The escape behaviour of the brown shrimp, *Crangon crangon* (L.).

Kathryn Hazel Smith, B.Sc. (Glasgow)

A Thesis submitted for the degree of Master of Science to the Faculty of Science at the University of Glasgow.

June 1993

Department of Zoology
University of Glasgow, G12 8QQ

University Marine Biological Station
Millport, Isle of Cumbrae, Scotland KA28 0EG

Declaration.

I hereby declare that this thesis represents work carried out by myself, except where a note is made to the contrary. It has not been previously submitted for any degree.

Kathryn H. Smith

30th June 1993

ABSTRACT

The escape behaviour of the brown shrimp, *Crangon crangon* (L.) was investigated in the laboratory. The kinematics of the tail-flip were examined using high speed video techniques, and the parameters of the escape movements measured. A typical tail-flip at 10°C was found to last around 211 ms and cover a distance of around 63 mm. In the course of the tail-flip the shrimp was found to accelerate at 48 $m.s^{-2}$ and reach a maximum velocity of 1 $m.s^{-1}$.

High speed video recording techniques were used to compare the escape responses of animals exposed to rapid changes in temperature of between 5 and 25°C with animals exposed to temperatures of 5 - 20°C after acclimation. Parameters of movement such as velocity, acceleration and angular velocity showed an increase with an acute increase in temperature. After acclimation shrimps at 5° to 20°C produced similar escape performance, suggesting some adaptation had occurred. Mechanisms for adaptation were discussed.

The extinction of tail-flipping behaviour and its subsequent recovery were examined in a behavioural study, using repeated mechanical stimuli. The timing of these behavioural events appears to correspond with the findings of previous biochemical studies. These behavioural data suggest that partial recovery is sufficient for escape behaviour to occur.

A histochemical and biochemical analysis of the abdominal musculature of *Crangon crangon* was carried out. Results indicate that *C. crangon* shares the basic body-plan of the macrurous decapods and show similarities with other documented tail-flippers. The implications of these findings are discussed in terms of the behavioural ecology of the shrimp.

The strategy employed by the shrimp in the execution of a tail-flip and the cues which may trigger it, were examined. Results suggest that the timing of the tail-flip is extremely important to its success, with shrimps waiting until the final stages of the approach of the predator before tail-flipping to gain most benefit from the rapid acceleration of the tail-flip. A number of cues appear to influence the timing of the tail-flip.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This study was carried out in the Zoology Department at the University of Glasgow and at Dunstaffnage Marine Laboratory and I am grateful to the directors of these establishments, Prof. G. Coombs and Prof. J. Matthews. In addition I would particularly like to thank Prof. J. Davenport at the University Marine Biological Station at Millport for the provision of equipment and facilities at U.M.B.S. in Millport where the bulk of my work was carried out.

I would also like to thank my supervisors Dr D. M. Neil and Dr A. D. Ansell for their guidance and encouragement throughout this project and also for their considerable editorial skills.

There are a number of people whom I wish to thank for their advice and assistance. In Glasgow, Willie Fowler and Graham Tobasnick and at D.M.L., Bob Batty and Karen Fretwell. In Millport I wish to thank Shona McGill, Pete Wilson, Andrew Rogerson, Ian Napier, David Henning, Jeremy Thomason and Tom Stevenson, all of whom were extremely generous with their equipment, time and skill.

I am indebted to staff, students and friends at all three laboratories who made the whole project a pleasant experience.

Finally I wish to thank my family. My sister Jennifer welcomed me whenever I was in Glasgow, either going to or coming from Millport, often at very short notice, and provided a much appreciated second home. My parents, as always, encouraged and supported me in my studies and once again gave me the financial backing which enabled me to carry them out. I am indebted to them both. Lastly, I wish to thank Tom, who has been a tower of strength, kindness and understanding.

