Behavioural and Physiological Studies of Fighting in the Velvet Swimming Crab, *Necora puber* (L.) (Brachyura, Portunidae)

by

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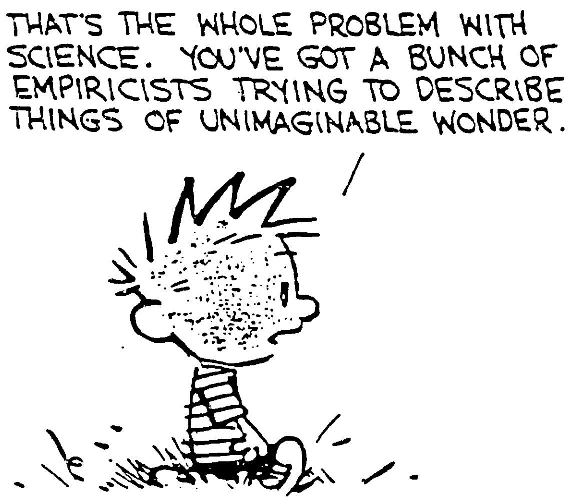
Dedicated to the memory of my Dad,
Ian Grenville Thorpe.


THAT'S THE WHOLE PROBLEM WITH SCIENCE. YOU'VE GOT A BUNCH OF EMPIRICISTS TRYING TO DESCRIBE THINGS OF UNIMAGINABLE WONDER.

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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.

K. Elaine Thorpe
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SUMMARY

The velvet swiming crab, *Necora puber* (L.), is a marine brachyuran commonly found in shallow, rocky, sublittoral areas on the Atlantic coasts of Europe. In Britain, the commercial importance of *N. puber* has increased over the last fifteen years, following the collapse of the Spanish fishery for this species, and the crab now forms the basis of an important fishery based mainly off the west coast of Scotland. *N. puber* has traditionally been regarded as "aggressive", due mainly to its rapid defensive response of striking and grasping humans who attempt to handle them, but relatively little was known of their intra-specific agonistic behaviour until the work of Smith (1990; Smith & Taylor 1993; Smith *et al.* 1994) on males. The aims of the work in this thesis were: to describe the intra-specific agonistic behaviour of females in relation to the size of the interactants, and to investigate the initiation, outcome and content of fights; to describe the processes of escalation and communication during fights in males and females; to investigate the metabolic consequences of fighting and exercise in males; and, to investigate the behavioural consequences of any metabolic costs incurred by the contestants during agonistic behaviour by examining the effects of prolonged fights in subsequent agonistic responses. The agonistic behaviour of *N. puber* is compared with the predictions of game theory.

The agonistic displays of female *N. puber* are similar to those described for other portunids, particularly the closely related *Liocarcinus depurator*, and identical to those of male *N. puber*. Female *N. puber* fight readily in the laboratory, with interactions being initiated equally often by the larger and smaller of two opponents, but with the larger usually being victorious. In some interactions, however, a smaller crab won against a larger opponent, and possible reasons for this are discussed. In two respects, the results are surprising in the context of insights gained from game theory:- firstly, the fights do not show a gradual pattern of escalation through display to overt physical violence. Secondly, fights do not become more costly in terms of either potential for injury (intensity) or duration as the contestants became more evenly matched; indeed, as the contestants became more evenly matched, fight duration decreased.

In both males and females, a short term escalatory process was apparent in intra-individual sequences, with winners and losers tending to follow their own previous low risk acts with high risk acts. However, this process was weaker and shorter lived in losers, which tended to de-escalate after performing high risk acts; winners sustained high intensity fighting by matching or escalating following the performance of high risk acts. Looking at inter-individual sequences to examine the process whereby crabs react to the behaviour of their opponent, one clear trend was a tendency for both winners and losers to match the behaviour of the opponent when this involved low risk acts, but winners and losers respond differently to high risk acts with the winners escalating or matching more frequently than losers. The different communicatory significance of the agonistic acts are discussed.

The metabolic consequences of agonistic behaviour in male *N. puber* were quantified by measuring the concentrations of L-lactate and D-glucose in the haemolymph, and L-lactate, D-glucose and glycogen in the walking leg muscle. There were no significant differences between fought and unfought crabs in terms of the parameters measured, but concentrations in fought and unfought crabs were significantly different from those in crabs forced to exercise. There appears, therefore, to be a very limited metabolic cost associated with agonistic behavour in *N. puber*. There were no significant differences between winners and losers in the metabolic parameters measured, and it can be inferred from this that there is no metabolic threshold reached that forces crabs to give up.

Previous agonistic behaviour did not alter the behaviour of male crabs in subsequent fights. Fought crabs were as likely to initiate a subsequent interaction as unfought crabs, and fought crabs were also equally likely to be successful as unfought crabs. First fights and subsequent re-fights were of similar duration, content and intensity. Again, this suggests that agonistic behaviour in male *N. puber* is not sufficiently metabolically expensive to impose constraints on any subsequent behaviour.

The results of these studies are discussed on a functional basis in relation to the predictions arsing from game theory and on a causal level in relation to possible mechanisms underlying the behaviour.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Why fight?

The success of an animal in terms of survival and reproduction depends on its ability to gain and use requisite resources. Individuals of the same species have similar resource requirements and if any of these resources should become limiting they may be contested by means of agonistic behaviour (Huntingford & Turner 1987). The term "agonistic behaviour" (Scott 1958) refers to the range of acts that may be used during interactions between individuals and that result in one gaining immediate or future access to a resource at the expense of other individuals. Agonistic behaviour includes acts designed to injure an opponent, as well as non-injurious displays, defensive acts and retreat. Intraspecific agonistic interactions vary widely in intensity from situations where one individual simply avoids another, through contests that are resolved by display, to fights that result in fatal injuries.

For agonistic behaviour to occur to any significant degree, a minimum of three pre-requisites have to be met (Dingle 1983). First, the resource must be defendable or quickly consumable once acquired. A resource that can be occupied (*e.g.*, a burrow or a nest), sequestered (*e.g.*, stored food or a guarded mate) or that occurs within a limited and well-defined space may be worth competing for, while more scattered resources may be too costly to gather and/or impossible to defend (Zahavi 1971; Rubenstein 1981). Similarly, it may be worth competing for access to a resource that can be quickly consumed or used, but not worth the cost if possession invites continued attacks by competitors.

Second, the animal must possess the wherewithal to acquire/defend the resource by increasing its cost to competitors. By altering the cost/benefit balance to the point that competitors are unwilling to incur greater costs to obtain or maintain the resource, an aggressive animal can increase the probability that its opponent will defer. Parker (1974) called this ability the "resource holding potential" (RHP); this is a measure of an individual's absolute fighting ability and consists of a combination of its morphological and physiological traits, such as body size, age, size of weapons, armour, greater energy reserves, strength and endurance, and the behavioural repertoire needed to effectively use them during a contest (Caldwell & Dingle 1975; Geist 1977; Caldwell 1987).

Finally, the contested resource must be worth more than the costs of engaging in aggressive encounters. Unless, on average, the extra costs of aggression are more than repaid by the additional resource gained, it would not be expected that aggressive strategies would evolve.

1.2 Effects of agonistic behaviour

When a resource is contested by agonistic behaviour, the form and outcome of such a dispute affects the use of the resource by the population. The way in which a resource is used affects its quality and quantity, which, in turn, influences the individuals using it. For example, dominant male American lobsters, *Homarus americanus*, evict males from other shelters near to their own, although they need only one in which to mate (Karnofsky *et al.* 1989a,b; Steneck 1991). Males are therefore dispersed more widely and sub-ordinates may be forced to occupy sub-optimal shelters. Agonistic behaviour, therefore, affects and is affected by the ecological and social environment in which it occurs.

As agonistic behaviour is used to compete for resources that increase the probability of survival and reproduction, the success of individuals engaged in agonistic interactions influences their lifetime reproductive output, or fitness (Davies & Krebs 1978). Agonistic interactions may result in a potential increase in fitness through gaining the resource, but there may also be an associated decrease in fitness. Agonistic behaviour, like any other activity, involves expenditure of time and energy, but may also result in injury or death, either directly through attack by an opponent, or indirectly through increased exposure to potential predators, for example. The net change in an individual's fitness is therefore the sum of these "costs" and "benefits". Furthermore, since the outcome of an agonistic interaction depends on the behaviour of both interactants, the influence of agonistic behaviour on the fitness of an individual depends on the behaviour of other individuals in the population (Archer 1988).

1.3 Agonistic behaviour & game theory

Many behavioural phenotypes are, at least partially, genetically determined (Hay 1985). Genetically determined phenotypes that influence individual fitness are potentially subject to evolutionary change through natural selection (Sheppard 1979). Natural selection results in organisms with collections of traits that promote individual fitness. It might be expected, therefore, that agonistic behaviour would evolve so that animals optimise their access to resources that can be contested in this way, subject to the constraints imposed by their morphology and physiology, and by their social and ecological environment.

Considerations of the costs and benefits of agonistic behaviour led Maynard Smith & Price (1973) to use game theory to develop an explanation for the non-injurious resolution of agonistic interactions in species that are capable of injuring each other. Previous explanations relied on the rationale of group selection, that escalated aggression would be damaging to the survival of the species (Lorenz 1966). Game theory was originally developed by economists to analyse human decisions in situations involving a conflict of interests. 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 1982a). Central to the application of game theory to animal behaviour is the concept of an evolutionarily stable strategy or ESS (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. The gene pool of a population in which most individuals adopt such a strategy is therefore postulated to be uninvadable by a genotype specifying another strategy within the available set of strategies.

Using simple models of animal contests, Maynard Smith & Price (1973) showed 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 evolutionarily 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 1982a), and agonistic behaviour in sexual populations (Maynard Smith 1982a). Asymmetries between opponents may be related to differences in fighting ability, *e.g.*, size, or differences in resource value assessment (Parker 1974). Differences between opponents that are unrelated to fighting ability or to resource value assessment (uncorrelated asymmetries, *e.g.*, prior ownership) may also be used to settle contests without recourse to escalation (Maynard Smith & Parker 1976; Hammerstein & Parker 1982).

Game theory generates testable predictions based on individual selection and these predictions have been supported by some empirical studies (Maynard Smith 1982a; Enquist & Leimar 1987). The game theory approach, however, is not without problems. Although the behaviour of animals can be compared with predicted behaviour, it is often difficult to determine the effects on fitness of a particular behavioural strategy. Variation in behaviour may not be genetically determined, and may be influenced by, for example, learning and other environmental factors. Although natural selection is thought to be the major agent of evolutionary change, in some circumstances, behavioural phenotypes may become prevalent in a population by other means, such as genetic drift (Gould & Lewontin 1979). The early game theory models were based on the assumptions that populations were asexual and infinite. Models of sexual populations indicate that in some circumstances ESSs do not exist (Maynard Smith 1982a), while there has been disagreement about the potential existence of ESSs in finite populations (Vickery 1987, 1988; Maynard Smith 1988). Even if the behavioural phenotypes prevalent in populations do tend towards evolutionary stability, any evolutionary peturbation will result in a prevalence of unstable phenotypes during an approach to stability. Perhaps the most important point arising from all the discussion about game theory is not that one particular model is the most successful in accounting for the form of animal fighting, but that development of such models should stimulate those studying behaviour to examine their data and assumptions in a new light. The game theory approach to animal fighting has received a great deal of attention from both theoretical and empirical biologists and this way of looking at the adaptive significance of aggression has clarified a number of complex theoretical issues and has stimulated empirical research (Huntingford 1984). This thesis describes a series of studies of agonistic behaviour in the velvet swimming crab (*Necora puber*) conceived and interpreted in the functional framework of game theory, but with causal implications as well.

1.4 The velvet swimming crab, *Necora puber* (L.) (Crustacea, Decapoda, Brachyura, Portunidae)

In the phylum Crustacea there are eight sub-classes and thirty extant orders. The phylum contains about 26 000 species, but the only sub-classes with more than 10% of this total are the Copepoda (4 500 species) and the Malacostraca (18 000 species), the latter containing some 70% of all crustacean species. Similarly, within the Malacostraca, most of the species are contained within three orders, the Amphipoda (3 600 species), the Isopoda (4 000 species) and the Decapoda (8 500 species). The crabs, shrimps and lobsters are contained within the Decapoda, but with a total of about 4 500 species, the majority of decapod species are crabs. Thus in terms of numbers of species, the crabs are one of the largest groups of the Crustacea. Due to their abundance, ease of collection and convenient size, more is known about the crabs than about any other single group of crustaceans. In addition, the true crabs, or brachyurans, also show the most advanced traits of all crustaceans in both morphology and social behaviour (Warner 1977).

Essentially, the brachyurans are members of a warm water fauna, since it is in tropical regions that crabs reach their greatest diversity in numbers of species and sub-species. A significant number of crab species, however, live in temperate waters. 67 species have been recorded around the coast of the British Isles, where they occupy a wide range of habitats and depths, from the intertidal to the continental shelf (Ingle 1980).

The Portunidae found in British waters have been subject to taxonomic revision in recent years (Holthuis 1987). *Necora puber* is the only species assigned to the new genus *Necora*, which was split off from the genus *Liocarcinus*. Previous generic names for this species include: *Macropipus*, *Portunus* and *Liocarcinus*.

Necora puber is a marine brachyuran found most often on hard ground in the shallow sub-littoral zone (Allen 1967; Ingle 1980), although the species has been recorded from a depth of 80m in the Firth of Clyde (Allen 1967). Other common names for this species are: velvet crab, velvet fiddler, lady crab and Kerry witch (Ingle 1980), and in Spain nécora (González Gurriarán 1978). As in all crabs, the sexes are distinguished by abdominal morphology. Males have a narrow, tapering abdomen, which has two pairs of pleopods, modified as accessory copulatory organs. The abdomen of females is wide and rounded, with four pairs of pleopods, developed for carrying eggs. There is sexual dimorphism in overall size and in the relative size of the chelipeds, males being larger in both respects.

Necora puber has a typical brachyuran reproductive cycle (Hartnoll 1969; González Gurriarán 1985). Prior to copulation there is a period of mate-guarding by the male, when the female is held, dorsal side uppermost, against the sternal surface of the male by his walking legs. Copulation occurs immediately after the female moults. During copulation, the sternal surfaces of the two crabs are closely apposed, with the abdomens extended, the female's overlapping the male's. The male's accessory copulatory organs are inserted into genital openings on the female's cephalothorax and are used to transfer spermatophores from muscular projections of the vas deferens to the spermathecae of the female. There is usually a period of post-copulatory mate-guarding by the male. Post-copulatory mate-guarding traditionally has been regarded as a behavioural mechanism that prevents predation on the soft post-moult female (van Engel 1958; Hartnoll 1969; Hazlett 1975a; Nelson & Hedgecock 1977; Wittenberger 1981). A study by Wilber (1989), however, indicates that sexual selection appears to be important in maintaining post-copulatory mate-guarding in stone crabs (Xanthidae, *Menippe*).

Following copulation, fluids secreted with the spermatophores harden to form a "sperm plug" in the spermathecae, extending to the genital openings of the female. The female's ovaries develop after this and eggs are fertilised at spawning, when they are extruded onto the pleopods, where they remain until hatching. Females may spawn more than once between copulations (sequential brooding) and may moult between spawnings (González Gurriarán 1985; Choy 1988; Norman 1989). The eggs hatch 1-3 months after spawning, depending on water temperature. The larvae are released into the plankton, where they develop through five zoeal stages to the megalopa, the final planktonic stage (Rice & Ingle 1975). After moulting from the megalopa to the first crab instar, the crab settles from the plankton. Planktonic development is also temperature dependent, but Rice & Ingle (1975) found that at 15°C, *N. puber* reached the first crab instar in 46-56 days after hatching. *N. puber* reach maturity about one year after settlement and may live for five years.

Direct observations and analyses of gut contents have shown *N. puber* to feed on algae, mainly laminarians (Choy 1986; Norman & Jones 1990), polychaetes (Choy 1986), gastropods (Ebling *et al.* 1964; Muntz *et al.* 1965; Choy 1986), bivalves (Kitching *et al.* 1959; Ebling *et al.* 1964; Muntz *et al.* 1965; Romero *et al.* 1982; Choy 1986), barnacles (Choy 1986), small crabs (Romero *et al.* 1982), including juvenile *N. puber* (Choy 1986), sea urchins (Muntz *et al.* 1965) and piscine carrion (Choy 1986).

Fishermen traditionally regarded *N. puber* as a pest species because they filled traps set for lobsters and edible crabs. This suggests that they are abundant at some locations (MacMullen 1983). Underwater observations, however, indicate that they do not often occur in large densities (Muntz *et al.* 1965). Occasional, localised fluctuations in numbers indicate that some populations may be highly mobile (Bell 1853). Norman (1989) found that there was migration into and out of his littoral study site, with crabs showing little site attachment. Large catches of *N. puber* in creels may be due to the attraction of crabs from a wide area.

Necora puber was previously caught in abundance off the Atlantic coast of Spain, particularly in rías (flooded river valleys) where mussels are cultured on rafts (Romero *et al.* 1982). Intense, unregulated fishing, however, caused the fishery to collapse, and in the last fifteen years, in response to demand from continental Europe, a fishery for the species has developed in Britain, based for the most part off the west coast of Scotland. The major markets for these crabs are in Spain, Portugal and France, where the crabs are sold live, at a price that is influenced by their condition (MacMullen 1983). Excessively injured crabs, usually in the form of missing legs, are rejected at market.

Necora puber has often been referred to as being a highly "aggressive" species (Campbell 1976), but this description seems to be mostly based on its rapid defensive reaction of spreading the chelipeds and striking and grasping humans who attempt to handle them, which is presumably an anti-predator response. Although *N. puber* do not defend territories *per se*, they appear to defend the area around themselves, enabling them to have access to any resources, such as mates and food, that may enter their "personal space". Despite frequent references to "aggression", there were no detailed, published accounts of intraspecific agonistic behaviour in this species until the work of Smith (1990).

Necora puber is a predator of sub-littoral and intertidal invertebrates and may be important in controlling the populations of some species (Kitching *et al.* 1959;

Ebling *et al.* 1964; Muntz *et al.* 1965; Choy 1986; Norman 1989). Information about the nature of resource competition in *N. puber* is important for a better understanding of the ecology of the species and the structure of the communities it may influence. From an applied aspect, agonistic behaviour may lead to size selectivity in catches and a reduction in efficiency of commercial traps set for other crustaceans (Miller 1973, 1979; Brown 1982; Bjordal 1986; Robertson 1989; Smith & Jamieson 1989).

The aims of the work described in this thesis were:-
1) To describe the intraspecific agonistic behaviour of females in relation to the size of interactants, and to investigate the initiation, outcome and content of fights.
2) To describe escalation and communication during fights.
3) To investigate the metabolic consequences of fighting and exercise in this species.
4) To investigate the behavioural consequences of any metabolic costs incurred by the contestants during agonistic behaviour by examining the effects of prolonged fights in subsequent aggressive responses.

The agonistic behaviour of females is described in chapter 2. Communication and escalation are discussed in chapter 3. Chapter 4 describes the metabolic consequences of agonistic behaviour and exercise, and in chapter 5 the behavioural constraints imposed by the metabolic consequences of agonistic behaviour are examined.

CHAPTER TWO

RELATIVE SIZE AND AGONISTIC BEHAVIOUR IN THE FEMALE VELVET SWIMMING CRAB, *NECORA PUBER*

2.1 INTRODUCTION

The agonistic behaviour of crustaceans has been intensively studied on account of their relatively large size, formidable weapons and conspicuous visual displays (Dingle 1983). In crustaceans, as in many animals, agonistic displays often result in an increase in the apparent size of the signaller, and in the presentation of potential weapons (*e.g.*, the chelae) to an opponent (Schöne 1968; Dingle 1983). Early studies of the agonistic behaviour of the Crustacea were descriptive (*e.g.*, Bovbjerg 1956) but some attempted to explain the evolution of this behaviour by comparing the displays of related species (Schöne 1968). Later studies investigated the factors that influence agonistic behaviour by experimental manipulation, and others investigated the communicative properties of agonistic acts (reviewed by Hyatt 1983). Recently, crustaceans have proved suitable subjects with which to test the predictions of game theory (Hyatt *et al.* 1979; Glass & Huntingford 1988; Gardner & Morris 1989; Adams & Caldwell 1990).

2.1.1 Agonistic displays of the Crustacea

The weapons displayed during agonism in many species of crustaceans have evolved in the first place for food gathering, for example, the chelipeds of decapods and the modified raptorial appendages of stomatopods (Dingle 1983). These appendages are often modified to increase their effectiveness in agonistic or sexual displays. In some cases, these modifications are morphological, such as the snapping chela of the Alpheidae (Ritzman 1973) or the enlarged chela of the male ocypodid fiddler crabs (Crane 1975), while in others bright colouration of the display appendages is an integral part of the visual stimulus transmitted by the display (Hazlett 1966, 1969, 1972c, 1979; Dunham 1978a; Dingle 1983). To the human observer, crustacean agonistic displays are visually striking, but non-visual stimuli may also be important. Chemical cues have been implicated in the social behaviour of crayfish (Ameyaw-Akumfi & Hazlett 1975), in the stomatopod *Gonodactylus festae* (Caldwell 1979), in shore crabs *Carcinus maenas* (Eales 1974), in blue crabs *Callinectes sapidus* (Gleeson 1980), and in American lobsters *Homarus americanus* (reviewed by Atema 1986). Acoustic cues are important in the nocturnal displays of fiddler crabs, *Uca* spp. (Salmon & Astaides 1968; Salmon & Hyatt 1979) and tactile cues are important in the agonistic behaviour of snapping shrimps, *Alpheus heterochelis* (Schein 1975) and the crayfish *Orconectes rusticus* (Bruski & Dunham 1987). In snapping shrimps, artificial water currents elicited threat postures by males (Hazlett & Winn 1962). Jachowski (1974) suggested that the strong respiratory currents produced by *Callinectes sapidus* during agonistic interactions might act as a physical stimulus or as a carrier of pheromones. Barron & Hazlett (1989) have reported that the hermit crabs *Clibanarius zebra* and *Calcinus* spp. also produce strong respiratory currents during agonistic interactions which apparently influence the outcome of contests.

In those species using the chelipeds as weapons during physical contests, the size of these appendages might be expected to be a good predictor of agonistic ability. In many species there is indirect evidence that cheliped size is important, as males have larger chelipeds than females and are agonistically superior (Hyatt 1983). Chela size correlates well with the probability of success in fights in the American lobster *Homarus americanus* (Scrivener 1971; O' Neill & Cobb 1979), while Berzins & Caldwell (1983) found that loss of the raptorial appendages adversely affected the agonistic ability of the stomatopod *Gonodactylus bredini.*

The morphology of brachyuran crabs limits the number of body positions that may be used in agonistic displays. As a consequence, there are broad similarites in the agonistic displays of a range of brachyuran families (Schöne 1968). A type of display commonly seen among the brachyuran crabs is the meral spread display, in which the chelipeds are raised and wide apart, increasing the apparent size of the animal. Among the Portunidae, intraspecific agonistic behaviour has been described in *Carcinus maenas* (Schöne 1968; Jensen 1972), *Callinectes sapidus* (Jachowski 1974), *Liocarcinus depurator* (Glass & Huntingford 1988) and male *Necora puber* (Smith 1990). Jachowski (1974) described cheliped extension, shielding with the chelipeds, and a variety of cheliped movements directed at the other crab, *e.g.*, poking. Other agonistic acts included raising the body from the ground, angling the anterior margin upwards, and grasping and striking with the chelae (Jachowski 1974). Similar agonistic acts were described for *L. depurator* (Glass & Huntingford 1988) and *N. puber* (Smith 1990), though shielding was not noted for *N. puber.* In addition, cheliped extension in these species is sometimes accompanied by extreme elevation of the body above the ground by extending the walking legs, and beating of the swimming paddles (flattened dactyli of the 5th pereiopods). Other cheliped displays are known for some semi-terrestrial crabs, for example a complex agonistic and sexual behavioural repertoire is associated with the very pronounced sexual dimorphism of ocypodid fiddler crabs (Crane 1975).

2.1.2 Application of game theory to agonistic behaviour

For two decades, game theory has provided the major theoretical basis for the analysis of the adaptive consequences of animal fights (Maynard Smith 1982a). A major insight resulting from this approach is that, if fighting is costly, encounters are likely to be resolved at an early stage on the basis of the relative fighting ability of the opponents. If one of them is clearly larger or stronger, *i.e.*, has superior fighting ability or resource holding potential (RHP, Parker 1974), the outcome of a fight is predictable and it would pay both animals for the dispute to be settled quickly and with minimum cost. Animals should base their decisions about whether or not to engage in a contest on relative RHP (Parker 1974; Maynard Smith & Parker 1976; Caldwell & Dingle 1979; Hammerstein & Parker 1982; Leimar & Enquist 1984). If the differences between opponents are small and consequently not obvious from the start, fights will involve a process of gradual acquisition of information about relative fighting ability (Parker & Rubenstein 1981; Enquist & Leimar 1983). Where contestants are more evenly matched, more intense contests are more likely to occur (Parker 1974; Maynard Smith & Parker 1976). Higher levels of intensity allow for the transfer of increasingly more accurate information. Intensity can be measured in terms of fight duration or risk of injury.

Early game theory models (War of Attrition, Maynard Smith 1974) suggest that, while exchange of information about fighting ability will be favoured, evenly matched animals engaged in aggressive display should not provide their opponent with any information about intention (Maynard Smith 1982a) or local strategy (Enquist 1985) (*i.e.*, how long or how fiercely they intend to fight); that is to say, up until the moment when a fight is resolved, the eventual loser should not reveal by its behaviour its likelihood of giving up. This questions the classic ethological view that fighting animals provide their opponents with information about intention (Lorenz 1966; Smith 1977). More recent models predict the opposite to the war of attrition model. The answer seems to lie in the fact that models predict that contestants should adjust their behaviour according to the value they place on the resource. In these cases the differences in behaviour between eventual winners and losers will be evident, assuming that the winner places the highest value on the resource.

Agonistic behaviour generally involves stereotyped actions, and exchange of these agonistic signals is often sufficient to resolve an encounter, implying assessment of a potential opponent's fighting ability. In other situations, however, especially in encounters between evenly matched pairs, fights escalate through increasingly intense displays to overt physical violence. Pushing, grasping and striking with the chelipeds occur during fights in a number of species of crab, for example, *Callinectes sapidus* (Jachowski 1974) and *Pagurus bernhardus* (Elwood & Glass 1981). Fights are still usually resolved without injury, but where valuable resources are to be gained, wounds can be inflicted. For example, 25% of male fiddler crabs (*Uca bergersi*), which fight over burrows needed for mating, are found to be injured (Jones 1980), and in mantis shrimps (*Gonodactylus* spp.) fights over moist shelters on exposed beaches can be fatal (Dingle 1983).

2.1.3 Effect of relative size on agonistic behaviour in crustaceans

The most widely reported factor to influence agonism in crustaceans is the relative size of the opponents: larger animals usually defeat smaller ones (Hyatt 1983). Although there are numerous references to the importance of contestant size in determining the outcome and content of contests, there have been few quantitative studies of these effects. Hazlett (1968) found that in agonistic interactions between hermit crabs, *Clibanarius vittatus*, the larger crab usually won and the probability of winning for the larger crab increased with the size differential between opponents. Hermit crab agonistic interactions are, however, frequently related to the acquisition of snail shells, and the effects on the interaction of relative size of the opponents and the suitability of their shells to each other are difficult to separate (Dowds & Elwood 1985; Elwood & Neil 1992). Caldwell & Dingle (1979) found that a size difference of as little as 10% influenced the outcome of contests between stomatopods (*Gonodactylus bredini*). Animals that were less than 70% of the length of their opponents did not win any contests. That study also indicated that smaller contestants attempted to bluff their opponents into retreat by performing exaggerated displays, although ultimately, they avoided their opponents.

Similar results were reported by Glass & Huntingford (1988) for *Liocarcinus depurator*. Crabs only defeated individuals larger than themselves when the size difference between them was small. Interactions between size-matched pairs were of longer duration than interactions between mis-matched crabs, but there was no obvious difference in the types of acts in interactions between crabs of various size differentials. Unexpectedly, crabs seemed equally likely to initiate interactions with individuals larger or smaller than themselves.

Despite frequent references to "aggression" in *N. puber*, there were no detailed, published accounts of intraspecific agonistic behaviour until the work of Smith (1990). Smith (1990; Smith *et al.* 1994) investigated the agonistic behaviour of males with respect to size, hunger, and in competition for mates. They found that the relative size of interacting crabs had a major influence on the content, duration and outcome of interactions. Smaller crabs were as likely to initiate interactions as were larger crabs, although the outcome was usually in favour of the larger crab. As the size difference decreased, the proportions of interactions won by the smaller crab approached 50%, and interactions became longer and involved potentially more injurious behaviour (Smith *et al.* 1994). Contests between males over sexually receptive females involved a high risk of injury, though smaller males were able to defend against larger males (Smith 1990). Food deprivation over a period of up to five days increased contest intensity, though after twelve days deprivation, contest intensity underwent only a small further increase or even a slight decrease (Smith 1990). Thus males, for the most part, obeyed the predictions of game theory. Females are also known to engage in agonism, and as different selection pressures may be operating, different decision rules may be possible.

2.1.4 Aims

The aim of the work presented in this chapter was to describe the agonistic behaviour of female *N. puber* and to investigate the effect of relative size on the initiation, content, duration and outcome of agonistic interactions, and to investigate the behaviour of winners and losers.

2.2 MATERIALS & METHODS

2.2.1 Experimental animals

Female *N. puber* were supplied by a shellfish exporter on Benbecula, Western Isles, at the end of May 1991. On arrival in Glasgow the crabs were transferred to individual holding tanks which consisted of grey PVC tanks (37x57x21cm) subdivided into quarters (18x21x23cm) by grey PVC dividers which had holes drilled in them to facilitate the circulation of water within the tank. The tanks had a substratum of gravel and were supplied by recirculating seawater (salinity 32-34‰), maintained at 10±2°C. The crabs were kept in these conditions for at least 7 days prior to any behavioural observations and were fed every 3 days with whitebait. The carapace width of the crabs was measured to the nearest mm between the 4th and 5th lateral spines using vernier callipers and from these measurements the size ratio of the interactants was calculated by dividing the carapace width of the smaller crab by that of the larger crab. 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 the behavioural observations the crabs were held for a further 2 weeks to ensure that none were in proecdysis (Stevenson 1985). None moulted in this time.

44 crabs were used in this study. Crabs had to be used more than once due to the constraints of obtaining animals and the limited space available to house them. Crabs were allocated into dyads according to the following criteria: they had not fought each other previously and lived in separate holding tanks. Crabs from separate holding tanks were used to prevent the possibility of chemical communication between the crabs prior to the staged encounters. A rest period of 7 days was allowed between each fight.

2.2.2 Observation of agonistic behaviour

The dyads were placed in a glass observation tank (68x43x34cm) with a gravel substratum. They were separated by a vertically sliding opaque partition to allow them a settling time of 15 minutes in continued isolation. The tank was screened from visual disturbance, and was supplied with aerated seawater at 10±2°C. There was a possibility of chemical communication between the crabs during the settling period in the observation tank, and, to reduce this chance, the air and water pumps were switched off when crabs were present in the tank. The tank was illuminated by an 18 watt fluorescent bulb the intensity of which was reduced by a tracing paper shade. The light intensity was 1.75-2.68 $\mu E.m^{-2}s^{-1}$ at the surface, and 0.91-1.42 $\mu E.m^{-2}s^{-1}$ at the bottom. Observations were made through a small opening in the screening. After the settling period and when both crabs were stationary, the partition was raised from outside the screening via a pulley mechanism. After raising the partition the actions of both crabs were recorded using a BBC-B microcomputer programmed as an event recorder.

2.2.3 Agonistic behaviour of female *Necora puber*

Female *N. puber* engaged in agonistic interactions in the observation tank when they encountered each other as a result of the movement of one or both crabs. Of 76 pairings, 65 resulted in an interaction, and in the remaining 8 pairings no interaction had occurred within 30 minutes of raising the partition and data recording was stopped. Interactions were composed of 12 recognisable acts, although there was variability in the exact positioning of the body and legs during their execution. These acts have been defined as follows (Figure 2.1):-

1) **Stand** The crab was stationary with its chelipeds partially flexed and directed downwards and towards each other. All legs were in contact with the ground and supported the body above the ground (Figure 2.1a).

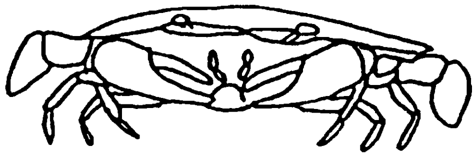
2) **Crouch** The crab was stationary with its chelipeds fully flexed and held next to the branchiostegite region. The body rested on the ground and was approximately parallel to it. To the human observer, this act minimised the apparent size of the crab (Figure 2.1b).

3) **Move to** The crab moved slowly sideways towards the other crab, with the chelipeds flexed.

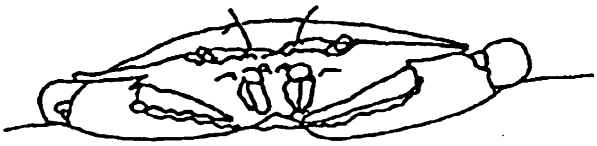
4) **Move away** The crab moved away from the other in a similar way to above.

5) **Cheliped display swimming paddles down** The chelipeds were extended, raised and wide apart. The chelae were usually partially open. The body was tilted with the anterior margin raised. The swimming paddles were not raised and were usually in contact with the ground. To an observer, the effect of this was to increase the apparent size of the animal and to present potential weapons (the chelae) to an opponent (Figure 2.1c).

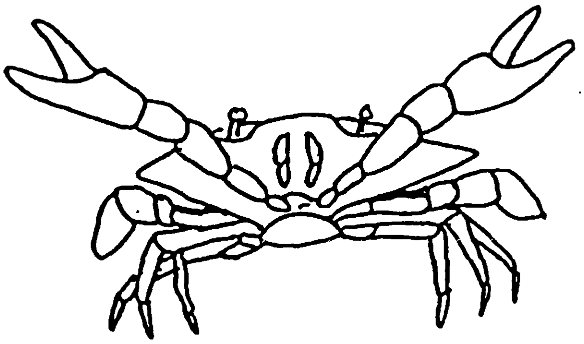
6) **Cheliped display swimming paddles up** Similar to 5) but with the body raised from the ground and the swimming paddles raised above the carapace. This act always occurred when the crabs were facing each other in close proximity. In this position they sometimes pushed against each other and gripped each others chelae.



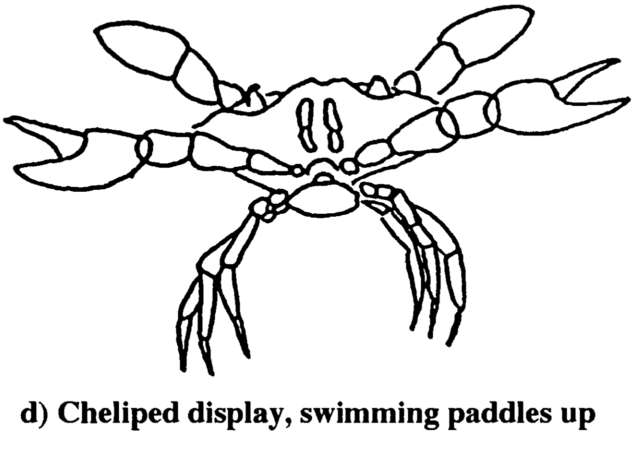
a) Stand



b) Crouch



c) Cheliped display, swimming paddles down



d) Cheliped display, swimming paddles up

Figure 2.1 Agonistic acts of *Necora puber* (after Glass 1985).

The chelae were usually open unless gripping the opponent's chelae (Figure 2.1d).

7) Cheliped display swimming paddles beating As for 6) but with the body raised, apparently to its full extent. The swimming paddles beat in swimming like movements. This activity appeared to be used to generate force during pushing bouts.

8) Approach in display The crab moved toward the other while adopting a cheliped display.

9) Retreat in display The crab moved away from the other, either backwards or sideways, whilst adopting a cheliped display. This behaviour always began within about five carapace widths of the other crab.

10) Retreat The crab moved from the other rapidly, often by swimming, with one or both chelipeds flexed. If a cheliped were held extended it was usually the one closest to the opponent. Again this behaviour always began within about five carapace widths of the other crab.

11) Strike The crab struck its opponent with its chelae. One or both chelipeds may be used. Strikes were performed with the chelae open or closed. If the chelae were open a grasp sometimes followed.

12) Grasp The crab gripped its opponent with one or both chelae. The walking legs of the opponent were usually targeted. This act could result in crushing and autotomy of the gripped limb, although this was never seen in the staged encounters.

2.2.4 Definition of initiation and duration of interactions

After the settling period, the crabs remained stationary or moved about the observation tank. At the start of agonistic interactions 3 situations could be identified (for ease of explanation the 2 crabs have been called crab 1 and crab 2):-

1) Crab 1 moved towards crab 2 which was still. Crab 1 adopted a cheliped display as it approached. Crab 2 either retreated or also adopted a cheliped display. In this case it seems clear that crab 1 was the initiator.

2) Crab 1 moved toward crab 2 which was stationary. Neither crab displayed and crab 2 moved away as the distance between them decreased. In this case crab 1 was designated as the initiator.

3) Crab 1 moved toward crab 2 which was stationary. During this approach, crab 1 did not display but as it neared crab 2, the latter adopted a cheliped display. In this situation it is not clear if crab 1 had:- i) detected crab 2 and was attempting to displace

it without display; ii) detected crab 2 and was about to display when crab 2 displayed first; iii) detected crab 2 and was not engaging it agonistic behaviour; or, iv) not detected crab 2.

In the first 2 situations crab 1 would have initiated the interaction, while in the latter 2, crab 2 would have been the initiator. These situations were, however, indistinguishable to the human observer. Therefore the initiator was designated as the first crab to adopt a cheliped display; or, in non-display interactions, the crab whose initial approach caused the retreat of the other crab. These designations allow precise definition of the beginning of an interaction for measuring the duration. However, for reasons stated previously, in some circumstances they may inaccurately reflect the relative agonistic motivation of the 2 crabs. The time since raising the partition until initiation of the interaction was designated the latency period.

One crab eventually elicited repeated retreats from its opponent. However, in some interactions a crab which initially retreated, subsequently caused its opponent to retreat consistently. The winner was therefore defined as the crab which elicited three or more retreats from its opponent. The duration of the interaction was taken as the time from initiation to the first of the repeated retreats by the loser.

2.2.5 Content of agonistic interactions

Interactions could be classified with respect to their behavioural content, according to the degree of involvement of the two crabs and the potential for injury. The interaction types have been subjectively placed in order of increasing intensity:-

1) The interaction ended after the approach of one crab caused the retreat of the other. Neither crab displayed.
2) One crab displayed and the other retreated without displaying.
3) One crab displayed and struck the other, which retreated without displaying.
4) Both crabs displayed. No strikes were performed by either crab.
5) Both crabs displayed. One retreated after a single strike by its opponent.
6) Both crabs displayed. One retreated after multiple strikes by its opponent.
7) Both crabs displayed. One retreated after strikes by both.

In some of the interactions involving physical contact, strikes or grasps were preceded by a progression from non-contact display, to displays in which the crabs pushed against each other, finally to displays with the swimming legs raised and then beating, apparently to make the push more forceful. Where this was the case, however, there was frequently a reversion to non-contact display before the resolution of the interaction. In other cases, strikes or grasps were not preceded by this sequence of displays (Figure 2.2). This is unusual since interactions usually follow a gradual pattern of escalation with resolution being preceded by the most intense acts (Clutton-Brock & Albon 1979; Enquist *et al.* 1990).

