141-147.

Thorpe K.E., Huntingford F.A. & Taylor A.C. 1994. Relative size and agonistic behaviour in the female velvet swimming crab, *Necora puber* (L.). *Behav. Proc.* 32, 235-246.

Thorpe K.E., Taylor A.C. & Huntingford F.A. 1995. How costly is fighting? Physiological effects of sustained exercise and fighting in the velvet swimming crab, Necora puber (L.). *Anim. Behav.* 50, 1657-1666.

van Aardt W.J. 1988. L-lactate metabolism and glucose patterns in the river crab, *Potamonautes warreni* (Calman) during anoxia and subsequent recovery. *Comp. Biochem. Physiol.* 91A, 299-304.

Wallace J.C. 1973. Activity and metabolic rate in the shore crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* 41A, 523-533.

Weinstein R.B., Hennessey T.M., Morovich J.D. & Herreid C.F. 1988. Does exercise change lipid and sugar levels in crab blood. *Am. Zool.* 28(4), 125A.

Zar J.H. 1984. *Biostatistical Analysis*. New Jersey, Prentice Hall.

Zebe E. 1982. Anaerobic metabolism in *Upogebia pugettensis* and *Callianassa californiensis. Comp. Biochem. Physiol.* 72B, 613-618.

9.6 Chapter Six

Barnett C.W. & Pankhurst N.W. 1996. Effect of density on the reproductive behaviour of the territorial male demoiselle, *Chromis dispilus*. *Environ. Biol. of Fishes* 46, 343-349.

Burke E.M. 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. *Biol. Bull. Mar. Biol. Lab., Woodshole* 156, 157-168.

Crothers J.H. 1964. The biology of the shore crab *Carcinus maenas* (L.) 2. The biology of the adult crab. *Fld Stud.* 2(5), 579-614.

Davies N.B. & Krebs J.R. 1978. Introduction: ecology, natural selection and social behaviour. In: *Behavioural Ecology: An Evolutionary Approach.* (Eds. Krebs J.R. & Davies N.B.), Blackwell Science Publications, p 1-18.

Eckert R., Randal D. & Augustine G. 1988. *Animal Physiology. Mechanisms and Adaptations*. 3rd Edition, New York, Freeman.

Ellington W.R. 1983. The recovery from anaerobic metabolism in invertebrates. *J. exp. Zool.* 228, 431-444.

Engel P.C. & Jones J.B. 1978. Causes and elimination of erratic blanks in enzymatic metabolite assays during the use of NAD+ in alkaline hydrazine buffers: improved conditions for the assay of L-glutamate, L-lactate and other metabolites. *Anal. Biochem.* 88, 475-484.

Full R.J., Herreid II C.F. & Assad J.A. 1985. Energetics of the exercising wharf crab, *Sesarma cinerum*. *Physiol. Zool.* 58(5), 605-615.

Gutmann I. & Wahlefeld A.W. 1974. L-lactate determination with lactate dehydrogenase and NAD+. In: *Methods of Enzymatic Analysis*. 2nd Edition, (Ed H.U. Bergmeyer), New York, Academic Press, p 464-468.

Hack M.A. 1997. The energetic costs of fighting in the house cricket, *Achetus domesticus* (L.). *Behav. Ecol.* 8(1), 28-36.

Hamilton N.M. & Houlihan D.F. 1992. Respiratory and circulatory adjustments during aquatic treadmill exercise in the common shore crab, *Carcinus maenas*. *J. exp. Biol.* 162, 37-54.

Hill A.D. 1989. The anaerobic metabolism of the shore crab, *Carcinus maenas*. Ph.D Thesis, University of Glasgow.

Hill A.D., Taylor A.C. & Strang R.H.C. 1991. Physiological and metabolic responses of the shore crab, *Carcinus maenas* during environmental anoxia and subsequent recovery. *J. Exp. Mar. Biol. Ecol.* 150, 31-50.

Houlihan D.F., Govind C.K. & El Haj A.J. 1985. Energetics of swimming in *Callinectes sapidus* and walking in *Hommarus americanus*. *Comp. Biochem. Physiol.* 82A(2), 267-279.

Houlihan D.F., Mathers E. & El Haj A. (1984) Walking performance and aerobic and anaerobic metabolism of *Carcinus maenas*. *J. Exp.Mar. Biol. Ecol.* 74, 211-230.

Jaeger R.G., Nishikawa K.C.B. & Barnard D.E. 1983. Foraging tactics of a terrestrial salamander: costs of territorial defence. *Anim. Behav.* 31, 191-198.