CHAPTER 1 : GENERAL INTRODUCTION

GENERAL INTRODUCTION

The brown or common shrimp, *Crangon crangon* (L.) is found throughout the North, Baltic and Mediterranean Seas, and along the Atlantic coasts of Europe. Its main distribution is in temperate regions between the latitudes 45°N and 57°N, mostly in shallow water in highly productive estuarine areas with strong tidal movement (Tiews, 1970). *C. crangon* has been reported to be found to maximum depths of 70, 90 and 150 metres (Allen, 1960; Lloyd & Yonge, 1947; Smaldon, 1982, respectively), mostly on sandy substrates where it is extremely well camouflaged (Lloyd & Yonge, 1947; Tiews, 1970; Tallmark & Evans, 1986).

Crangon crangon is euryhaline and is able to withstand very low salinities and as such it is often found in brackish water (Lloyd & Yonge, 1947; Tiews, 1970). It is also tolerant of a wide range of temperatures, from below 5°C up to 30°C. However, the combination of different salinities and temperatures affects this tolerance and the ability of *C. crangon* to osmo-regulate at low salinities appears to be reduced when exposed to low temperatures (Lloyd & Yonge, 1947). It is thought that it is due to this decline in osmo-regulatory ability, in response to physical changes in the estuary, that *C. crangon* exhibits seasonal migratory behaviour, moving to deeper, more saline water in winter (Henderson & Holmes, 1987). Evidence suggests that males are less tolerant of low salinities than females and are found to migrate to deeper water than the females in winter (Lloyd & Yonge, 1947 and Tiews, 1970).

Many studies report that *Crangon crangon* is nocturnal in aquaria, staying buried in the substrate during the day and feeding at night (Lloyd & Yonge, 1947; Tiews, 1970). However Lloyd & Yonge (1947) report that catch data from the Bristol Channel and the Severn Estuary show no variation between catches on night and day tides. Nonetheless, it is suspected that the turbidity of the water in this area causes the effect of light on behaviour to be reduced and that *C. crangon* are similar to many other Natantia in being nocturnal, with a peak activity period at dusk when feeding occurs (Lloyd & Yonge, 1947; Hughes, 1968; Tiews, 1970; Moller & Jones, 1975).

Crangon crangon is omnivorous, though it appears to favour an animal diet and is most frequently found to feed on worms, particularly *Nereis diversicolor* and *N. succinea*, amphipods, namely *Gammarus* and *Corophium*, and copepods. Cyprid larvae of *Balanus*, and young mussels and snails are also eaten (Tiews, 1970; Lloyd & Yonge, 1947). There is also evidence that *C. crangon* will feed on juvenile fish or fish larvae such as turbot (*Scophthalmus maximus*) and plaice (*Pleuronectes platessa*) (Pihl, 1985; Ansell & Gibson, in press). *C. crangon* is also known to eat filamentous and microscopic algae and diatoms (Tiews, 1970), as well as macro algae such as *Ulva lactuca* and *Enteromorpha intestinalis* (Lloyd & Yonge, 1947).

Crangon crangon has some importance as a commercially fished species, with fisheries along the German North Sea coast and the Netherlands. The Scottish shrimp fishery is based in the south west of the country with a handful of boats

sailing from the Solway Firth. Most of the catch has been from the Irish Sea in the recent years, but shrimp are still fished further up the west coast of Scotland. In 1990, the Scottish shrimp fishery landed forty-nine tonnes of shrimp with a market value of around £79,000 (S.O.A.F.D., 1990).

Crangon crangon is also an important prey item for a number of fish, many of which are important commercial species. Tiews (1970) found some of the main predators on the German North Sea coast to be sea snail (*Liparis vulgaris*), sand goby (*Pomatoschistus minutus*), whiting (*Merlangus merlangus*), and dab (*Limanda limanda*). Elliott *et al* (1990) found *C. crangon* to be an important prey species for cod (*Gadus morhua*), whiting (*M. merlangus*) and flounder (*Pleuronectes flesus*) in the Firth of Forth. Evidence shows that predation is an important limiting factor in the distribution and density of *C. crangon.* Tiews (1970) reported the loss of shrimp stock through predatory fish to be as high as 145×10^9 shrimp annually along the German North Sea coast between 1954 and 1963. This represents a biomass of 15400 tonnes per annum.