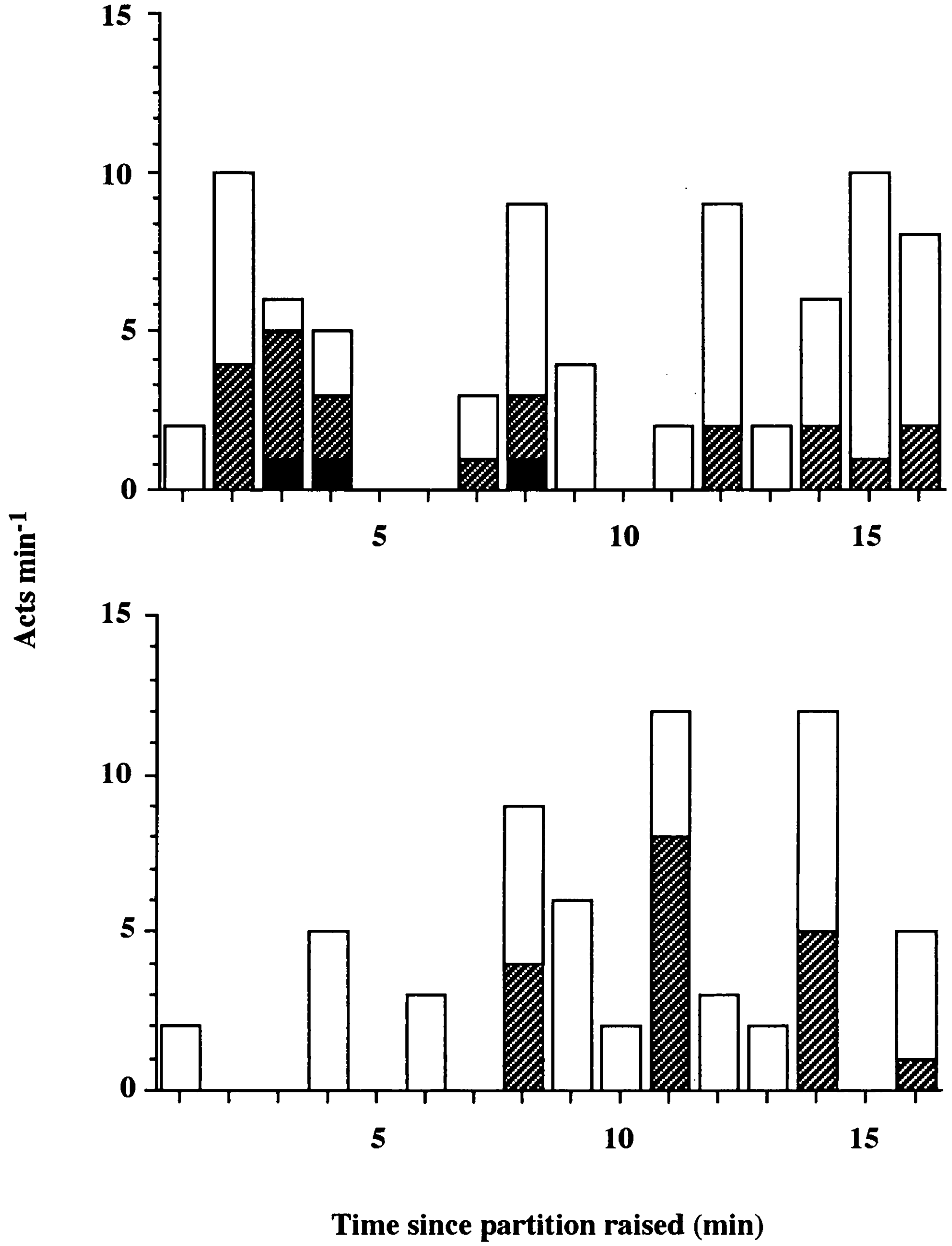


Figure 2.2 Plots of acts min^{-1} for successive minutes of two randomly selected fights. ▨ Display ■ Contact ☐ Other acts

2.2.6 Statistical Analyses

The effect of relative size on the initiation and outcome of interactions was investigated using χ^2 (Siegal & Castellan 1988). Possible differences between contests won by small crabs compared with contests won by large crabs were examined using Mann-Whitney tests (Siegal & Castellan (1988). Contest costs were evalauated by calculating Spearman rank order correlation coefficients (Siegal & Castellan 1988). The behaviour of winners and losers were compared using Wilcoxon matched-pairs signed-ranks tests (Siegal & Castellan 1988). In 56/65 cases, the interaction was not resolved after the first retreat of loser, and subsequent retreats were observed before the fight was finally resolved. The identity of the re-initiator was investigated using χ^2 (Siegal & Castellan 1988).

2.3 RESULTS

2.3.1 Effect of relative size on initiation and outcome

Relative size did not influence the tendency to display first (Figure 2.3a, $\chi^2 = 0.02$, $p > 0.8$). Comparisons of the relative size of opponents showed that the larger crab won on 48 out of 65 occasions (Figure 2.3b, $\chi^2 = 14.8$, $p < 0.001$). Both smaller and larger crabs were more successful when they initiated an interaction than when they responded (Figure 2.4, $\chi^2 = 4.77$, $p < 0.05$). Larger crabs won 87.5% of the interactions that they initiated, compared to 60.6% when they were the responder. Smaller crabs won 39.4% of interactions when they initiated compared to 12.5% when they were responding. So it appears that although the size of RHP discrepancy of the contestants does not influence the initiation of contests, it does influence the outcome of an interaction. Additionally, initiation itself is somehow associated with winning.

2.3.2 Contests won by small crabs

In 17 out of 65 interactions a smaller crab defeated a larger crab. In 13 of these interactions the smaller crab initiated the interaction. 12 of the interactions in which the smaller crab won were in the size ratio class 0.91-1.0; that is to say, they involved the most evenly-matched opponents. 2 were in the 0.81-0.90 size ratio class, and 3 were in the most disparately-matched class (0.71-0.80). Interactions in which the smaller crab won were not significantly different from interactions in which the larger crab won in terms of contest duration (Mann-Whitney, W = 1634.0, $p > 0.4$), length of latency period (Mann-Whitney, W = 1637.0, $p > 0.4$), initiation type (Mann-Whitney, W = 1629.0, $p > 0.5$), intensity (Mann-Whitney, W = 1585.0, $p > 0.9$) or proportion of display and contact (Mann-Whitney, W = 427.0, $p > 0.4$).

2.3.3 Evaluation of contest costs

It would be expected that interactions in which the contestants are more evenly matched would be of greater cost than interactions between less evenly matched crabs. Cost can be assessed in terms of either contest duration or intensity (potential for injury). To test this, Spearman rank order correlation coefficients were calculated between size ratio and interaction duration, interaction type (intensity) and proportion of display and contact, and also between interaction duration and interaction type.

Duration was not significantly correlated with intensity ($r_s = 0.172$, $z = 1.38$, $n = 65$, $p = 0.08$). Neither the proportion of display and contact in a contest, nor its intensity, were significantly correlated with size ratio ($r_s = 0.05$, $z = 0.4$, $n = 65$, $p >$

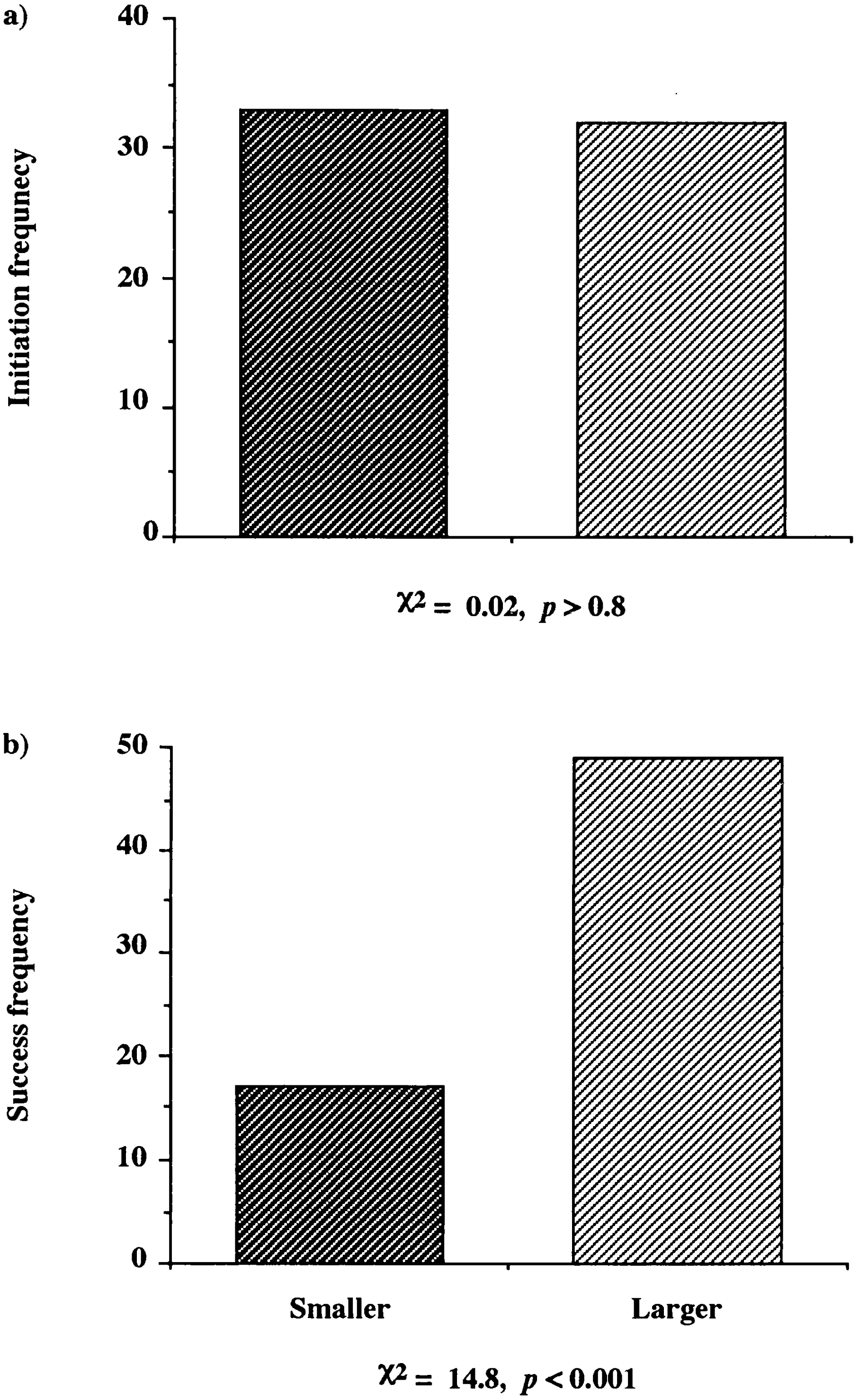
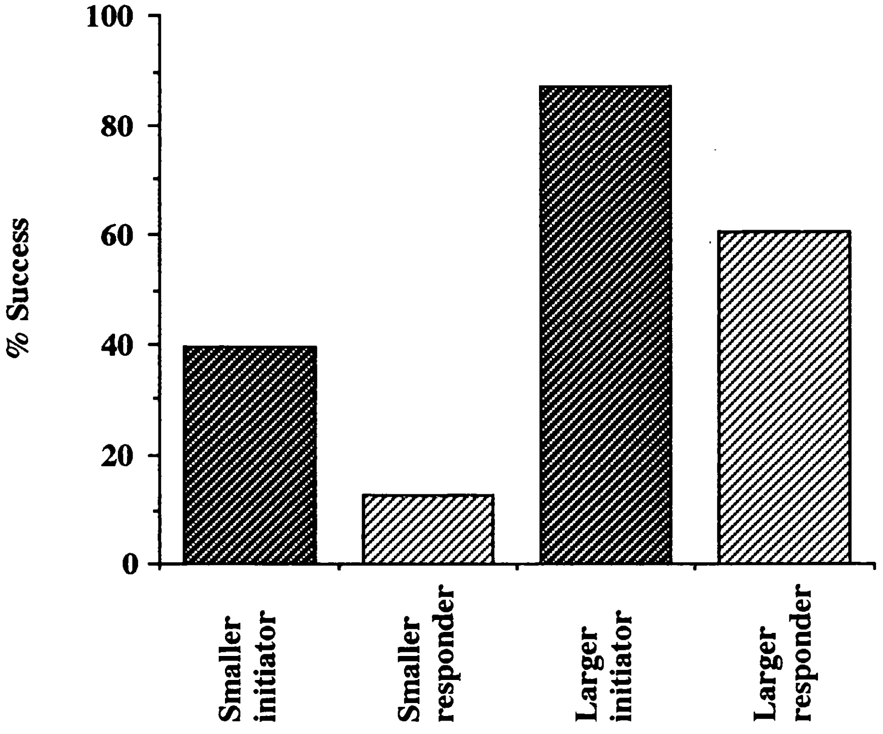


Figure 2.3 Effect of relative size on a) initiation and b) outcome of interactions between female *Necora puber*.



$\chi^2 = 4.77$, 1 df, $p < 0.05$

Figure 2.4 Effect of initiation on the outcome of fights between female *Necora puber*.

0.3, and $r_s = -0.015$, $z = 0.12$, $n = 65$, $p > 0.4$ respectively). Size ratio was significantly negatively correlated with duration ($r_s = -0.23$, $z = 1.84$, $n = 65$, $p = 0.033$) as was the proportion of display and contact in a contest ($r_s = -0.205$, $z = 1.38$, $n = 65$, $p = 0.05$).

2.3.4 Behaviour of winners and losers

The differences in behaviour of winners and losers are likely to be confounded by the differences between the contests in both duration and intensity. Therefore a Wilcoxon matched-pairs signed-rank test was used, with the 2 participants in a contest being taken to be the pair. The individual acts were pooled into categories as follows:- 'stationary', which included stand and crouch; 'move to'; 'move away'; 'display', which included the 3 types of stationary cheliped display and approach in display; 'contact', which included strike and grasp; and "retreat", which included retreat with and without display. In order to investigate at what stage during an interaction behavioural differences became apparent, each contest was divided into halves by time, and the behaviour of the crabs was compared for these 2 sections, along with the latency period and the time from the end of the interaction until data recording ceased.

Table 2.1 presents the results of the Wilcoxon tests. During the latency period, no acts were performed significantly more often by either the eventual winners or losers. In the first halves of the interactions, the eventual winners performed significantly more 'move to' and 'display', and the eventual losers performed significantly more 'move away' and 'retreat'. In the second halves of the interactions the eventual winners performed significantly more 'display' and 'contact', and the eventual losers significantly more 'move away' and 'retreat'. After resolution of the interactions, the winners performed significantly more 'move to' and 'display' and 'contact', and the losers performed significantly more 'retreat'. Thus significant differences in the behaviour of winners and losers are obvious in the first half of an interaction, and become more significant in the second half of an interaction.

Since there is some indication that losers may show more retreat in the first half of the fight it could be inferred that if the loser could have got away the interactions would have ended more quickly. In 9 out of 65 cases, the interaction was resolved after the first retreat of the loser. In the remaining 56 cases, the interaction was not resolved after the first retreat, and 2 or more retreats were observed prior to the resolution of the interaction, in other words rather like a series of bouts or skirmishes (Maynard Smith & Riechert 1984). To see if such fights might have ended at the first retreat the identity of the individual that re-initiated the encounter was noted. First of all, whether the winner or loser at the first retreat re-initiated was

Part of fight	Act	Test N	W	p	Direction
Latency	stationary	58	1025.5	0.189	-----
	move to	61	1121.0	0.209	-----
	move away	38	385.0	0.839	-----
	display	0	-----	-----	-----
	contact	1	0	1.0	-----
	retreat	6	3.0	0.142	-----
First half	stationary	44	613.0	0.170	-----
	move to	35	449.0	0.029	W
	move away	43	283.0	0.022	L
	display	45	826.0	0.001	W
	contact	7	15.5	0.866	-----
	retreat	26	42.0	0.001	L
Second half	stationary	45	496.0	0.813	-----
	move to	45	642.5	0.160	-----
	move away	46	135.5	<0.001	L
	display	31	404.5	0.002	W
	contact	15	99.0	0.029	W
	retreat	50	67.5	<0.001	L
After resolution	stationary	51	858.0	0.068	-----
	move to	49	1125.5	<0.001	W
	move away	55	566.0	0.088	-----
	display	44	990.0	<0.001	W
	contact	6	21.0	0.036	W
	retreat	38	0	<0.001	L

Table 2.1 Behavioural differences between winners and losers during interactions between female *Necora puber*. W= acts performed significantly more often by winners, and L= acts performed significantly more often by losers.

examined. If the loser of the first bout has, in effect, given up then skirmishes are unlikely to be re-initiated by the loser. From Figure 2.5a it can be seen that the winners at the first retreat re-initiated 40/56 of the subsequent skirmishes, significantly more ($X^2 = 10.3$, $p < 0.01$) than the losers at the first retreat. Considering eventual winners and losers, (Figure 2.5b) the eventual winner re-initiated 44/56 of the subsequent skirmishes, significantly more ($X^2 = 18.3$, $p < 0.01$) than the eventual losers.

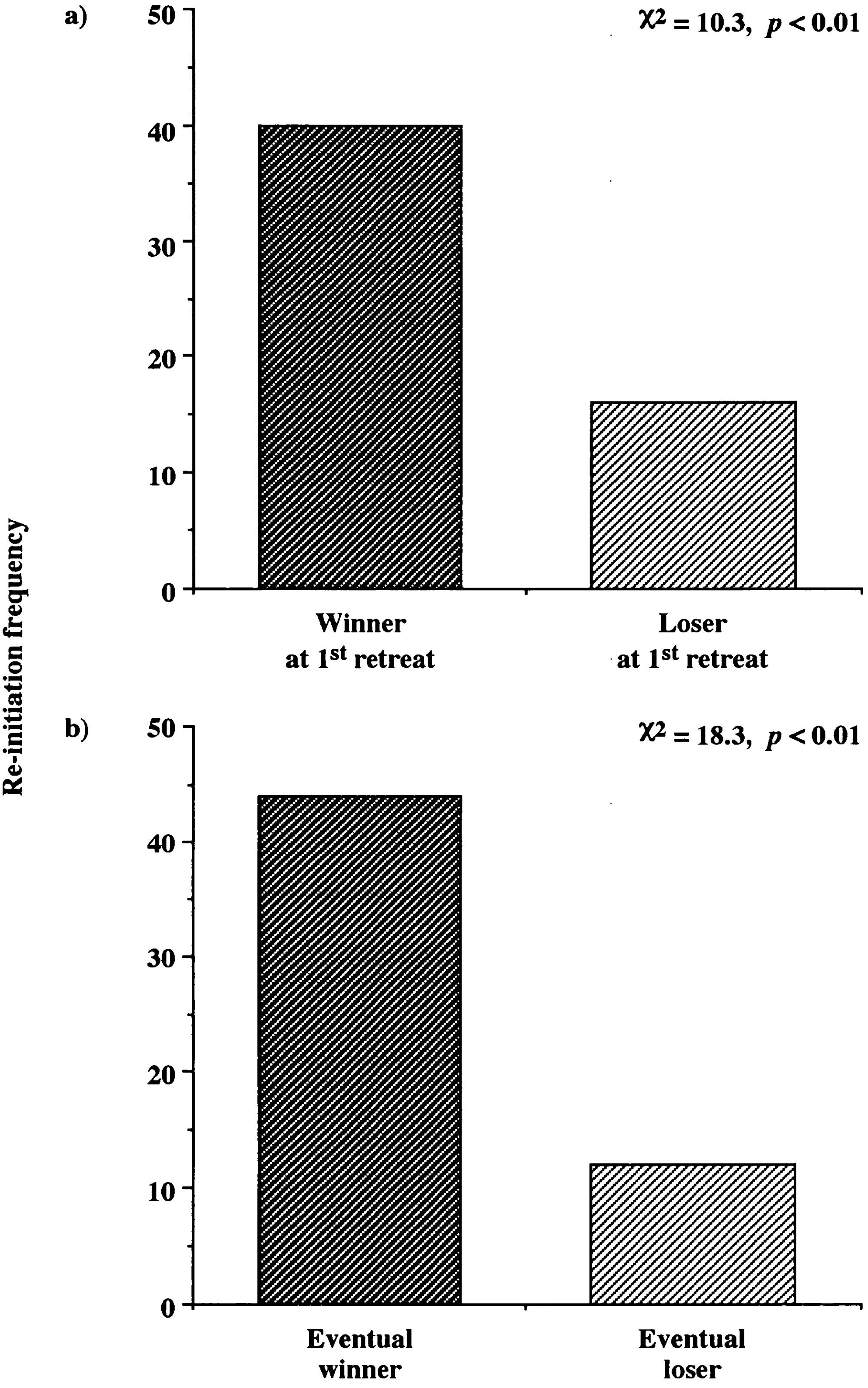


Figure 2.5 Initiation frequency of a) winners and losers at the first retreat, and b) the eventual winners and losers of the interaction, in 2nd skirmishes between female *Necora puber*.

2.4 DISCUSSION

Crustacea have been popular subjects for empirical tests of game theory because they have several attributes making them ideally suited for such studies (Dingle 1983). They possess RHP in the form of chelae, spines and claws, which makes them formidable opponents. Frequently a contested resource can be identified and its value can sometimes be quantified (Scully 1983). In addition, many species are readily observed in the field and can be easily maintained in the laboratory. Agonistic behaviour generally involves stereotyped actions, which involve an exchange of agonistic displays during which the body is raised off the ground and the walking legs are spread, increasing the apparent size of the animal, and which display potential weapons to an opponent (Schöne 1968; Dingle 1983). The intraspecific agonistic behaviour of female *N. puber* is similar to that of males of the same species (Smith *et al.* 1994) and to that of other portunids (Jachowski 1974; Glass & Huntingford 1988). In female *N. puber*, behavioural differences between winners and losers were obvious in the first halves of interactions. This is consistent 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), and agrees with other studies (Hazlett 1982; Bronstein 1985; Turner & Huntingford 1986).

Interactions in the present study occurred in the absence of any tangible resources. In other crustaceans, agonistic interactions occur in the context of competition for resources such as food or shelter, as well as resulting from chance encounters between individuals (Hazlett 1968, 1974; Warner 1970; Jachowski 1974; Rubenstein & Hazlett 1974; Abele *et al.* 1986; Glass & Huntingford 1988). In the observation tank, *N. puber* interacted agonistically when they found each other and this is representative of the situation in their natural environment (Smith 1990; pers. obs.). There may be several reasons why it is disadvantageous for crabs to be close to conspecifics. Aggregations may attract predators or facilitate the spread of disease or parasites, and conspecifics may compete for local resources and may represent a threat of cannibalism. American lobsters, *Homarus americanus*, become more aggressive prior to moulting, which suggests that exclusion of conspecifics from the vicinity by agonistic behaviour is a means of reducing the threat of cannibalism (Tamm & Cobb 1978). Evans & Shehadi-Moacdieh (1988) postulated that the individual space defended by female prawns, *Palaemon elegans*, functioned as a "food collector" in an environment where food items were small and randomly distributed in space and time.

As found for many crustaceans (hermit crabs, Hazlett 1978; fiddler crabs, Hyatt & Salmon 1978; snapping shrimps, Schein 1975; stomatopods, Caldwell &

Dingle 1979) larger individuals were more likely to defeat their opponents, and the success rate of smaller crabs increased as the contestants became more evenly matched. In this study, relative size did not influence the tendency to initiate an interaction, as a smaller crab was as likely to initiate as a larger one. The outcome of an interaction can therefore be predicted with some certainty if the relative size of the opponents is known, suggesting that the crabs themselves use this as a cue to decide. Size is likely to be related to strength and thus to reflect relative RHP. This is consistent with game theory predictions (Parker 1974; Maynard Smith & Parker 1976) and is found in many other species, *e.g.*, crayfish (*Procambarus alleni*, Bovbjerg 1956), snapping shrimps (Schein 1975), fiddler crabs (*Uca pugilator* & *U. pugnax*, Hyatt & Salmon 1978) and stomatopods (Caldwell & Dingle 1979). So it appears that, although the size of RHP discrepancy of the contestants does not influence the initiation of contests, it does influence the outcome of an interaction: this implies that assessment is occurring, but during rather than prior to the interaction.

The cheliped display may have several functions during an interaction. Raising the body off the ground and extending the chelipeds probably gives a visual cue of a crab's size to an opponent. When crabs of a similar size extend their chelipeds in close proximity to one another, they may have been able to use tactile cues to assess their relative sizes. Alternatively, when the crabs are facing up to each other they may actually be measuring up the relative reach of their chelipeds. When velvet swimming crabs engage in bilateral display and push each other, the exhalent channels and the attenules, which have a chemosensory function (Gleeson 1980), of the two crabs are very close. It is therefore likely that the crabs are able to detect the respiratory currents produced by their opponents. If the strength of these currents reflects the size and fitness of the contestants, then it is possible that these are used to assess RHP. In addition, it is probable that any olfactory stimulus carried in the respiratory current of one crab would be detected by its opponent. A rapid respiratory rate in response to intense activity would, however, inevitably result in a strong jet of water from the scaphognathites.

Why should crabs have initiated interactions that they had little chance of winning? In most cases the initiator was defined as the first crab to adopt a cheliped display, and this definition may not have always accurately reflected the relative aggressive motivation of the crabs, as cheliped displays could have been defensive as well as offensive. However, it seems likely that crabs cannot accurately assess the size of their opponent until it displays. Regardless of which crab initiated an interaction, in many cases the smaller continued offensively against an opponent it apparently had little chance of beating.

Crabs as small as 79% of the size of their opponent engaged in interactions involving strikes or grasps by both crabs. It is impossible to know if the smaller crab had accurately assessed the size of its opponent. Although closely matched crabs may have been unable to assess their size differential without recourse to pushing bouts or strikes, it seems likely that the more mis-matched crabs that engaged in escalated encounters could visually detect their size difference. If this is so, the decision about whether to continue or retreat was unaffected by the size of the opponent in some crabs. Possibly the smaller crab had detected some cue from the larger crab revealing its unwillingness to engage in an interaction. In this study, intermoult, intact females were used. In natural populations, however, injury, disease, gender and recent ecdysis may introduce substantial variation into the relationship between size and agonistic ability. Pavey & Fielder (1991) examined a combination of traits that may contribute to RHP in the freshwater crayfish, *Cherax cuspidatus*. In pairs where the smaller crayfish was dominant, measurements revealed that in each case the "smaller" animal was superior in at least one other potential RHP values (weight, chelae length or age). This suggests that a single RHP index has limited use for pairs when both members are superior in one or more morphological measurements. Such a situation is especially relevant to organisms with indeterminate growth, such as crustaceans. In their natural environment, therefore, small crabs may have greater success against larger opponents.

Analysis of asymmetric games (Maynard Smith & Parker 1976; Enquist & Leimar 1983) suggests that interactions should be of higher cost when the asymmetry is less pronounced. For male *N. puber*, interactions between closely matched crabs were of longer duration and involved more potentially injurious behaviour than those between disparate crabs (Smith *et al.* 1994), as was the case for male *Liocarcinus depurator* (Glass 1985). In contrast, the present study shows that for female *N. puber* the interactions of closely matched crabs were not more intense, and if anything were less intense, in terms of content or length. In neither male nor female *N. puber* are the interactions structured into a series of escalating phases. Intense acts could occur at any stage in an interaction and could be followed by a return to display only tactics. This is unusual since interactions usually follow a gradual pattern of escalation with resolution being preceded by the most intense acts (Clutton Brock & Albon 1979; Enquist *et al.* 1990). The assumptions of the sequential assessment game therefore do not hold true for interactions between *N. puber* of either sex. The same was true for interactions between *L. depurator* (Glass & Huntingford 1988). In their natural situation any fights probably take on one of two forms:- short exchanges to exclude a conspecific from the immediate vicinity; or, longer and more involved fights over valuable resources such as a carcass or a baited creel. Since population densities are low, and the inability of females to assess each other without recourse to fighting may be because they rarely fight in nature, and this may also explain why mismatched crabs fought for longer.

2.4 DISCUSSION

Crustacea have been popular subjects for empirical tests of game theory because they have several attributes making them ideally suited for such studies (Dingle 1983). They possess RHP in the form of chelae, spines and claws, which makes them formidable opponents. Frequently a contested resource can be identified and its value can sometimes be quantified (Scully 1983). In addition, many species are readily observed in the field and can be easily maintained in the laboratory. Agonistic behaviour generally involves stereotyped actions, which involve an exchange of agonistic displays during which the body is raised off the ground and the walking legs are spread, increasing the apparent size of the animal, and which display potential weapons to an opponent (Schöne 1968; Dingle 1983). The intraspecific agonistic behaviour of female *N. puber* is similar to that of males of the same species (Smith *et al.* 1994) and to that of other portunids (Jachowski 1974; Glass & Huntingford 1988). In female *N. puber*, behavioural differences between winners and losers were obvious in the first halves of interactions. This is consistent 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), and agrees with other studies (Hazlett 1982; Bronstein 1985; Turner & Huntingford 1986).

Interactions in the present study occurred in the absence of any tangible resources. In other crustaceans, agonistic interactions occur in the context of competition for resources such as food or shelter, as well as resulting from chance encounters between individuals (Hazlett 1968, 1974; Warner 1970; Jachowski 1974; Rubenstein & Hazlett 1974; Abele *et al.* 1986; Glass & Huntingford 1988). In the observation tank, *N. puber* interacted agonistically when they found each other and this is representative of the situation in their natural environment (Smith 1990; pers. obs.). There may be several reasons why it is disadvantageous for crabs to be close to conspecifics. Aggregations may attract predators or facilitate the spread of disease or parasites, and conspecifics may compete for local resources and may represent a threat of cannibalism. American lobsters, *Homarus americanus*, become more aggressive prior to moulting, which suggests that exclusion of conspecifics from the vicinity by agonistic behaviour is a means of reducing the threat of cannibalism (Tamm & Cobb 1978). Evans & Shehadi-Moacdieh (1988) postulated that the individual space defended by female prawns, *Palaemon elegans*, functioned as a "food collector" in an environment where food items were small and randomly distributed in space and time.

As found for many crustaceans (hermit crabs, Hazlett 1978; fiddler crabs, Hyatt & Salmon 1978; snapping shrimps, Schein 1975; stomatopods, Caldwell &

Dingle 1979) larger individuals were more likely to defeat their opponents, and the success rate of smaller crabs increased as the contestants became more evenly matched. In this study, relative size did not influence the tendency to initiate an interaction, as a smaller crab was as likely to initiate as a larger one. The outcome of an interaction can therefore be predicted with some certainty if the relative size of the opponents is known, suggesting that the crabs themselves use this as a cue to decide. Size is likely to be related to strength and thus to reflect relative RHP. This is consistent with game theory predictions (Parker 1974; Maynard Smith & Parker 1976) and is found in many other species, *e.g.*, crayfish (*Procambarus alleni*, Bovbjerg 1956), snapping shrimps (Schein 1975), fiddler crabs (*Uca pugilator* & *U. pugnax*, Hyatt & Salmon 1978) and stomatopods (Caldwell & Dingle 1979). So it appears that, although the size of RHP discrepancy of the contestants does not influence the initiation of contests, it does influence the outcome of an interaction: this implies that assessment is occurring, but during rather than prior to the interaction.

The cheliped display may have several functions during an interaction. Raising the body off the ground and extending the chelipeds probably gives a visual cue of a crab's size to an opponent. When crabs of a similar size extend their chelipeds in close proximity to one another, they may have been able to use tactile cues to assess their relative sizes. Alternatively, when the crabs are facing up to each other they may actually be measuring up the relative reach of their chelipeds. When velvet swimming crabs engage in bilateral display and push each other, the exhalent channels and the attenules, which have a chemosensory function (Gleeson 1980), of the two crabs are very close. It is therefore likely that the crabs are able to detect the respiratory currents produced by their opponents. If the strength of these currents reflects the size and fitness of the contestants, then it is possible that these are used to assess RHP. In addition, it is probable that any olfactory stimulus carried in the respiratory current of one crab would be detected by its opponent. A rapid respiratory rate in response to intense activity would, however, inevitably result in a strong jet of water from the scaphognathites.

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CHAPTER THREE

ESCALATION AND COMMUNICATION DURING AGONISTIC BEHAVIOUR IN *NECORA PUBER*

3.1 INTRODUCTION

3.1.1 Escalation in animal fights

Fighting is widespread in the animal kingdom (Huntingford & Turner 1987; Archer 1988), occurring most often over access to valuable but limited resources such as food or mates. The exact form of the behaviour is variable, but there are some features that are common to the great majority of animal fights, one of these being the occurrence of escalation.

Most species have a number of different aggressive actions in their repertoire and these are deployed in a characteristic sequence. Fights often begin when the opponents are at a distance from one another, exchanging movements or displays that are of relatively low intensity and of therefore low cost in terms of energy consumption and the likelihood of damage. If these low intensity actions do not cause one or other contestant to withdraw, then patterns of behaviour that are progressively more energetic and more risky come into play, culminating in the most damaging and intense acts in the animal's repertoire. These are the most effective in inducing retreat or submission in the opponent. This progressive increase in the intensity and cost of behaviour as a fight proceeds is referred to as escalation.

Escalation has been described in detail in many ethological studies of animal fights. One example is a laboratory study of the cichlid fish *Nannacara anomala* by Jakobsson *et al.* (1979), which fight over status in a social group. In the initial phases of the fight the fish usually engage in actions such as lateral display and tail beats that are performed at distance and involve low risk of injury. As the fight progresses, these actions are replaced by mouth wrestling and biting, which involve direct physical contact and are more intense. In this species escalated fighting carries a higher chance of injury, in the form of lost scales and damaged fins. Fights stop when one animal, the loser, gives up, thus leaving its opponent victorious. The behaviours shown by the winner and loser are similar and indicative of aggressive motivation until near the end of the fight; the eventual loser then reverts rapidly to low cost actions (de-escalation) and withdraws from the encounter. Jakobsson *et al.* (1979) found that early on in fights between *N. anomala* both the eventual winner and eventual loser tended to respond to a bite (high cost) by retaliating in kind. However, just before the end, one animal started to respond to a bite with tail-beating and frontal displays (low cost) and this was the eventual loser.

3.1.2 Explaining and predicting the occurrence of escalation - the use of game theory

Ethological studies may provide detailed descriptions of the process of escalation, but they do not explain why escalation occurs, either in terms of its evolution or in terms of the behavioural mechanisms responsible for it. Fighting takes up time and energy, but these costs are usually trivial compared with those of injury or death, including the risk of predation (Hammerstein & Riechert 1988). Whether it is better to settle a contest by signalling or fighting depends on the value of the resource, the costs of fighting, and the behaviour of opponents. Because the best thing to do depends on what a rival does, the consequences of fights lend themselves to the game theory approach.

The simplest game theory model of animal fighting is the hawk-dove game (Maynard Smith 1976, 1982a), where the animals adopt one of two strategies, hawks, which always launch in to fierce high cost fighting, and doves, which display, but retreat it attacked by an opponent. A population of doves can be invaded by hawks because doves alway lose to hawks without incurring injury. If the costs of fighting are less that the value of the contested resource, hawk is a pure ESS and animals should always fight fiercely. If injury costs more than resouce value, however, doves can invade a population of hawks. This means that the ESS is a mixture of both strategies: play hawk with a probability of p, and dove with a probability of $1-p$. This ESS could be reached in two ways; first, individuals could adopt a mixed strategy, fighting with a probability p; and, second, the population could become genetically polymorphic, with proportion p of animals playing hawk, and a proportion $1-p$ always playing dove. By showing that, because of the costs of injury, hawk is not always an ESS, the hawk-dove model explains the existence of low and high intensity forms of fighting. The hawk-dove model, however, was set up to investigate the fitness-related consequences of using displays rather than overtly aggressive actions and so does not depict the progressive increase in intensity described in ethological studies of animal fights (Caryl 1981).

There is great variability in whether and to what extent animal conflicts become escalated. In some cases, one animal may flee relatively quickly without engaging in intense, costly fighting: in other encounters involving the same species, and perhaps even the same individuals on a different occasion, fights may be long and fierce. Game theory models can help predict the circumstances in which fights are expected to escalate to high cost actions by specifying in functional terms when it will no longer be advantageous for an animal to continue fighting.

The hawk-dove game assumes that animals can only differ in strategy, but many games models have asked whether other differences between opponents can resolve contests without fighting. Three types of asymmetry have been considered:- opponents may differ in RHP; opponents may differ in the value they place on the contested resource (*e.g.*, food is worth more to a hungrier animal); and finally, opponents may also differ in ways that in principle do not cause differences in the net benefit of fighting (*i.e.*, uncorrelated asymmetries, *e.g.*, owner/intruder).

3.1.3 Assessment of fighting ability and resource value

The game theory models most relevant to predicting the occurrence of escalated fights are those which incorporate assessment of RHP and/or resource value (Parker 1974; Parker & Rubenstein 1981). In his assessment strategy model, Parker (1974) argued that any individual that has the ability to assess how its own RHP compares with that of its opponent will be at a selective advantage, since it can withdraw without damage when its own RHP is lower, but attack (with a good chance of winning) when its own RHP is higher. The concept of RHP enables broad predictions as to the circumstances when escalated fights will occur. These exist when opponents' RHPs are reasonably well matched; when they are not, the one with the lower RHP will withdraw after an initial assessment or "sizing-up" phase (Parker 1974; Maynard Smith & Parker 1976; Maynard Smith 1982a).

Empirical evidence generally supports the prediction that animals assess relative RHP and that longer, fiercer fights occur between opponents whose fighting abilities are similar (Glass & Huntingford 1988; Smith *et al.* 1994). Neverthless, as Parker (1974) recognised, a model based only on relative RHP is a naive one. Even though the concept of RHP has proved one of the most important insights to come from the application game theory to animal fights, it is not the whole story. Several other factors must be considered. All game theory models are based on calculations of the pay-offs to the participants, given that certain costs and benefits are involved. RHP involves one very important aspect of the costs of fighting, but the benefits to be obtained from winning a fight have to be considered.

Animal fights are competitive in the sense that the contestants are competing for a resource such as food, territory, nest sites or potential mates (Wilson 1975). It will often be the case that opponents differ both in RHP and in the value they place on the disputed resource. These considerations have been introduced into game theory models representing contests which are asymmetrical in terms of both RHP and resource value. The most satisfactory model to incorporate RHP and resource value was developed by Parker & Rubenstein (1981). Such interactions could either be mutually reinforcing, when the animal with the most to gain also has the highest RHP, or contradictory, when the one with the highest RHP has the least to gain. The first case is straightforward and will follow the rules of the assessor model (Parker 1974). Contradictory interactions were considered in terms of whether an opponent occupies a particular "assessment" role. If both animals have full information about the value of the resource (V), and about RHP and hence costs (K), the one with the lesser value of *V*/*K* should retreat first, and a convention based on this assessment rule was shown to be the only ESS.

The assumption that even inaccurate information about asymmetries is available from the start of an encounter is unlikely to be valid if differences in RHP or resource value are small, or if they depend on properties that cannot be assessed by distance cues. In such cases, after an exchange of displays performed at a distance, animals may engage in trials of strength, usually some form of pushing or pulling contest, before escalating to more damaging forms of fighting. Whenever fighting is costly, exchange of information about pay-off asymmetries is favoured. Contests should escalate gradually in a way that increases the probability that any asymmetry is detected before injury is risked (Parker & Rubenstein 1981; Enquist & Leimar 1983, 1987). More recent game theory models not only assume initially imperfect information but also include the effects of gradually accumulating information during an encounter.

The sequential assessment model of Enquist & Leimar (1983, 1987), and Enquist *et al.* (1990) explains the occurrence of escalation, in functional terms, as the result of a process of gradual acquisition of information about RHP. In this model, two opponents with different RHPs come into conflict. At the start of the encounter, the opponents have little or no information about their relative fighting abilities, but during successive aggressive exchanges they accumulate this information. The animals have available to them a number of different behaviour patterns and at any point in the fight the two contestants tend to perform the same kinds of action. Low intensity actions give only inaccurate information about RHP, but are cheap to perform. On the other hand, high intensity actions provide reliable information about RHP, but are costly both in terms of energy and risk of injury. The purpose of a fight is to acquire sufficiently accurate information about RHP at the lowest possible cost.

What happens during a fight is similar to a process of statistical sampling, in that there is a random error in an animal's assessment of its relative RHP. This error is reduced as more exchanges occur and assessments are based on a larger sample size. At any point in a fight an animal bases its decisions about whether to continue a fight, or to withdraw, on its current assessment of RHP and on the accuracy of that estimate. The choice of what action to use if it does continue is based on how much information has been acquired up to this point: if the animal is still uncertain in spite of extensive low level display, it will switch to actions that are more effective at gaining information, even though these are more costly.

The model makes several predictions about how animals should behave. For example, the number of different behavioural elements used during a fight should increase as the difference in RHP decreases. Another, more complex prediction is that when animals enter the final round of the fight, regardless of the level of cost to which the fight has escalated, the length of the time spent fighting up to the switch to the final phase should be independent of relative RHP. This is because contestants switch to an escalated level only when they have failed to acquire an accurate estimate of relative RHP, and so this cannot influence their behaviour. By the rules of the game - only escalate if you do not have an accurate assessment of relative RHP - this is the case for all rounds except the final one in which the fight is resolved. In contrast, the model predicts that the length of this final round will be negatively related to the difference in RHP, because if the animals are nearly equally matched they need to exchange many actions of the highest, most informative, level before their estimates are sufficiently accurate to allow one to give up. Support for this hypothesis was provided by a study of cichlids by Enquist *et al.* (1990).

3.1.4 Behavioural mechanisms underlying escalation

All the game theory models discussed above are functional in focus and do not address the mechanisms by which information about asymmetries is converted into behaviour. To explain in causal terms what is now known about the strategic importance of RHP and resource value in fights, explanations must be extended to provide a mechanism whereby these factors can influence aggression and fear.