Keppler D. & Decker K. 1974. Glycogen determination with amyloglucosidase. In: *Methods in Enzymatic Analysis*. 2nd Edition (Ed Bergmeyer H.U.), New York, Academic Press. p1129-131.

Kitching J.A., Sloane J.F. & Ebling F.J. 1959. The ecology of Lough Ine VIII. Mussels and their predators. *J. Anim. Ecol.* 28, 331-341.

Kunst A., Draeger B & Ziegerhorn J. (1981) UV methods with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis*. 3rd Edition (Ed Bergmeyer H.U.), New York, Academic Press, p163-172.

Lynch M.P. & Webb K.L. 1973. Variations in serum constituents of the blue crab, *Callinectes sapidus* : Glucose. *Comp. Biochem. Physiol.* 45A, 127-139.

Marden J.H. & Waage J.K. 1990. Escalated damselfly territorial contests are energetic wars of attrition. *Anim. Behav.* 39, 954-959.

McNicol R.E. & Noakes D.L.G. 1984. Environmental influences on territoriality of juvenile brook charr, *Salvelinus fontinalis*, in a stream environment. *Environ. Biol. Fishes* 10, 29-42.

Neat F.C., Taylor A.C. & Huntingford F.A. 1998. Proximate costs of fighting in male cichlid fish: the role of injury and energy metabolism. *Anim. Behav.* 55, 875-882.

Plaistow S. & Siva-Jothy M.T. 1996. Energetic constraints and male mate-securing tactics in the damsel fly *Calopteryx splendens xanthostoma* (Charpentier). *Proc. Roy. Soc. Lond.: B* 263, 1233-1238.

Pough F.H. & Taigen T.L. 1990. Metabolic correlates of the foraging and social behaviour of dart poison frogs. *Anim. Behav.* 39, 145-155.

Prestwich K.N. Brugger K.G. & Topping M. 1989. Energetics and communication in 3 species of hylid frogs : power input, power output and efficiency. *J. exp. Biol.* 144, 53-80.

Reid D.G. & Naylor E. 1993. Different free running periods in split components of the circatidal rhythm in the shore crab, *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* 102, 295-302.

Robertson J.D. 1989. Physiological constraints upon marine organisms. *Earth Sciences* 80, 225-234.

Simmons L.W., Teale R.J., Maier M., Standish R.J. Bailey W.J. & Withers P.C. 1992. Some costs of reproduction for male bush crickets *Requena verticalis*: allocating resources to mate attraction and nuptial feeding. *Behav. Ecol. Sociobiol.* 31, 57-62.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997. The influence of resource value on the agonistic behaviour of the shore crab, *Carcinus maenas* (L.). *Mar. Freshw. Behav. Physiol.* 30, 225-237.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1998. Impact of an ecological factor on the costs of resource acquisition: fighting and metabolic physiology of crabs. *Functional Ecol.*, in press.

Taigen T.L. & Wells K.D. 1985. Energetics of vocalisation by anuran amphibians. *J. comp. Physiol.* 155B, 163-170.

Taylor A.C. 1988. The ecophysiology of decapods in a rock pool environment. In: *Aspects of the biology of decapod Crustacea* (Eds

Fincham AA. & Rainbow P.S.). Oxford University Press, Oxford, 227-261.

Taylor E.W. 1982. Control and co-ordination of ventilation and circulation in the crustacean response to hypoxia and exercise. *J. exp. Biol.* 100, 289-319.

Thorpe K.E., Taylor A.C. & Huntingford F.A. 1995. How costly is fighting? Physiological effects of sustained exercise and fighting in the velvet swimming crab, *Necora puber* (L.). *Anim. Behav.* 50, 1657-1666.

van Aardt W.J. 1988. L-lactate metabolism and glucose patterns in the river crab, *Potamonautes warreni* (Calman) during anoxia and subsequent recovery. *Comp. Biochem. Physiol.* 91A, 299-304.

Wallace J.C. (1973) Activity and metabolic rate in the shore crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* 41A, 523-533.

Weinstein R.B., Hennessey T.M., Morovich J.D. & Herreid C.F. 1988. Does exercise change lipid and sugar levels in crab blood. *Am. Zool.* 28(4), 125A.

9.7 Chapter Seven

Abello P., Warman C.G., Reid D.G. & Naylor E. 1994. Chela loss in the shore crab, *Carcinus maenas* and its effect on mating success.*Mar. Biol.* 121, 247-252.

Atema J., Jackobson S., Karnofsky E., Olesko-Szuts S. & Stein L. 1979. Pair formation in the lobster, *Hommarus americanus*: behavioural development, pheromones and mating. *Mar. Behav. Physiol.* 6, 277-296.