Maynard Smith & Riechert (1984) developed a model of fights over feeding territories in spiders (*Agelenopsis aperta*) that does exactly this. The spiders use several different aggressive behaviour patterns during fights and go through a process of escalation from locating movements performed at a distance, through threats performed closer together to intense contact fighting. Previous studies within the functional framework of game theory (Riechert 1979, 1982, 1984) had shown that, all other things being equal, the owner of a territory will win a fight, but if there is a size discrepancy of more than 10% a larger intruder can take over the territory. The more closely matched the opponents are the more fiercely they fight, and the more valuable the territory, the more intensely the owner will defend it. These findings were used in constructing the model.

The model has two main features:- firstly, what a spider does during a fight depends on the level of two independent, varying, motivational systems attack (A) and fear (F). Briefly, if the strength of the fear tendency is greater than that of attack tendency, *i.e.*, $F > A$, then the spider retreats. If $A > F$, the spider engages in a fight, the actual behaviour chosen depending on the absolute strength of A. The owner starts with a higher A than the intruder, the size of this advantage depending on the value of the territory. The second major feature of the model is that it views fights as consisting of a series of behavioural exchanges in each of which one animal is the performer and the other is the observer, whose *A* and *F* levels are altered by seeing a rival perform an agonistic action. After one spider has performed a given action, the two animals switch roles and the next performer choses its next action on the basis of its new, reset values of *A* and *F*. If *A* increases sufficiently the fight will escalate; if the new value of *A* is less than the new value of *F*, the spider withdraws and the fight ends. The model was used to simulate many hypothetical fights, whose nature and outcome were found to be very similar to those of real fights. This suggests that the critical features of the mechanisms that determine how fighting spiders behave are correctly envisaged in the model.

3.1.5 Communication during aggressive encounters

The purpose of aggressive displays is to obtain something, *i.e.*, it is goal-directed (Wilson 1975). However, it is not always clear why displays are effective in achieving these goals. An approaching rival may reveal its hostile intentions by preparing its weapons, or reveal its fear by hesitating, and these cues can be used by the receiver to select an appropriate response. In recent years there has been a great deal of controversy about how behaviour can effect the resolution of contests. The development and testing of hypotheses based on the concept of the evolutionarily stable strategy (ESS) has helped considerably the understanding of how asymmetries between opponents in non-behavoural variables such as size, fighting ability, prior ownership or resource value can settle contests (Maynard Smith 1982a). However, the simplicity of the models may have slowed progress in elucidating how behaviour patterns reveal asymmetries in many contests (Enquist & Leimar 1983; Enquist *et al.* 1990). The models have had a tendency to generate very restrictive predictions about the kinds of behaviour patterns that are used. This has generated controversy by contradicting the results of empirical research and has inhibited research on the function of many forms of behaviour (Enquist *et al.* 1985).

Prior to the application of adaptationist principles, it was believed that displays revealed information on what the signalling animal was likely to do next. The classical ethological view of threat displays was that they accurately reflected the animal's underlying motivational status, and therefore its likelihood of particular actions in the future (Lorenz 1966; Cullen 1966; Smith 1977). Both early game theory (Maynard Smith 1974, 1979, 1982a,b; Maynard Smith & Parker 1976; Caryl 1979, 1981) and gene selection (Dawkins & Krebs 1978; Krebs & Dawkins 1984) approaches suggested that signals will rarely provide information about intentions. In contrast, Zahavi (1975, 1977, 1987) concluded that animal signals must be honest. Zahavi's argument rests on the assumption that signals such as loud calls or displays that consume energy are costly to produce and so the signaller can demonstrate its quality by its ability to survive despite the handicap of the signal (formally modelled by Grafen 1990a,b). High quality animals suffer lower cost for a given level of signal than lower quality animals so at the ESS, the signal level adopted will correlate with underlying quality. Hinde (1981) argued that the differences between game theory and classical ethological approaches to agonistic displays had been over-emphasised. It may be naive to expect a signal to predict accurately a single behaviour (Hinde 1981). Signals may occur in sequences that require more complex analysis. Signals may offer inaccurate information ("I will attack or stay put, but not retreat"), conditional information ("I will retaliate if attacked") or attempts to gain information ("What will you do if I escalate briefly?"). The relationship between a signal and the signaller's subsequent behaviour could thus be very complex. Hinde (1981) viewed the lack of predictability in displays as a consequence of the signaller being uncertain about its next action because this depends partly on the response of the receiver. In this way, Hinde (1981) emphasised the changing and interactive nature of agonistic sequences.

Early game theory models indicated that settling contests through variation in behaviour can be evolutionarily stable only if the variation is constrained by non-behavioural variables, so that no invading mutant can win by producing behaviour that does not correspond to these variables (*i.e.*, dishonesty). This constrained behaviour has been called communication through the performance of behaviour (Enquist 1985) or assessment signalling (Maynard Smith & Harper 1988) and it appears to resolve contests (*e.g.*, Riechert 1978; Clutton-Brock & Albon 1979; Arak 1983; Robertson 1986). In a classic example, croak pitch, which can settle contests between toads (*Bufo bufo*) reflects body size because only a large resonating chamber can produce low-pitched croaks (Davies & Halliday 1978).

In contrast, many empirical studies have indicated that contests can be settled by more labile behaviour such as repertoires of calls or postures, which can be fully performed by any individual (*e.g.*, Stokes 1962; Dunham 1966; Dingle 1969; Andersson 1976; Enquist *et al.* 1985; Turner & Huntingford 1986), suggesting that some honesty is caused by selection, not constraints. This has been called communication through choice of behaviour (Enquist 1985) or conventional signalling (Maynard Smith & Harper 1988; Dawkins & Guilford 1991). This kind of behaviour can respond to changeable aspects of a contests, such as an animal's assessment of resource value or its relative RHP. Early models predicted that conventional signalling could not be an ESS because dishonest mutants could easily arise and invade honest populations (Maynard Smith 1974; Maynard Smith 1976; Dawkins & Krebs 1978; Krebs & Dawkins 1984). More recent models indicate that conventional signalling can be an ESS if effectiveness at winning covaries with a sufficiently large cost for some individuals (Enquist 1985; Enquist *et al.* 1985; Maynard Smith & Harper 1988; Grafen 1990a; Maynard Smith 1991).

Recently, Dawkins & Guilford (1991) have argued that dishonest signals are likely to be a widespread component of signalling systems concerned with quality advertisement. Dawkins & Guilford (1991) stated that previous discussions neglected a vital evolutionary consideration, namely the cost to the receiver of eliciting and evaluating honest signals. If both signaller and receiver pay costs, it will be to their mutual advantage to reduce them wherever the value of the extra information contained in a costly (rather than less costly) signal is outweighed by the costs of giving and receiving the costly signal. Dawkins & Guilford (1991) argued that honest assessment will be replaced by conventional signalling. The conventional signals are open to dishonesty, but it will be kept at low frequencies by the frequency-dependent benefits of probing, so dishonest signalling remains stable. Johnstone & Grafen (1993) formally presented this idea in a modification of Maynard Smith's (1991) 'Philip Sidney' game.

3.1.6 Information availability

Whether RHP is communicated to the opponent once a contest is underway is not clear from game theory models. Both the assessment model (Parker 1974) and the WOA model (Maynard Smith 1974) assume that information is available before the animal enters the aggressive encounter. Subsequent models, such as that of Parker & Rubenstein (1981) and the sequential assessment model of Enquist & Leimar (1983), have emphasised the gradual acquisition of information during an interaction. If information becomes available only as a consequence of interacting with the opponent, it would seem unrealistic, in motivational terms, to regard an animal as being able to decide in advance the detailed course of its aggressive behaviour.

The modified WOA model predicts that the maximum cost each animal is prepared to pay is set in advance, but that it will not be communicated to the opponent. The cost an animal is prepared to pay in a particular contest depends on the value of the resource, and in those cases where one or both animals do not have perfect information about resource value at the start of a contest, the cost they are prepared to pay may change as further information becomes available. In other cases, resource value itself may vary as the fight progresses, again altering the maximum cost.

These considerations indicate that the WOA model will apply only to a restricted range of conditions. However, if it is assumed that the conditions of the model are met (*i.e.*, perfect symmetry), its prediction does have implications for a motivational analysis. One possibility is that motivational balance (level of *A*/*F*) at which the animal will yield is set in advance, yet exerts little or no influence on the exact nature of the fighting sequence until just prior to giving up. This corresponds to Maynard Smith (1972) in his first article on the game theory approach. Maynard Smith's initial argument was as follows: during an aggressive encounter, each animal is under competing motivation to continue attacking or retreat, the precise strength of each depending on a combination of internal and external causal factors. However, it will not pay either contestant to reveal the strength of this motivation, since it could be used to predict the giving up point.

3.1.7 Resolution of fights

Fights often end abruptly, in contrast to their slow build up. Until the final stages of the fight, the behaviour of the eventual winner and loser is very similar. In functional terms, this is as predicted by the war of attrition model (Maynard Smith 1974). Natural selection would favour a sharp switch at a threshold level of motivation. As Maynard Smith (1982b) pointed out, retreat is the one intention that it is advantageous to communicate, thereby avoiding the possibility of further attack. As long as the motivational balance still favours attack, it should occur at full intensity, but once the threshold level is reached, there should be a sudden change to escape behaviour. Therefore the resolution of a fight occurs as a consequence of rapid de-escalation by one of the opponents, namely the loser.

The WOA model predicts that the cost an animal is prepared to pay is set in advance, but it is not communicated to the opponent through signals indicating motivation or intentions. There would be selection for a sharp switch in motivation from attack to flee at the point where one animal has reached the highest point to which it is prepared to go. This implies that as long as the motivational balance between attack and flee favours attack, this will be maintained at full intensity. However, once escape becomes the dominant motivation there will be a sudden shift from attack to escape. Therefore the resolution of a fight occurs as a consequence of rapid de-escalation by one of the protagonists.

3.1.8 Describing & analyising sequences of behaviour

Some insights into these kinds of processes (escalation and communication) can be gained by sequence analysis, a method commonly used in the 1960s but now out of fashion and rarely used in game theory studies. Sequence analysis is an objective way of examining empirically associations between acts, but one must be aware of the limitations of the method. The mechanisms underlying the sequence of acts shown by an animal are likely to be complex, and to analyse them using methods appropriate to simpler situations will involve some rather sweeping assumptions. The less valid the assumptions, the less realistic will be the results (Slater 1973). Methods used for analysing sequences of behaviour vary according to the complexity of the data and how structured the behaviour appears to be at first sight. Basically, the methods can be split into two groups depending on whether or not they involve comparison with a random model (Slater 1973).

1) Analysis of transition frequencies & conditional probabilities The simplest type of sequencing events is a deterministic sequence: in this, the events always follow each other in a fixed order, so that the nature of the preceding act defines precisely the nature of the act that will follow. Such sequences are rarely studied in normal behaviour, partly because two acts alway occurring in a particular order tend to be regarded as a single behaviour pattern.

Most behavioural sequences are probabilistic rather than deterministic in form, meaning that while the probability of a given act depends on those preceding it, it is not possible to predict at a particular point exactly which behaviour will follow. Some such sequences are less precisely ordered than deterministic ones, and in these cases the probability of a particular event is so markedly altered by the nature of the event before it that a flow diagram (kinematic graph), indicating the frequencies with which different transitions occur, provides a good impression of the organisation of the behaviour. Where sequences are not so highly ordered, some transitions may be observed between almost every behaviour and every other, so that simple flow diagrams become complex and hard to interpret. A way around this is to include only those transitions which have a high probability of occurrence, a method which was used by Fabricius & Jannsen (1963). For each type of act, an impression is thus given of those other behaviours most likely to follow.

2) Comparison with a random model The short-comings of the descriptions based on frequencies and conditional probabilities led to the adoption of Markov chain analysis. A sequence of behaviour can be described as a first-order Markov chain if the probabilities of different acts depend only on the immediately preceding act and not on any earlier ones (Cane 1961), *i.e.*, in the sequence $A \rightarrow B \rightarrow C$, the probability that C will follow B is not in any way altered by the nature of A or events prior to A. Models in which more than one of the preceding acts affects the probability of a given event are called r^{th}-order Markov chains, where the probability of a given event is significantly affected by the r preceding events.

Most sequence analysis of behaviour is concerned with establishing the existence of, and identifying, first-order dependencies. Here, the matrix of observed transition freqencies is compared with that which would be expected if all acts were independent of one another. For these first-order transitions, the expected values are calculated as for an r x c contingency table (assuming that it is possible for any of the behaviours considered to follow any other), and comparison between the matrices can then be made either using χ^2 or information theory (Lemon & Chatfield 1970). If the difference is found to be significant, the hypothesis that behaviour consists of a sequence of independent acts can be rejected, but further analysis is required before any conclusions about the behaviour can be drawn, and here two different approaches are possible.

First, the sequence can be analysed more closely as a whole to decide whether the first-order model is adequate or whether a second-order model is more precise (see Chatfield & Lemon 1970 & Lemon & Chatfield 1971). A second approach to finer analysis is to attempt to find those first-order transitions which are significantly more common than their expected values. It is necessary to examine the discrepancy between the observed and expected values in the individual cells of the transition matrix. Because many of the cells often contain low figures and because the observed value in each cell depends on those in the other cells, this is difficult both to carry out and interpret. The commonest method is partitioning, the condensation of the whole matrix into a 2 x 2 table about the cell of interest, followed by a χ^2 test to detect whether that particular transition is more frequent than expected (Maxwell 1961; Stokes 1962; Blurton Jones 1968).

Possible problems include:-

i) Complete *vs* incomplete matrices: The comparison with a random model method is easy if the matrix considered is complete, *i.e.*, all cells have a value. A complete matrix includes transitions which are repetitions of the same behaviour. This is readily understandable for inter-animal transitions, where one animal can respond to an act by another animal with any act in its repertoire, *e.g.*, by responding to movement toward with movement toward as well. In intra-animal transitions, however, this is not the case, since it is difficult to decide when one act ends and the same act begins if the acts are carried out sequentially. For each behaviour the observer must develop a criterion with which it can be decided when one act ends and the next begins. This is relatively easy for some behaviours which are discrete, *e.g.*, striking, or a consistent time interval may separate succceeding events so that they can be simply distinguished. Other behaviours pose more difficult problem; when, for instance it is difficult to distinguish the end of one act of locomotion end from the beginning of the next.

ii) Stationarity: A stationary sequence is one in which the probabilistic structure does not change with time (Cox & Lewis 1966; Delius 1969). The non-stationarity of act probabilities from one time to another during a communicative interaction has presented a major problem in assessing the transmission of information during contests. Non-stationarity can lead to complex transition matrices that are very hard to interpret.

3.1.9 Communication in Crustacea

Most detailed, quantitative analyses of animal communication have been done with invertebrates. Crustaceans have proven especially amenable to study, and species that have been studied in this way include:- hermit crabs (Hazlett & Bossert 1965), mantis shrimps (Dingle 1969), crayfish (Rubenstein & Hazlett 1974), spider crabs (Hazlett & Estabrook 1974), snapping shrimps (Schein 1975), stone crabs (Sinclair 1977) and fiddler crabs (Hyatt & Salmon 1979). Aggressive behaviour is the most common modality chosen for study, with courtship next (Salmon & Astaides 1968; Crane 1975; Warner 1977).

In many quantitative studies of crustacean agonistic behaviour, data recorded over the duration of the entire contest was pooled and analysed as one unit. Several authors, however, have demonstrated non-stationarity in the data during contests when they were divided into several segments on an arbitrary time scale (Dingle 1969, 1972; Lemon & Chatfield 1971), or on the basis of serial act number (Hazlett & Estabrook 1974; Rubenstein & Hazlett 1974). Salmon & Hyatt (1983) analysed fiddler crab contests following division of contests on the basis of a biologically meaningful event, namely the submission of one of the contestants. Barki *et al.* (1991) demonstrated that hour-long symmetrical fights between male *Macrobrachium rosenbergii* were complex, dynamically changing processes, which may be divided into several phases. Contests may include a phase of escalated fighting, and two additional phases, one before and one following it (Barki *et al.* 1991).

With the Crustacea, as with all other animals, there is a need to understand the functional dynamics of their aggressive behaviour at the signal level, sender level and receiver level. Fortunately the Crustacea have enjoyed a good deal of attention from behavioural biologists. Most studies have focused on descriptive analyses of agonistic ethograms and probability of inter-indidvidual fight sequences. Some have used information theory (Hazlett & Bossert 1965; Hazlett & Estabrook 1974; Dingle 1969, 1972), character analysis, an extension of information theoretic analysis which can quantify the inherent variability in both the static (size, sex, colour, *etc*) and behavioural factors of an interaction (movement, signals, *etc*), (Rubenstein & Hazlett 1974), and elementary game theory (Hyatt *et al.* 1979) to explore various aspects of combat. Practically all have examined conflict behaviour at the signal level, with only correlative speculation into the "causes", "meanings" and "functions" of the behaviour in signaller and receiver. Some experiments with models (*e.g.*, Hazlett 1966, 1975b;

Rubenstein & Hazlett 1974; Dunham 1978a) have uncovered the relevant stimuli acted on by receivers.

3.1.10 Aims

The aim of the work presented in this chapter was to use sequence analysis to examine and quantify the processes of escalation and communication during fights between *Necora puber* by: (i) comparing the processes in winners and losers who, by definition, are making different assessments during the course of a fight; (ii) by comparing the processes in males to those in females where the processes are less intense.

3.2 MATERIALS & METHODS

3.2.1 Experimental design

No separate experiments were carried out for the work presented in this chapter. Instead the fight data generated during the course of the work on females (chapter 2), the work on the metabolic consequences of agonistic behaviour in males (chapter 4), and the work on the behavioural constraints of agonistic behaviour in males (chapter 5, first fights only) were used. The maintenance of the crabs prior to the experimental work, and descriptions of how the fights were conducted are contained within the Materials & Methods of the appropriate chapters. Fights between females were between mixed size ratio dyads, *i.e.*, both evenly and mis-matched crabs were fought, and the crabs were used on more than one occasion (chapter 2). Fights between males occurred between crabs matched in size (carapace width, to the nearest mm) and the crabs were allowed to fight only once (chapters 4 & 5). Due to the destructive nature of the tissue sampling regime used in chapter 4, some of these males were not kept after resolution of the fights. Thus there was no way of telling if any of these crabs were in pro-ecdysis (Stevenson 1985). At the time of year the experiment was conducted (Autumn) males were unlikely to be moulting and so it should not have influenced the aggressive motivation of the crabs concerned.

The agonistic repertoire of both males and female *N. puber* is described in section 2.2.3, and the initiation and resolution points of the fights are defined in section 2.2.4. Only the exchanges occurring between the initiation of a fight and its subsequent resolution were included in the analyses. Exchanges during the latency period and after the fight had been resolved were not used in order to reduce any effects of non-stationarity in the data.

3.2.2 Statistical analyses

The frequencies of the different acts used by one crab in response to a given act by the other crab (responding acts) were counted and cast into inter-individual transition matrices. This was carried out for winners responding to losers and for losers responding to winners (loser-winner and winner-loser transition matrices respectively), for both fights between males and fights between females. The frequencies of the different acts used by a crab following its own previous act (following acts) were also counted and cast into intra-individual transition matrices. This was carried out for winners and losers, again for both males and females. The frequencies in the transition matrices were converted into percentages, and those acts whose occurrence was greater than 10% or 5% were used to plot fight sequence diagrams (kinematic graphs) for inter-individual and intra-individual transition matrices respectively.

Due to the low frequencies of some of the cells in the transition matrices, the frequencies of responding/following acts of the contestants were further condensed into 3 categories - de-escalatory, matching and escalatory acts - as defined in table 3.1. χ^2 tests (Siegal & Castellan 1988) were performed to investigate the possibility that a particular category of acts might have been used frequently in response to/following a given act. Opposing condensed inter-individual and intra-individual transition matrices, *i.e.*, loser-winner and winner-loser, and winner-winner and loser-loser, were compared directly using χ^2 tests and partitioning (Siegal & Castellan 1988) to investigate whether any category of acts was used more frequently by one crab compared to another.

	Responding/Following Acts		
Initial Act	**De-escalatory**	**Matching**	**Escalatory**
Crouch	Move Away Retreat	Crouch Stand	Move To App. in Disp. CP Disp. P. Down CP Disp. P. Up & Beat Contact
Stand	Crouch Move Away Retreat	Stand	Move To App. in Disp. CP Disp. P. Down CP Disp. P. Up & Beat Contact
Move To	Crouch Stand Move Away Retreat	Move To	App. in Disp. CP Disp. P. Down CP Disp. P. Up & Beat Contact
Move Away	Crouch Retreat	Stand Move Away	Move To App. in Disp. CP Disp. P. Down CP Disp. P. Up & Beat Contact
App. in Disp.	Crouch Stand Move Away Retreat	Move To App. in Disp.	CP Disp. P. Down CP Disp. P. Up & Beat Contact
CP Disp. P. Down	Crouch Stand Move Away Retreat	CP Disp. P. Down	Move To App. in Disp. CP Disp. P. Up & Beat Contact
CP Disp. P. Up & Beat	Crouch Stand Move Away Retreat	CP Disp. P. Down CP Disp. P. Up & Beat	Move To App. in Disp. Contact
Contact	Crouch Stand Move Away Retreat	CP Disp. P. Down CP Disp. P. Up & Beat Contact	Move To App. in Disp.
Retreat	Crouch Stand Move Away Retreat		Move To App. in Disp. CP Disp. P. Down CP Disp. P. Up & Beat Contact

Table 3.1 Classification of responding/following acts into categories of de-escalatory, matching & escalatory acts for each preceding act for *Necora puber*. Key: App. in Disp. = Approach in Display; CP Disp. P. Down = Cheliped Display, Swimming Paddles Down; CP Disp. P. Up & Beat = Cheliped Display, Swimming Paddles Up & Beat; Contact = Strike & Grasp; Retreat = Retreat in Display & Retreat. For descriptions of individual acts see section 2.2.3.

3.3 RESULTS

3.3.1 Males

3.3.1.1 Inter-individual transitions: winners

The frequencies of the different acts used by the winner in response to a given act by the loser in 101 fights between male *Necora puber* are shown in Table 3.2, and the percentages of the different responding acts used by winners derived from these frequencies are shown in Table 3.3. The fight sequence diagram based on these percentages is presented in Figure 3.1. Only acts with percentages greater than 10% were plotted on Figure 3.1. The results of the χ^2 tests on the condensed inter-individual (loser-winner) transition matrix are shown in table 3.4. See Table 3.1 for definitions of escalatory, matching and de-escalatory acts.

From Figure 3.1 and Table 3.3, it can be seen that winners used considerably more escalatory acts than either matching or de-escalatory acts in response to acts by losers, as indicated by the greater number and thickness of red arrows compared with green and blue arrows. Going through act by act, winners responded to 'crouch' by losers by adopting escalatory acts ('move to', 'approach in display' & 'cheliped display, swimming paddles down') and the matching act, 'stand', in 57.2% and 35.7% of cases, respectively (Table 3.3). Escalatory acts ('move to', 'approach in display', 'cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat', & 'contact') accounted for 70.8% of the response by winners to 'stand' by losers. 'Stand' was the only matching act seen, and the de-escalatory acts comprised 'move away' and 'retreat'. Winners responded to 'move to' by losers by adopting escalatory acts ('approach in display', 'cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat' & 'contact') in 56.7% of cases, matching acts ('move to' only), and de-escalatory acts ('stand', 'move away' & 'retreat') in 29.6% and 13.7% of cases respectively. Winners responded to 'move away' by losers by adopting escalatory acts ('move to', 'approach in display', 'cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat' & 'contact'), in 78.5% of cases, and matching acts ('stand' & 'move away') in 21.5%. No de-escalatory acts were adopted by winners in response to 'move away' by losers. Escalatory acts ('cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat' & 'contact') accounted for 42.9% of the response by winners to 'approach in display' by losers. De-escalatory acts ('crouch', 'stand', 'move away' & 'retreat') and matching acts ('move to' & 'approach in display') accounted for 33.7% and 23.4% of the response respectively. Winners resonded to 'cheliped display, swimming paddles down' by losers by adopting escalatory acts ('move to', 'approach in display', 'cheliped display, swimming paddles up & beat' & 'contact'), matching acts (cheliped display, swimming paddles down'), and de-escalatory acts ('crouch', 'stand', 'move away' & 'retreat') in 51.0%, 33.5% and 15.5% of cases respectively. Matching acts ('cheliped display, swimming paddles down' & 'cheliped display, swimming paddles up & beat') accounted 61.4% of the response of winners to 'cheliped display, swimming paddles up & beat' by losers. Escalatory acts ('approach in display' & 'contact') and de-escalatory acts ('stand', 'move away' & 'retreat') constituted 34.3% and 4.3% of the response respectively. Matching acts ('cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat' & 'contact') constituted 86.2% of the response of winners to 'contact' by losers. Escalatory acts ('move to' & 'approach in display') comprised only 8.9% of the response. The remaining 4.9% of the response were accounted for by the de-escalatory act 'retreat'. The majority of the response of winners to 'retreat' by the losers (91.3%) was composed of escalatory acts ('move to', 'approach in display', 'cheliped display, swimming paddles down', 'cheliped display, swimming paddles up & beat', & 'contact'). The remaining response (8.7%) comprised de-escalatory acts ('crouch', 'stand', 'move away' & 'retreat').

From Table 3.4, it can be seen that winners did not show any significantly non-random response to 'crouch' by the losers, though winners used matching acts significantly more often than other categories of response to 'cheliped display, swimming paddles up & beat' and 'contact' by the losers. Winners adopted escalatory acts significantly more frequently in response to 'stand', 'move away', 'cheliped display, swimming paddles down', 'retreat', 'move to' and 'approach in display' by the losers.

Response by Winner → Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		5	3	1	2	3				14
Stand		68	68	35	104	93	14	10	16	408
Move To		5	6	6	10	11	2	2	2	44
Move Away		17	6	4	31	23	16	1		98
App. in Disp.	2	15	1	11	40	59	4	12	31	175
CP Disp. P. Down	2	49	28	19	139	203	84	59	24	607
CP Disp. P. Up & Beat		3		2	24	41	59	32	2	163
Contact			1		8	24	9	55	5	102
Retreat	2	7	2	1	51	57	11	15	3	149

Table 3.2. Inter-individual (loser-winner) transition matrix of fights between male *Necora puber*.

Response by Winner → Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		35.7	21.4	7.2	14.3	21.4			
Stand		16.7	16.7	8.6	25.5	22.8	3.4	2.4	3.9
Move To		11.4	13.7	13.7	22.7	25.0	4.5	4.5	4.5
Move Away		17.4	6.1	4.1	31.6	23.5	16.3	1.0	
App. in Disp.	1.1	8.6	0.6	6.3	22.8	33.7	2.3	6.9	17.7
CP Disp. P. Down	0.3	8.1	4.6	3.1	22.9	33.5	13.8	9.7	4.0
CP Disp. P. Up & Beat		1.9		1.2	14.7	25.2	36.2	19.6	1.2
Contact			1.0		7.9	23.5	8.8	53.9	4.9
Retreat	1.3	4.7	1.3	0.7	34.2	38.3	7.4	10.1	2.0

Table 3.3 Percentages of the different responding acts used by winners in fights between male *Necora puber*.

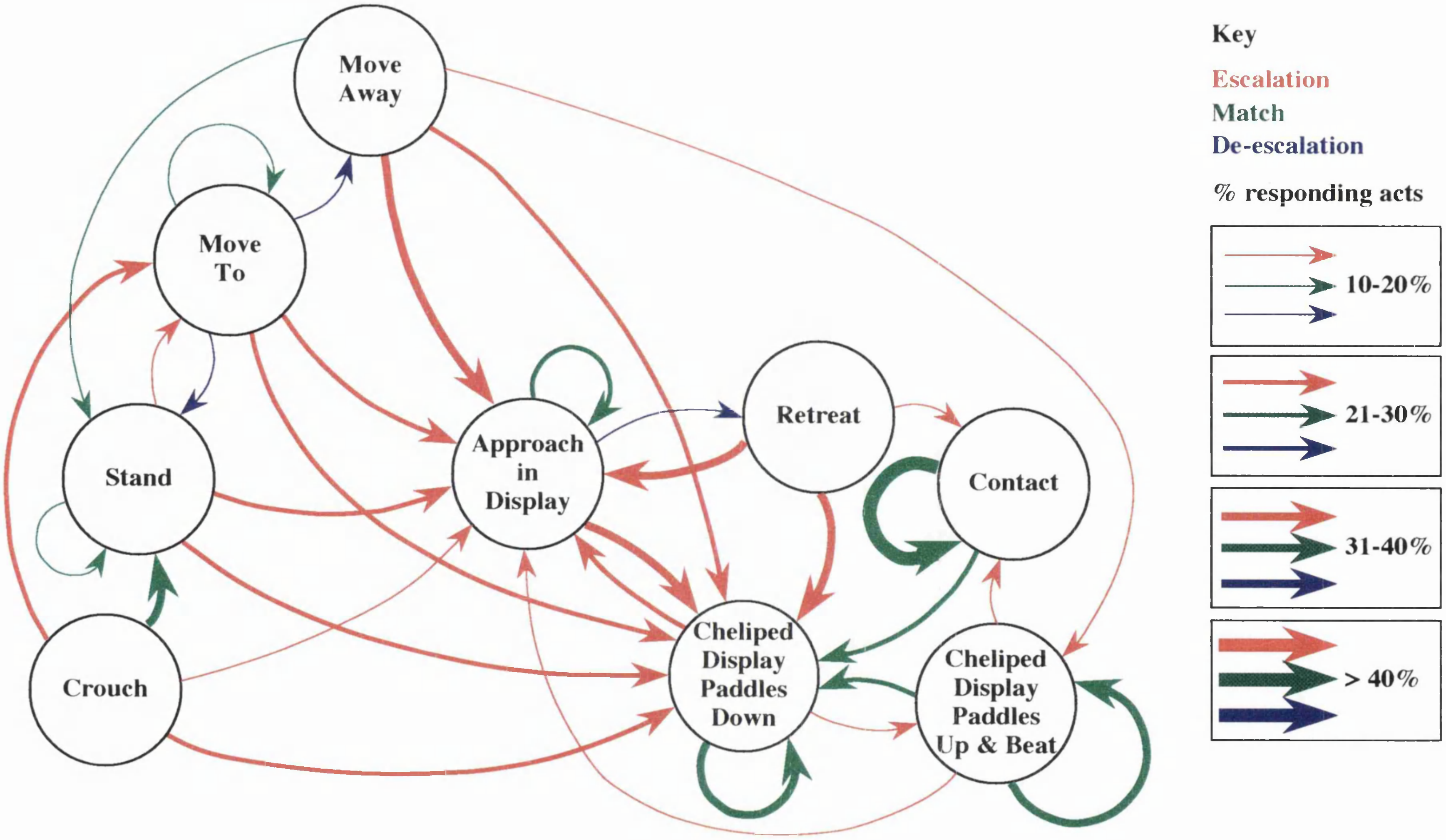


Figure 3.1 Inter-individual (loser-winner) fight sequence diagram for fights between male *Necora puber*.

	Responding Act by Winner					
Act by Loser	**D**	**M**	**E**	χ^2	**df**	p
Crouch	1	5	8	5.3	2	> 0.05
Stand	51	68	289	168.1	2	<< 0.001
Move To	13	6	25	12.6	2	< 0.01
Move Away	0	21	77	96.9	2	<< 0.001
App. in Disp.	59	41	75	9.9	2	< 0.01
CP Disp. P. Down	94	203	310	115.3	2	<< 0.001
CP Disp. P. Up & Beat	7	100	56	79.7	2	<< 0.001
Contact	5	88	9	128.9	2	<< 0.001
Retreat	13	N/A	136	101.5	1	<< 0.001

Table 3.4 Results of the χ^2 tests on the condensed inter-individual (loser-winner) transition matrix of fights between male *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.1.2 Inter-individual transitions: losers

The frequencies of the different following acts used by losers in response to acts by winners are shown in Table 3.5, and the percentages in Table 3.6. The fight sequence diagram for the inter-individual (winner-loser) transition matrix is presented in Figure 3.2, (only acts with occurrences greater than 10% were plotted) and the results of the χ^2 tests on the condensed inter-individual (winner-loser) transition matrix are summarised in Table 3.7. For definitions of categories of escalatory, matching and de-escalatory acts see Table 3.1.

From examining Figure 3.2 and Table 3.6, it can be seen that losers also used escalatory acts more frequently than either matching or de-escalatory acts. The usage of matching and de-escalatory acts was about equal. Losers responded to 'crouch' by winners by adopting escalatory and matching acts in 66.6% and 33.3% cases respectively. Escalatory acts accounted for 65.4% of the response of losers to 'stand' by winners. Matching and de-escalatory acts accounted for 24.6% and 9.9% of response respectively. Losers responded to 'move to' by winners by adopting escalatory and de-escalatory acts in 51.4% and 43.0% of cases respectively. 'Move to' (5.6%) was the only matching act seen. Escalatory acts accounted for 58.6% of the response of losers to 'move away' by winners. Matching and de-escalatory acts accounted for 37.9% and 3.4% of the response respectively. Losers responded to 'approach in display' by winners by adopting de-escalatory acts in 62.6% of cases. Matching acts accounted for the remaining 8.4% of the response. Escalatory, de-escalatory and matching acts accounted for 38.7%, 32.4% and 28.9% respectively of the response of losers to 'cheliped display, swimming paddles down' by the winners. Losers responded to 'cheliped display, swimming paddles up & beat' by winners with matching acts in 66.6% of cases. Escalatory and de-escalatory acts accounted for 17.0% and 16.4% of the response of losers to 'cheliped display, swimming paddles up & beat' by winners respectively. Matching and de-escalatory acts accounted for 59.1% and 38.8% of the response of losers to 'contact' by winners. 'Approach in display' was the only escalatory act seen in response to 'contact' by winners, and accounted for 1.8% of the response of the losers. Finally, escalatory and de-escalatory acts accounted for 75.6% and 24.3% of the response of losers to 'retreat' by the winners. By definition, no matching acts were seen.

From Table 3.7, it can be seen that losers tended to use de-escalatory acts significantly more often in response to 'approach in display' by the winners. Losers responded to 'cheliped display, swimming paddles up & beat' with significantly more matching acts. Losers responded to 'contact' by the winners by using both de-escalatory and matching acts significantly more frequently. Losers responded significantly more often with escalatory acts after 'crouch', 'stand', 'move to', 'move away', 'cheliped display, swimming paddles down', and 'retreat' by the winners.

Response by Loser → Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		3			2	3	1			9
Stand		35	25	11	32	29	2	5	3	142
Move To		11	4	15	16	19	2		5	72
Move Away		7	4	4	8	4		1	1	29
App. in Disp.		64	6	72	41	135	22	7	216	563
CP Disp. P. Down	3	92	14	44	74	164	91	40	45	567
CP Disp. P. Up & Beat		7		1	13	49	49	12	16	147
Contact		9		12	3	28	8	62	43	165
Retreat		6			11	14	3		3	37

Table 3.5 Inter-individual (winner-loser) transition matrix of fights between male *Necora puber*.

Response by Loser → Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P.Down	CP Disp. P Up & Beat	Contact	Retreat
Crouch		33.3			22.2	33.3	11.1		
Stand		24.6	17.6	7.8	22.5	20.4	1.4	3.5	2.1
Move To		15.3	5.6	20.8	22.2	26.4	2.8		6.9
Move Away		24.1	13.8	13.8	27.6	13.8		3.4	3.4
App. in Disp.		11.4	1.1	12.8	7.3	24.0	3.9	1.2	38.4
CP Disp. P. Down	0.5	16.2	2.5	7.8	13.1	28.9	16.0	7.1	7.9
CP Disp. P. Up & Beat		4.8		0.7	8.8	33.3	33.3	8.2	10.9
Contact		5.5		7.3	1.8	17.0	4.8	37.6	26.1
Retreat		16.2			29.7	37.8	8.1		8.1

Table 3.6 Percentages of the different responding acts used by losers in fights between male *Necora puber*.

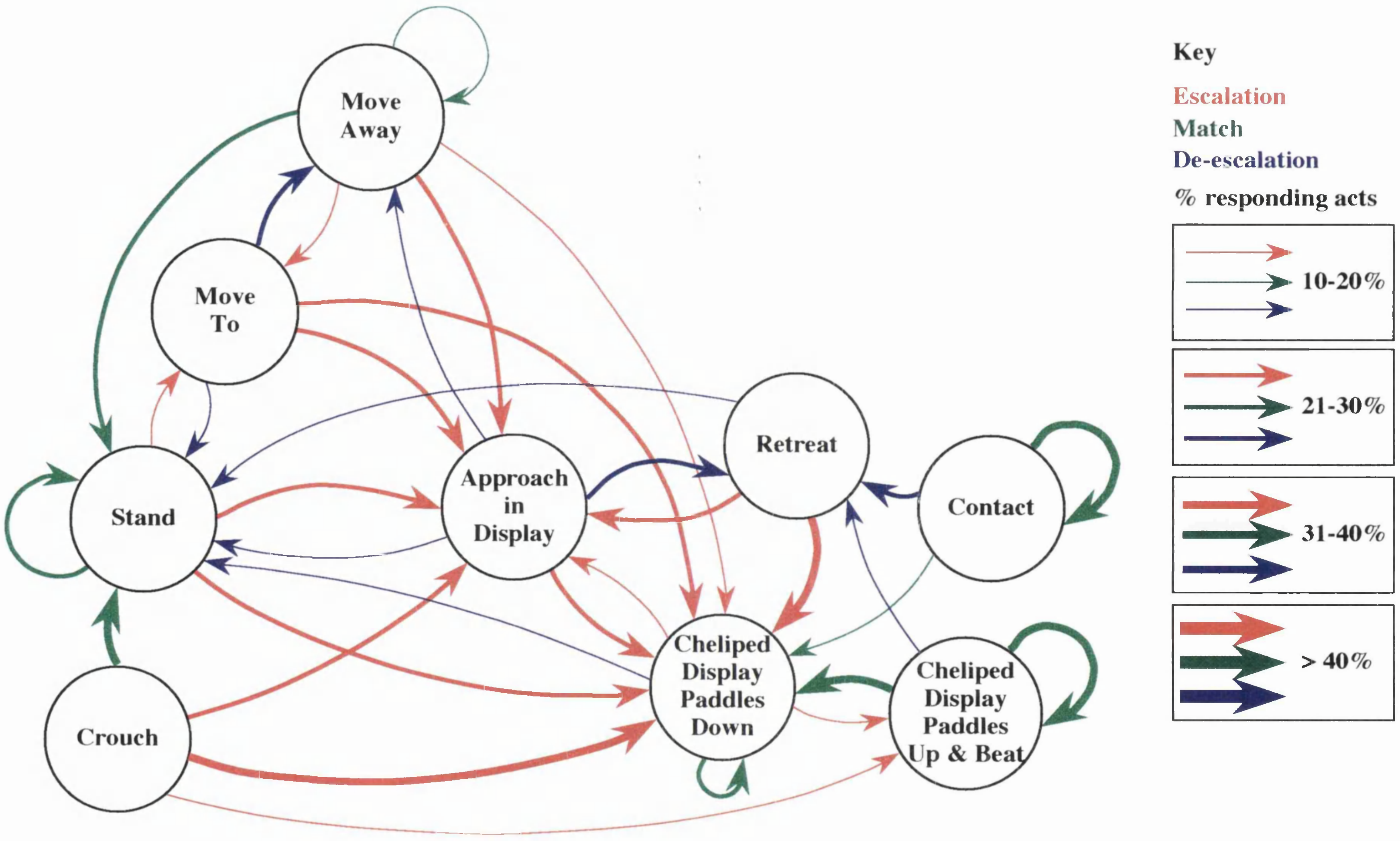


Figure 3.2 Inter-individual (winner-loser) fight sequence diagram for fights between male *Necora puber*.

	Responding Act by Loser					
Act by Winner	**D**	**M**	**E**	χ^2	**df**	p
Crouch	0	3	6	6.0	2	< 0.05
Stand	14	35	93	71.0	2	$<< 0.001$
Move To	29	4	42	29.8	2	$<< 0.001$
Move Away	1	11	17	13.5	2	< 0.01
App. in Disp.	352	47	164	251.8	2	$<< 0.001$
CP Disp. P. Down	184	164	219	7.6	2	< 0.05
CP Disp. P. Up & Beat	24	98	25	73.5	2	$<< 0.001$
Contact	64	88	3	74.3	2	$<< 0.001$
Retreat	9	N/A	28	17.1	1	< 0.001

Table 3.7 Results of the χ^2 tests on the condensed inter-individual (winner-loser) transition matrix of fights between male *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.1.3 Comparison of inter-individual (winner & loser) transition matrices

Next the 2 inter-individual transition matrices, *i.e.*, loser-winner and winner-loser, were compared directly, and the results are shown in Table 3.8. Winners and losers did not show any significant deviation from expected values in response to 'crouch', 'stand' and 'move to'. Both winners and losers, however, used escalatory acts most frequently in response to 'move away', though the tendency was strongest in the winners. Losers responded to 'approach in display' by the winner with de-escalatory acts, whereas the winners responded to losers adopting the same act with escalatory acts. Escalatory acts were used most often by both winners and losers responding to 'cheliped display, swimming paddles down', but the losers also had a tendency to use de-escalatory acts in response to this act by the winners. Both winners and losers used matching acts most frequently in response to 'cheliped display, swimming paddles up & beat', but losers also had a tendency to use de-escalatory acts, whereas winners also tended to use escalatory acts in response to this act. Winners and losers used matching acts most commonly in response to 'contact', but losers again had a tendency to use de-escalatory acts. Both winners and losers used escalatory acts most frequently in response to 'retreat', but the tendency was strongest in the winners, whereas losers had a greater tendency to use de-escalatory acts.

In summary, both winners and losers of fights between male *Necora puber* tended to adopt high risk, escalatory acts in response to low risk, de-escalatory acts, *e.g.*, crouch and stand. Winners tended to respond to high risk acts by losers with matching/escalatory acts, whereas losers responded to winners by adopting matching/de-escalatory acts.

Act	Transition	D	M	E	χ^2	df	*p*	Explanation of results
Crouch	W-L L-W	0 1	3 5	6 8	0.7	2	> 0.05	N/A
Stand	W-L L-W	14 51	35 68	93 289	4.6	2	> 0.05	N/A
Move To	W-L L-W	29 13	4 6	42 25	2.9	2	> 0.05	N/A
Move Away	W-L L-W	1 0	11 21	17 77	7.0	2	< 0.05	E is the commonest response for both winners & losers, but the tendency is strongest in winners ($\chi^2 = 4.6$ $p < 0.05$).
App. in Disp.	W-L L-W	352 59	47 41	164 75	53.0	2	<< 0.001	D is the commonest response for losers ($\chi^2 = 41.6, p << 0.001$) & E is the commonest response for winners ($\chi^2 = 8.2, p < 0.01$).
CP Disp. P. Down	W-L L-W	184 94	164 203	219 310	47.6	2	<< 0.001	E is the commonest response for both winners & losers, but losers have a strong tendency to adopt D ($\chi^2 = 46.7, p << 0.001$).
CP Disp. P. Up & Beat	W-L L-W	24 7	98 100	25 56	21.6	2	<< 0.001	M is the commonest response for both winners & losers, but losers tend to adopt D ($\chi^2 = 8.4$ $p < 0.01$) & winners tend to adopt E ($\chi^2 = 8.0, p < 0.01$).
Contact	W-L L-W	64 5	88 88	3 9	44.3	2	<< 0.001	M is the commonest response for both winners & losers, but losers have a stronger tendency to adopt D ($\chi^2 = 41.5$ $p << 0.001$)
Retreat	W-L L-W	9 13	N/A	28 136	15.3	1	< 0.001	E is the commonest response for both winners & losers, but the tendency is strongest in winners, whereas losers have a tendency to adopt D.

Table 3.8 Comparison of the condensed inter-individual (winner-loser & loser-winner) transition matrices of fights between male *Necora puber*. Key: W-L = winner-loser transitions; L-W = loser-winner transitions; D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable.

3.3.1.4 Intra-individual transitions: winners

The frequencies of the following acts used by winners in 101 fights between male *Necora puber* are shown in Table 3.9, and the percentages are shown in Table 3.10. The fight sequence diagram is presented in Figure 3.3. Only acts whose occurrence was greater than 5% were plotted. The results of the χ^2 tests on the condensed intra-individual (winner) transition matrix, *i.e.*, the category of acts used by the winner following a given act by the winner, are summarised in Table 3.11. See Table 3.1 for definitions of categories of escalatory, matching and de-escalatory acts.

Winners tended to follow a given preceding act with escalatory acts, as indicated by the number and thickness of red arrows in Figure 3.3. Matching acts appeared infrequently due to the difficulty of defining the start and finish of, for example, 'stand' followed by 'stand'. Winners always followed 'crouch' with the matching act 'stand'. Winners followed 'stand' with escalatory acts in 76.4% of cases, and de-escalatory acts in 23.6% of cases. Winners followed 'move to' with escalatory and de-escalatory acts in 46.6% and 53.5% of cases respectively. Winners followed 'move away' with the matching act, 'stand', in 84.0% of cases. Escalatory acts accounted for 12.0% of the acts following 'move away', and 'retreat' (4.0%) was the only de-escalatory act observed. Winners followed 'approach in display' with escalatory acts in 93.1% of cases and de-escalatory acts constituted the remaining 7.0%. 'Cheliped display, swimming paddles down' was followed in 74.7% of cases by escalatory acts and the remaining 25.3% was accounted for by de-escalatory acts. Winners followed 'cheliped display, swimming paddles up & beat' with escalatory acts in 53.8% of cases, and with matching and de-escalatory acts in the remaining 39.2% and 6.9% of cases respectively. 'Contact' was followed with matching acts in 69.3% of cases. 'Approach in display' was the only escalatory act seen, accounting for 21.5% of the following acts, and de-escalatory acts constituted the remaining 9.2%. 'Retreat' was followed by escalatory and de-escalatory acts in 75.0% and 25.0% of cases respectively.

From Table 3.11, it can be seen that winners did not follow 'move to' with any particular category of acts, but they did have a tendency to follow 'crouch', 'move away', and 'contact' significantly more frequently with matching acts. 'Stand', 'cheliped display, swimming paddles down' and 'retreat' were followed significantly more often with escalatory acts, as was 'approach in display'. 'Cheliped display, swimming paddles up & beat' was followed significantly more often by both matching and escalatory acts.

2nd Act by Winner → 1st Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		10								10
Stand	5		146	77	27	80	10	12	3	360
Move To	2	98		5	64	27	2	1	3	202
Move Away		84	3		4	4		1	4	100
App. in Disp.		19	1	2		234	71	28	4	359
CP Disp. P. Down	2	62	20	5	225		67	46	53	480
CP Disp. P. Up & Beat		7		1	56	62		29	3	158
Contact		8			28	79	11		4	130
Retreat		14		4	7	45	1	1		72

Table 3.9 Intra-individual (winner) transition matrix of fights between male *Necora puber.*

2nd Act by Winner → 1st Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		100.0							
Stand	1.4		40.6	21.4	7.5	22.2	2.8	3.3	0.8
Move To	1.0	48.5		2.5	31.7	13.4	1.0	0.5	1.5
Move Away		84.0	3.0		4.0	4.0		1.0	4.0
App. in Disp.		5.3	0.3	0.6		65.2	19.8	7.8	1.1
CP Disp. P. Down	0.4	12.9	4.2	1.0	46.9		14.0	9.6	11.0
CP Disp. P. Up & Beat		4.4		0.6	35.4	39.2		18.4	1.9
Contact		6.2			21.5	60.8	8.5		3.1
Retreat		19.4		5.6	9.7	62.5	1.4	1.4	

Table 3.10 Percentages of different following acts used by winners in fights between male *Necora puber*.

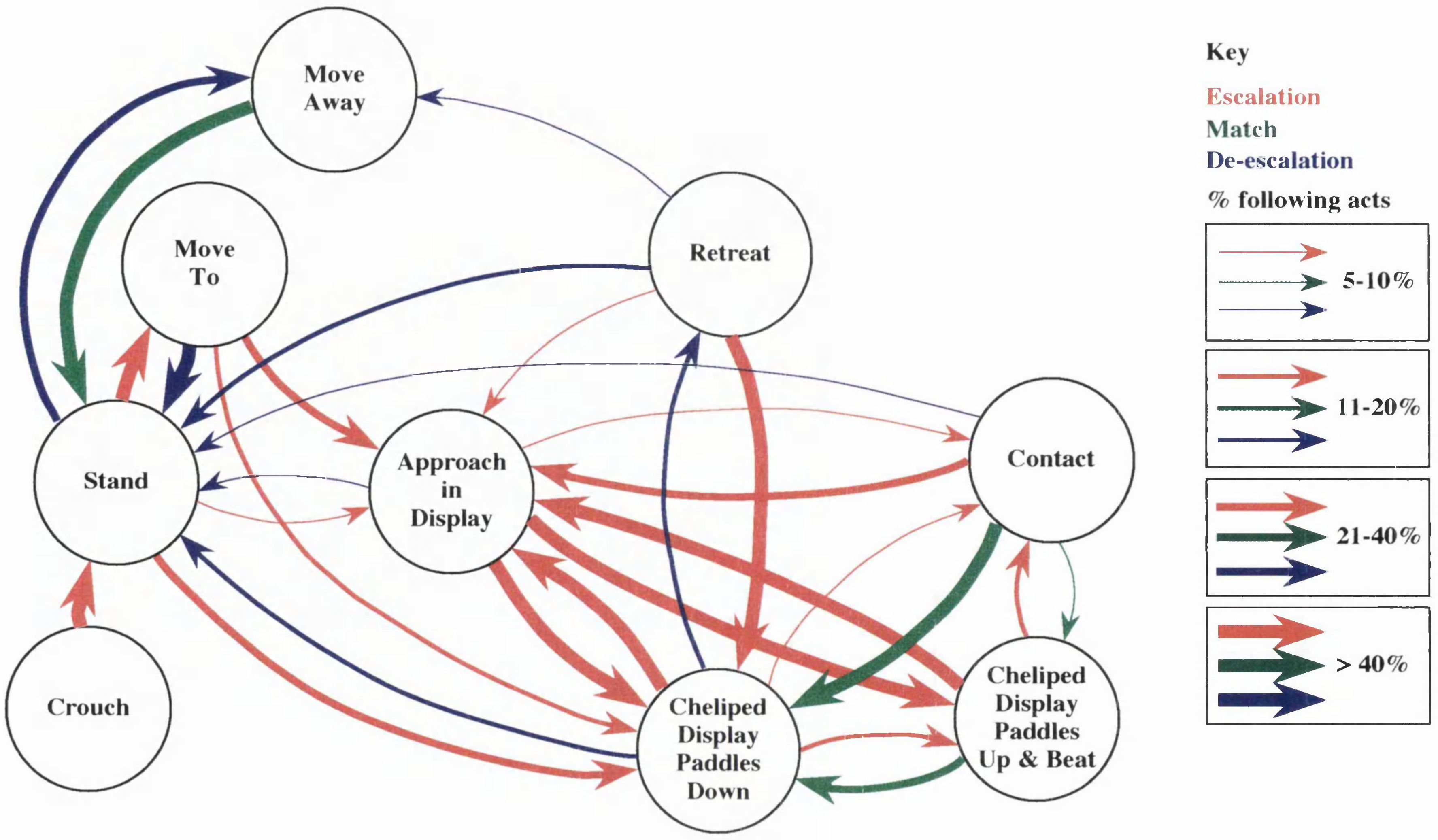


Figure 3.3 Intra-individual (winner) fight sequence diagram for fights between male *Necora puber*.

	Following Act by Winner					
Act by Winner	**D**	**M**	**E**	χ^2	**df**	***p***
Crouch	0	10	0	20.2	2	<< 0.001
Stand	85	N/A	275	100.3	1	<< 0.001
Move To	108	N/A	94	1.0	1	> 0.05
Move Away	4	84	12	116.6	2	<< 0.001
App. in Disp.	25	1	333	527.7	2	<< 0.001
CP Disp. P. Down	122	N/A	358	116.0	1	<< 0.001
CP Disp. P. Up & Beat	11	62	85	54.4	2	<< 0.001
Contact	12	90	28	78.4	2	<< 0.001
Retreat	18	N/A	63	18.0	1	<< 0.001

Table 3.11 Results of the χ^2 tests on the condensed intra-individual (winner) transition matrix of fights between male *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.1.5 Intra-individual transitions: losers

The frequencies of the different following acts used by the losers are summarised in Table 3.12, and the percentages are shown in Table 3.13. The fight sequence diagram derived from these percentages is presented in Figure 3.4. Only acts whose occurrence was greater than 5% were plotted. The results of the χ^2 tests on the condensed intra-individual (loser) transition matrix are shown in Table 3.14. See Table 3.1 for definitions of escalatory, matching and de-escalatory acts.

From Figure 3.4 and Table 3.13, it can be seen that losers tended to use escalatory and de-escalatory acts equally often, as indicated by the approximate equal number and thickness of red and blue arrows. The only following act seen after 'crouch' was 'stand'. Losers followed 'stand' with escalatory and de-escalatory acts in 59.9% and 40.1% of cases respectively. 'Move to' was followed by de-escalatory acts in 56.7% of cases, with escalatory acts constituting the remaining 43.5% of following acts. Losers mainly followed 'move away' with the matching act 'stand' (55.4%), and de-escalatory and escalatory acts accounted for the remaining 26.3% and 18.4% respectively. 'Approach in display' was followed mainly by escalatory acts (85.1%). De-escalatory acts accounted for a further 14.0% of the following acts used by losers after 'approach in display', and 'move to', the only matching act, the remaining 1.0%. Losers followed 'cheliped display, swimming paddles down' with escalatory acts in 52.7% of cases and de-escalatory acts accounted for the remaining 47.4%. 'Cheliped display, swimming paddles up & beat' was followed by one matching act in 48.2% of cases, specifically 'cheliped display, swimming paddles down'. De-escalatory and escalatory acts accounted for the remaining 30.6% and 27.0% respectively. Losers followed 'contact' with matching acts in 59.4% of cases and de-escalatory acts accounted for a further 33.4%. The only escalatory act seen was 'approach in display', accounting for the remaining 7.3%. Finally, 'retreat' was followed by approximately equal percentages of escalatory and de-escalatory acts, 52.3% and 47.7% respectively.

From Table 3.14, it can be seen that losers did not follow 'move to', 'cheliped display, swimming paddles down' and 'retreat' with any particular category of following acts. Losers followed 'crouch' and 'cheliped display, swimming paddles up & beat' significantly more often with matching acts, as was the case with 'move away' and contact'. Losers followed 'stand' and 'approach in display' significantly more frequently with escalatory acts.

2^{nd} Act by Loser → 1^{st} Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		6								6
Stand	3		73	105	27	98	1	4	28	339
Move To	1	54		11	24	24	1	3	2	120
Move Away	1	97	1		8	22	1		45	175
App. in Disp.		6	2	3		113	51	7	19	201
CP Disp. P. Down		52	4	23	108		64	54	132	437
CP Disp. P. Up & Beat		3		5	18	66		19	26	137
Contact		2		5	5	30	11		16	69
Retreat		61		22	1	83	6	1		174

Table 3.12 Intra-individual (loser) transition matrix of fights between male *Necora puber*.

2nd Act by Loser → 1st Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		100.0							
Stand	0.9		21.5	31.0	8.0	28.9	0.3	1.2	8.3
Move To	0.8	45.0		9.2	20.0	20.0	0.8	2.5	1.7
Move Away	0.6	55.4	0.6		4.6	12.6	0.6		25.7
App. in Disp.		3.0	1.0	1.5		56.2	25.4	3.5	9.5
CP Disp. P. Down		11.9	0.9	5.3	24.7		14.7	12.4	30.2
CP Disp. P. Up & Beat		2.2		3.6	13.2	48.2		13.9	19.0
Contact		2.9		7.3	7.3	43.5	15.9		23.2
Retreat		35.1		12.6	0.6	47.7	3.4	0.6	

Table 3.13 Percentages of the different following acts used by losers in fights between male *Necora puber*.

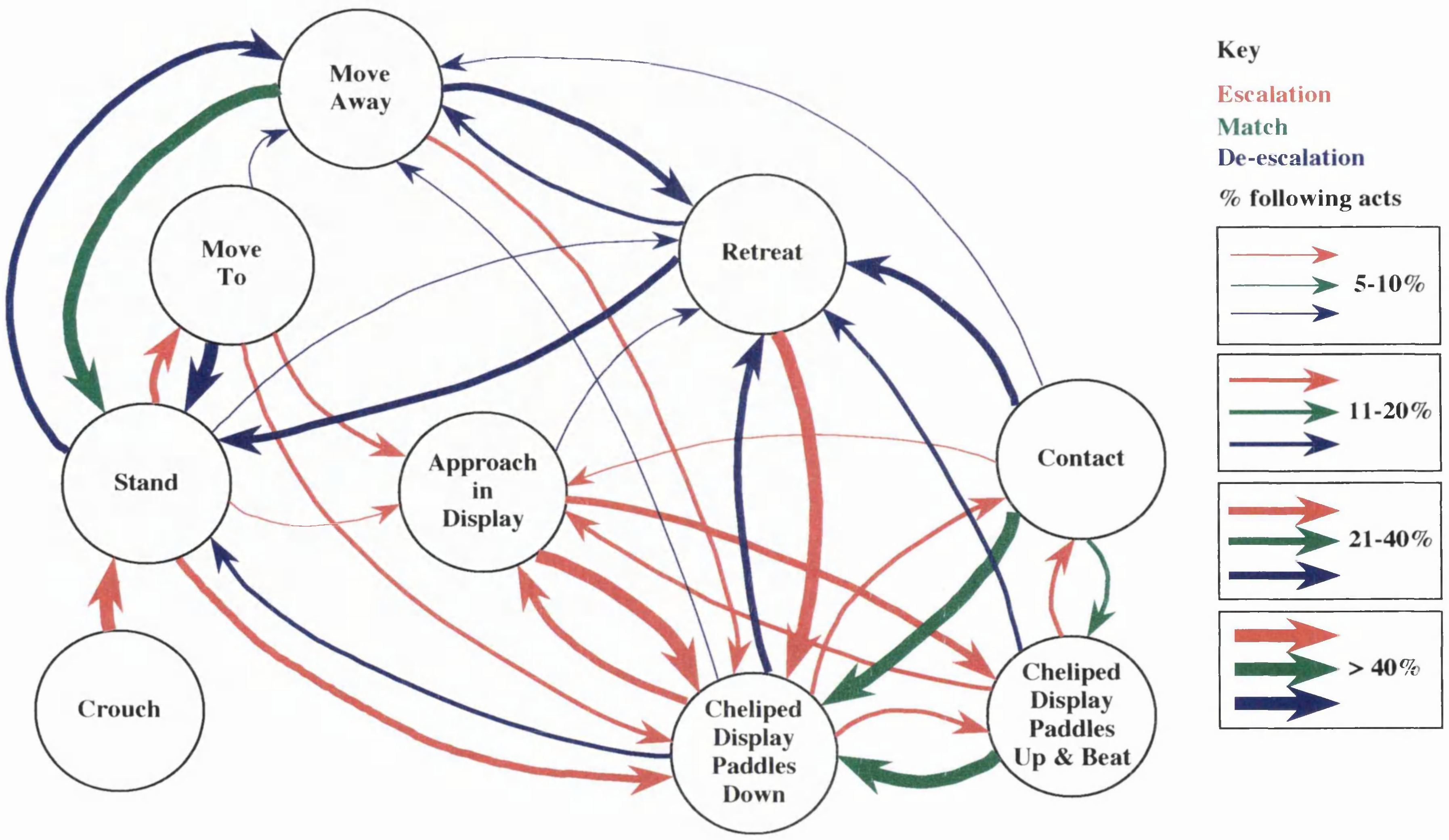


Figure 3.4 Intra-individual (loser) fight sequence diagram for fights between male *Necora puber*.

	Following Act by Loser					
Act by Loser	**D**	**M**	**E**	χ^2	**df**	*p*
Crouch	0	6	0	12.0	2	< 0.01
Stand	136	N/A	203	13.2	1	< 0.001
Move To	67	N/A	52	1.9	1	> 0.05
Move Away	46	97	32	40.1	2	<< 0.001
App. in Disp.	28	2	171	247.2	2	<< 0.001
CP Disp. P. Down	207	N/A	230	1.2	1	> 0.05
CP Disp. P. Up & Beat	34	66	37	13.7	2	< 0.01
Contact	23	41	5	28.2	2	<< 0.001
Retreat	83	N/A	90	0.3	1	> 0.05

Table 3.14 Results of the χ^2 tests on the condensed intra-individual (loser) transition matrix of fights between male *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.1.6 Comparison of intra-individual (winner & loser) transition matrices

The results of the direct comparison of the 2 intra-individual (winner and loser) transition matrices are summarised in Table 3.15. Neither winners or losers followed 'crouch' and 'move to' with any particular category of acts. Escalatory acts were used most frequently by both winners and losers following 'stand', but the tendency was strongest in winners, whereas losers had a tendency to follow 'stand' with de-escalatory acts. Winners and losers followed 'move away' most commonly with matching acts, but losers also had a tendency to follow with de-escalatory acts. Escalatory acts were used most often by both winners and losers following 'approach in display', but the tendency was strongest in the winners. Both winners and losers followed 'cheliped display, swimming paddles down' with escalatory acts, but the tendency was strongest in the winners. Losers had a tendency to follow this act with de-escalatory acts. Winners followed 'cheliped display, swimming paddles up & beat' most frequently with escalatory acts, whereas losers followed this act most commonly with matching acts, but losers also tended to use de-escalatory acts as well. Both winners and losers followed 'contact' most often with matching acts, but the losers also had a great tendency to use de-escalatory acts. Escalatory acts were used most commonly by winners and losers following 'retreat', but the tendency was greatest in winners, whereas the losers also tended to use de-escalatory acts.

Overall, winners and losers tended to follow their own previous low risk, de-escalatory act with similar acts. Winners followed their previous high risk, escalatory act with similar acts, whereas losers tended to follow with de-escalatory acts.

Act	Transition	D	M	E	χ^2	df	p	Explanation of results
Crouch	W-W L-L	0 0	10 6	0 0	0	2	> 0.05	N/A
Stand	W-W L-L	85 136	N/A	175 203	4.8	1	< 0.05	E is the commonest action for both winners & losers following "Stand", but the tendency is stronger in winners. Losers have a greater tendency to adopt D.
Move To	W-W L-L	108 67	N/A	94 52	2.3	1	> 0.05	N/A
Move Away	W-W L-L	4 46	84 97	12 32	26.9	2	$<< 0.001$	M is the commonest following action for both winners & losers, but losers have a stronger tendency to adopt D ($\chi^2 = 25.0$, $p << 0.001$).
App. in Disp.	W-W L-L	25 28	1 2	333 171	8.7	2	< 0.02	E is the commonest following action for both winners & losers, but the tendency is stronger in winners ($\chi^2 = 8.5$, $p < 0.01$)
CP Disp. P. Down	W-W L-L	122 207	N/A	358 230	47.9	1	$<< 0.001$	E is the following commonest action for both winners & losers, but the tendency is stronger in winners. Losers have a greater tendency to adopt D.
CP Disp. P. Up & Beat	W-W L-L	11 34	62 66	85 37	29.5	2	$<< 0.001$	Winners tend to adopt E ($\chi^2 = 11.3$, $p < 0.001$). Losers tend to adopt M, but they also have a tendency to adopt D ($\chi^2 = 7.7$, $p < 0.01$).
Contact	W-W L-L	12 23	90 41	28 5	21.1	2	$<< 0.001$	M is the commonest following action for both winners & losers, but losers have a stronger tendency to adopt D ($\chi^2 = 14.4$, $p < 0.001$)
Retreat	W-W L-L	18 83	N/A	63 90	15.3	1	< 0.001	E is the commonest following action for both winners & losers, but the tendency is strongest in winners, whereas losers have a tendency to adopt D.

Table 3.15 Comparison of the condensed intra-individual (winner & loser) transition matrices for fights between male *Necora puber*. Key: W-W = winner-winner transitions; L-L = loser-loser transitions; D = de-escalation; M = match; E = escalation; N/A = not applicable.

3.3.2 Females

3.3.2.1 Inter-individual transitions: winners

The frequencies of the different responding acts used by winners in 65 fights are shown in Table 3.16, and the percentages are shown in Table 3.17. The fight sequence diagram for the loser-winner transition matrix, based on percentages greater than 10%, is presented in Figure 3.5. The results of the χ^2 tests on the condensed inter-individual (loser-winner) transition matrix are shown in Table 3.18. See Table 3.1 for definitions of escalatory, matching and de-escalatory acts.

From Figure 3.5 and Table 3.17, it can be seen that the winners of fights between female *Necora puber* appeared to use matching acts (green arrows) most frequently in response to losers. De-escalatory acts were the next most frequently used category of acts, followed by escalatory acts. Winners responded to 'crouch' by losers by adopting the matching act, 'stand', in 36.4% of cases De-escalatory and escalatory acts each accounted for 31.8% of the response of winners to 'crouch'. Escalatory acts accounted for 46.5% of the response of winners to 'stand' by losers. 'Stand' was the only matching act seen, accounting for 32.5% of the response and de-escalatory acts accounted for the remaining 21.1% of the response. Winners responded to 'move to' by losers by adopting de-escalatory acts in 47.2% of cases, and escalatory and matching acts in the remaining 33.6% and 19.3% of cases, respectively. Matching acts accounted for 55.7% of the response of winners to 'move away' by losers, escalatory acts 43.1% and de-escalatory acts the remaining 1.2%. Winners responded to 'approach in display' by losers by adopting de-escalatory, matching and escalatory acts in 44.3%, 31.5% and 24.2% of cases respectively. Matching acts accounted for 39.3% of the response of winners to 'cheliped display, swimming paddles down' by the losers, escalatory acts a further 37.5% and de-escalatory acts the remaining 23.2% of the response. Winners responded to 'cheliped display, swimming paddles up & beat' with matching acts in 45.6% of cases. Escalatory and de-escalatory acts accounted for 31.7% and 22.8% of the remaining response respectively. De-escalatory and matching acts accounted for 53.9% and 46.2% of the response of winners to 'contact' by losers respectively. No escalatory acts were observed. Winners responded to 'retreat' by losers with approximately equal amounts of escalatory and de-escalatory acts, 51.2% and 48.7% respectively.

From Table 3.18, winners did not show any significantly more or less frequent response to 'crouch', 'approach in display', 'cheliped display, swimming paddles down' and 'retreat' by the losers. Winners responded to 'stand' by using escalatory acts significantly more frequently and they responded to 'move away' by using significantly more escalatory, and particularly matching acts. Winners responded to 'cheliped display, swimming paddles up & beat' by using matching acts significantly more often, as was the case with 'contact', though the winners responded equally as often with de-escalatory acts. Finally, winners responded to 'move to' by the loser significantly most frequently with de-escalatory acts.

Response by Winner → Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		8	6	7	1					22
Stand	10	156	164	90	38	10	8	3	1	480
Move To	1	28	27	33	28	9	6	4	4	140
Move Away	1	77	27	16	28	11	6		1	167
App. in Disp.		9	6	18	16	11	5	1	4	70
CP Disp. P. Down		7	3	4	11	22	4	3	2	56
CP Disp. P. Up & Beat		14	5	4	13	16	20	7		79
Contact	1	1		4		2	3	1	1	13
Retreat	1	16	1	3	7	12		1		41

Table 3.16 Inter-individual (loser-winner) transition matrix of fights between female *Necora puber*.

Response by Winner → Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		36.4	27.3	31.8	4.5				
Stand	2.1	32.5	34.2	18.8	7.9	2.1	1.7	0.6	0.2
Move To	0.7	20.0	19.3	23.6	20.0	6.4	4.3	2.9	2.9
Move Away	0.6	46.1	16.2	9.6	16.7	6.6	3.6		0.6
App. in Disp.		12.9	8.6	25.7	22.9	15.7	7.1	1.4	5.7
CP Disp. P. Down		12.5	5.4	7.1	19.6	39.3	7.1	5.4	3.6
CP Disp. P. Up & Beat		17.7	6.3	5.1	16.5	20.3	25.3	8.9	
Contact	7.7	7.7		30.8		15.4	23.1	7.7	7.7
Retreat	2.4	39.0	2.4	7.3	17.1	29.3		2.4	

Table 3.17 Percentages of different responding acts used by winners in fights between female *Necora puber*.

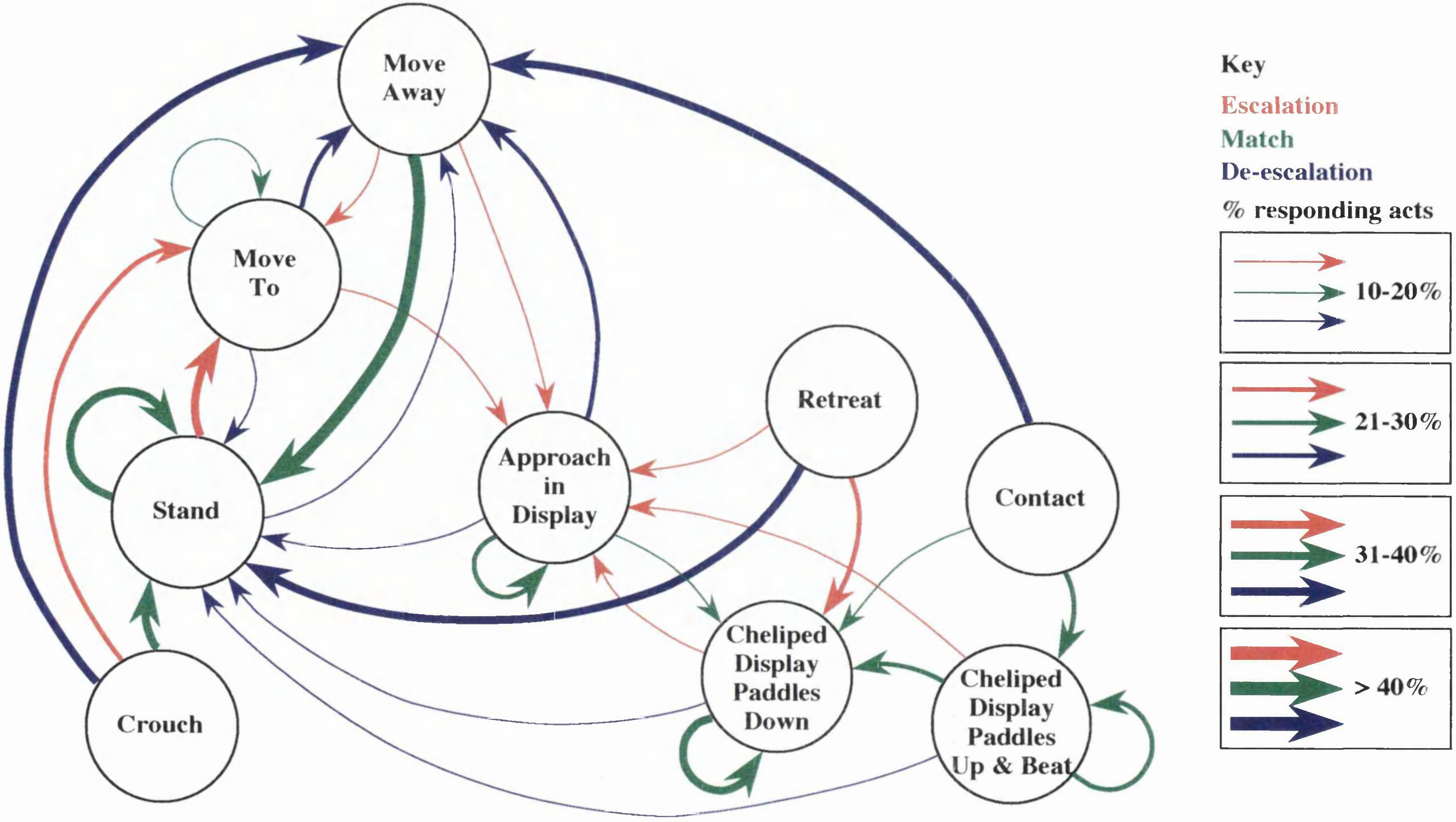


Figure 3.5 Inter-individual (loser-winner) fight sequence diagram for fights between female *Necora puber*.

	Response by Winner					
Act by Loser	**D**	**M**	**E**	χ^2	**df**	p
Crouch	7	8	7	0.08	2	> 0.05
Stand	101	156	223	46.7	2	<< 0.001
Move To	66	27	47	16.3	2	< 0.001
Move Away	2	93	72	81.5	2	<< 0.001
App. in Disp.	31	22	17	4.3	2	> 0.05
CP Disp. P. Down	13	22	21	2.6	2	> 0.05
CP Disp. P. Up & Beat	18	36	25	6.3	2	< 0.05
Contact	7	6	0	6.7	2	< 0.05
Retreat	20	N/A	21	0.02	1	> 0.05

Table 3.18 Results of the χ^2 tests on the condensed inter-individual (loser-winner) transition matrix of fights between female *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.2.2 Inter-individual transitions: losers

The frequencies of the different acts used by losers in response to winners are shown in Table 3.19, and the percentages are shown in Table 3.20. The fight sequence diagram for the inter-individual (loser-winner) transition matrix is presented in Figure 3.6. Only acts with occurrences greater than 10% were plotted. The results of the χ^2 tests on the condensed winner-loser transition matrix are summarised in Table 3.21. Definitions of escalatory, matching and de-escalatory acts are contained in Table 3.1.

It can be seen from Figure 3.6 and Table 3.20 that losers responded to winners by using de-escalatory acts most frequently, indicated by the greater number and thickness of blue arrows. Escalatory and matching acts were used equally as often as each other. Losers responded to 'crouch' by winners by adopting equal amounts of matching and escalatory acts (36.8%), and the remaining 26.4% of the response was accounted for by de-escalatory acts. The matching act, 'stand', accounted for 39.3% of the response of losers to 'stand' by winners. Escalatory acts accounted for a further 33.4% of the response and de-escalatory acts constitued the remaining 27.3%. Losers responded to 'move to' by winners with de-escalatory acts in 71.7% of cases and escalatory acts accounted for a further 18.3% of the response. 'Move to' (matching act) accounted for the remaining 10.0% of the response. Matching acts constituted 56.5% of the response of losers to 'move away' by winners, and escalatory and de-escalatory acts accounted for 37.7% and 5.9% respectively. Losers responded to 'approach in display' by winners by adopting de-escalatory acts in 74.6% of cases. Escalatory and matching acts accounted for 13.4% and 12.0% respectively. De-escalatory, matching and escalatory acts accounted for 54.8%, 31.0% and 14.3% of the response of losers to 'cheliped display, swimming paddles down' by winners. Matching acts comprised 50.0% of the response of losers to 'cheliped display, swimming paddles up & beat' by winners. De-escalatory acts accounted for a further 41.0% of the response and escalatory acts formed the remaining 9.0%. Losers responded to 'contact' by winners with de-escalatory acts in 90.5% of cases, and with matching and escalatory acts accounting for 4.8% each of the remaining response. Finally, losers responded to 'retreat' by winners by adopting escalatory and de-escalatory acts in 54.6% and 45.5% of cases respectively.

As seen in Table 3.21, losers did not show any particular category of act in response to 'crouch' or 'retreat' by the winners. Losers resonded to 'move away' by using significantly more escalatory, and particularly matching acts. Losers used significantly more matching acts in response to 'stand'. Losers responded to 'cheliped display, swimming paddles up & beat' by using de-escalatory, and particularly matching acts, significantly more often. Losers responded to 'move to', 'approach in display', 'cheliped display, swimming paddles down' and 'contact' by using significantly more de-escalatory acts.

Response by Loser → Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		7	7	4					1	19
Stand	13	168	106	95	18	10	6	3	9	428
Move To	2	32	18	79	16	6	8	3	16	180
Move Away	1	35	10	13	11	4	5	2	4	85
App. in Disp.		16	4	44	12	7	11		40	134
CP Disp. P. Down	1	24	1	13	3	26	8		8	84
CP Disp. P. Up & Beat		10	1	11	3	11	22	2	6	66
Contact		1	1	4		1			14	21
Retreat				5	2	3	1			11

Table 3.19 Inter-individual (winner-loser) transition matrix of fights between female *Necora puber*.

Response by Loser → Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		36.8	36.8	21.1					5.3
Stand	3.0	39.3	24.8	22.2	4.2	2.3	1.4	0.7	2.1
Move To	1.1	17.8	10.0	43.9	8.9	3.3	4.4	1.7	8.9
Move Away	1.2	41.2	11.8	15.3	12.9	4.7	5.9	2.4	4.7
App. in Disp.		11.9	3.0	32.8	9.0	5.2	8.2		29.9
CP Disp. P. Down	1.2	28.6	1.2	15.5	3.6	31.0	9.5		9.5
CP Disp. P. Up & Beat		15.2	1.5	16.7	4.5	16.7	33.3	3.0	9.1
Contact		4.8	4.8	19.0		4.8			66.7
Retreat				45.5	18.2	27.3	9.1		

Table 3.20 Percentages of different responding acts used by losers in fights between female *Necora puber*.

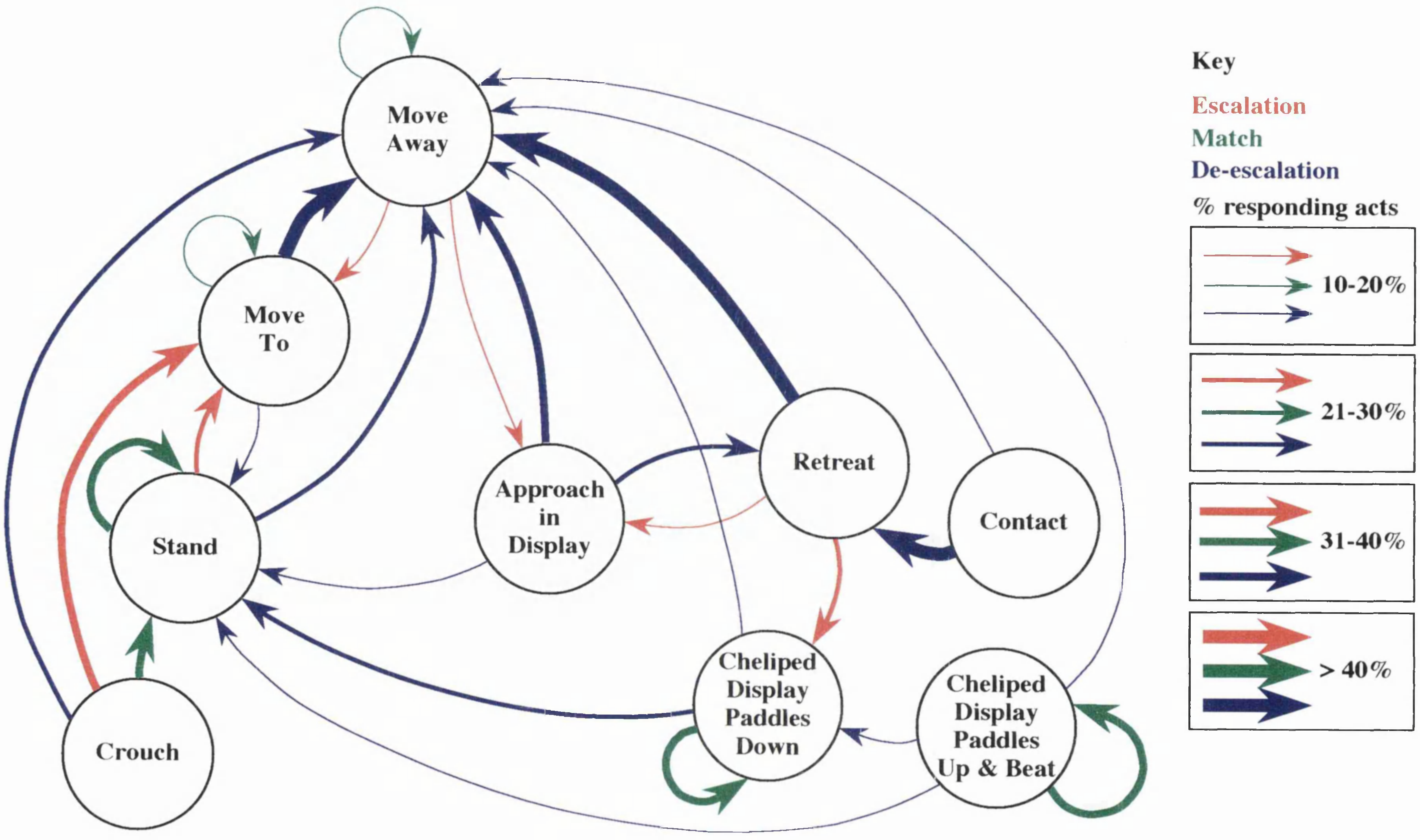


Figure 3.6 Inter-individual (winner-loser) fight sequence diagram for female *Necora puber*.