Barnett C.W. & Pankhurst N.W. 1996. Effect of density on the reproductive behaviour of the territorial male demoiselle, *Chromis dispulis*. *Environ. Biol. of Fishes* 46, 343-349.

Bjordal A. 1986. The behaviour of Norway lobsters towards baited creels and size selectivity of creels and trawl. *Fisk. Dir. Skr. HavUnders.* 18, 131-137.

Caine E.A. 1989. Caprellid amphipod behaviour and predatory strikes by fish. *J. exp. Mar. Biol. Ecol.* 126, 173-180.

Chapman C.J. & Howard F.G. 1979. Field observations on the emergence rhythm of the Norway lobster, *Nephrops norvegicus* using different methods. *Mar. Biol.* 51, 157-165.

Crowley P.H., Gillett S. & Lawton J.H. 1988. Contests between larval damselflies: empirical steps toward a better ESS model. *Anim. Behav.* 36, 1496-1510.

Dingle H. 1983. Strategies of agonistic behaviour in crustaceans. In Studies in Adaptation: the behaviour of the higher Crustacea. (Ed Dunham D.W.). Wiley Interscience. p85-111.

Engel P.C. & Jones J.B. 1978. Causes and elimination of erratic blanks in enzymatic metabolite assays during the use of NAD+ in alkaline hydrazine buffers: improved conditions for the assay of L-glutamate, L-lactate and other metabolites. *Anal. Biochem.* 88, 475-484.

Green A.J. 1990. Determinants of dominance participation and the effects of size, weight and competition on advertisement calling in the Tungara frog, *Physalaemus pustulosus*. *Anim. Behav.* 39, 620-638.

Gutmann I. & Wahlefeld A.W. 1974. L-lactate determination with lactate dehydrogenase and NAD+. In: *Methods of Enzymatic Analysis.* 2nd Edition, (Ed Bergmeyer H.U.), New York, Academic Press, p 464-468.

Hill A.D. 1989. The anaerobic metabolism of the shore crab, *Carcinus maenas*. Ph.D Thesis, University of Glasgow.

Hill A.D., Taylor A.C. & Strang R.H.C. 1991. Physiological and metabolic responses of the shore crab, *Carcinus maenas* during environmental anoxia and subsequent recovery. *J. Exp. Mar. Biol. Ecol.* 150, 31-50.

Houlihan D.F., Govind C.K. & El Haj A.J. 1985. Energetics of swimming in *Callinectes sapidus* and walking in *Hommarus americanus*. *Comp. Biochem. Physiol.* 82A(2), 267-279.

Karnofsky E.B. & Price H.J. 1989. Dominance, territoriality and mating in the lobster Homarus americanus: a mesocosm study. *Mar Behav. Physiol.*

Keppler D. & Decker K. 1974. Glycogen determination with amyloglucosidase. In: *Methods in Enzymatic Analysis*. 2nd Edition (Ed Bergmeyer H.U.), New York, Academic Press. p1129-131.

Kunst A., Draeger B & Ziegerhorn J. 1981. UV methods with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis*. 3rd Edition (Ed Bergmeyer H.U.), New York, Academic Press, p163-172.

Lima S.L. & Dill L.M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619-640.

Loew E.R. 1976. Light and photoreceptor degeneration in Norway lobster, *Nephrops norvegicus* (L.). *Proc. Roy. Soc. Lond:B* 193, 31-44.

McNicol, R.E. & Noakes, D.L.G. 1984. Environmental influences on territoriality of juvenile brook charr, *Salvelinus fontinalis*, in a stream environment. *Environ. Biol. Fishes* 10, 29-42.

Reid D.G., Abello P., Warman C.G. & Naylor E. 1994. Size related mating success in the shore crab, *Carcinus maenas*. *J. Zool.* 232, 397-407.

Sekkelsten G.M. 1988. Effect of handicapping on the mating success in the male shore crab, *Carcinus maenas*. *Oikos* 51, 131-134.

Smith I.P. 1990. The agonistic behaviour of the velvet swimming crab, *Liocarcinus puber* (L.). Ph.D. Thesis, University of Glasgow.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997a. The influence of resource value on the agonistic behaviour of the shore crab, *Carcinus maenas* (L.). *Mar. Freshw. Behav. Physiol.* 30, 225-237.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1997b. Weapon size versus body size as a predictor of winning fights between shore crabs, *Carcinus maenas* (L.). *Behav. Ecol. Sociobiol.* 41, 237-242.

Sneddon L.U., Huntingford F.A. & Taylor A.C. 1998. Impact of an ecological factor on the costs of resource acquisition: fighting and metabolic physiology of crabs. *Functional Ecol.*, in press

Stein L., Jackobson S & Atema J. 1975. behaviour of lobsters in a semi-natural environment at ambient temperature and under thermal stress.*Woods Hole Oceanogr. Instit. Tech. Rep.* 75-48.

Thorpe K.E., Taylor A.C. & Huntingford F.A. 1995. How costly is fighting? Physiological effects of sustained exercise and fighting in the velvet swimming crab, *Necora puber* (L.). *Anim. Behav.* 50, 1657-1666.

Wallace J.C. (1973) Activity and metabolic rate in the shore crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* 41A, 523-533.

Warner G.F. 1970. Behaviour of two species of grapsid crab during intraspecific encounters. *Behav.* 36, 9-19.

9.8 Chapter Eight

Andersson M.B. 1994. *Sexual Selection.* Princeton University Press, Princeton.

Archer J. 1988. *The Behavioural Biology of Aggression.* Cambridge, Cambridge University Press.

Barnett C.W. & Pankhurst N.W. 1996. Effect of density on the reproductive behaviour of the territorial male demoiselle, *Chromis dispulis. Environ. Biol. of Fishes* 46, 343-349.

Bishop D.T., Cannings C. & Maynard Smith J. 1978. The war of attrition with random rewards. *J. Theoret. Biol.* 74, 377-388.

Bronstein P.M. 1985. Predictions of dominance in male *Betta splendens. J. Comp. Psychol.* 99, 56-59.

Crothers J.H. (1964) The biology of the shore crab *Carcinus maenas* (L.) 2. The biology of the adult crab. *Fld Stud.* 2(5), 579-614.

Dingle H. 1983. Strategies of agonistic behaviour in Crustacea. In: *Studies in adaptations: the behaviour of the higher Crustacea* (Eds Rebach S. & Dunham D.W.), Wiley Interscience p85-111.

Gabbanini F., Gherardi F. & Vannini M. 1995. Force and dominance in the agonistic behaviour of the freshwater crab *Potamon fluviatile. Aggr. Behav.* 21, 451-462.

Glass C.W. & Huntingford F.A. 1988. Initiation and resolution of fights between swimming crabs (Liocarcinus depurator). *Ethology* 77, 237-249.

Hammerstein P. & Parker G.A. 1982. The asymmetric war of attrition. *J. Theoret. Biol.* 96, 647-682.

Hazlett B.A. 1982. Resource value and communication strategy in the hermit crab *Pagurus berhardus* (L.). *Anim. Behav.* 30, 135-139.

Hill A.D., Taylor A.C. & Strang R.H.C. 1991. Physiological and metabolic responses of the shore crab, *Carcinus maenas* (L.) during environmental anoxia and subsequent recovery. *J. Exp. Mar. Biol. Ecol.* 150, 31-50.

Huntingford F.A. & Turner A.K. 1987. *Animal Conflict.* Chapman & Hall, 448pp.

Hyatt G.W. 1983. Qualitative and quantitative dimensions of crustacean aggression. In: *Studies in adaptations: the behaviour of the higher Crustacea* (Eds Rebach S. & Dunham D.W.), Wiley Interscience.

Jaeger R.G., Nishikawa K.C.B. & Barnard D.E. 1983. Foraging tactics of a terrestrial salamander: costs of territorial defence. *Anim. Behav.* 31, 191-198.

Maynard Smith J. 1982. *Evolution and the theory of games.* Cambridge University Press, 224pp.

Maynard Smith J. & Parker G.A. 1976. The logic of animal conflict. *Anim. Behav.* 24, 154-175.

McNicol R.E. & Noakes D.L.G. 1984. Environmental influences on territoriality of juvenile brook charr, *Salvelinus fontinalis*, in a stream environment. *Environ. Biol. Fishes* 10, 29-42.

Neat F.C., Taylor A.C. & Huntingford F.A. 1998. Proximate costs of fighting in male cichlid fish: the role of injury and energy metabolism. *Anim. Behav.* 55, 875-882.

Parker G.A. 1974. Assessment strategy and the evolution of animal conflict. 47, 223-243.

Smith I.P.1990. The agonistic behaviour of the velvet swimming crab, *Liocarcinus puber* (L.) (*Brachyura, Portunidae*). Ph. D Thesis. University of Glasgow. 252pp.

Smith I.P., Huntingford F.A., Taylor A.C. & Atkinson R.J.A. 1994. Strategic decisions during agonistic behaviour in the velvet swimming crab, *Necora* (=*Liocarcinus*) *puber* (L.). *Anim. Behav.* 47, 885-894.

Thorpe K.E. 1994. Behavioural and physiological studies of agonistic behaviour in the female swimming crab, *Necora puber* (L.). Ph.D Thesis. University of Glasgow. 185pp.