	Response by Loser					
Act by Winner	**D**	**M**	**E**	χ^2	**df**	p
Crouch	5	7	7	0.4	2	> 0.05
Stand	117	168	143	9.1	2	< 0.02
Move To	129	18	33	120.9	2	<< 0.001
Move Away	5	48	32	33.4	2	<< 0.001
App. in Disp.	100	16	18	102.8	2	<< 0.001
CP Disp. P. Down	46	26	12	20.9	2	<< 0.001
CP Disp. P. Up & Beat	27	33	6	18.3	2	< 0.001
Contact	19	1	1	30.9	2	<< 0.001
Retreat	5	N/A	5	0	1	> 0.05

Table 3.21 Results of the χ^2 tests on the condensed inter-individual (winner-loser) transition matrix of fights between female *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.2.3 Comparison of inter-individual (winner & loser) transition matrices

The results of the direct comparison of the two opposing transition matrices are summarised in Table 3.22. Winners and losers did not show any significant deviation from expected values in response to 'crouch', 'move away' and 'retreat'. Both winners and losers used de-escalatory acts significantly more frequently in response to 'move to', 'approach in display' and 'contact', though in all cases the tendency was strongest in the losers. Matching acts were used most commonly by losers responding to 'stand', but the winners responded significantly more often with escalatory acts. Losers responded most frequently to 'cheliped display, swimming paddles down' with de-escalatory acts, whereas winners tended to respond with matching or escalatory acts. Winners and losers used matching acts most frequently in response to 'cheliped display, swimming paddles up & beat', but winners had a strong tendency to use escalatory acts.

In summary, winners and losers of fights between female *Necora puber* showed similar responses to low risk, de-escalatory acts. Winners responded to static displays by the losers by adopting matching/escalatory acts, but winners adopted matching/de-escalatory acts in response to movement toward and contact by losers. Losers tended to respond to display, movement toward and contact by winners with matching/de-escalatory acts.

Act	Transition	D	M	E	χ^2	df	p	Explanation of results
Crouch	W-L L-W	5 7	7 8	7 7	0.2	2	> 0.05	N/A
Stand	W-L L-W	117 101	168 156	143 223	16.2	2	<< 0.001	M is the commonest response for losers, & E is the commonest response for winners ($\chi^2 = 16.0, p << 0.001$).
Move To	W-L L-W	129 66	18 27	33 47	19.9	2	<< 0.001	D is the commonest response for both winners & losers, but the tendency is stronger in losers ($\chi^2 = 19.9, p << 0.001$).
Move Away	W-L L-W	5 2	48 93	32 72	4.7	2	> 0.05	N/A
App. in Disp.	W-L L-W	100 31	16 22	18 17	19.3	2	<< 0.001	D is the commonest response for winners & losers, but the tendency is stronger in losers ($\chi^2 = 18.4\ p << 0.001$).
CP Disp. P. Down	W-L L-W	46 13	26 22	12 21	16.3	2	<< 0.001	D is the commonest response for losers ($\chi^2 = 13.7, p < 0.001$), whereas the winners tend to adopt M or E.
CP Disp. P. Up & Beat	W-L L-W	27 18	33 36	6 25	12.5	2	< 0.001	M is the commonest response for both winners & losers, but winners have a strong tendency to adopt E ($\chi^2 = 10.9, p < 0.001$).
Contact	W-L L-W	19 7	1 6	1 0	8.6	2	< 0.02	D is the commonest response for both winners & losers, but the tendency is stronger in losers ($\chi^2 = 6.0 p < 0.02$).
Retreat	W-L L-W	5 20	N/A	5 21	0	1	> 0.05	N/A

Table 3.22 Comparison of the condensed inter-individual (winner-loser & loser-winner) transition matrices for fights between female *Necora puber*. Key: W-L = winner-loser transitions; L-W = loser-winner transitions; D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable.

3.3.2.4 Intra-individual transitions: winners

The frequencies of the different following acts used by winners are shown in Table 3.23, and the percenatges are shown in Table 3.24. The fight sequence diagram derived from the intra-individual (winner) transition matrix is presented in Figure 3.7. Only percentages greater than 5% were plotted. The results of the χ^2 tests on the condensed intra-individual (winner) transition matrix are shown in Table 3.25. See Table 3.1 for definitions of escalatory, matching and de-escalatory acts.

From Figure 3.7 and Table 3.24, it can be seen that escalatory and de-escalatory acts were used equally as often as following acts by the winners. Winners followed 'crouch' with the matching act 'stand' in 60.0% of cases, the escalatory act 'move to' in 25.0%, and the de-escalatory act 'move away' in the remaining 15.0% of cases. Winners followed 'stand' with escalatory acts in 67.6% of cases and the remaining 32.4% was accounted for by de-escalatory acts. De-escalatory and escalatory acts each accounted for 79.4% and 20.5% of the following acts used by winners after 'move to'. Winners followed 'move away' with the matching act 'stand' in 86.8% of cases. Escalatory acts accounted for 10.2% of cases and de-escalatory acts made up the remaining 3.0%. Winners followed 'approach in display' with escalatory acts in 62.9% of cases. De-escalatory and matching ('move to' only) acts constituted 33.7% and 3.5% respectively of the remaining cases. Winners followed 'cheliped display, swimming paddles down' with 44.3% de-escalatory acts and 55.7% escalatory acts. Winners followed 'cheliped display, swimming paddles up & beat' with de-escalatory acts in 43.9% of cases. Escalatory acts were used in 36.6% of cases and the matching act 'cheliped display, swimming paddles down' in the remaining 19.5%. Winners followed 'contact' with equal amounts of de-escalatory and matching acts (46.2% each), and the escalatory act 'move to' constituted the remaining 7.7%. Winners followed 'retreat' with the de-escalatory act 'stand' in 85.7% of cases, and the escalatory act 'move to' in the remaining 14.3%.

As seen in Table 3.25, winners did not follow 'contact', 'cheliped display, swimming paddles up & beat' and 'cheliped display, swimming paddles down' with any particular category of act, but they followed 'stand' and 'approach in display' with significantly more escalatory acts. 'Crouch' and 'move away' were followed significantly more frequently with matching acts, whereas 'move to' and 'retreat' were followed significantly more often by de-escalatory acts.

2nd Act by Winner → 1st Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		12	5	3						20
Stand	15		301	154	37	20	5	3	6	541
Move To	1	257		20	54	11	6	1		350
Move Away	1	171	7		4	6	2	1	5	197
App. in Disp.		30	4	6		43	21	7	2	113
CP Disp. P. Down		27	5	8	25		8	6		79
CP Disp. P. Up & Beat		13	2	5	11	8		2		41
Contact		3	1	2		5	1		1	13
Retreat		12	2							14

Table 3.23 Intra-individual (winner) transition matrix of fights between female *Necora puber*.

2^{nd} Act by Winner → 1^{st} Act by Winner ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		60.0	25.0	15.0					
Stand	2.8		55.6	28.5	6.8	3.7	0.9	0.6	1.1
Move To	0.3	73.4		5.7	15.4	3.1	1.7	0.3	
Move Away	0.5	86.8	3.6		2.0	3.1	1.0	0.5	2.5
App. in Disp.		26.6	3.5	5.3		38.1	18.6	6.2	1.8
CP Disp. P. Down		34.2	6.3	10.1	31.7		10.1	7.6	
CP Disp. P. Up & Beat		31.7	4.9	12.2	26.8	19.5		4.9	
Contact		23.1	7.7	15.4		38.5	7.7		7.7
Retreat		85.7	14.3						

Table 3.24 Percentages of different following acts used by winners in fights between female *Necora puber*.

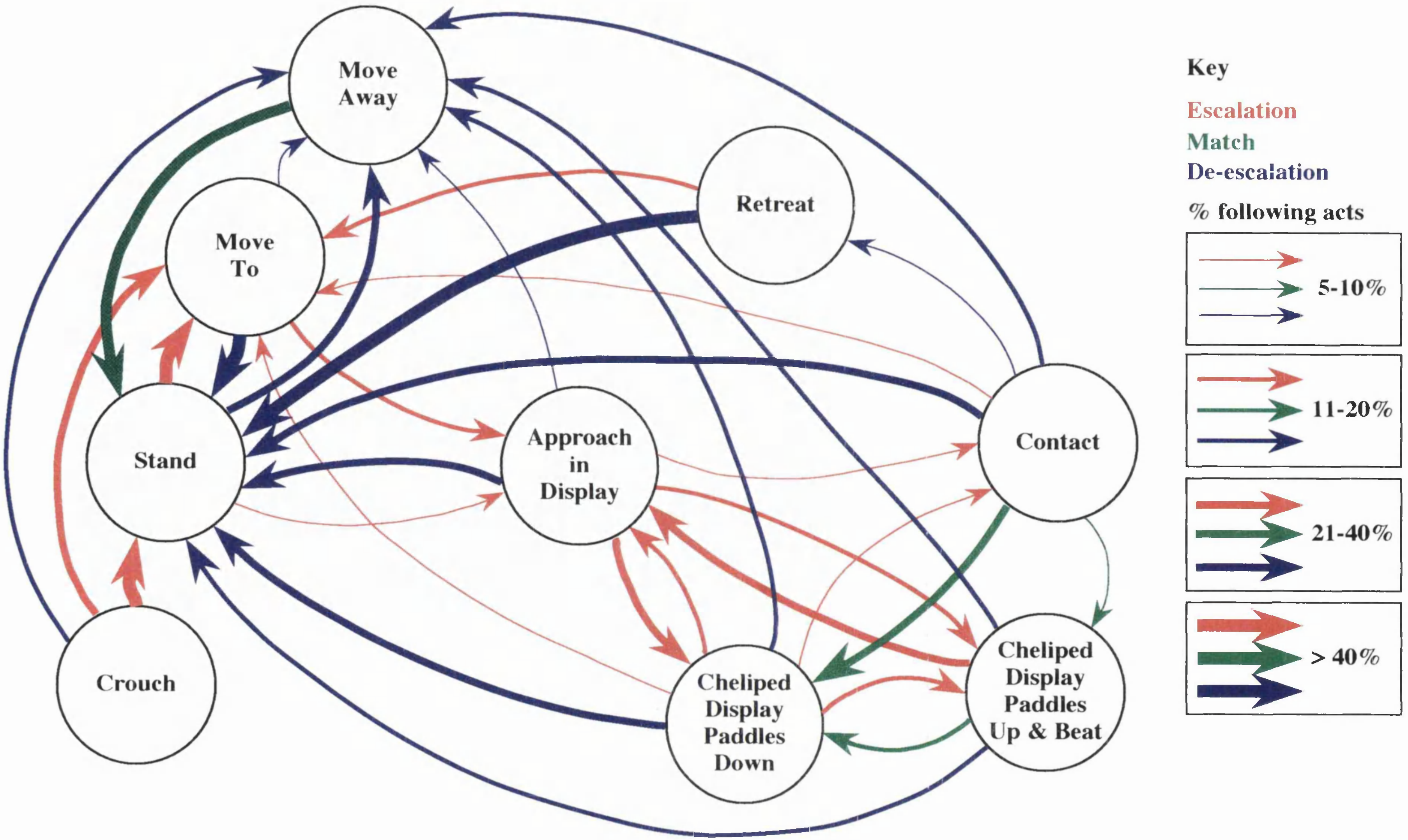


Figure 3.7 Intra-individual (winner) fight sequence diagram for fights between female *Necora puber*.

	Following Act by Winner					
Act by Winner	**D**	**M**	**E**	χ^2	**df**	p
Crouch	3	12	5	6.7	2	< 0.05
Stand	175	N/A	366	67.4	1	$<< 0.001$
Move To	278	N/A	72	121.3	1	$<< 0.001$
Move Away	6	171	20	254.8	2	$<< 0.001$
App. in Disp.	38	4	71	59.5	2	$<< 0.001$
CP Disp. P. Down	35	N/A	44	0.6	1	> 0.05
CP Disp. P. Up & Beat	18	8	15	3.8	2	> 0.05
Contact	6	6	1	3.8	2	> 0.05
Retreat	12	N/A	2	7.1	1	< 0.01

Table 3.25 Results of the χ^2 tests on the condensed intra-individual (winner) transition matrix of fights between female *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.2.5 Intra-individual transitions: losers

The frequencies of the following acts used by the losers are shown in Table 3.26. and the corresponding percentages are shown in Table 3.27. The fight sequence diagram for the intra-individual (loser) transition matrix is presented in Figure 3.8. The results of the χ^2 tests on the condensed loser-loser transition matrix are shown in Table 3.28. See Table 3.1 for definitions of catogories of escalatory, matching and de-escalatory acts.

Losers tended to follow their preceding act most frequently with de-escalatory acts, as indicated by the considerable amount of blue on Figure 3.8. Losers followed 'crouch' with the matching act 'stand' in 59.1% of cases. Escalatory acts were used in a further 27.3% of cases, and the de-escalatory act 'move away' was used in the remaining 13.6% of cases. Losers followed 'stand' with escalatory and de-escalatory acts in 51.7% and 48.3% of cases respectively. Losers followed 'move to' with de-escalatory acts in 86.8% of cases. The remaining 13.3% consisted of escalatory acts. Losers followed 'move away' with de-escalatory acts in 93.1% of cases and escalatory acts constituted the remaining 6.8%. Escalatory acts were used by losers to follow 'approach in display' in 56.3% of cases, de-escalatory acts in 40.7%, and the matching act 'approach in display' in 3.1% of cases. 'Cheliped display, swimming paddles down' was followed by losers with 55.4% de-escalatory and 44.7% escalatory acts. Losers followed 'cheliped display, swimming paddles up & beat' with 68.0% de-escalatory, 20.0% matching ('cheliped display, swimming paddles up & beat' only) and 12.0% escalatory acts. Losers followed 'contact' with de-escalatory acts in 62.5% of cases and with matching acts in 25.0% of cases. The remaining 12.5% was accounted for by the escalatory act 'approach in display'. Losers followed 'retreat' in 90.5% of cases with de-escalatory acts and escalatory acts accounted for the remaining 9.6%.

Losers did not follow 'stand' and 'cheliped display, swimming paddles down' with any particular category of acts, as was the case with 'contact'. 'Approach in display' was followed significantly more frequently with escalatory acts, whereas 'crouch' and 'move away' were followed significantly more often with matching acts. Losers followed 'move to', 'cheliped display, swimming paddles up & beat' and 'retreat' with significantly more de-escalatory acts.

2nd Act by Loser → 1st Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat	Total
Crouch		13	5	3		1				22
Stand	15		223	218	29	13	6	2	22	528
Move To	2	177		34	20	4	7	3	10	257
Move Away	1	235	10			9	1		35	291
App. in Disp.		8	2	12		18	17	1	6	64
CP Disp. P. Down		15	2	7	9		12	2	9	56
CP Disp. P. Up & Beat		11	3	11	3	10			12	50
Contact	1	1		2	1	1	1		1	8
Retreat		31	1	7		1	2			42

Table 3.26 Intra-individual (loser) transition matrix of fights between female *Necora puber*.

2^{nd} Act by Loser → 1^{st} Act by Loser ↓	Crouch	Stand	Move To	Move Away	App. in Disp.	CP Disp. P. Down	CP Disp. P. Up & Beat	Contact	Retreat
Crouch		59.1	22.7	13.6		4.6			
Stand	2.8		42.2	41.3	5.5	2.5	1.1	0.4	4.2
Move To	0.8	68.9		13.2	7.8	1.6	2.7	1.2	3.9
Move Away	0.3	80.8	3.4			3.1	0.3		12.0
App. in Disp.		12.5	3.1	18.8		28.1	26.6	1.6	9.4
CP Disp. P. Down		26.8	3.6	12.5	16.1		21.4	3.6	16.1
CP Disp. P. Up & Beat		22.0	6.0	22.0	6.0	20.0			24.0
Contact	12.5	12.5		25.0	12.5	12.5	12.5		12.5
Retreat		73.8	2.4	16.7		2.4	4.8		

Table 3.27 Percentages of different following acts used by losers in fights between female *Necora puber*.

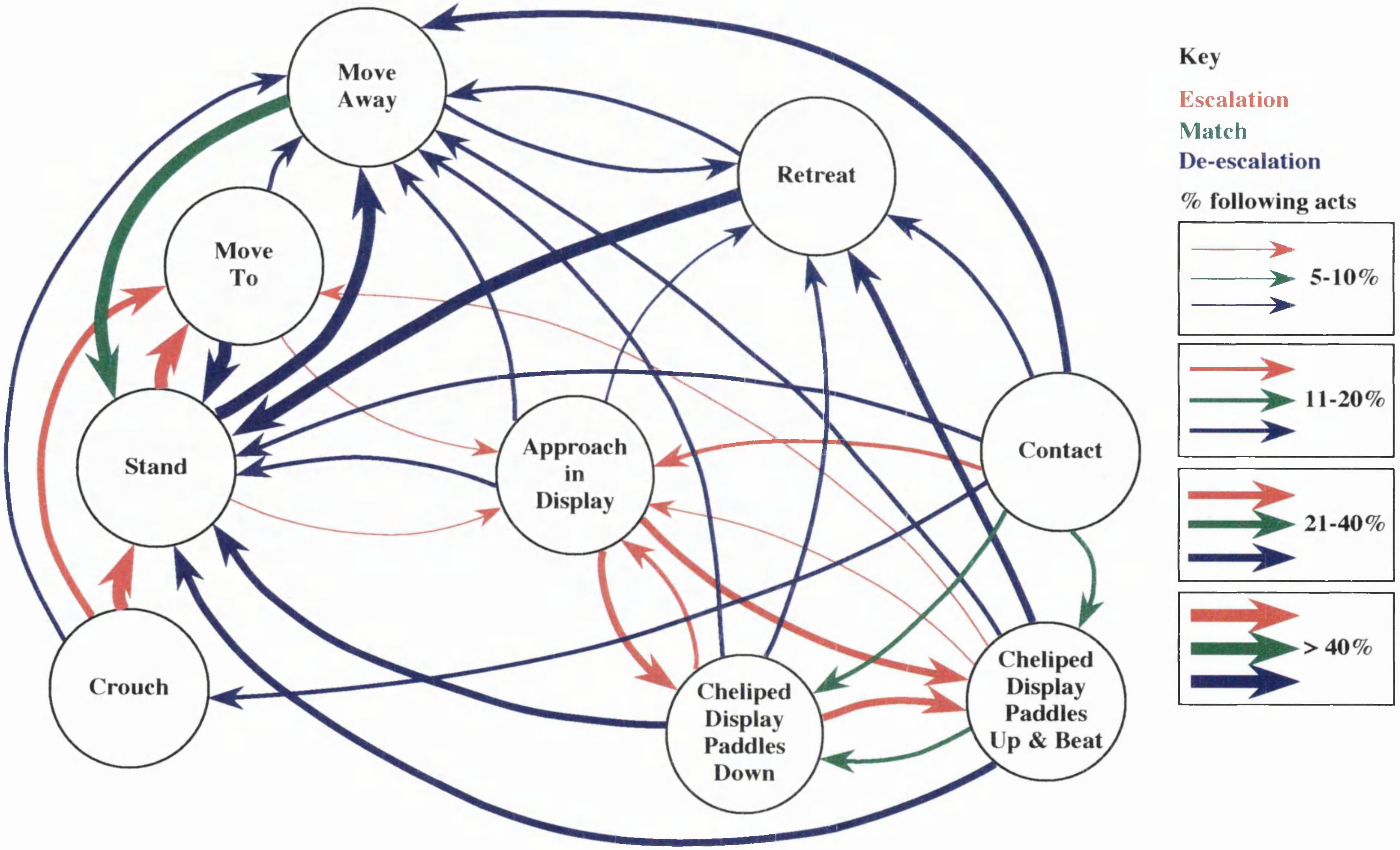


Figure 3.8 Intra-individual (loser) fight sequence diagram for fights between female *Necora puber*.

	Following Act by Loser					
Act by Loser	**D**	**M**	**E**	χ^2	**df**	*p*
Crouch	3	13	6	7.2	2	< 0.05
Stand	255	N/A	273	0.6	1	> 0.05
Move To	223	N/A	34	139.0	1	$<< 0.001$
Move Away	36	235	20	295.8	2	$<< 0.001$
App. in Disp.	26	2	46	39.3	2	$<< 0.001$
CP Disp. P. Down	31	N/A	26	0.4	1	> 0.05
CP Disp. P. Up & Beat	34	10	6	27.5	2	$<< 0.001$
Contact	5	2	1	3.2	2	> 0.05
Retreat	38	N/A	4	27.5	1	$<< 0.001$

Table 3.28 Results of the χ^2 tests on the condensed intra-individual (loser) transition matrix of fights between female *Necora puber*. Key: D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable. See table 3.1 for definitions of D, M & E acts for each preceding act.

3.3.2.6 Comparison of intra-individual (winner & loser) transition matrices

The results of the comparison between the condensed intra-individual (winner and loser) transition matrices are summarised in Table 3.29. Winner and losers did not show any significant deviation from expected values for acts following 'crouch', 'approach in display', 'contact', 'cheliped display, swimming paddles down' and 'retreat'. Both winners and losers followed 'stand' mainly with escalatory acts, but the tendency was greatest in the winners. Losers also tended to follow this act with de-escalatory acts. Winners and losers most frequently followed 'move to' with de-escalatory acts, but the tendency was strongest in the losers. Matching acts were most often used by winners and losers following 'move away', but losers also had a tendency to follow with de-escalatory acts. Winners and losers most commonly followed 'cheliped display, swimming paddles up & beat' with de-escalatory acts, though winners also had a strong tendency to follow with escalatory acts.

Overall there were fewer differences between winners and losers, but there was a tendency for winners to escalate after high risk acts whereas losers tended to de-escalate.

3.3.1 Comparison of males and females

Looking at the intra-individual transitions, in both males and females a short term escalation was obvious, with winners and losers tending to follow their own previous low risk acts with high risk acts. The process was, however, weaker and shorter lived in losers which tended to de-escalate after performing high risk acts. In contrast, winners tended to escalate or match following high risk acts. The tendency was weaker and shorter lived in females than in males.

From the inter-individual transitions it can be seen that in both males and females, winners and losers tended to match the behaviour of their opponent when this was in response to low risk acts. Winners and losers responded differently to high risk acts, with winners escalating or matching more frequently than losers. Again, the tendency was less pronounced in females than in males.

Act	Transition	D	M	E	χ^2	df	p	Explanation of results
Crouch	W-W	3	12	5	0.02	2	> 0.05	N/A
	L-L	3	13	6				
Stand	W-W	175	N/A	366	28.3	1	<< 0.001	E is the commonest action for both winners & losers following "Stand", but the tendency is stronger in winners. Losers have a greater tendency to adopt D.
	L-L	255		273				
Move To	W-W	278	N/A	72	5.6	1	< 0.02	D is the commonest following action for both winners & losers, but the tendency is strongest in losers.
	L-L	223		34				
Move Away	W-W	6	171	20	14.1	2	< 0.001	M is the commonest following action for both winners & losers, but losers have a stronger tendency to adopt D ($\chi^2 = 13.0, p < 0.001$).
	L-L	36	235	20				
App. in Disp.	W-W	38	4	71	0.2	2	> 0.05	N/A
	L-L	26	2	46				
CP Disp. P. Down	W-W	35	N/A	44	1.3	1	> 0.05	N/A
	L-L	31		26				
CP Disp. P. Up & Beat	W-W	18	8	15	8.1	2	< 0.02	D is the commonest following action for both winners & losers. Winners have a tendency to adopt E ($\chi^2 = 7.7, p < 0.01$).
	L-L	34	10	6				
Contact	W-W	6	6	1	0.9	2	> 0.05	N/A
	L-L	5	2	1				
Retreat	W-W	12	N/A	2	0.3	1	> 0.05	N/A
	L-L	38		4				

Table 3.29 Comparison of the condensed intra-individual (winner & loser) transition matrices for fights between female *Necora puber*. Key: W-W = winner-winner transitions; L-L = loser-loser transitions; D = de-escalatory acts; M = matching acts; E = escalatory acts; N/A = not applicable.

3.4 DISCUSSION

A short term escalatory process was apparent in the intra-individual sequences in fights involving males, with both winners and losers tending to follow their own previous low risk acts with high risk acts. However, this process was weaker and shorter-lived in losers, which tended to de-escalate after performing high risk acts; winners sustained high intensity fighting by matching or escalating following the performance of high risk acts. Key acts whose sequential relationship was different between winners and losers were those involving cheliped display, which winners followed with escalatory acts and losers tended to follow with matching or de-escalatory acts. Winners were more likely to re-initiate an interaction, as they tended to escalate again after their 'retreat'. In contrast, losers tended to continue de-escalating.

A similar tendency was observed in fights between females, with low risk acts leading into escalated acts, especially in the winners. Key actions were 'stand' after which winners tended to escalate whereas losers were equally likely to escalate or de-escalate; and cheliped display after which winners escalated or de-escalated equally often, and losers tended to de-escalate. Overall, the tendency to escalate was much less marked in the intra-individual sequences involving females.

Using the inter-individual sequences to examine the process whereby crabs react to the behaviour of their opponent, one clear trend was a tendency by both winners and losers to match the behaviour of their opponent when this involved low risk actions, such as 'crouch', 'stand' and 'move away'. Matching probably allows for easier assessment of relative RHP. Winners and losers responded differently to high risk acts such as cheliped display, with winners matching or escalating more frequently than losers. The same actions therefore elicit different responses when performed by a winner to a loser, by effecting a general reduction in the level of aggression, than when performed by a loser to a winner. A similar tendency was seen in the inter-individual sequences between females. This presumably reflects in part a difference in underlying motivational state and in part a difference in size or some other aspect of RHP on the stimulus effect of the same act performed by the two categories of crabs.

Behavioural acts have been used as variables for quantitative analyses in many ethological studies. The major components of agonistic acts in crustaceans are the position and movement of the chelipeds and other limbs, the elevation of the body, and orientation towards the opponent. In numerous crustacean species, the chelipeds were described as the main component of several agonistic acts (reviewed by Dingle 1983). The cheliped display may have several functions during an interaction. Raising the body off the ground and extending the chelipeds probably gives a visual indication of a crab's size to an opponent. When similarly sized crabs adopted a cheliped display in close proximity to one another and engaged in pushing bouts, they may have been able to use tactile cues to assess their relative sizes. Another possibility is that when the crabs are facing up to each other they may actually be measuring up the relative reach of their chelipeds. The communicative value of cheliped position and colouration have been demonstrated in several experimental studies (Hazlett 1972a,b,c, 1975c, 1979; Caldwell & Dingle 1976; Dunham 1978a,b, 1981). The raising of one or more walking legs during agonistic encounters is common (Warner 1977) but rarely involves the last pair of legs. In the frontal approach commonly used by hermit and spider crabs, a pair of legs, usually the 2nd or 3rd walking legs, may be raised together, which presents a cross-shaped posture to the recipient (Hazlett 1972 b,d). In crabs which approach sideways, the raised leg is usually directed towards the other crab and similarly the 2nd or 3rd walking legs are used. This occurs in the European hairy crab (*Pilumnus hirtellus*, Lobb 1972) and the mangrove crab (*Goniopsis*, Warner 1970).

Conspicuous postures and vigorous movements of the swimming paddles constitute a striking feature of agonistic behaviour in many brachyurans, including the swimming crabs (Portunidae). Due to the flattened nature of the dactylus (or paddle) which is held face onto an opponent, an increase in frontal area presented to an opponent is achieved, and an estimate of visual size is obtained by the opponent. Lobb (1972) suggested that leg raise postures may have arisen by evolution from an exploratory movement performed as a prelude to locomotion. Although the swimming paddle display in portunids involves different limbs, its origins may be similar. When disturbed *N. puber* and *Liocarcinus depurator* run/swim rapidly sideways over the substratum while beating their swimming paddles above their carapaces (pers. obs.), movements which are characteristic of swimming (Hartnoll 1971). It is quite possible, therefore, that swimming paddles provide extra propulsion, thereby enabling the crab to move more quickly over the substratum than running alone would permit. If this was the case, then raising the swimming paddles in readiness for escape when faced with a novel stimulus may have considerable adaptive significance. It is possible therefore that the swimming paddles raised posture shown by *N. puber* and *L. depurator* may have arisen from movements performed in readiness for rapid locomotion just as the walking leg raise has in other crabs (Lobb 1972).

Body elevation has been less investigated, although mentioned in many ethograms as a component of agonistic repertoires. In some studies, low or high body positions were defined as discrete behavioural acts (*e.g.*, Jachowski 1974; Jacoby 1981). Hazlett (1975b), using models, demonstrated in two species of xanthid crab that three levels of body elevation had a different effect on crab reaction. Glass &

Huntingford (1988) suggested that agonistic postures in *L. depurator*, including height and angle of the body, make actual size more evident, thus enabling assessment of relative size. Barki *et al.* (1991) found that the participants in a fight changed their body elevation during a contest. In *N. puber*, winners of fights between males responded to low body elevation ('cheliped display, swimming paddles down') with escalatory and matching acts, whereas losers responded to the same act with escalatory and de-escalatory acts. In response to high body elevation ('cheliped display, swimming paddles up & beat'), winners either to matched or escalated, whereas losers were equally likely to escalate or de-escalate. In females, winners responded to low body elevation by adopting more escalatory or de-escalatory acts when compared to losers. Losers mainly de-escalated in response to high body elevation whereas winners mostly matched or escalated.

Body orientation during crustacean agonistic behaviour has so far not been studied in detail. In *Callinectes sapidus* orientation during contests was analysed but without reference to winning or losing (Jachowski 1974). Barki *et al.* (1991) found that the frequency of "approach to a frontal position" and "approach to a non-frontal position" differed between winners and losers. As for body elevation, the orientation of the two interactants also changed during contests (Barki *et al.* 1991). An escalated fight occurred only when the two prawns faced each other (Barki *et al.* 1991). The resolution of the fight was marked by a change in the orientation of the loser to a non-frontal position. In the present study, winners of fights between males responded to non-frontal movement towards them (*i.e.*, 'move to') and frontal movement towards them (*i.e.*, 'approach in display') with escalatory acts. Losers also adopted escalatory acts in response to frontal or non-frontal movement, but they also adopted de-escalatory acts in response to frontal movement. In females, winners responded to non-frontal movement by losers with de-escalatory acts, but did not exhibit any particular response to frontal movement. Losers, in contrast, responded to both non-frontal and frontal movement with de-escalatory acts.

The production of strong respiratory currents during agonistic behaviour has been noted in some crustaceans. Jachowski (1974) observed that *Callinectes sapidus* produced a strong jet of water directed at the opponent during agonistic encounters and suggested that this might be a tactile or olfactory stimulus. The same phenomenon has been noted in the river crab *Potamon potamios* (Erpenbeck & Altevogt 1966), the crayfish *Orconectes virilis* (Rubenstien & Hazlett 1974) and several hermit crab species (Barron & Hazlett 1989). Barron & Hazlett (1989) observed that hermit crabs produced currents directed downwards during agonistic interactions, compared with normally upwardly directed respiratory currents. This, and the fact that production of a downwards current by a hermit crab often resulted in the retreat of its opponent, led Barron & Hazlett (1989) to suggest that these currents were hydrodynamic displays. The intertidal crab, *Grapsus grapsus*, aims jets of water from nephridial pores at opponents (Kramer 1967). When *N. puber* engage in bilateral display and push each other, the exhalent channels and attenules - which have a chemosensory function (Gleeson 1980) - of the two crabs are in close proximity. It is therefore likely that crabs are able to detect the current produced produced by their opponents. If the strength of the ventilatory currents reflects the size and fitness of crabs, then it is possible that these are used to assess RHP. In addition, it is probable that any olfactory stimulus carried in the ventilatory current of one crab would be detected by its opponent. These suggestions, however, remain speculative. A rapid respiratory rate in response to intensive activity would inevitably result in a strong jet of water from the scaphognathites. The mouth parts of *N. puber* beat rapidly during agonistic behaviour (pers. obs) and it is probable that a reasonably strong current is produced, which may serve as some kind of RHP signal to opponents.

During agonistic interactions, there was a tendency for the intensity of agonistic acts to increase, albeit in the short term. For example, contact was generally preceded by non-contact display. However, often there was not a sequential progression of acts of increasing intensity: crabs often reverted to from high to low risk acts during the course of an interaction. These complexities in the structure of interactions mean that the assumptions of the sequential assessment game (Enquist & Leimar 1983) were not valid for either males or females, although the predictions from that model and others (Parker 1974; Maynard Smith & Parker 1976; Hammerstein & Parker 1982) of longer, more intense interactions between closely matched crabs was supported in males (Smith *et al.* 1994) though not in females (chapter 2).

In *N. puber* there is sexual dimorphism in cheliped size and males are more active than females (Smith 1990), particularly ovigerous females (Choy 1986). Jacoby (1983) found that large male *Cancer magister*, which had proportionately larger and more powerful chelipeds than females or juveniles, used fewer potentially injurious agonistic acts. Possession of less dangerous weapons reduces the potential costs of escalated interactions. Application of game theory to such situations gives rise to predictions that escalated interactions should occur more often and over less valuable resources (Maynard Smith 1982a) and the process of escalation should be shorter.

During the initial stages of an agonistic interaction, an exchange of cheliped displays allows each crab to assess the relative RHP of the other. One crab, presumably with an assessment of superior RHP, initiates the interaction, however, the initial assessment will change as further infomation is gained during the fight, and perhaps confirm or enhance the initiator's perception of superiority in RHP (Enquist & Leimar 1983). Assessment will continue throughout the fight, not only visually but by tactile means during the pushing bouts and wrestling sessions which may follow. An assessment strategy implies that the animal attempts to predict the cost of a fight (without actually incurring that cost) (Parker 1984) so that the information gained will alter motivational state.

Functional models of agonistic behaviour predict that the important features that determine fight duration and intensity are the value of the resource (*V*) and the likely cost of the fight (*K*) as assessed by each opponent (Parker 1974; Maynard Smith & Parker 1976; Enquist & Leimar 1983, 1987). M^{c}Farland & Houston (1981) proposed bringing these two changing estimates into a single motivational state space. The motivational model of Maynard Smith & Riechert (1984) assumed that conflicting tendencies for attack and fear produced a variety of outcomes according to their relative levels. The axes in this model were were labelled 'attack' and 'fear' but the terms were ones of convenience (Riechert & Maynard Smith 1989), and can be substituted for *V* and *K*. A similar model can be applied to contests between *N. puber*, as shown below in Figure 3.9. The axes are the tendency for attack and the tendency for retreat. The former is determined by information available to each crab about the benefits to be gained from winning the interaction, and the latter by the relative RHP of the opponents and the accuracy of the estimation.

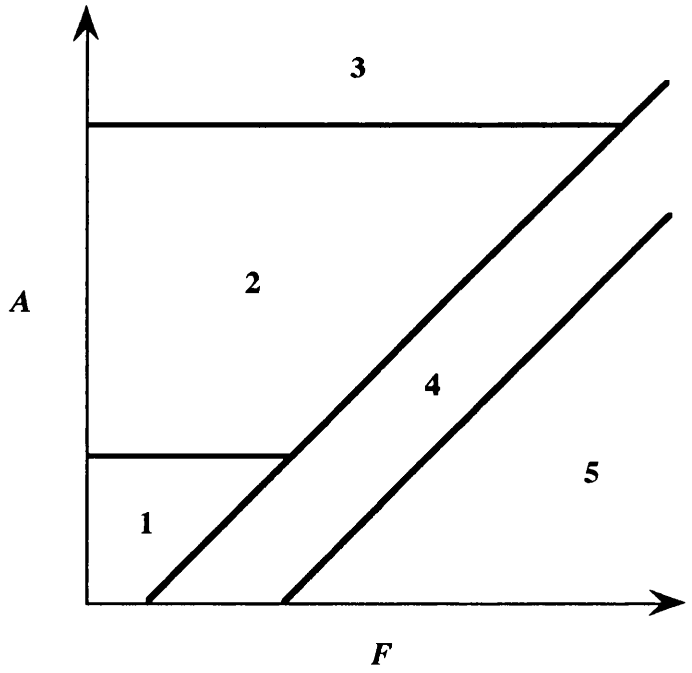


Figure 3.9 Graphical representation of the interaction between the tendencies for attack (*A*) and fear (*F*) in *Necora puber*, to produce a series of agonistic acts differing in motivational state. Key to zones:- 1 = Approach; 2 = Display; 3 = Physical Attack; 4 = Temporary Withdrawal; 5 = Retreat.

The model assumes that the behaviour during fights is controlled by two independent motivational systems, attack (*A*) and fear (*F*). Below a certain relative level of aggression a crab will retreat permanently (zone 5), while at intermediate levels of attack and fear tendencies, a crab may withdraw temporarily (zone 4). Above a certain level of aggression (zone 2), the behaviour of the crab depends on the absolute level of *A*, with behaviour ranging from low risk displays to contact display and pushing bouts. If the tendency for aggression becomes sufficiently large relative to the tendency for fear, then a crab will physically attack its opponent (zone 3).

In some of the fights involving physical attack, strikes and grasps were preceded by a gradual progression of approach to non-contact display, through displays in which the crabs pushed against each other, finally to displays with the swimming paddles raised and then beating, apparently to make the push more powerful. Where this was the case, there was often a return to non-contact agonistic acts, including sometimes the temporary withdrawal of the eventual winner, before the resolution of the contest. In other fights, physical attack was not preceded by this sequence of acts, and occasionally the crabs launched straight into overt physical attack without any display. This is unusual since interactions are thought usually to follow a process of gradual escalation, with, resolution being preceded by the highest risk acts (Enquist & Leimar 1983, 1987; Enquist *et al.* 1990). The short term and non-gradual nature of the escalation process in fights between *N. puber* is taken into account by the model. The levels of motivation for attack and retreat in the crabs may change rapidly and irregularly during the course of an interaction, *e.g.*, from zone 1 to zone 3, and the behaviour of the crabs may move rapidly between high and low risk agonistic acts. The escalation process in fights between *N. puber* is in contrast to the territorial disputes between *Agelenopsis aperta*, where the fights go through a gradual process of escalation from locating movements performed at a distance, through threats performed closer together to intense contact fighting (Maynard Smith & Riechert 1984).

The model assumes that the estimate of *F* to be relatively accurate. However, this may not always be the case and the estimate *F* is revised as more information becomes available. This is the situation modelled in the sequential assessment game (Enquist & Leimar 1983, 1987). The motivational model of Maynard Smith & Riechert (1984) and also the sequential assessment game (Enquist & Leimar 1983, 1987), both studies originating in the functional framework of game theory, have generated causal explanations of the process of escalation in animal fights. By focusing on the interaction of the costs and benefits of fighting and the way they can influence decisions, game theory has precipitated studies on the behavioural mechanisms that determine how animals behave during fights.

CHAPTER FOUR

METABOLIC CONSEQUENCES OF AGONISTIC BEHAVIOUR AND EXERCISE IN MALE *NECORA PUBER*

4.1 INTRODUCTION

4.1.1 Costs of agonistic behaviour

Predictions about animal behaviour arising from optimisation models, such as evolutionary game theory, are based on the assumption that behavioural phenotypes evolve through selection for those that maximise the fitness of the animal. The overall change in fitness due to a particular behaviour or activity is a combination of beneficial and deleterious effects, or costs and benefits (Maynard Smith 1982a). Interpretation of agonistic behaviour on this basis therefore requires an understanding of the nature of the costs and benefits accrued by competitors. It is often impossible to measure directly the changes in lifetime reproductive success due to a particular behaviour, but the short term consequences of behaviour can be recorded and their probable effects on fitness estimated. For example, injury and death, which are obvious risks of fighting, and enhanced predation on animals engaged in fights and displays, are quantifiable consequences of behaviour and have a directly adverse influence on the reproductive output of an animal. 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 or reproduction. Animals often grow structures, such as antlers or horns, for use as weapons, and develop protective structures such as manes or fat deposits (Lott 1979). That this is costly is suggested by the fact that such structures are sometimes lost when they are no longer required, *e.g.*, the antlers of deer. The more aggressive an animal is, the more benefits it may gain, but if an animal is too aggressive it might face unacceptably high costs (for example, serious injury) so the animal must weigh up the relative costs and benefits of its actions and choose an optimal level of aggression, *i.e.*, maximise the benefits. If the costs are too high and the benefits too low, avoiding a fight may be preferable to competing. In other cases it may be worth fighting vigorously for a valuable resource.

Animals store energy in organic compounds. The cost of an activity can therefore also be considered as the resulting net reduction in these stores. The energetic cost of behaviour may also have consequences for the fitness of an animal. Energy spent on agonistic behaviour is not available for feeding or reproduction and therefore represents a reduction in fitness. Additionally, depletion of energy reserves and accumulation of certain metabolites, such as lactate, may limit 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 escaping from a predator, is required (Ellington 1983).

4.1.2 Measurement of the physiological costs associated with behaviours

It is usually impossible to measure directly the energy expenditure associated with a particular activity. When an activity is performed using aerobic metabolism by an animal in isolation, the energetic cost may be estimated by measuring oxygen consumption in a respirometer. This approach has been used to estimate the energetic costs of social behaviour in some amphibians (Taigen & Wells 1985; Prestwich *et al.* 1989; Pough & Taigen 1990). Respirometry, however, does not incorporate the measurement of any anaerobic component of energetic expenditure, and is of limited use in studying behaviour that occurs when two animals are in close proximity. Recently, whole-body calorimetry has been used to determine the energetics of different behaviours (Gnaiger 1980; Shick *et al.* 1988; Byrne *et al.* 1990; Hill *et al.* 1991a). The advent of calorimeters capable of measuring heat fluxes enables the quantification of total heat dissipation in air and water (Pamatmat 1983). The coordinated measurement of both oxygen consumption and heat produced enables partitioning of the latter into its aerobic and anaerobic components. The doubly-labelled water technique for measuring carbon dioxide production has been used to estimate energy expenditure in birds and mammals, but the temporal resolution of this method is insufficiently fine to estimate the costs of individual behaviour patterns (Tatner & Bryant 1989). In some studies, the rate of energy expenditure during an activity measured in the laboratory has been used to estimate the cost of behaviour involving that activity in the animal's natural habitat (*e.g.*, Gill & Wolf 1975). Physiological correlates of oxygen consumption, such as heart rate, have been used to study behavioural energetics in the field (Priede & Young 1977; Armstrong *et al.* 1989). Wolcott & Hines (1989) employed ultrasonic biotelemetry of muscle activity to study foraging in free-ranging blue crabs, *Callinectes sapidus.*

4.1.3 Physiological consequences of various activities in non-crustacean species

4.1.3.1 Anuran amphibians

Investigations of the natural activities of poikilothermic vertebrates have revealed that, though the behaviour of these animals is often surprisingly complex, the energetic costs of most activities are quite low, especially in comparison with mammalian or avian energetics, *e.g.*, the metabolic rates of salamanders engaged in courtship behaviour are barely elevated above resting values (Bennett & Houck 1983). Even continuous, active foraging by lizards at high body temperatures entails only moderate rates of oxygen consumption that are less than 4 times resting metabolism (Bennet & Gleeson 1979). In contrast to these results, studies of anuran vocal behaviour indicate that the energetic cost of calling of male frogs to attract females can be very high, as great as 25 times resting rates (Taigen & Wells 1985). Anuran reproductive activities, especially calling to attract mates, are energetically demanding, suggesting that the behaviour of male frogs at a breeding pond may be limited by their metabolic capacities. In a study of reproductive behaviour and aerobic capacities of male American toads (*Bufo americanus*), Wells & Taigen (1984) tested the hypothesis that individual variation in calling and searching behaviour by males in a chorus were correlated with differences in aerobic capacity. Toads were observed on the night of peak breeding activity, followed by measurements of aerobic capacity during forced activity. The toads displayed significant variation among individuals in the amount of movement, calling and number of clasping attempts (Wells & Taigen 1984). Males also differed in aerobic capacity, but aerobic capacity was not correlated with any behavioural variable. Wells & Taigen (1984) therefore concluded that the differences in behaviour of males in a chorus were not due to differences in their physiological capacities to use energy.

In addition to documenting the high cost of calling, previous analyses of vocalisation energetics in the grey tree frog (*Hyla versicolor*) produced an unexpected result - rates of oxygen consumption while calling were considerably higher than those attained during vigorous locomotor exercise (Taigen & Wells 1985). These data were not consistent with many previous studies of anuran exercise physiology in which oxygen consumption during forced locomotion was assumed to represent the maximum metabolic rate the animal can sustain (Taigen & Pough 1981; Taigen *et al.* 1982; Taigen & Beuchat 1984). An explanation suggested by Taigen & Wells (1985) for these unusual data was that vocalisation and locomotion involved the use of muscles with different oxidative capacities. Understanding anuran vocalisation indicates that the internal and external oblique muscles (located in the trunk) are used to create the pressure in the thoracic cavity necessary for sound production (Martin & Gans 1972; Gans 1973). High metabolic rates in calling frogs may be the result of sustained intense contraction of large trunk muscles with high catabolic enzyme activities. Taigen *et al.* (1985) therefore investigated the energetics of sound production in the spring peeper (*Hyla crucifer*), together with the size and enzyme profiles of the muscles used in calling. They found that male trunk muscles were very large, accounting for 15% of total body mass. Citrate synthase activity in male trunk muscle, indicative of oxidative capacity, was 6 times the citrate synthase activity in leg muscle, and 17 times the citrate synthase activity in female trunk muscle. Prestwich *et al.* (1989) examined lactate accumulations in the whole body, legs and remainder of the body (trunk) in *Hyla squirella.* Lactate accumulation increased significantly over resting values in the legs and whole body as a result of forced exercise. Trunk lactate levels did not increase significantly during calling but did during forced hopping. Thus it appears that anaerobic metabolism was not a significant factor in sustained calling whereas it was in sustained, maximal hopping. The use of different groups of muscles for calling and locomotion also explains the lack of a correlation between calling and searching behaviour and aerobic capacity in male *Bufo americanus* (Wells & Taigen 1984), since the correlation was calculated using data for aerobic capacity derived from forced locomotory activity and not calling.

4.1.3.2 Birds

Current models of sexual selection involving female choice presume that male advertisement generates increased costs for males performing that advertisement. Two major classes of costs have been proposed: predation risks (Ryan *et al.* 1982; Marler & Moore 1988) and physiological trade-offs (Halliday 1987; Partridge & Endler 1987). Vehrencamp *et al.* (1989) designed a study to investigate whether display performance in male sage grouse (*Centrocercus urophasianus*) was limited by energetic constraints. The sage grouse is a classical lek-breeding species with extreme behavioural and morphological differentiation between the sexes and clear female choice of mates. Approximately 10% of the males on a lek obtain 80% of the matings, and the best predictor of male success in this species is a ritualised display behaviour, the strut, rather than morphological traits (Gibson & Bradbury 1985). Successful males tend to be those with high daily attendance rates on the same lek, high strut rates and certain acoustical properties in their strut performance. Overall predation rates on males are low and there are no significant correlations between male success and risk of predation on the lek (Bradbury *et al.* 1989).

That display by males is energetically costly is indicated by the following points. First, males lose mass during the display season (Beck & Braun 1978). Second, males appear to tire during the morning display sessions, as strut rate declines linearly over time independently of female attendance (Gibson & Bradbury 1985). Third, male attendance on leks is reduced on days after exceptionally low ambient temperatures, suggesting that increased thermoregulatory activity may deplete reserves needed for display (Bradbury *et al.* 1989). Finally, the types of cues preferred by females (stereotyped production of certain sounds, lek attendance time and strut rate) all require continuous energy expenditure, rather than one-time, pre-season or maturational costs, such as growth of vocal sacs or plumage. A continuous high energy expenditure during the breeding season might be expected to reduce future mating success or survival by causing weight loss, general loss of condition, increased parasite loads and/or increased predation off the lek due to longer foraging bouts or weaker condition.

Vehrencamp *et al.* (1989) measured the energetic expenditure of 18 displaying male sage grouse in the field using the doubly-labelled water technique (Tatner & Bryant 1989). Vehrencamp *et al.* (1989) found that the daily energy expenditure (DEE) increased significantly with increased display rate, increased time spent on the lek, and decreased ambient temperature. DEE for the most vigorously displaying males was twice that of non-displaying males, and four times higher than basal metabolic rate (BMR). Vehrencamp *et al.* (1989) estimated the instantaneous rate of energy expenditure during display to be 13.9-17.4 times BMR. The effort devoted to display differed markedly among males and was correlated with certain other male characteristics. Males that attended leks were in better condition (higher body weight in relation to size) than non-attenders, but among lek-attenders condition was negatively correlated with increased display effort. Active displayers lost less weight per day and foraged further from the lek than less active males, suggesting that differences in foraging and food intake affected daily energy output.

4.1.4 Crustacean metabolism

A prerequisite for life is a continual supply of energy in the form of adenosine triphosphate (ATP) molecules. Until the mid-1940s it was believed that, in multicellular tissues, energy was in general, produced only by aerobic metabolic pathways. It has become apparent, however, that at certain times, some animals are unable to meet their energy demands solely by the complete oxidation of carbohydrates, fats and proteins to carbon dioxide and water. At such times, animals have to rely partly or totally on anaerobic metabolism.

The two functions of anaerobic metabolism are:- to produce energy; and, to maintain the redox balance within a cell. In the normal aerobic glycolytic pathway, glyceraldehyde-3-phosphate is oxidised with the concomitant reduction of the co-factor NAD^+ and then phosphorylated to 1,3-diphosphoglycerate. If glycolysis is to be maintained, the resulting NADH must be constantly reoxidised. Under aerobic conditions the reoxidation of the NADH by the mitochondrial electron transport chain is not a problem, but where oxygen is limited, alternative mechanisms are needed. Therefore a central feature of all anaerobic pathways is their capacity to reoxidise NADH, thereby maintaining the redox balance.

Amongst air-breathing animals, anaerobic metabolism is usually confined to particular tissues that have been unable to meet, by aerobic metabolism, a transient increase in energy demand. This often occurs in actively contracting muscle fibres, in which oxygen demand exceeds the rate of oxygen delivery, and is termed "functional anaerobiosis". Aquatic animals experience anoxia more often than air-breathers since water contains far less oxygen per unit volume than air and the oxygen diffuses more slowly. Aquatic animals are, therefore, periodically exposed to hypoxic conditions that are potentially very serious, and may result in what is called "environmental anaerobiosis".

Crustaceans use anaerobic energy metabolism under a variety of physiological and ecological circumstances. Environmental anoxia can occur during exposure to air at low tides, during burrowing in reducing substrata, or in other situations where environmental oxygen concentrations are insufficient to support aerobic energy metabolism. In addition to anoxia at the organism level, specific tissues may become anoxic even though the whole organism itself remains at normoxic levels. The respiratory system of crustaceans is potentially limited in its ability to supply respiring tissues with oxygen, due to the relative impermeability of the chitinous exoskeleton to gaseous exchange and the low oxygen carrying capacity of the circulatory system (Taylor 1982). During functional anaerobiosis, such as burst contractile activity in muscles, oxygen concentrations fall, due to the inadequacy of the circulatory system. Under these conditions the muscles operate under anoxic conditions.

The biochemical pathways by which crustaceans derive ATP appear similar to those of vertebrates in which these processes have been more extensively studied (Chang & O' Connor 1983). In the past, several studies have dealt with the influence of anoxia and hypoxia on the metabolism of crustaceans, particularly decapods (Uglow 1973; Taylor 1976, 1977; Burke 1979; Whyte *et al.* 1986; Johnson & Rees 1988; Greenaway *et al.* 1988; Hill *et al.* 1991a). As most members of this group are capable of rapid swimming, they may not actually be confronted with environmental anaerobiosis. It is more likely, however, that intensive muscular work may cause functional anaerobiosis, *i.e.*, the necessary production of energy is derived largely or even exclusively by mechanisms not requiring oxygen. More recently research has concentrated on exercise physiology (Houlihan *et al.* 1984, 1985; Forster *et al*, 1989; van Aardt 1990, 1991; Hamilton & Houlihan 1992). The exercise regimes in these studies have differed widely. In most studies of aquatic species, movement has been induced by prodding the animals, either for a specified period of time or until exhaustion, which is defined as a state of unresponsiveness to tactile stimulation or loss of a righting response. *Callinectes sapidus* were made to swim for up to an hour by suspending them above the substratum (Booth *et al.* 1982). In studies on terrestrial or semi-terrestrial crabs it has been possible to control the intensity of exercise more carefully by "walking" the animals on a treadmill. Hamilton & Houlihan (1992) recently developed an aquatic version of the treadmill.

Not surprisingly, the use of different species, varying experimental conditions and exercise regimes employed in these studies have produced a variety of respiratory responses. These range from a predominantly aerobic response to sustained exercise (*Ocypode gaudichaudii*, Full & Herreid 1983) to the primarily anaerobic escape response in macrurous decapods (*Cherax destructor*, England & Baldwin 1983; *Orconectes limosus*, Gäde 1984). The aerobic response is characterised by a rapid rise in the rate of oxygen consumption at the onset of exercise to a steady state which is maintained throughout the period of exercise and which may be several times the resting rate. On cessation of exercise there is a return of the rate of oxygen consumption to resting values.

A variety of processes take place during the recovery phase after anaerobic metabolism. In the first place, the arginine phosphate and ATP pools, depleted during anoxia, are typically replaced. Second, end products of metabolism are cleared from the tissues, either by excretion into the external environment, or by oxidation of substrates by aerobic metabolism, or by conversion back to anaerobic substrates. Finally, a number of other adjustments probably take place during recovery. For instance, intracellular pH, reduced by the accumulation of acidic end products, must return to the preanoxic value. An organismal manifestation of all of the above, essentially cellular, processes is the oxygen debt phenomenon. That is, during recovery from anoxia there is usually a period of supranormal oxygen consumption.

L-lactate is the major end product of anaerobic metabolism in crustaceans (Morris & Greenaway 1989; Hill *et al.* 1991a). Fumarate and alanine also accumulate during anoxia, but the amounts accumulated indicate that alternative metabolic pathways are of only very limited importance (Hill *et al.* 1991a). L-lactate is produced via the glycolytic pathway by the anaerobic reduction of pyruvate catalysed by L-lactate dehydrogenase (LDH) (Gäde & Grieshaber 1986; Lallier & Walsh 1992). L-lactate production takes place at a high rate in muscle fibres during exhaustive exercise, and it is released to the haemolymph (M^{c}Donald *et al.* 1979; Booth *et al.* 1984; Booth & M^{c}Mahon 1985). The routes of its elimination or reprocessing during recovery from exhaustive activity or hypoxia, however, are not known (Gäde & Grieshaber 1986). Direct excretion of L-lactate does not seem to occur (Bridges & Brand 1980; Gäde *et al.* 1986; Hill *et al.* 1991b), but the exact pathway used - that is, oxidation or gluconeogenesis - and the tissues involved are still a matter of controversy (Ellington 1983). So far, most studies have detected L-lactate conversion by both pathways in crustaceans, but on an organism level (Phillips *et al.* 1977; Gäde *et al.* 1986; van Aardt 1988). By following the fate of labelled L-lactate, these workers have found labelled carbon dioxide, glycogen and D-glucose in the blood, but they have been unable to locate the source of these products. Several tissues have been put forward as sites of possible gluconeogenesis in crustaceans:- hepatopancreas (Munday & Poat 1971), gills (Thabrew *et al.* 1971), and haemocytes (Johnston *et al.* 1971). Some authors have claimed that the hepatopancreas of decapod crustaceans does not have hepatic functions at all (van Weel 1974; Phillips *et al.* 1977). Lallier & Walsh (1992) investigated the LDH activity of *Callinectes sapidus* hepatopancreas cells *in vitro*, and concluded that the hepatopancreas plays a limited role in the initial post-activity metabolism of L-lactate. Lallier & Walsh (1992) suggest that the dark mitochondria-rich fibres found in the muscles and gills maybe the site of initial L-lactate metabolism.

The duration and effectiveness of the recovery process is of great functional importance. For instance, intertidal species may regularly be exposed to air. Thus, the recovery from anaerobic metabolism must be sufficiently rapid and complete for the organism to cope with the next period of exposure to air. In the case of muscles powering escape responses, this process of recovery must be very effective to allow the organism to evade additional predators. Similarly, the metabolic or energetic consequences of agonistic behaviour may place constraints on the subsequent behaviours an animal may perform, thus rendering the animal temporarily susceptible to predation or unable to forage.

4.1.5 Responses of brachyuran crabs to exercise

The migratory portunid, *Callinectes sapidus*, is the aquatic decapod studied so far which is most capable of sustained exercise in the laboratory (Booth *et al.* 1982; Booth *et al.* 1984; Houlihan *et al.* 1985). This species also attained a stable rate of oxygen consumption quite quickly (≈ 30 seconds). Despite these aerobic characteristics, anaerobic metabolism played a significant role in energy production during exercise, as there was a 14-fold increase in haemolymph L-lactate concentration (Booth *et al.* 1982). Booth *et al.* (1982) reported that the rate of oxygen consumption was still increased 30 minutes after exercise and that return to resting levels took several hours. Subsequent analysis of the recovery period indicated that L-lactate levels took 9 to 10 hours to return to pre-exercise values (Booth *et al.* 1984). *Carcinus maenas* also reached a steady state of oxygen consumption whilst exercised at 5.8 ms^{-1} for 5 minutes (Hamilton & Houlihan 1992), and had a relatively quick recovery from exercise (8 to 25 minutes) (Houlihan *et al.* 1984). The amount of L-lactate accumulated during 5 minutes of activity at this rate suggested that *Carcinus maenas* was approaching burst speed (Hamilton & Houlihan 1992).

In contrast, most other decapods studied so far have a limited aerobic capacity and are only able to raise their rates of oxygen consumption to 3 to 5 times the resting rate (McMahon 1981). A steady state of oxygen consumption is never reached even during mild exercise and fatigue times are short. These species rely on stored oxygen and arginine phosphates, and on anaerobic metabolism, accumulating large quantities of L-lactate during short periods of activity. Recovery from exercise in these species is characterised by a period of increased oxygen consumption which is long in comparison with the exercise period. L-lactate concentrations may also take many hours to decline. This response to exercise has been recorded from *Cancer magister* (McDonald *et al.* 1979; McMahon *et al.* 1979), *Cardisoma guanhumi* (Herreid *et al.* 1979), *Cardisoma carnifex* (Wood & Randall 1981) and *Gecarcinus lateralis* (Herreid *et al.* 1983).

The reasons why some species of crustaceans show markedly different physiological responses to sub-maximal exercise remain unclear. One contributory factor may be whether the animal relies upon rapid neurophysiological or much slower acting humoral mechanisms to initiate the ventilatory responses during activity (Herreid 1981). The oxygen diffusion rate across the gills will also be a limiting factor during activity. The amount of oxygen that can diffuse into the haemolymph will be a function of the magnitude of the oxygen diffusion gradient, the area available for diffusion, the diffusion pathlength and the barrier to diffusion created by the physical structure of the gaseous exchange apparatus. *Callinectes sapidus* has a relatively large gill area (Gray 1957) and a short diffusion pathlength (Aldridge & Cameron 1979) compared with many other species of crabs (Gray 1957), including *Carcinus maenas* (Butler 1976; Taylor & Butler 1978). This may explain why *C. sapidus* is capable of sustained activity in the laboratory. *C. sapidus* is an active benthic forager (Williams 1984) and is capable of impressive feats of high speed sideways swimming ($\geq 1 ms^{-1}$, Spirito 1972) and long distance migrations (Judy & Dudley 1970). The mode of locomotion used for migration is not known, but it seems likely that walking, running and swimming are all used.

4.1.6 Physiological consequences of fighting in *Necora puber*

Aquatic crustaceans have a limited capacity for aerobic activity, and recovery from anaerobic metabolism is prolonged in most species studied (M^cMahon 1981, Ellington 1983). The energetic and metabolic cost of behaviour may therefore be influential in determining the strategies used by aquatic crustaceans in agonistic behaviour, particularly when the risk of serious injury is low. The costs of agonistic behaviour in crustaceans have been analysed in terms of the duration of contests and the risk of injury (Berzins & Caldwell 1983; Dingle 1983; Steger & Caldwell 1983; Glass & Huntingford 1988; Shuster & Caldwell 1989; Adams & Caldwell 1990), but there is little information on the energetic and metabolic costs of this behaviour.

Smith & Taylor (1993) examined the energetic cost of agonistic behaviour in male *Necora puber*. They found a linear relationship between the rate of oxygen consumption and the rate of beating of the ventilatory pumps (scaphognathites). An irregular pattern of scaphognathite activity was recorded during agonistic behaviour, incorporating periods of extreme hyperventilation as well as apnoea (cessation of beating of the scaphognathites). Maximum scaphognathite rates were amongst the highest reported for a range of similar decapod crustaceans. During agonistic behaviour, the respiratory activity of the interactants was related both to the degree of escalation and to the duration of the interaction. Recovery was prolonged in some cases. There was no significant difference between the estimates of energy expenditure of winners and losers. As injuries are rare, energy expenditure may account for a large proportion of the total cost of agonistic behaviour in this crab.

4.1.7 Aims

Smith & Taylor (1993) did not, however, investigate the metabolic consequences of fighting or exercise in male *N. puber*, and so the aim of the work presented in this chapter was to quantify further the metabolic consequences of agonistic behaviour in winners and losers of fights between male *Necora puber* by measuring the concentrations of L-lactate and D-glucose in the haemolymph, and L-lactate, D-glucose, and glycogen in the walking leg muscle. The metabolic effects of agonistic behaviour were compared with those of forced exercise and of very limited activity. Finally, the metabolic events in different tissue types during recovery from forced exercise were investigated. Information about the metabolic events in different body compartments is needed to understand fully the implications of metabolite accumulation/depletion for future behavioural potential.

4.2 MATERIALS & METHODS

4.2.1 Experimental animals

Male *Necora puber* were supplied by a shellfish exporter on Benbecula, Western Isles, in September, October and December 1992. On arrival in Glasgow the crabs were transferred into individual holding tanks as described in section 2.2.1. The crabs were kept in these conditions for at least 7 days prior to any experimental work. The crabs were fed every 3 days with whitebait prior to the experiment, but were deprived of food for 7 days before haemolymph or tissue samples were taken. This was done in order to reduce variation in metabolite concentrations between individuals (Hill 1989). Only animals without missing or recently regenerated legs, and without excessive epifaunal growth, were used in experiments.

4.2.2 Experimental development

The enzymatic assays described below took several months of preliminary work until the methods were considered accurate and reliable to be used on experimental samples.

4.2.3 Experimental design

4.2.3.1 Metabolic consequences of agonistic behaviour

The September batch of crabs were allocated into 4 groups and treated as follows:-

1) **Resting crabs** - these crabs were removed from their individual holding tanks and haemolymph samples were immediately taken, by piercing the arthrodial membrane at the base of the 3rd walking leg with a hypodermic needle (21G) attached to a syringe (1ml) and treated as described below in section 4.2.4.1. Samples were taken as quickly as possible so as to cause minimum disturbance to the crabs.

2) **Partition raised crabs** - these crabs were placed in the observation tank described in section 2.2.2, and allowed 24 h to recover. The separating partition in the tank was then raised and immediately lowered. Haemolymph samples were taken immediately as above.

3) **Exercised crabs** - crabs were individually placed in a tank and exercised by a combination of tapping them on the carapace with a glass rod, causing them to run or swim, or by turning them on their backs, forcing them to right themselves, until the crab was unwilling to move further. This generally took between 3 and 7 minutes.

Haemolymph samples were immediately taken as above.

4) Fought crabs - crabs, matched in size to the nearest mm, were placed in a glass observation tank, as described in section 2.2.2. The crabs were allowed a 24 h recovery period, after which time the partition separating the crabs was raised and they were allowed to fight. The actions of both crabs were recorded using an Epson HX 120 laptop computer programmed as an event recorder. Once the fight had been resolved the partition was lowered, and haemolymph samples were taken immediately as above.

All haemolymph samples were treated as described in section 4.2.4.1.

All crabs were kept for a further 2 weeks to make sure none were in proecdysis (Stevenson 1985), which may have an effect on aggressive motivation. This was unlikely at that time of year, and none moulted in this time. All crabs were then set free into the Clyde Sea at Farland Head.

The October batch of crabs was allocated into experimental groups as above. This time, however, tissue samples in the form of walking leg muscle were taken. Crabs were killed by semi-submerging them, "headfirst", in liquid nitrogen (N_2). The largest walking legs were removed and snap frozen by submerging them in liquid N_2. Samples were stored at -70°C prior to PCA extraction and enzymatic analysis (see sections 4.2.4.2 and 4.2.5.3).

4.2.3.2 Metabolic consequences of exercise

The December batch of crabs was randomly allocated into groups of 4. Each crab was individually placed in a tank and exercised as above. The crabs were then returned to their individual holding tanks, and haemolymph samples were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12 and 16 h after the end of exercise.

The crabs were fed whitebait and allowed to recover for one week, being fed again on the 4th and 7th days. The crabs were again deprived of food for 7 days and exercised as before, but this time various tissue samples were taken. The crabs were killed as above, and the chelipeds, walking legs and swimming paddles were removed and kept. The carapace was rapidly cut open and the hepatopancreas removed and kept. All tissue samples were treated as below.

4.2.4 Preparation of haemolymph & tissue samples using perchloric acid (PCA) extraction

4.2.4.1 Haemolymph samples

i. 75 μl haemolymph was taken and immediately mixed with 75 μl chilled 0.6 M PCA, to precipitate out the proteins and deactivate the enzymes.
ii. After mixing thoroughly, the mixture was centrifuged at 10 000g for 20 minutes, and the resulting supernatant was removed.
iii. 1/20 2 M potassium hydrogen carbonate was added dropwise to neutralise the supernatant. The pH was checked using full range indicator paper.
iv. The mixture was then frozen and thawed slowly to precipitate out the potassium perchlorate formed, which was removed by centrifugation at 10 000g for 10 minutes.
v. The clear supernatant was separated off and aliquoted out into several Eppendorf tubes, and stored at -20°C until enzymatic analysis for L-lactate and D-glucose was carried out.

4.2.4.2 Tissue samples

i. The exoskeleton was removed from the leg muscle samples and the frozen tissue was placed in a mortar of liquid N_2 and ground into a powder using a pestle.
ii. Once all the liquid N_2 had evaporated away, 50 mg of the powder was transferred to an Eppendorf tube and 500 μl chilled 0.3 M PCA was added.
iii. After mixing thoroughly on a vortex mixer, the mixture was centrifuged at 10000g for 10 minutes, and the resultant mixture was decanted off and stored on ice.
iv. A further 500 μl 0.3 M PCA was added to the pellet, mixed again and centrifuged at 10 000g for 20 minutes.
v. The resulting supernatant was then added to the first supernatant, and after mixing, neutralised with 2 M potassium hydrogen carbonate.
vi. The mixture was kept on ice for 10 minutes and then centrifuged at 10 000g for 10 minutes to remove the precipitate of potassium perchlorate. The supernantant was aliquoted into several Eppendorf tubes and stored at -20°C until analysed for L-lactate and D-glucose content. (Hill 1989; Gäde *et al.* 1978).

4.2.5 Enzymatic analyses

All reagents were supplied by Sigma Chemical Company Ltd.

4.2.5.1 L-lactate determination

This method is based on that of Gutmann & Wahlefeld (1974) with the modification suggested by Engel & Jones (1978), and was used on both tissue and haemolymph PCA extracted samples. The L-lactate is oxidised to pyruvate in a reaction catalysed by L-lactate dehydrogenase (LDH):

$$\text{L-lactate} + \text{NAD}^+ \leftrightarrow \text{pyruvate} + \text{NADH}$$

Reagents: Glycine-hydrazine buffer - 3.75 g glycine, 0.5 g EDTA & 2 ml hydrazine hydrate were mixed with 98 ml distilled water. pH was adjusted to 9.0 with 1 M sodium hydroxide.

L-lactate - the following standards were run: 2 mM, 1 mM, 0.5 mM & 0.25 mM (38.4 mg.200 ml^{-1} = 2 mM).

NAD^+ - 26.5 mg.ml^{-1} (40 mM).

LDH - dilution of 1 in 2, *i.e.*, 600 U.mg^{-1} protein.

Procedure: The analysis was carried out using a fluorimeter (Shimadzu, Japan) set at wavelengths of 340 nm and 457 nm for excitation and emission respectively. The following reagents were mixed in a 1.5 ml Eppendorf tube:

Glycine-hydrazine buffer	1 ml
NAD^+	50 μl
Sample/standard	50 μl
LDH	5 μl

The contents of the tube were mixed thoroughly and then incubated in a waterbath at 37°C for 2 h. The contents of the Eppendorf tubes were transferred to a quartz 1.5 ml semi-microcuvette and the fluorescence read in the fluorimeter, and the concentration of L-lactate calculated from calibration curves.

4.2.5.2 D-glucose determination

This method, based on that of Kunst *et al.* (1981) involves a 2 step reaction using hexokinase and glucose-6-phosphate dehydrogenase (G-6-PDH). The hexokinase catalyses the phosphorylation of D-glucose, whilst G-6-PDH catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate:

$$\text{D-glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \rightarrow \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

Reagents: Tris buffer - 2.42 g Tris (100 mM) & 0.24 g magnesium sulphate (10 mM) were added to 20 ml distilled water. pH was adjusted to 7.4 with 5 M hydrochloric acid.

D-glucose - the following standards were run: 1 mM, 0.5 mM, 0.25 mM, 0.1 mM & 0.05 mM (18 mg.100 ml^{-1} = 1 mM).

Hexokinase - dilution of 1 in 25, *i.e.*, 180 U.mg^{-1} protein.

G-6-PDH - dilution of 1 in 25, *i.e.*, 300 U.mg^{-1} protein.

ATP - 121 mg.10 ml^{-1}.
NADP$^+$ - 168 mg.10 ml^{-1}.

Procedure: The following reagents were added to a 1.5 ml Eppendorf tube:

Tris buffer	700 μl
ATP	100 μl
NADP$^+$	100 μl
Sample/standard	100 μl
G-6-PDH	10 μl

The contents of the tubes were mixed and left for 10 minutes to incubate at room temperature, then 10 μl hexokinase was added. Once more the tubes were agitated and left for a further 10 minutes to incubate at room temperature. The contents of the tubes were then transferred into a quartz 1.5 ml semi-microcuvette and the fluorescence read in a fluorimeter set at 340 nm (excitation) and 457 nm (emission). D-glucose concentrations were calculated from calibration curves.

4.2.5.3 Glycogen determination

This method is based on that of Keppler & Decker (1974) and involves the hydrolysis of 1-4 and 1-6 glycosidic bonds of glycogen by 1-4,1-6-amyloglucosidase. The glucosyl units formed are then assayed using hexokinase and glucose-6-phosphate dehydrogenase as described in section 4.2.4.2:

$$\text{Glycogen (glycosyl)}_n + H_2O \rightarrow \text{Glycogen (glucosyl)}_{n-1} + \text{D-glucose}$$
$$\text{D-glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$$
$$\text{Glucose-6-phosphate} + \text{NADP}^+ \rightarrow \text{6-phosphogluconate} + \text{NADPH} + H^+$$

Reagents: Liquid N_2
Potassium hydroxide - 30 g.100ml^{-1} (30%).
Absolute ethanol
Acetate buffer - 2.4 ml 96% acetic acid & 4.87 g sodium acetate (35 mM) were made up to 1000 ml with distilled water. pH was adjusted to 4.8 with 5 M sodium hydroxide.
Tris buffer - as in section 4.2.4.2.
Glycogen - the following standards were run: 1 mM, 0.5 mM, 0.25 mM, 0.1 mM & 0.05 mM (16.2 mg.100 ml^{-1} = 1 mM).
ATP - as in section 4.2.4.2.
NADP$^+$ - as in section 4.2.4.2.

Hexokinase - as in section 4.2.4.2.
G-6-PDH - as in section 4.2.4.2.
1-4,1-6-amyloglucosidase - used without dilution, *i.e.*, 77 U.mg^{-1}
protein).

Procedure:

Preparation of frozen tissue:

i. The exoskeleton was removed from the leg muscle samples and the frozen tissue was placed in a mortar of liquid N_2 and ground into a powder using a pestle.

ii. Once the liquid N_2 had evaporated away, 50 mg of the powdered tissue was transferred to an Eppendorf tube and 400 μl potassium hydroxide was added.

iii. After mixing, the tubes were placed in a boiling waterbath. The lids of the Eppendorf tubes were pierced to prevent a build up of pressure within the tubes. After 20 minutes the tubes were removed and allowed to cool.

iv. After the addition 1ml absolute ethanol to give a final concentration of 70%, the Eppendorf tubes were left on ice for 2 h to allow the glycogen to precipitate out.

v. After centrifuging the tubes at 10 000g for 10 minutes the supernatant was decanted off and discarded.

vi. The pellet was stored at -20°C prior to analysis.

Glycogen estimation:-

i. 1ml acetate buffer and 10 μl amyloglucosidase were added to the pellet obtained in vi. above, mixed thoroughly and incubated at 37°C for 2 h.

ii. 100 μl of this mixture was added to 700 μl Tris buffer, 100 μl ATP and 100 μl NADP$^+$, and analysed as for D-glucose (section 4.2.4.2).

4.2.6 Statistical analyses

The assumptions neccessary for the application of parametric statistics were not met, hence non-parametric statistical tests were applied. Data presented graphically are therefore plotted as medians.

4.2.6.1 Metabolic consequences of agonistic behaviour

Metabolite levels in resting crabs and partition raised crabs, and levels in winners and losers, were compared using Mann-Whitney tests (Siegal & Castellan 1988). If there were no significant differences the groups were combined and analysed along with the forced exercise group using Kruskal-Wallis ANOVA and multiple comparisons between treatments (Siegal & Castellan 1988).

Spearman's rank order correlation coefficients were calculated between haemolymph and walking leg muscle metabolite concentrations of (winners+losers)/2 and the following:- the proportion of display and contact in a fight, and fight duration. Haemolymph D-glucose and L-lactate concentrations were correlated with each other, as were D-glucose, L-lactate and glycogen concentrations in the walking leg muscles.

4.2.6.2 Metabolic consequences of exercise

Metabolite levels in the resting controls and in animals during the course of recovery from exercise were compared using Kruskal-Wallis ANOVA and comparisons of treatments with controls (Siegal & Castellan 1988).

4.3 RESULTS

4.3.1 Comparison of the metabolic consequences of agonistic behaviour, exercise & low activity

4.3.1.1 Haemolymph metabolites

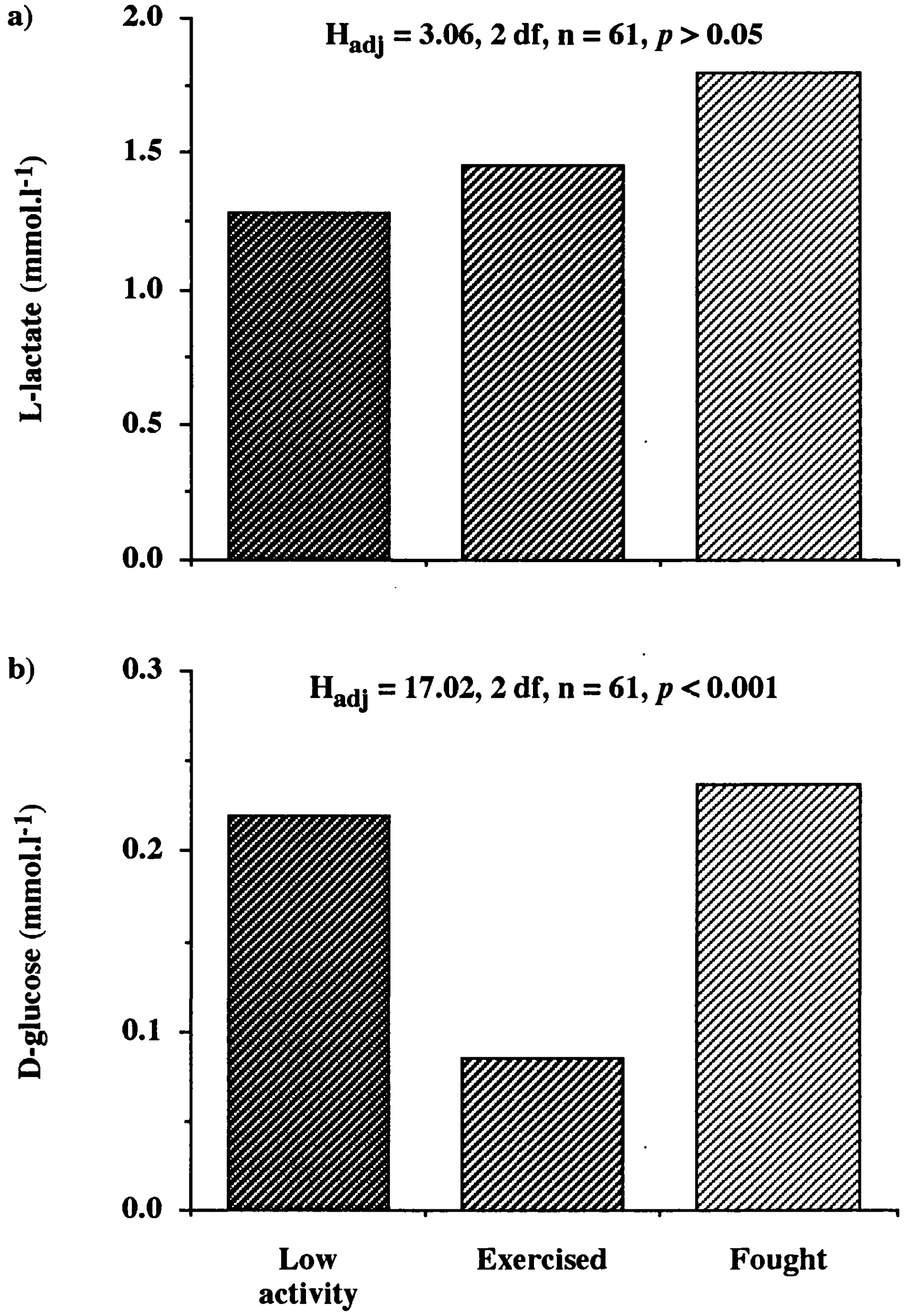
No significant differences were found between haemolymph metabolite concentrations of winners and losers ($p > 0.37$ & 0.61 for D-glucose & L-lactate respectively) and hence they were grouped together as fought crabs. There were also no significant differences between resting and partition raised crabs ($p > 0.85$ & 0.41 for D-glucose & L-lactate respectively) and they were grouped together as low activity crabs.

There were no significant differences in haemolymph L-lactate concentrations between fought, low activity and exercised crabs (Figure 4.1a, KW ANOVA, $H_{adj} = 3.06$, 2 df, $n = 61$, $p > 0.05$). The concentration of D-glucose in the haemolymph varied significantly between fought, low activity and exercised crabs (Figure 4.1b, KW ANOVA, $H_{adj} = 17.02$, 2 df, $n = 61$, $p < 0.001$). Multiple comparisons between treatments revealed that low activity crabs had significantly higher concentrations of glucose in the haemolymph than exercised crabs (Figure 4.1b). Fought crabs also had significantly higher haemolymph D-glucose concentrations than exercised crabs, and there was no significant difference between low activity and fought crabs (Figure 4.1b). That is, the haemolymph D-glucose concentrations in low activity and fought crabs were statistically similar, and both were significantly higher than the concentration in exercised crabs.

4.3.1.2 Tissue metabolites

There were no significant differences between winners and losers in terms of D-glucose, L-lactate and glycogen concentrations in the walking leg muscles ($p >$ 0.56, 0.64 & 0.53 respectively), as was the case for resting and partition raised crabs ($p >$ 0.6, 0.11 & 0.41 respectively), and so the groups were combined as above.

The concentration of L-lactate in the walking leg muscle varied significantly between the 3 categories (Figure 4.2, KW ANOVA, $H_{adj} = 25.3$, 2 df, $n = 65$, $p < 0.001$). Multiple comparisons between treatments revealed that the concentrations of L-lactate in the muscles of low activity crabs were significantly lower than those of exercised crabs (Figure 4.2). There was no significant difference in the muscle L-lactate concentrations between low activity and fought crabs (Figure 4.2). The L-lactate concentrations of exercised crabs were significantly higher than those of fought crabs (Figure 4.2). That is, the accumulation of L-lactate in the walking leg

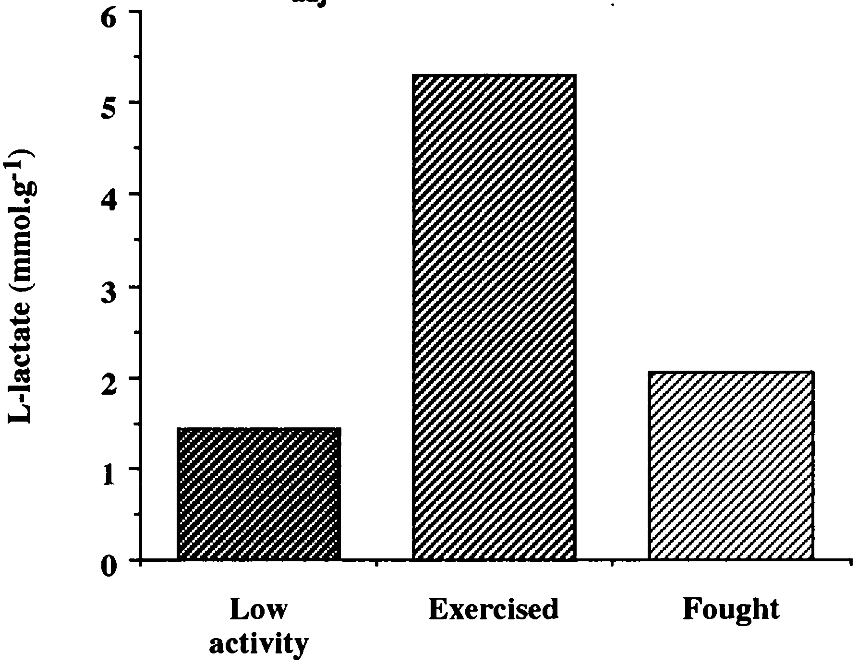


Multiple comparisons between treatments

	$/\bar{R}_x-\bar{R}_y/$	Critical value	p
Low activity *vs* exercised	21.9	16.47	< 0.05
Low activity *vs* fought	4.8	12.02	> 0.05
Exercised *vs* fought	26.7	15.59	< 0.05

Figure 4.1 Haemolymph a) L-lactate & b) D-glucose concentrations in low activity, exercised & fought *Necora puber* (medians plotted).

$H_{adj} = 25.3$, 2 df, n = 65, $p < 0.001$



Multiple comparisons between treatments

	$/\bar{R}_x-\bar{R}_y/$	Critical value	p
Low activity *vs* exercised	35.2	16.76	< 0.05
Low activity *vs* fought	9.0	12.21	> 0.05
Exercised *vs* fought	26.2	16.68	< 0.05

Figure 4.2 L-lactate levels in walking leg muscle of low activity, exercised & fought *Necora puber* (medians plotted) .

muscle in low activity and fought crabs was statistically similar, and both were significantly lower than the accumulation of L-lactate in the walking leg muscle due to exercise.

The concentration of D-glucose in the leg muscle also varied significantly between the three groups (Figure 4.3 KW ANOVA, $H_{adj} = 17.23$, 2 df n = 65, $p < 0.001$). Multiple comparisons between treatments revealed that low activity crabs had significantly lower muscle D-glucose concentrations than exercised crabs (Figure 4.3). The D-glucose concentrations in low activity crabs were also significantly lower than those in fought crabs (Figure 4.3). The muscle D-glucose concentrations in exercised crabs were not significantly different from those of fought crabs (Figure 4.3). In summary, muscle D-glucose concentrations in fought and exercised crabs were statistically similar, and both were significantly higher than those of low activity crabs.

The amount of glycogen in the walking leg muscle in the 3 groups also varied significantly (Figure 4.4, KW ANOVA, $H_{adj} = 24.11$, 2 df, n = 65, $p < 0.001$). Examining the data more closely, multiple comparisons between treatments revealed that low activity crabs had a significantly higher concentration of glycogen in the walking leg muscle than exercised crabs (Figure 4.4). There was no significant difference in the amount of leg muscle glycogen of low activity and fought crabs (Figure 4.4). The amount of muscle glycogen in exercised crabs was significantly lower than the amount in fought crabs (Figure 4.4). In summary, the amount of glycogen in the walking leg muscle of low activity and fought crabs were statistically similar, and both were significantly higher than the amounts in exercised crabs.

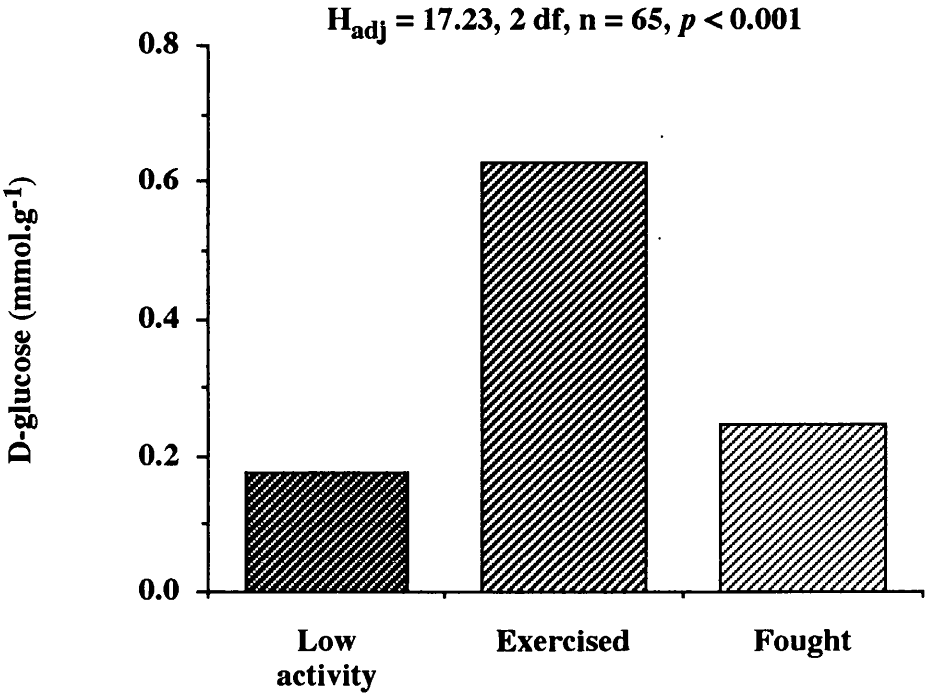
4.3.1.3 Relationship between physiological & behavioural variables

The only significant correlation found was between haemolymph L-lactate and D-glucose concentrations in fought crabs which were negatively correlated ($r_S = -0.582$, n = 15, $0.05 > p > 0.02$).

4.3.2 Metabolic events during recovery from forced exercise

4.3.2.1 Haemolymph metabolites

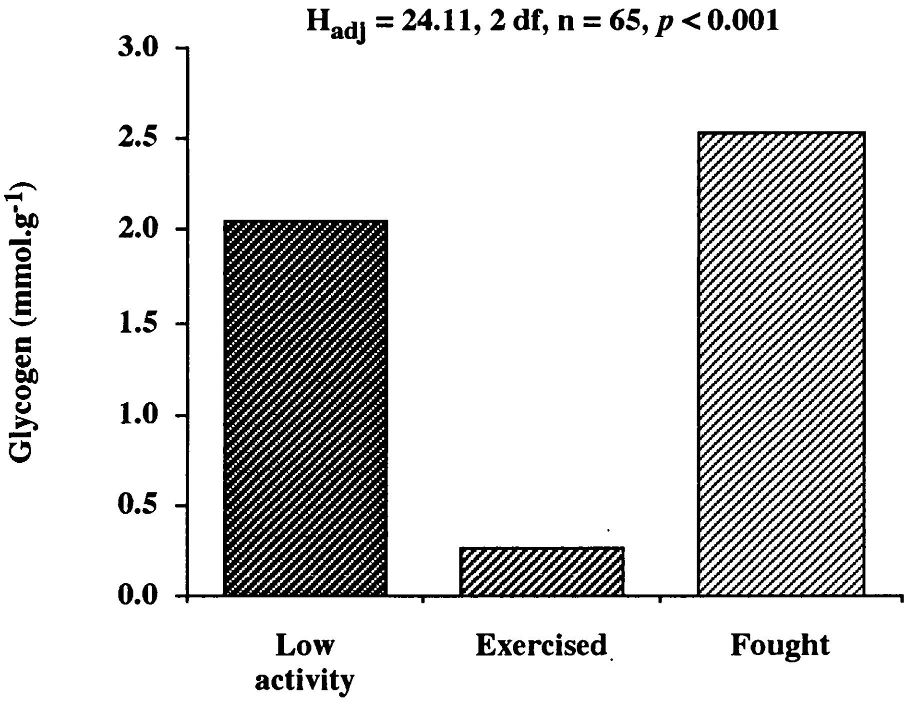
The median concentrations of L-lactate in the haemolymph during recovery from exercise are shown in Figure 4.5a. The maximum accumulation of L-lactate (7.36 $mmol.l^{-1}$) did not occur until 1 h after the end of exercise, and declined to resting concentrations by 8 h after the end of exercise. L-lactate concentrations were significantly different from the control value at 1, 1.5, 2 & 2.5 h after the cessation of exercise (KW ANOVA, $H_{adj} = 41.4$, 14 df, n = 60, $p < 0.001$; critical value =



Multiple comparisons between treatments

	$/\bar{R}_x-\bar{R}_y/$	Critical value	p
Low activity *vs* exercised	28.0	16.76	< 0.05
Low activity *vs* fought	12.8	12.21	< 0.05
Exercised *vs* fought	15.2	16.68	> 0.05

Figure 4.3 D-glucose levels in walking leg muscle of low activity, exercised & fought *Necora puber* (medians plotted).



Multiple comparisons between treatments

	$/\bar{R}_x-\bar{R}_y/$	Critical value	p
Low activity *vs* exercised	30.1	16.76	< 0.05
Low activity *vs* fought	3.1	12.21	> 0.05
Exercised *vs* fought	33.2	16.68	< 0.05

Figure 4.4 Glycogen levels in walking leg muscle of low activity, exercised & fought *Necora puber* (medians plotted).

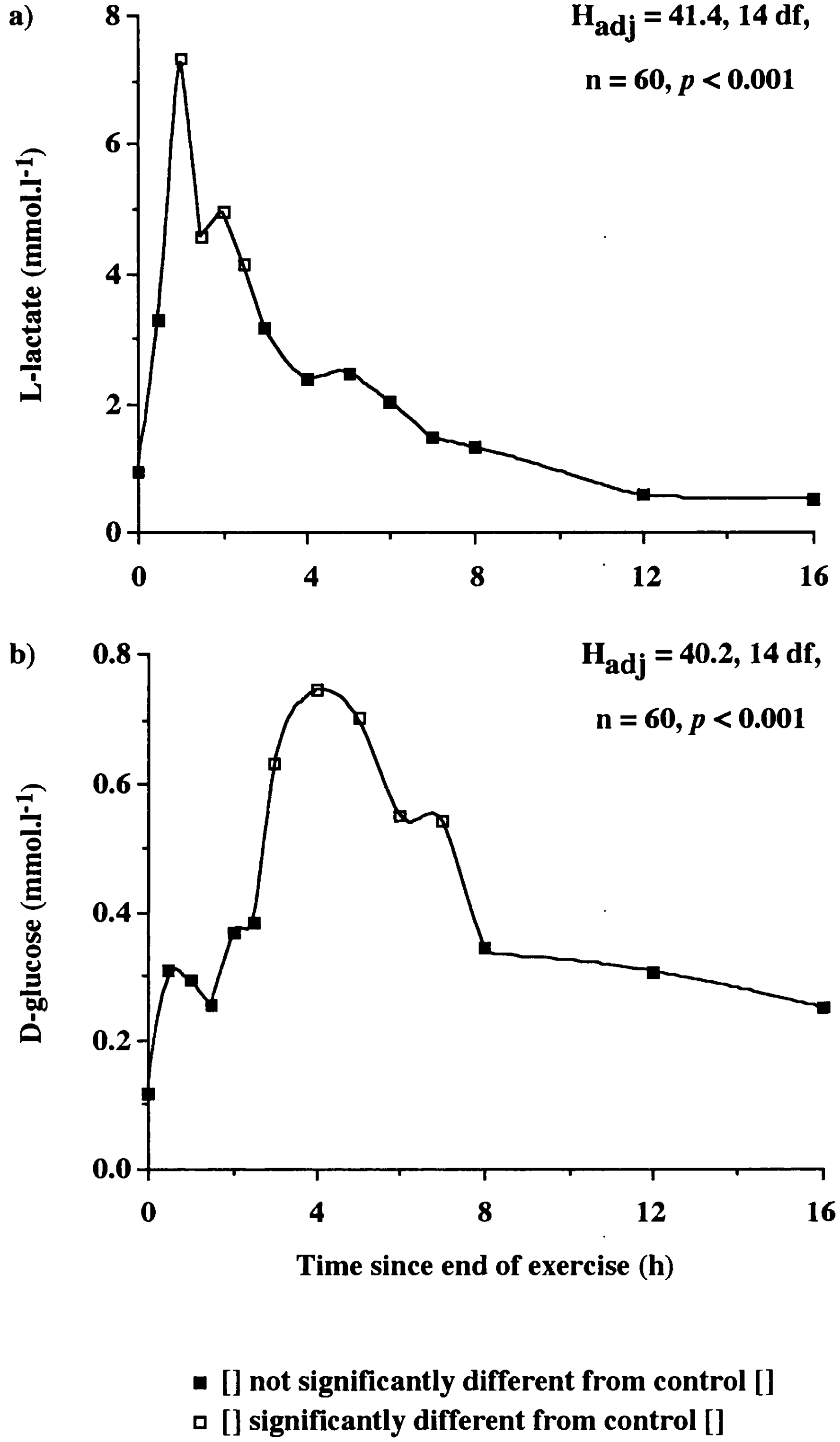


Figure 4.5 Haemolymph a) L-lactate and b) D-glucose concentrations during recovery from forced exercise in *Necora puber* (medians plotted).

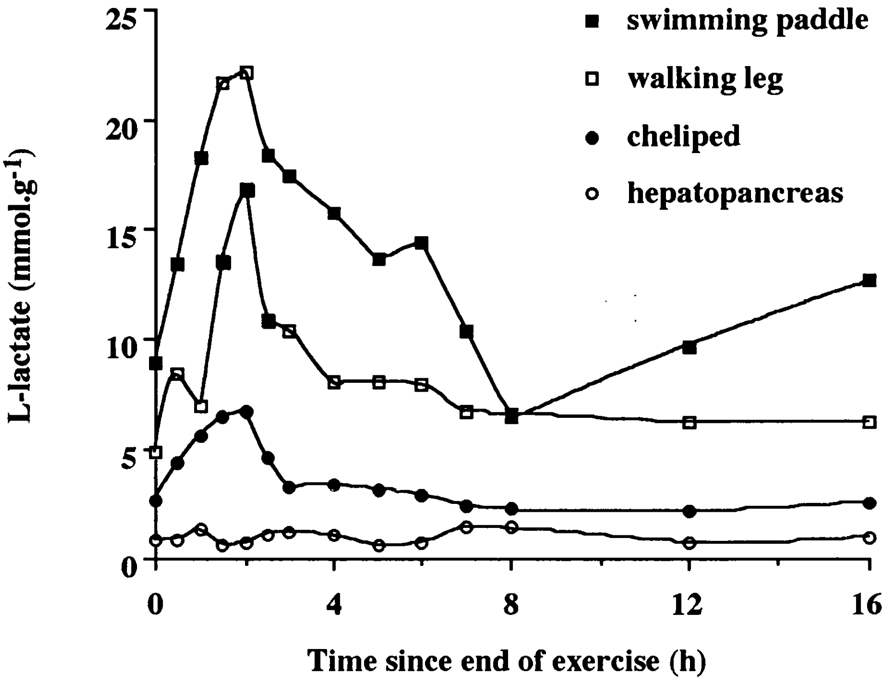
$/R_c\text{-}R_u/ = 40.5, 40.0, 39.2$ & 35.7 respectively, $p < 0.05$).

Figure 4.5b shows the median concentrations of D-glucose in the haemolymph during recovery from exercise. The maximum accumulation of D-glucose was 0.744 $mmol.l^{-1}$, and occured 4 h after the end of exercise. The concentration of D-glucose declined to resting concentrations about 16 h after the end of exercise. D-glucose concentrations were significantly different from the control value at 3, 4, 5, 6 & 7 h after the end of exercise (KW ANOVA, $H_{adj} = 40.2$, 14 df, n = 60, $p < 0.001$; critical value = 34.9, $/R_c\text{-}R_u/ = 40.1, 48.3, 46.2, 39.3$ & 35.3 respectively, $p < 0.05$).

4.3.2.2 Tissue Metabolites

The median L-lactate concentrations in the 3 different muscle types and hepatopancreas are shown in Figure 4.6. The greatest concentration of L-lactate occurred in the muscle of the swimming paddle and amounted to 21.18 $mmol.g^{-1}$, and occurred 1.5 h after the end of exercise. Concentrations of L-lactate were significantly different from the control value at 1.5 & 2 h after the end of exercise (KW ANOVA, $H_{adj} = 39.1$, 14 df, n = 60, $p < 0.001$; critical value = 34.9, $/R_c\text{-}R_u/ = 37.5$ & 35.0 respectively, $p < 0.05$). The greatest concentration of L-lactate in the walking leg muscle was 16.80 $mmol.g^{-1}$, and occurred 2 h after the end of exercise. The concentrations of L-lactate in the walking leg muscle differed significantly from the control value at 1.5, 2 & 2.5 h after the end of forced exercise (KW ANOVA, $H_{adj} =$ 35.4, 14 df, n = 60, $p = 0.001$; critical value = 34.9, $/R_c\text{-}R_u/ = 38.4, 48.2$ & 40.3 respectively, $p < 0.05$). In the cheliped muscle, L-lactate concentrations reached a maximum of 6.67 $mmol.g^{-1}$ 1.5 h after the end of exercise. KW ANOVA revealed a significant difference within the data set (KW ANOVA, $H_{adj} = 32.0$, 14 df, n = 60, $p < 0.005$), however, comparisons of treatments *versus* control was unable to reveal the location of the significantly different concentrations. This was possibly because of an anomalous value for the control group which affected the rest of the comparison. L-lactate concentrations returned to resting values in all 3 muscle types within approximately 8 h of the end of exercise. The concentration of L-lactate in the hepatopancreas remained constant at approximately 1.0 $mmol.g^{-1}$ (KW ANOVA, $H_{adj} = 18.9$, 14 df, n = 60, $p > 0.05$).

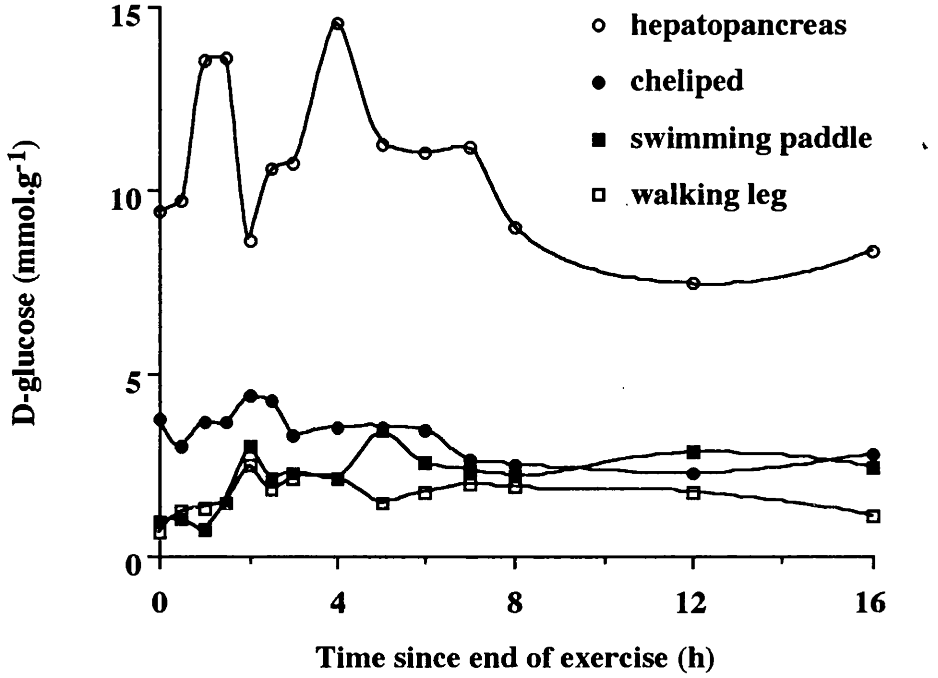
As can be seen in Figure 4.7, the median concentrations of D-glucose in the tissues during recovery from exercise did not show such a clear pattern as the L-lactate concentrations. There was an increase in the concentration of D-glucose in the walking leg muscle from 0.62 $mmol.g^{-1}$ at the end of exercise, up to 2.47 $mmol.g^{-1}$ 2 h after the end of execise, however, this was not a significant increase (KW ANOVA, $H_{adj} = 14.9$, 14 df, n = 60, $p > 0.05$). The amount of D-glucose then slowly declined to approximately 1.13 $mmol.g^{-1}$ 16 h after the end of exercise. The D-glucose



KW ANOVA, 14 df, n = 60

Tissue	H_{adj}	p
Swimming paddle	39.0	< 0.001
Walking leg	35.4	0.001
Cheliped	32.0	< 0.005
Hepatopancreas	18.9	> 0.05

Figure 4.6 L-lactate levels in the four tissue types during recovery from exercise in *Necora puber* (medians plotted). Opposite-shaded plot symbols indicate significantly different concentrations.



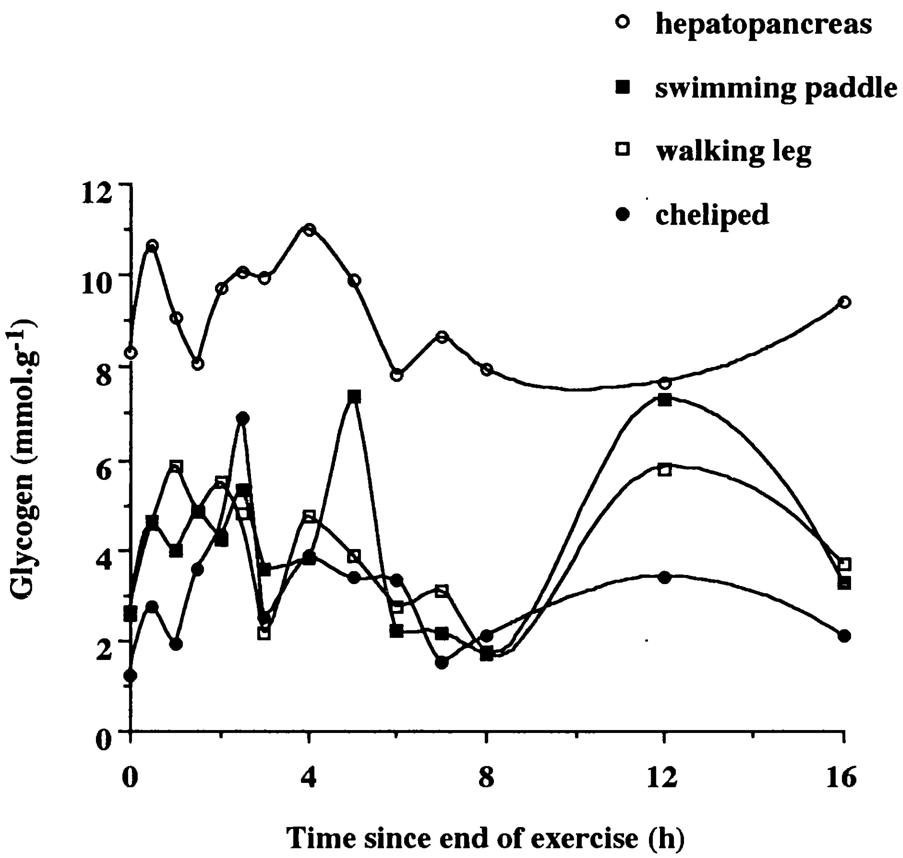
KW ANOVA, 14 df, n = 60

Tissue	H_{adj}	p
Hepatopancreas	17.9	> 0.05
Cheliped	10.5	> 0.05
Swimming paddle	19.8	> 0.05
Walking leg	22.3	> 0.05

Figure 4.7 D-glucose levels in the four tissue types during recovery from exercise in *Necora puber* (medians plotted).

concentrations in the cheliped muscles remained fairly constant throughout the 16 h after the end of exercise. Again, there was a slight peak at 2.5 h after the end of exercise, though again this was not significant (KW ANOVA, $H_{adj} = 10.1$, 14 df, n = 60, $p > 0.05$). The concentrations of D-glucose in the muscles of the swimming paddles showed a similar pattern to that of the walking leg muscles. At the end of the exercise period D-glucose concentrations were at a minimum of 0.95 $mmol.g^{-1}$. There was then a slow, but non-significant, accumulation of D-glucose up to a maximum of 3.10 $mmol.g^{-1}$ 5 h after the end of exercise (KW ANOVA, $H_{adj} = 21.8$, 14 df, n = 60, $p > 0.05$), and a decline to a constant concentration of about 2.20 $mmol.g^{-1}$ at 8 h after the end of exercise. The concentrations of D-glucose in the hepatopancreas were much higher than the concentrations in the 3 muscle types. After the end of exercise, the D-glucose concentrations fluctuated but generally increased to 14.57 $mmol.g^{-1}$ at 4 h after the end of exercise, and then declined to approximately 8.00 $mmol.g^{-1}$ at 8 h after the end of exercise. These fluctuations, however, were not significant (KW ANOVA, $H_{adj} = 17.9$, 14 df, n = 60, $p < 0.05$).

The median concentrations of glycogen in the tissues during recovery from exercise are shown in Figure 4.8. There was no clear pattern to, and no significant accumulations of, glycogen in the walking leg, cheliped and swimming paddle muscle, and hepatopancreas during recovery from exercise (KW ANOVA, H_{adj} = 22.3, 10.5, 19.8 & 15.1 respectively, 14 df, n = 60, $p > 0.05$).



KW ANOVA, 14 df, n = 60

Tissue	H_{adj}	p
Hepatopancreas	15.1	> 0.05
Swimming paddle	19.8	> 0.05
Walking leg	22.3	> 0.05
Cheliped	10.5	> 0.05

Figure 4.8 Glycogen levels in the four tissue types during recovery from exercise in *Necora puber* (medians plotted).

4.4 DISCUSSION

Predictions arising from game theory about how animals should behave in a variety of different situations requires understanding of the costs associated with different strategies, as well as the possible benefits. Game theory models predict that, on average, animals will incur greater costs when competing for resources of higher value (Hammerstein & Parker 1982; Maynard Smith 1982a; Enquist & Leimar 1987). Testing such predictions requires measurable costs that have a bearing on the behaviour of the animal. Aquatic crustaceans have a limited ability to undertake aerobic activity, and recovery from anaerobic metabolism is prolonged in most species studied (M^{c}Mahon 1981; Ellington 1983). The physiological costs of behaviour may therefore be influential in determining the strategies used by aquatic crustaceans during fighting, especially when the risk of serious injury is low. There is, however, little information available about the metabolic costs of fighting. In this study, the metabolic consequences of fighting in winners and losers were investigated, and compared to those of forced exercise and very limited activity.

There were no significant differences in the metabolic parameters measured between winners and losers of fights between male *Necora puber*, *i.e.*, in terms of haemolymph D-glucose and L-lactate, and D-glucose, L-lactate and glycogen in the walking leg muscle. It can be inferred from this that there is no metabolic threshold, *e.g.*, a certain accumulation of L-lactate or depletion of D-glucose from the haemolymph or muscles, that cues one crab to give up. It is possible that the enzymatic assays used were not sufficiently sensitive to detect the differences between winners and losers. The methods can, however, detect the differences between exercised, fought and low activity crabs. It is more likely that fighting is not that metabolically expensive, at least in terms of the parameters measured, and so there are no differences between winners and losers. Smith & Taylor (1993) found no significant difference between the estimates of energy expenditure, as indicated by oxygen consumption and scaphognathite activity, of winners and losers in fights between male *N. puber*. This agrees with the findings of the present study in that there were no differences between winners and losers in terms of the haemolymph and muscle metabolite concentrations measured. Smith & Taylor (1993) also found that the respiratory activity of crabs in a fight was related both to the intensity of the interaction and to its duration. No such relationship was found in the present study between metabolite concentrations and the proportion of display and contact in a fight, and fight duration. During most interactions, eventual winners and losers performed similar activities. Behavioural differences were apparent between winners and losers in the early stages of fights (chapter 2), but it was not until near the resolution of an interaction that winners tended to strike more and losers tended to retreat from their opponents by rapid sideways running and/or swimming. These differences, however, do not seem to have been sufficient to cause a difference in metabolic expenditure between winners and losers. Other parameters that were not quantified, for example, intra- and extracellular pH, hormone release and arginine phosphate concentrations, may shed more light on the possibility of a physiological cue to giving up.

No significant difference was found in the concentrations of L-lactate in the haemolymph of fought, exercised and low activity crabs. The lack of a difference is possibly due to a delay in the movement of L-lactate produced in the muscles into the haemolymph. This delay has been reported in other crustacean species:- *Palaemon elegans* (Taylor & Spicer 1987), *Leptograpsus variegatus* (Forster *et al.* 1989) and *Carcinus maenas* (Hill *et al.* 1991a).

L-lactate accumulates significantly in the walking leg muscle of *N. puber* as a result of forced exercise. There was also a slight accumulation of L-lactate as a result of fighting, but this was not significant when compared to the concentrations in low activity crabs. This suggests that fighting is not as metabolically costly as forced exercise, or activities in other crustaceans, such as the tail flip escape response of macrurous decapods, *e.g.*, *Crangon crangon* (Onnen & Zebe 1983) and *Cherax destructor* (Head & Baldwin 1986). *Cherax destructor* undergoes a behavioural switch from escape to defensive behaviour once available arginine phosphate is depleted (Head & Baldwin 1986).

Haemolymph D-glucose was significantly depleted as a result of forced exercise, but the haemolymph D-glucose concentration of fought crabs was similar to that of low activity crabs and significantly higher than that of exercised crabs. Again, this suggests that agonistic behaviour is not as metabolically expensive as forced exercise. D-glucose concentrations in the walking leg muscles were significantly higher in crabs that had undergone forced exercise or fought, than in low activity crabs. This may be because functional anaerobiosis in active muscle causes metabolic acidosis that may alter the efficiency of enzymes in the appropriate metabolic pathway and may slow down the regeneration of co-factors (NAD^+). This may result in an accumulation of D-glucose in the muscles due to a reduced ability to carry out glycolysis. Another possible explanation is that there is a hormonal response caused by the low haemolymph D-glucose concentration. Low haemolymph D-glucose concentrations (and high L-lactate concentrations) trigger the release of crustacean hyperglycaemic hormone (CHH) and possibly also glucagon. CHH was first discovered by Abramowitz *et al.* (1944), and as its name suggests produces elevation of the haemolymph D-glucose concentration. A glucagon-like substance was discovered by van Deijnen *et al.* (1985). These hormones stimulate the mobilisation of D-glucose from glycogen stored in the muscles and hepatopancreas respectively.

Glycogen was significantly depleted from the walking leg muscles of crabs that had performed forced exercise. The concentrations in fought and low activity crabs were similar, and significantly higher than that of exercised crabs. It seem likely that the glycogen stored in the muscles may have been broken down into D-glucose and metabolised to provide energy for continued activity until it was completely depleted. Once again, this suggests that fighting is not as metabolically expensive as exercise in *N. puber*. To continue a theme, this glycogen catabolism may have been in response to CHH release caused by low haemolymph D-glucose and high L-lactate concentrations. In the present study, the metabolic consequences of fighting were compared only with those of exhaustive activity and undisturbed animals. Estimates of the metabolic cost of a range of activities are also required so that the exertion of crabs during fighting may be better interpreted.

Necora puber could be induced into activity for 3-7 minutes by turning the crabs onto their carapaces and forcing the crabs to right themselves, and by tapping the legs or carapace, causing the crabs to walk, run or swim. This compares to the 3-8 minutes of swimming that Smith & Taylor (1993) induced by suspending the crabs above the substratum. During the course of the exercise period the crabs moved less quickly, and their righting response slowed, although they still attempted both activities. Eventually the crabs could not be induced to move further or to right themselves. It is unlikely that habituation to the stimuli occurred during the short duration of the exercise period and also because the stimuli were applied at different points on the crabs. Preliminary observations of the crabs indicated that once they had become unresponsive to tactile stimulation they would not respond to severe disturbance either. It is probable, therefore, that exercise ceased due to fatigue rather than habituation.

The short fatigue times of *Necora puber* when undergoing forced exercise are in contrast to the energetic capacity of the related swimming crab, *Callinectes sapidus* (Booth *et al.* 1982, 1984; Houlihan *et al.* 1985). *Callinectes sapidus* was capable of swimming for up to one hour in laboratory conditions and are known to perform long distance migrations to breeding grounds (Judy & Dudley 1970). The endurance of *N. puber* is similar to that of less active crabs such as *Cancer magister* (M^{c}Mahon *et al.* 1979) and *Carcinus maenas* (Burke 1979; Hamilton & Houlihan 1992) which can sustain only short periods of strenuous activity. The rate of ATP production may be impaired by acid-base disturbances affecting enzymes involved in ATP producing pathways or by the depletion of the substrates of these enzymes. Anaerobic metabolism results in the accumlation of acidic end-products and inefficient, and therefore rapid, utilisation of substrates. Onset of fatigue is therefore more rapid when the supply of oxygen to the muscles is inadequate to sustain the energy demand aerobically. Aquatic crustaceans are limited in their ability to maintain a rapid rate of oxygen delivery to the tissues by the relative impermeability of the chitinous gills, the "open" circulatory system and the low oxygen-carrying capacity of the haemolymph (Taylor 1982). These limitations may be compensated to some extent by stores of oxygen in the haemolymph (M^{c}Mahon & Wilkens 1983), and also by the increased oxygen-affinity of the haemocyanin at increased L-lactate concentrations (Truchot 1980; Booth *et al.* 1982; Graham *et al.* 1983; Mangum 1983).

The maximum accumulation of L-lactate in the haemolymph after exercise occurred 1-2.5 hours into the recovery period. The same pattern was seen in the swimming paddle and walking leg muscles, and also, but to a lesser extent, in the cheliped muscles. Since L-lactate is produced by the muscles and exported into the haemolymph, it seems likely that the maximum accumulation of L-lactate seen in the muscles is actually less than the total amount of L-lactate produced by them. The elevated L-lactate concentrations indicate that anaerobic metabolism makes a significant contribution to energy production in some muscle types during forced exercise in male *N. puber.* The fact that L-lactate concentrations increase in the muscles during the initial stages of recovery from exercise suggests that anaerobic glycolysis may be important in meeting the initial energy demand after the end of exercise (Hill *et al.* 1991a).

The L-lactate produced by the muscles is not excreted (Bridges & Brand 1980; Hill *et al.* 1991b) but instead is converted into glycogen via gluconeogenesis (Hill *et al.* 1991b; Lallier & Walsh 1992). The site of reprocessing of L-lactate is not known but part of the L-lactate load may be disposed of by *in situ* metabolism by the muscle fibres (Milligan *et al.* 1989), and it has been found that *in vitro* isolated hepatopancreas cells play a definite but moderate role in the removal of L-lactate during the initial stages of recovery from exercise (Lallier & Walsh 1992).

The muscles of the swimming paddles accumulated the largest maximum concentration of L-lactate, followed by the muscles of the walking legs and chelipeds respectively. The largest accumulations of L-lactate after 30 minutes of exercise were also seen in the swimming paddle muscles of *Callinectes sapidus* (Milligan *et al.* 1989). Swimming paddles and walking legs, as their names suggest, are used mainly in locomotion as well as for other activities such as the righting response and to generate force during pushing bouts in fights. Chelipeds are not used to the same extent during exercise as the other limbs. It is probable that the swimming paddles and walking legs are performing a greater work load during exercise, and possibly also during fighting, than the chelipeds, and so accumulate higher concentrations of L-lactate. Alternatively, it could be that the chelipeds are actually doing most work but have a greater capacity for aerobic metabolism than the swimming paddles and walking legs and hence do not accumulate L-lactate to the same extent. A similar phenomenon was seen in spring peeper, *Hyla crucifer* (Taigen *et al.* 1985). The citrate synthase (indicative of oxidative capacity) activity in the trunk (calling) muscles was six times that of the leg (locomotion) muscles, and Prestwich *et al.* (1989) found that there was no accumulation of L-lactate during calling in *Hyla squirella*.

D-glucose was depleted from the the haemolymph of crabs that had been forced to exercise. D-glucose concentrations increased up to a maximum four hours into the recovery period and then declined. A similar increase has been reported for crustaceans that had undergone anoxia (*Potamonautes warreni*, van Aardt 1988; *Palaemon elegans*, Taylor & Spicer 1987). Johnson & Uglow (1985) also found an increase in haemolymph D-glucose concentrations in *Necora puber* after 24 hours of aerial exposure. Taylor & Spicer (1987) also reported a slight reduction in the glycogen content of the tissues. In the present study, however, there appears to be no pattern to the glycogen concentrations in the tissues.

As postulated previously, the low concentration of D-glucose in the haemolymph immediately after exercise may trigger a hormonal response. CHH seems to act in a similar way to adrenaline which acts on muscles and stimulates the catabolism of glycogen into D-glucose, and inhibits the uptake of D-glucose by the muscles, resulting in the release of D-glucose into the blood/haemolymph. D-glucose concentrations do increase up to a maximum four hours into the recovery period, and there is no significant uptake of D-glucose by the muscles during the recovery period. Glucagon acts in a similar way but glycogen stored in the liver/hepatopancreas is broken down in to D-glucose and released into the haemolymph. This glucagon-type response may be seen in the D-glucose profile of the hepatopancreas up to four hours into the recovery period, followed by a decline back to resting concentrations, presumably as the D-glucose is exported to the haemolymph.

CHAPTER FIVE

POSSIBLE CONSTRAINTS IMPOSED BY AGONISTIC BEHAVIOUR IN MALE *NECORA PUBER*

5.1 INTRODUCTION

The literature on behavioural constraints imposed by the physiological consequences of behaviour is sparse. Consequently, the wider literature was browsed and examples mostly from taxa other than Crustatcea are presented here.

5.1.1 Effect of aggression on reef-building corals

Sessile colonial organisms often occur in multispecies assemblages in which all species have similar resource requirements. As a result of growth and competition for resources, these organisms may come into contact with one another when they grow. Unlike mobile solitary organisms, they cannot respond to such interactions by removing themselves to a more favourable environment. Response to interactions with neighbours must be in the form of either specific aggressive mechanisms, such as allelopathy or by employing specialised structures such as nematocysts, or through morphological changes, such as redirection of growth.

Reef-building corals have evolved many different mechanisms for obtaining and maintaining space in an environment which is often space-limited. One mechanism used in aggressive interactions is extra-coelenteric digestion, whereby mesenterial filaments from the polyps of one coral are spread over the soft tissue of a neighbouring coral (Lang 1971, 1973). Mesenterial filaments contain large numbers of nematocysts (Thomason & Brown 1986) and secrete digestive enzymes (Muscatine 1973). They are capable of damaging or killing tissues with which they come into contact (Lang 1973). Extra-coelenteric digestion allows an aggressive coral to either release space into which it may slowly grow or to halt the progress of another colony encroaching on its space (Lang 1973; Sheppard 1982). Interactions involving extra-coelenteric digestion may occur between different species of corals when they come into contact with each other as they expand across the substratum (Lang 1973). These interactions are aggressive in that they result in the destruction of the tissue of one coral by the other.

Romano (1990) carried out experimental manipulations to determine the short term and long term effects of interspecific aggression by means of extra-coelenteric digestion between two species of reef-building corals, *Cyphastrea ocellina* and *Pocillopora damicornis.* Short term interactions between these species resulted in tissue damage to *P. damicornis* by *C. ocellina.* However, the long term effects of aggression between the two species were quite different. The short term winner, *C. ocellina,* had lower growth and lower skeletal weight increase over 11 months when interacting with *P. damicornis* than when growing alone. The growth of the short term loser, *P. damicornis,* was not quantitatively affected by interactions, although it did redirect growth away from the zone of interaction. This suggests that aggression among corals is energetically costly.

The growth patterns of *C. ocellina* and *P. damicornis* were very different - growth of *C. ocellina* occured over the entire colony whereas *P. damicornis* grew only at the tips of the branches. When in an interaction with *C. ocellina*, *P. damicornis* died in the interaction zone but the rest of the colony continued growing, and indeed, the growth rate of the branches furthest away from the zone of contact was even greater than average rates. However, this redirection of growth did not affect the overall growth rate of *P. damicornis*: the interaction itself was not energetically costly.

Redirection of growth effectively permits *P. damicornis* to escape a menacing neighbour without the energetic demands of extra-coelenteric digestion. *C. ocellina*, because of its morphology and relatively low growth rate, is behaviourally constrained in that it is not able to effectively redirect its growth, *i.e.*, it is not able to escape a threatening neighbour by growing away from it. However, superior extra-coelenteric digestive ability permits *C. ocellina* to survive an interaction. The energetic cost of this aggressive mechanism may be high, but corals using it are able to survive. *C. ocellina*, because of its morphology and low growth rate is constrained to use the very energetically costly extra-coelenteric digestion, with concommitant reduction in growth, to survive in the presence of a superior growth competitor.

5.1.2 Behavioural constraints of reproduction in crickets - calling *versus* courtship gifts in males

Communication by acoustic signals is widespread in the animal kingdom where signals are used in mate attraction. It is usually the male that calls and the female searches for the caller. The cost of producing sound is additional to other costs of mating (Calow 1979). In those animals where components of reproductive investment by the male consist of more than just the donation of its sperm, any metabolic cost of calling could energetically constrain the males' mating behaviour (Arak 1983). Additionally, where there is variation among males in the use of metabolic energy for calling, perhaps resulting in a louder, longer or more complex call, females may prefer these more energy-requiring calls as an indicator of superior male quality (Rand & Ryan 1982; Arak 1988; Bailey *et al.* 1990).

Reducing the amount of time spent calling should reduce the male's metabolic requirements and these can be diverted to other activities. Prestwich & Walker (1981) calculated the portion of the daily metabolic budget used for singing by two species of cricket, *Anurogryllus arboreus* and *Oecanthus celerinctus*, to be 26-56 % and 2.5-25.0 % respectively. Although there was no indication of the energy reserves of the crickets, starvation over several days lead to no dimunition of singing, and Prestwich & Walker (1981) assumed that metabolic reserves were not depleted. However, Simmons *et al.* (1992) demonstrated that sound production in *Requena verticalis* incurred a metabolic cost that affected the energy expended for the production of spermatophyla - protein gifts for coupled females. Recently-mated males, fed on a low-nutrient diet, took the same time to replenish their accessory glands as did males fed a high-nutrient diet, but had a reduced time spent in calling. A hypothesis derived from the studies of Prestwich & Walker (1981) and Simmons *et al.* (1992) is that tettigoniid crickets that produce large energy-rich spermatophyla may be under strong selection to reduce the costs of calling. *R. verticalis* produces a spermatophylax equivalent to about 20 % of its body weight (Davies & Dadour 1989) and has a very low metabolic cost of calling. The males' calls consist of discrete pulses of sound separated by intervals of silence. By comparison, conehead crickets, which produce a very small spermatophylax (Gwynne 1990), may be expected to invest more in calling, and indeed call more or less continuously.

Bailey *et al.* (1993) measured the metabolic costs of calling in *R. verticalis* by measuring oxygen consumption. *R. verticalis* emits chirps formed of several syllables within which are discrete sound pulses. Wing stroke rates, when the insect was calling at its maximum rate, were much slower than those observed in conehead tettigoniids. In *R. verticalis* there appears to be a trade-off between the metabolic cost of spermatophylax production and that of acoustically attracting a mate. However, there are other reasons for reducing time spent calling. One may be to reduce conspicuousness to predators (Belwood & Morris 1987; Belwood 1990). In other species, calling time is extremely short because the call of the male elicits an acoustical response by the female towards which the male will move in search of a mate (Zimmerman *et al.* 1989).

5.1.3 Metabolic constraints on spider web relocation

Spiders webs may form effective prey traps but they require energy for construction. Some spiders build very simple webs that consist of a single or just a few threads of silk, but others make rather complex webs. The energetic cost of web building would be expected to vary among web types, depending on the amount of silk used and the activity necessary for construction.

The profitability of web sites affects survivorship and reproductive success (Riechert 1981). Parameters of web site quality, such as prey availability, may vary spatially and temporally. Spiders can respond to variation in web site quality by leaving the site and building a web at another site (Olive 1982). Web relocation, however, requires energy expenditure, and Tanaka (1989) assumed that the energy spent on the construction of a new web was the major cost of web relocation, and hypothesised that the energetic cost of web construction would affect the decision as to whether to relocate at another site. More specifically, spiders that build more complicated, and hence more costly, webs would relocate less frequently. To clarify the effect of the cost of web construction on web relocation, Tanaka (1989) estimated the energy cost of web construction and the rate of web relocation for the spider *Agelena limbata* (Araneae, Agelenidae), and compared them to those of other spiders that produce webs of varying complexity and cost.

The web of *A. limbata* is non-adhesive and consists of a flat sheet, a funnel retreat and barrier web. The barrier web is an irregular network of silk strands which serves to knock down flying insects onto the sheet or to prevent prey from escaping. The sheet and funnel are characterised by a very tight mesh of silk threads and require a considerable amount of silk in their construction, and this implies that the web of *A. limbata* is costly in terms of energy for construction. In contrast, the webs of orbweaving spiders, such *Araneus diadematus*, consist of a sparse mesh of silk threads and thus require a small amount of silk (Janetos 1982).

Tanaka (1989) found that the energetic cost of web construction (C_W) for *A. limbata* was high, ranging from 9-19 times the daily standard metabolic rate (DSMR). The C_W:DSMR ratio in the orbweaver, *A. diadematus*, was 0.021 (Peakall & Witt 1976). There are two possible reasons for the low cost of the *A. diadematus* web:- first, orbwebs consist of a relatively sparse mesh of silk threads compared with other webs and hence requires less silk; and secondly, *A. diadematus* eats its old web and recycles the silk for a new web in a process that is over 90% efficient (Peakall 1971). Tanaka (1989) concluded that the differences in web construction costs between the species or web types may be due to total web weight, which is associated with mesh size and web size, and to whether the species recycles silk.

The daily rate of web relocation in *A. limbata* was less than 1%, indicating very high site tenacity (Tanaka 1989). The sheet-funnel weavers, which produce the most costly webs show much higher site tenacity than orbweavers which make the least costly webs. Factors other than the cost of web building may affect web relocation, such as prey availability (Olive 1982), kleptoparasitic load (Rypstra 1981), wind damage to the web (Eberhard 1971), and territorial disputes with conspecifics (Riechert 1982). *Agelena limbata* has a high web site tenacity because of the high costs of web construction. Since spiders search for good web sites by web relocation, *A. limbata* may not be able to respond well to variation in profitability of web sites.

5.1.4 Metabolic constraints on behaviour in decapods

The freshwater crayfish, *Cherax destructor*, displays escape behaviour in which bursts of tail flipping are used to propel the animals rapidly backwards. The initial stage of this behaviour consists of about 30 tail flips in rapid succession, following which the animal adopts a stationary threat posture and attempts to defend itself with its large chelipeds. The tail musculature has a limited capacity for aerobic metabolism, and ATP utilised during the initial phase of tail flipping is obtained from anaerobic glycolysis. The switch from rapid tail flipping to defensive behaviour occurs when arginine phosphate reserves are depleted and the energy charge in muscle fibres has fallen (England & Baldwin 1983). Presumably, the chelipeds have not incurred any metabolic costs during the tail flipping stage, and are able to function at full capacity.

Recovery involves regenerating muscle arginine phosphate and high-energy phosphate (AMP, ADP & ATP) reserves, and the removal of anaerobic end products, namely L-lactate. When *C. destructor* are left to rest in well-oxygenated water following a burst of tail flipping, they recover within about 60 minutes to a state when the behaviour can be repeated. This recovery is accompanied by a slow increase in haemolymph L-lactate, which reaches a maximum after about 50 minutes and then falls slowly. Behavioural recovery corresponded with the repletion of high-energy phosphates after about 60 minutes (Head & Baldwin 1986).

5.1.5 Aims

Cherax destructor shows a clear behavioural switch in response to a physiological cue, in this case depletion of arginine phosphate reserves in the tail muscles. The aim of the work presented in this chapter was to investigate the possibility of behavioural constraints imposed by the physiological consequences of previous agonistic behaviour in male *Necora puber*. The work of the previous chapter found that fighting was not as metabolically expensive as forced exercise in male *N. puber*, at least in terms of the parameters measured. It is possible, however, that agonistic behaviour may be costly in terms of other parameters that were not quantified. Hence, the work in this chapter consists of a preliminary test of whether fighting in this species is in any way sufficiently metabolically costly to compromise further behaviour.

If *N. puber* is metabolically constrained in any way by previous agonistic activity, it would be expected to see behavioural differences in subsequent agonistic encounters. If a crab is at all metabolically stressed after a fight, that crab may be expected to be less willing to initiate a subsequent interaction, and be less likely to be successful if an interaction did occur. Subsequent fights are also likely to be less intense (and less costly), at least on the part of the previously fought crab, which may be expected to retreat from the encounter, hence second fights would be of shorter duration as well.

5.2 MATERIALS & METHODS

5.2.1 Experimental Animals

Male *Necora puber* were supplied by a shellfish exporter on Benbecula, Western Isles, in April 1993. On arrival in Glasgow the crabs were transferred into individual holding tanks as described in section 2.2.1. The crabs were kept in these conditions for at least 7 days prior to any behavioural work. The crabs were fed whitebait every 3 days, but were deprived of food for 5 days prior to any experimental work. Only animals with no missing or recently regenerated limbs, and without excessive epifaunal growth, were used in experiments.

5.2.2 Experimental Design

Briefly, groups of 3 crabs were used. Two crabs were placed in one observation tank, and the third crab was placed in a second observation tank. The crabs in the first observation tank were allowed to fight and the winner (fought crab) was transferred to the second observation tank, where it was allowed to fight with the third (unfought) crab. More experimental details are given below.

Crabs were allocated into groups of 3, all matched in size (carapace width) to the nearest millimetre. Size-matched crabs were used in order to induce the most intense fights (Smith *et al.* 1994). 2 crabs of a group were placed in a glass observation tank (as described in section 2.2.2), separated by the vertically sliding opaque partition. The 3rd crab (unfought crab) was placed in a 2nd observation tank, confined to one half by the sliding partition. The crabs were left to recover for 24 h, after which time the partition in the 1st observation tank was raised and the crabs were allowed to fight (1st fight). The actions of both crabs were recorded on an Epson HX 120 laptop computer programmed as an event recorder. Once the fight had been resolved the partition was lowered, and the winner (fought crab) transferred to the 2nd observation tank with minimal disturbance. Here the crabs underwent 1 of 2 fates (2nd fights):-

1) **Immediate Re-fight** Once both the crabs were still, the separating partition was raised and the crabs were allowed to fight, with the actions of the crabs being recorded as before. Once the fight was resolved, the partition was lowered and the crabs separated.

2) **Delayed Re-fight** After 2 h had elapsed since the end of the 1st fight, and when both crabs were still, the partition was raised and the crabs were allowed to fight, as above.

The winner was transferred so as to obviate the effect of defeat on subsequent agonistic behaviour. Delayed fights were held 2 h after the 1st fight in order to investigate the effect of the delay in the export of L-lactate produced in the muscles into the haemolymph.

5.2.3 Statistical Analyses

Initiation and success were compared in crabs made to fight repeatedly and control crabs (unfought) using χ^2 tests (Siegal & Castellan 1988). The intensity (interaction type, section 2.2.5) of fights and the initiation type (certain *versus* uncertain motivation, section 2.2.4) were compared between 1st and 2nd fights with Mann-Whitney tests (Siegal & Castellan 1988). Fight duration, pre-fight latency and the proportion of display and contact used by the dyad were analysed using Wilcoxon matched pairs signed ranks tests (Siegal & Castellan 1988), with the 1st and 2nd fights for each triplet of crabs taken to be the pair. The intensity of the behaviour of the fought crab, in terms of the proportion of display and contact used, was compared between 1st and 2nd fights using Wilcoxon matched pairs signed ranks tests (Siegal & Castellan 1988).

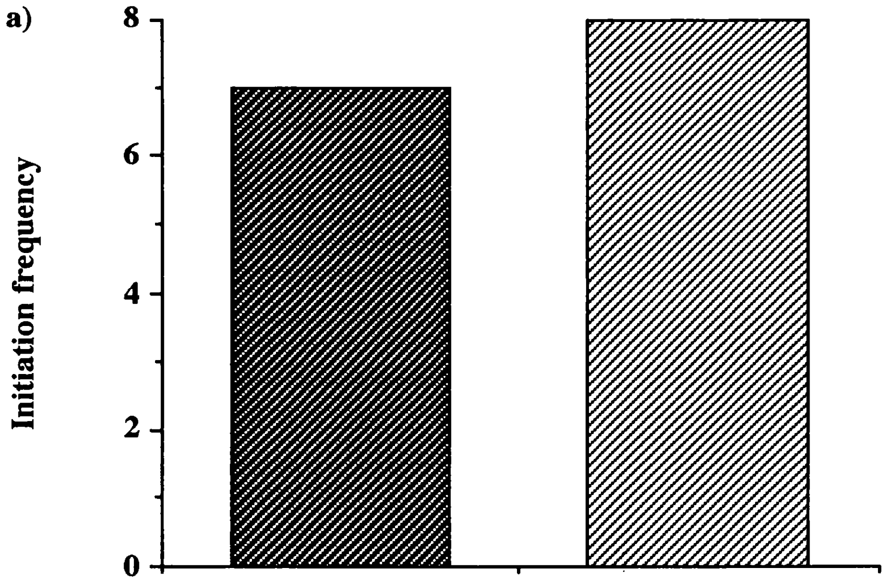
5.3 RESULTS

5.3.1 Immediate re-fights

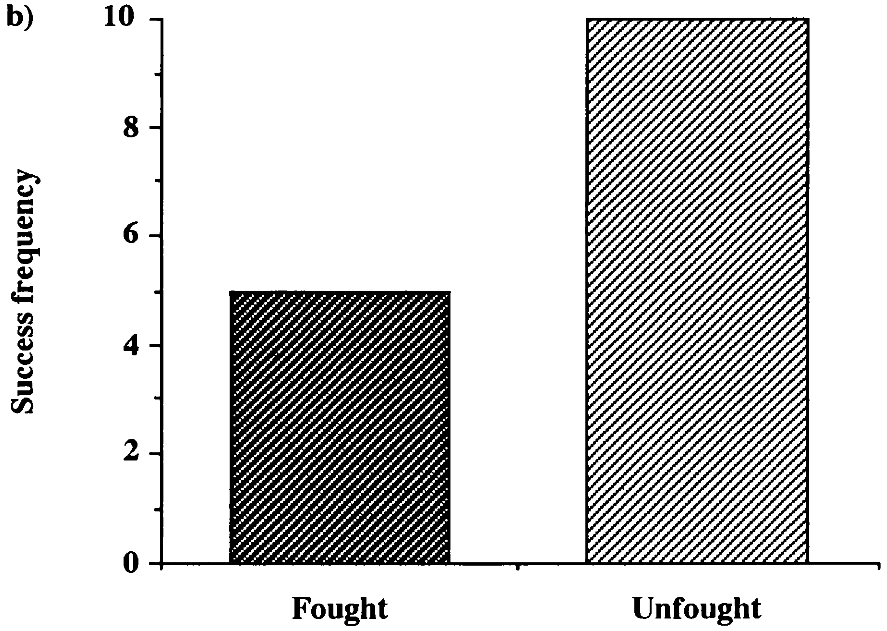
As seen in Figure 5.1, fought crabs did not differ from unfought crabs in terms of initiation frequency (Figure 5.1a, $\chi^2 = 0.07$, n = 15, 1 df, $p > 0.05$) and success (Figure 5.1b, $\chi^2 = 1.7$, n = 15, 1 df, $p > 0.05$). Initiation type did not differ between 1st and 2nd fights (Mann-Whitney, $p > 0.05$), and nor did intensity (or interaction type) (Mann-Whitney, $p > 0.05$). There were no differences between 1st and 2nd fights in terms of latency (Figure 5.2, W = 70.0, test n = 15, $p > 0.05$), interaction duration (Figure 5.2, W = 33.0, test n = 15, $p > 0.05$), or the proportion of display and contact (W = 138.0, test n = 27, $p > 0.05$). The proportion of display and contact used by the fought crabs did not differ significantly between 1st and 2nd fights (W = 61.0, test n = 13, $p > 0.05$).

5.3.2 Delayed re-fights

Fought crabs did not differ from unfought crabs in either frequency of initiation (Figure 5.3a, $\chi^2 = 0.07$, n = 15, 1 df, $p > 0.05$) or success (Figure 5.3b, $\chi^2 = 0.9$, n = 15, 1 df, $p > 0.05$). Initiation type and intensity did not differ between 1st and 2nd fights (Mann-Whitney, $p > 0.05$). No differences were observed between 1st and 2nd fights in terms of latency (Figure 5.4, W = 69.0, test n = 15, $p > 0.05$), interaction duration (Figure 5.4, W = 72.0, test n = 15, $p > 0.05$), and the proportion of display and contact (W = 146.0, test n =27, $p > 0.05$). The proportion of display and contact used by fought crabs did not differ significantly between 1st and 2nd fights (W = 47.0, test n = 13, $p > 0.05$).



$\chi^2 = 0.07$, n = 15, 1 df, $p > 0.05$



$\chi^2 = 1.7$, n = 15, 1 df, $p > 0.05$

Figure 5.1 Effect of agonistic behaviour on the frequency of a) initiation and b) outcome of subsequent fights, held immediately after the first fight.

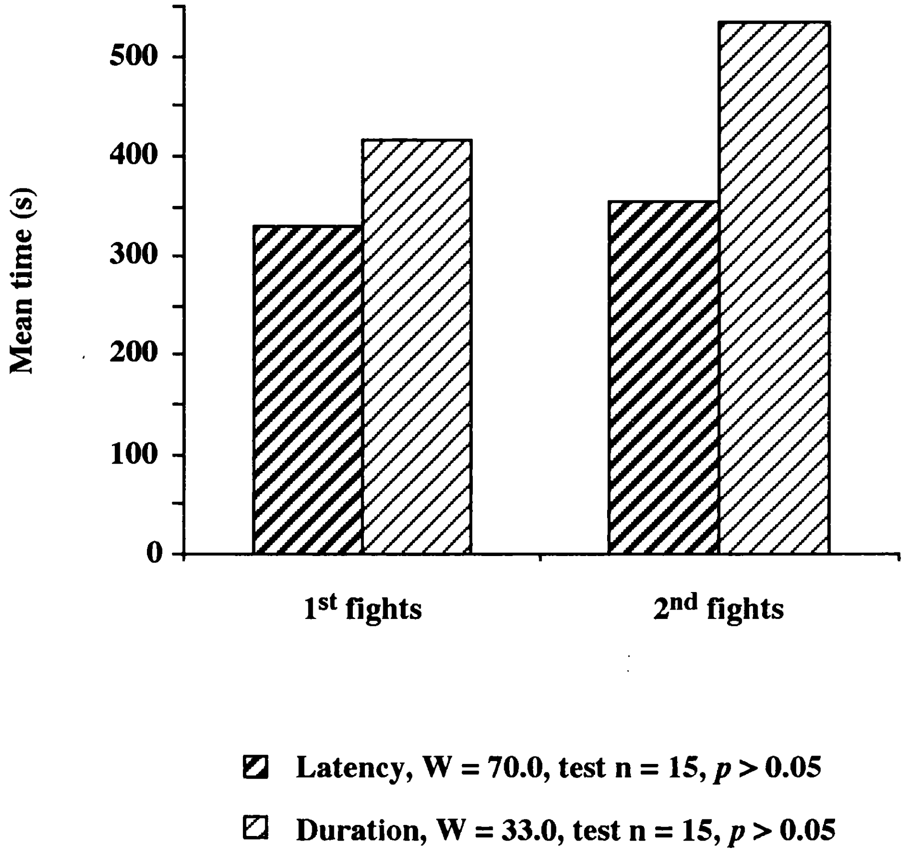
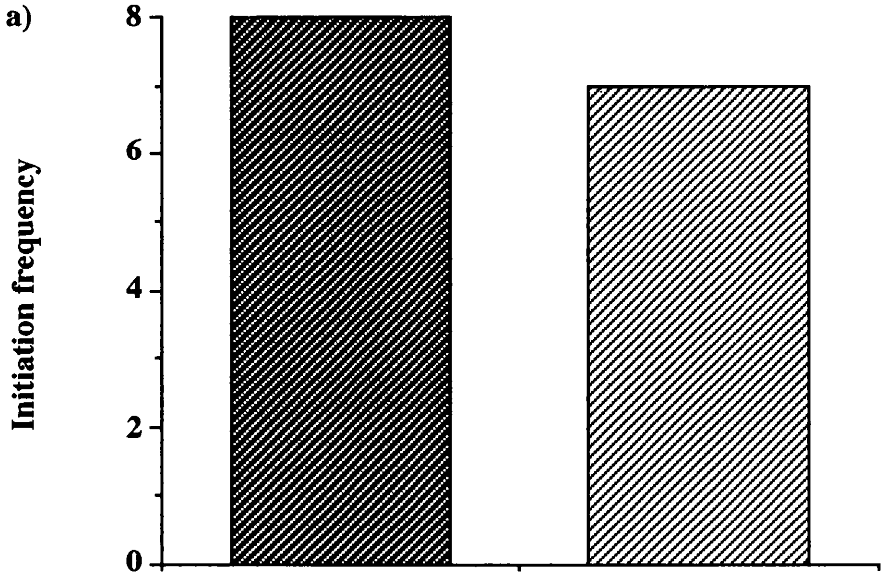
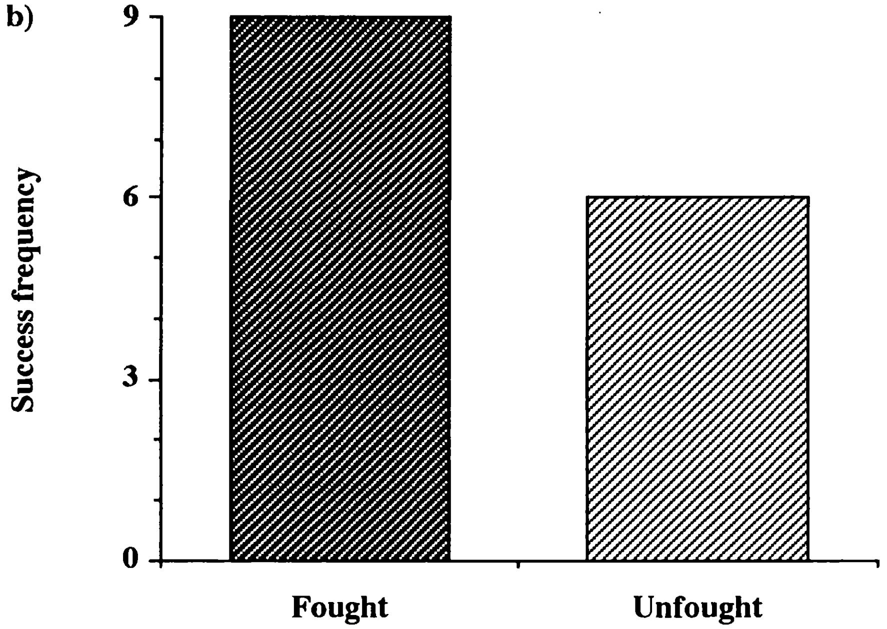


Figure 5.2 Latency & duration of immediate re-fights in male *Necora puber*.



$\chi^2 = 0.07$, n = 15, 1 df, $p > 0.05$



$\chi^2 = 0.9$, n = 15, 1 df, $p > 0.05$

Figure 5.3 Effect of agonistic behaviour on the frequency of a) initiation and b) outcome of subsequent fights, held two hours after the first fight.

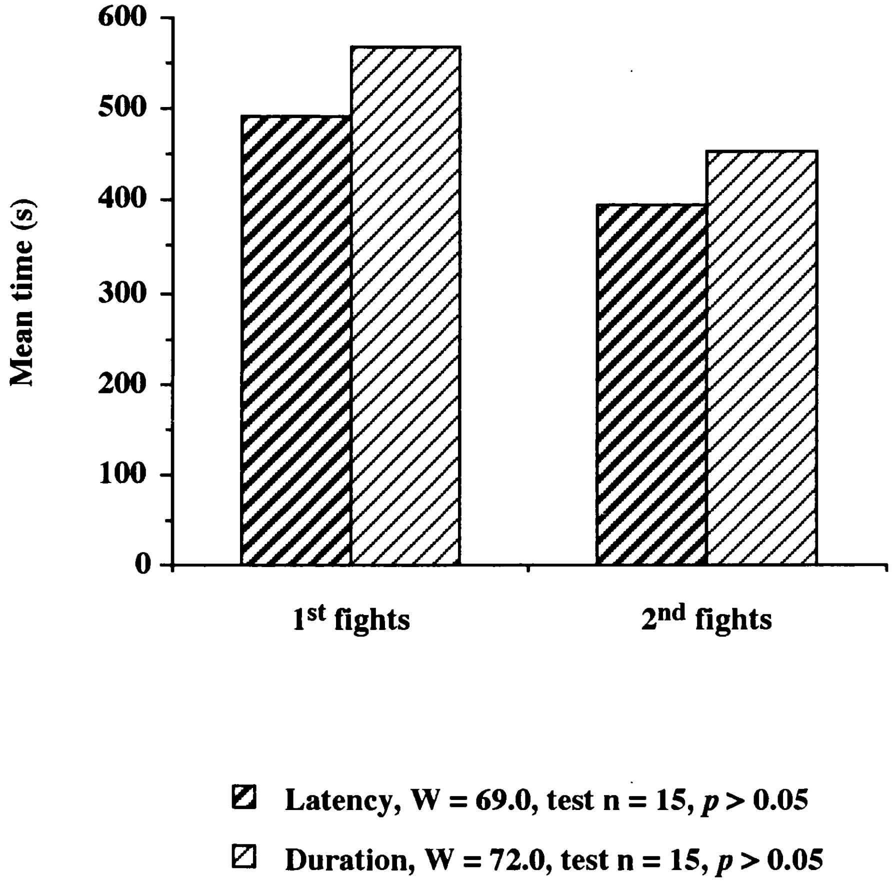


Figure 5.4 Latency & duration of delayed re-fights in male *Necora puber*.

5.4 DISCUSSION

Previous agonistic behaviour did not alter the behaviour of crabs in subsequent fights, held immediately after the first fight or two hours later. Fought crabs were as likely to initiate an interaction as unfought crabs. First fights and immediate and delayed re-fights were of similar duration and intensity (interaction type). Fought crabs were also equally as likely to be successful as unfought crabs. The results of this chapter reinforce those of the previous chapter (chapter 4), in that agonistic behaviour in male *Necora puber* is not sufficiently metabolically expensive to impose constraints on any subsequent behaviour.

Smith & Taylor (1993) found that the recovery time from agonistic behaviour estimated from changes in the rate of beating of the scaphognathites (correlated with oxygen consumption) was approximately 2 h, and approxmately 4 h for recovery from exhaustive swimming. This timescale compares well with the metabolic events during recovery from forced exercise (chapter 4). It is evident that while agonistic behaviour in *N. puber* is not exhausting, the respiratory consequences of fighting last much longer than the duration of the interaction itself. It seem likely, however, that the very limited metabolic costs of agonistic behaviour do not persist, and do not compromise further activity.

This contrasts with the escape behaviour shown by macrurous decapods. The freshwater crayfish, *Cherax destructor*, performs an escape behaviour (tail flipping), that although it may propel the animal rapidly backwards out of harm's way, may render it temporarily unable to escape in a similar way from subsequent threats. In these circumstances the animal is metabolically constrained to switch to defensive behaviour involving the use of the chelipeds, which have not yet been involved in any escape behaviour and should not be metabolically constrained.

Another possible physiological constraint imposed on the behaviour of crustaceans arises from the effects of ecdysis. Most crustaceans are subject to continual periodic ecdysis throughout their lives. Diverse physiological processes such as hormone production, calcium deposition, limb regeneration and protein metabolism all undergo profound changes during the moult cycle. Stomatopods undergo a dramatic change in behaviour during moulting. For several days after moulting, without body armour, in the form of a hardened exoskeleton, and unable to strike, stomatopods are essentially unable to defend themselves against cavity takeover attempts by conspecifics. Many newly moulted animals, however, are able to retain their cavities by adopting an alternative defensive strategy. Newly moulted animals give a meral spread display, an aggressive display that involves the spreading and extension of the raptorial appendages. In intermoult animals, this display generally provides accurate information about the aggressiveness of the signaller

(Dingle 1969) and inhibits attacks by intruders (Dingle 1969; Dingle & Caldwell 1969), and also is an effective display of an animal's size and weapons. Newly moulted animals tend to use this display more frequently than intermoult animals. Steger & Caldwell (1983) argued that the display served as a bluff of both RHP and intent, however, the display can also be viewed as being imposed on the animal by the physiological constraints of ecdysis. The bluff remains resistant to probing because about 80% of the animals using the display are in intermoult, and fully capable of striking.

Animals will incur greater costs when competing for resources of higher value (Hammerstein & Parker 1982; Maynard Smith 1982a; Enquist & Leimar 1987). Animals evenly matched for fighting ability are also expected to incur greater costs (Enquist & Leimar 1983; Leimar & Enquist 1984; Enquist *et al.* 1990). The costs of escalated fighting can be assessed in several ways. Increased risk of injury and death, and enhanced predation on animals in the act of fighting are obvious potential costs. Time spent on fighting, especially if mutually exclusive with feeding and reproduction, is another type of cost. Similarly, energy diverted to agonistic behaviour is not available for feeding or reproduction, and depletion of energy reserves and accumulation of metabolites may compromise an animal's subsequent activity. It is generally assumed that escalated fights are energetically costly, and in some species this may well be the case. There is, however, sparse literature on the physiological costs and consequences of agonistic behaviour. In *N. puber*, and probably other decapod crustaceans, escalated fighting is not energetically expensive and does not impose constraints on subsequent behaviour.

CHAPTER SIX

GENERAL DISCUSSION

In male *Necora puber* (Smith *et al.* 1994), as in several crustacean species (Hyatt 1983) and other taxonomic groups (Archer 1988; Huntingford & Turner 1987), the size difference between opponents in agonistic interactions greatly influences the content, duration and outcome of these interactions. This, however, was not the case for female *N. puber* (chapter 2): fights between evenly matched crabs were not more intense or of longer duration than those between more disparately matched individuals. Relative size was correlated with agonistic ability in females, as in males, as fights were usually won by the larger crab, but the correlation was not perfect as some fights were won by the smaller crab, including some fights that involved strikes and grasps by both crabs. In males, smaller crabs were successful only when the size difference between opponents was small (Smith *et al.* 1994) but, in females crabs as small as 79% the size of their opponent won fights involving strikes and grasps.

Although smaller crabs mostly tended to win fights when the size difference was small, both males (Smith *et al.* 1994) and females (chapter 2) initiated interactions or continued aggressively against opponents, which by the predictions of game theory (Parker 1974; Maynard Smith & Parker 1976; Hammerstein & Parker 1982), they had apparently little chance of beating. It is probable that crabs cannot accurately judge the size of their opponent until it displays. Although similarly sized crabs may not have been able to assess size difference without resorting to contact, it is likely that the more unevenly matched crabs were able to see the size difference. If this is so, the decision as to continue or retreat was unaffected by the size of the opponent in some cases. This suggests that a single index of RHP is of limited use in situations where both members of a dyad are superior in one or more morphological measurements, *e.g.*, mass or chelae length. However, it is possible that the smaller crab had detected unwillingness on the part of the larger crab to engage in a fight. Differences not obvious to an observer, such as recent injury, stage in moult cycle, disease and gender, may introduce considerable variation into the relationship between size and RHP. This behaviour is not unique to *N. puber*. Glass & Huntingford (1988) found that smaller *Liocarcinus depurator* were as likely to initiate a fight as a larger crab. This behaviour is not in agreement with predictions from game theory, which indicates that interactions should be resolved according to asymmetries that are associated with the ability to inflict costs . In addition, there may be circumstances where an ESS stipulates that contests should be settled by uncorrelated asymmetries (Maynard Smith & Price 1973; Maynard Smith & Parker 1976; Hammerstein & Parker 1982). To compare the behaviour of an animal with this prediction one must know the relative costs of assessment and escalation and the accuracy with which individuals can assess asymmetries (Archer 1988). In *N. puber* escalated contests can result in injury, particularly to the limbs. Damaged limbs are usually autotomised, leading to a potential loss of agility and agonistic ability

(Berzins & Caldwell 1983), diminished growth rate (Norman & Jones 1991), and for males, a possible reduction in mating opportunities (Sekkelsten 1988) during regeneration of the autotomised limb. Escalated interactions also involve greater expenditure in terms of time (males only, Smith 1990), increased oxygen consumption (Smith & Taylor 1993) but not metabolic currency (chapter 4).

An often contested resource for crustaceans is shelter (Dingle 1983). *N. puber* are usually found under rocks or in crevices (Smith 1990; pers. obs.), although little is known about shelter use by this species. Shelters may be in short supply in certain areas at dawn when crabs may have been seeking shelter after night time foraging bouts, *e.g.*, where the substratum is mainly bedrock with few cracks and crevices. Norman (1989) noted agonistic interactions in such areas at dawn.

Shelter is presumably necessary for protection from predators. However, the types of predators and the intensity of predation on *N. puber* are not known. In the sublittoral zone, potential predators of *N. puber* are cephalopods, such as the octopus, *Eledone cirrhosa* (Boyle *et al.* 1986), large fish such as Conger eels, *Conger conger*, birds such as eider ducks, *Somateria* spp. (Cramp *et al.* 1977), or mammals such as otters, *Lutra lutra* (Chanin 1985). Glass (1985) speculated that seals (*Halichoerus grypus* or *Phoca vitulina*) preyed on *Liocarcinus depurator* in Loch Sween. Seals are often present in areas where *N. puber* are common, but there is no evidence that they are important predators. Octopuses are important in some localities, and a number of fish species are capable of preying on juvenile *N. puber*, but adults are probably safe from all but the largest fish. All of these suggested predators are visual hunters, and while the agonistic displays of *N. puber* are visually conspicuous, there is no evidence that the displays render the crabs vulnerable to predation. Increased predation risk resulting from certain types of behaviour are known for other crustaceans. Caprellid amphipods are more susceptible to fish predation when engaged in conspicuous feeding behaviour (Caine 1989). Lawton (1987) found that subordinate juvenile American lobsters, *Homarus americanus* were exposed to greater predation risk than dominant individuals, as they spent more time in the open, or in inadequate shelters.

Interactions in this study occurred in the absence of any material resources, such as shelter or food. In the observation tank, *N. puber* engaged in agonistic behaviour when they encountered each other, and this is representative of the situation in the field (Smith 1990; pers. obs.). Although *N. puber* may not defend territories *per se*, they may defend their immediate vicinity, where there may be competition for local resources in an environment where food items are randomly distributed in space and time (Evans & Shehadi-Moacdieh 1988). However, there may be several reasons why it is disadvantageous for crabs to be close to conspecifics, such as spread of disease and parasites, attraction of predators, and the threat of cannibalism at ecdysis.

Comparison of animal contest behaviour with game theoretic predictions requires a knowledge of the relative costs associated with alternative strategies. 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 *N. puber* and may have adverse effects on fitness, they do not appear to be a common result of agonistic behaviour in brachyuran crabs since losers usually managed to flee. Spivak & Poliltis (1989) found the highest incidence of autotomy in *Cyrtograpsus angulatus* occurred in the longest and most exposed limbs, namely the third and fourth walking legs with the chelipeds showing a reduced incidence. This suggests that *C. angulatus* did not lose its limbs as a consequence of agonistic behaviour, but during escape from predators (Spivak & Politis 1989).

The main costs associated with the agonistic interactions of *Necora puber* may therefore be time and energy expenditure. Investigation of the energetic expenditure of crabs during agonistic behaviour is hindered by the difficulty of making physiological measurements without disrupting the animals' behaviour. Smith & Taylor (1993) used an impedence technique (Hogarth & Trueman 1967; Ansell 1973) to measure scaphognathite rate that can be used as an indicator of the oxygen consumption of the interacting crabs. Scaphognathite rate is one of the few respiratory variables that can be measured from individual crabs without severely restricting their behaviour. The respiratory activity of crabs in a fight was related to the intensity of the fight and to its duration (Smith & Taylor 1993), but no such relationship existed between metabolite concentrations and fight content, intensity or duration (chapter 4). Energy expenditure by the crabs should therfore be closely related to oxygen consumption and scaphognathite activity. The physiological costs of fighting in male *N. puber*, and possibly other brachyuarn crustaceans, are not as great as generally supposed even in escalated contests (*e.g.*, Archer 1988; Huntingford & Turner 1987). Metabolic costs of fighting, such as accumulation of end products of anaerobic metabolism, depletion of substrates and constraints on further behaviour, may not be as great as once assumed, at least in some taxa, and such parameters should be measured directly to assess more accurately the actual metabolic currency of fighting. From a more proximate point of view, the quantification of the metabolic consequences of agonistic behaviour may shed some light on the mechanisms that cue an animal to retreat from an interaction. Although the parameters measured in this study did not reveal any such physiological cue that triggers a behavioural switch in *N. puber*, different metabolic parameters measured in other crustacean species have revealed such a cue (*e.g.*, arginine phosphate, Head & Baldwin 1986).

Rapid scaphognathite beating *per se* has an associated energetic cost, regardless of the behaviour that causes it. Wilkens *et al.* (1984) estimated that the energetic efficiency of the scaphognathites of *Carcinus maenas* was 3.15%, and they suggested that the oxygen consumption of the scaphognathite muscles at high rates of beating might limit the animal's capacity for aerobic exercise. If this is the case, it is possible that a more pronounced physiological cost due to fighting might be seen in littoral species, such as *Carcinus maenas*, in tidal rock pools which may become hypoxic or even anoxic. Another possible cause of increased physiological costs associated with fighting may be parasite burden or the occurrence of ectocommensal organisms. Many gill breathers are hosts to parasites and ectocommensals that could affect gaseous exchange. Gannon & Wheatly (1992) assessed the effect of the ectocommensal gill barnacle, *Octolasmis muelleri*, on gaseous exchange in *Callinectes sapidus* by comparing respiratory parameters of infested crabs with those of uninfested controls. Infested crabs maintained their oxygen uptake at the same level as controls, but heart and scaphognathite rates increased. Heavily infested crabs did not differ from uninfested crabs in terms of haemolymph pH, and carbon dioxide and oxygen concentrations. This suggested that the crabs could compensate for the presence of the barnacle in their branchial chambers. However, moderately infested crabs exhibited respiratory parameters with values similar to those of *Callinectes sapidus* during exercise. Circulating L-lactate levels were similar for all crabs, indicating that an increase in anaerobic metabolism was not required for compensation (Gannon & Wheatly 1992).

Necora puber and other crab species of the west coast of Scotland may be infested by the eunicid polychaete worm *Iphitime cuenoti* (Comely & Ansell 1989). Three or more worms were commonly found in the branchial chambers of each *N. puber* host, the highest number comprising nine adults and 98 juveniles, which could reach up to 80mm in length (Comely & Ansell 1989). Abelló *et al.* (1988), studying crab populations off the Catalan coast, NW Mediterranean, also observed *I. cuenoti* in the branchial chambers of some brachyuran crab species including *Liocarcinus depurator*. Analysis of the gut contents of the worms suggested that the relationship between the worm and crab was commensal rather than parasitic (Abelló *et al.* 1988). It would be interesting to assess whether the presence of *I. cuenoti* in the branchial chambers of *N. puber* would cause an increase in the physiological costs associated with fighting in this species. Although evident branchial damage associated to the presence of *I. cuenoti* has not been detected, the presence of the worms may have a deleterious effect (Abelló *et al.* 1988), possibly in the form of competition regarding oxygen uptake, possible minor damage to the branchiae and accumulation of toxic waste products. A host crab may, therefore, have reduced RHP compared with an uninfected crab. If the infestation is not superficially obvious, then the host crab, even though its RHP is reduced, may be able to deceive its opponent as to its fighting ability.

Problems in animal behaviour can be addressed at two different levels of analysis: proximate causation and ultimate causation. Studies of proximate causation focus on the processes and mechanisms that generate behaviour, whereas studies of ultimate causation consider the functional and adaptive significance of those behaviour patterns (Hinde 1975). Although these approaches are widely viewed as complimentary, in practice most studies concentrate on one of the two levels of analysis (Barlow 1989).

Behavioural ecologists construct theoretical models that begin by assuming that particular behaviour patterns have evolved to optimize some goal related to fitness (Wilson 1975), *e.g.*, feeding rate, number of mates, territory size *etc.* These models are then tested using experimental or correlative data from the laboratory or field. Often the empirical data support some but not all of the predictions of the model, leading to re-examination of the assumptions on which the original model is based. Behavioural ecology relies heavily on the currency of energetic costs and benefits, as well as on the risk of predation. This often translates into a detailed study of time-energy budgets, *e.g.*, Gill & Wolf 1975; M^cCann 1983. The activities of the animals are divided up into behavioural components which range from continuously variable actions, such as locomotion, to discrete events such as displays. Time spent engaged in each activity is converted to energy expended, usually employing some relatively crude estimate, such as the known cost of locomotion under specified conditions, *e.g.*, Feldmeth 1983; Wells & Taigen 1984. The different behavioural components are often assumed to have the same costs. When such behaviour is measured directly, it can show remarkably high metabolic costs (Ryan 1985; Wells & Taigen 1986), or in the case of the present study, very limited metabolic costs. That displays can be energetically expensive has implications for arguments concerning signalling as manipulation (*i.e.*, Dawkins & Krebs 1978). Equally, the cost of receiving the signal must also be considered (Dawkins & Guilford 1991; Guilford & Dawkins 1991).

There is one area in particular where theory has moved ahead of data, and that is the application of game theory, particularly in the analysis of aggression. The game theory approach to animal aggression has received considerable attention from theoretical and empirical biologists, and this way of looking at the functional significance of agonistic behaviour has clarified a number of complex theoretical issues and stimulated some empirical research (Huntingford 1984). These models are, however, all ultimate in focus and do not address the mechanisms by which information about asymmetries is translated into behaviour. It may be, from a functional point of view, that there is no need to know about the mechanisms by which this is acheived. On the other hand, motivational studies suggest that other important things are going on during fights: a number of studies have shown that decisions depend critically on the the animal's motivational state, on the current level of aggression of its rival, on whether this is increasing or decreasing, and on the part the animal's own previous actions have played in bringing about such changes. Equally, existing theories about the internal mechanisms underlying agonistic behaviour may not take account of the kind of functionally relevant variables identified by game theory models. Maynard Smith & Riechert's (1984) motivational model of spider fights over feeding territories achieves just that. With this model, studies in the essentially functional framework of game theory have generated causal explanations of the process of escalation during animal fights.

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