In a shallow soft bottomed marine environment vegetation increases the complexity of the habitat and reduces mortality of shrimps (Minello & Zimmerman, 1983). However, in the type of habitat in which *Crangon crangon* is most frequently found, on bare sand or sand with sparse vegetation, there is little or no shelter from predation and *C. crangon,* like other species, has developed anti-predator behaviour to suit its environment. Like many benthic decapods, *C. crangon* takes refuge in the

substrate by burying (although it does not actually construct a burrow). It achieves this by shuffling the pereiopods outwards and beating the pleopods rapidly until a small hollow is formed. The animal is then able to sink into the hollow while forcing water out of its gills which pushes more sand out of the hollow and up around its sides. Finally the shrimp uses its antennae to flick and smooth sand over its back and around its sides (Lloyd & Yonge, 1947; Pinn & Ansell, 1992).

Another predator avoidance mechanism utilized by *Crangon crangon* is the tail-flip characteristic of many decapods. This involves a very rapid flexion and extension of the abdomen which forces the shrimp backwards through the water at high speed. This type of escape behaviour has been studied extensively in the Norway lobster, *Nephrops norvegicus* (Newland *et al*, 1988; Newland & Neil, 1990, I & II), the crayfish, *Procambarus clarkii* (Kennedy & Takeda, 1965; Wine & Krasne, 1972), and in the prawn, *Pandalus danae* (Daniel & Meyhöfer, 1989). However, much of this work is concerned with the detail of the nervous control of the tail-flip mechanism or the mechanism itself, and few studies have considered the tail-flip in the context of the behavioural ecology of the animal exhibiting the behaviour.

The aim of this study is to examine in detail the anti-predator behaviour of *Crangon crangon* with particular reference to the tail flip and its importance in predator prey interactions. Initially, the kinematics of the tail-flip response are described in detail from analysis of high-speed video recordings, and the effect of

acute and acclimated temperature changes on the tail-flip response examined. Later sections evaluate the effectiveness of the tail-flip response in terms of the ability of the shrimp to perform repeated tail-flips, the recovery time needed to restore performance, and the strategy used to optimize escape success. A number of different techniques have been used so that the information gained provides a broad general view of the escape capabilities of *Crangon crangon* and the way in which these influence the behavioural ecology of the animal.

CHAPTER 2 : KINEMATICS OF THE TAIL-FLIP ESCAPE RESPONSE

INTRODUCTION

During predator-prey interactions, the ability of the prey animal to escape a predator is all important and the evolution of a fast and effective escape response is crucial to the survival of potential prey. As with many other marine crustaceans, the escape response of *Crangon crangon* comprises a tail-flip which propels the animal backwards through the water at high speed, away from a potential predator. The tail-flip results from an alternate flexion and extension of the abdomen.

A number of studies have been carried out to describe and quantify the tail-flip in different species. Newland *et al* (1988) examined the escape response of *Nephrops norvegicus*, and obtained measures of the parameters of the tail-flip such as duration, distance and the mean and maximum velocities of movement. Similar measures have been recorded from the tail-flips of the Antarctic krill, *Euphausia superba* (Kils, 1982) and the prawn, *Pandalus danae* (Daniel & Meyhöfer, 1989). Daniel & Meyhöfer (1989) also considered the body movement, physical forces and muscle stresses involved in the mechanism of the tail-flip. Similarly, Webb (1979) examined the forces and mechanics of the tail-flip of the crayfish, *Orconectes virilis*. Despite the sizable body of work examining the ecology of *Crangon crangon* (Lloyd & Yonge, 1947; Tiews, 1970; Henderson & Holmes, 1987), there is no comparable information on the escape response of the brown shrimp.

The escape movements of the tail-flip of *Crangon crangon* are extremely rapid and the details of the flip are missed by the human eye. This study utilises modern high speed video techniques which can resolve the details of the tail-flip and facilitate the analysis of the kinematics of the escape reaction. The use of video analysis in conjunction with the "Movias" software package allows measurement of some of the parameters of movement of the tail-flip such as velocity and acceleration.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS.

Shrimps were obtained by trawl from Kames Bay, Millport and transported in oxygenated seawater to the Dunstaffnage Marine Laboratory (D.M.L.), where, within 24 hours, they were transferred to a tank (0.5m deep, 0.5m diameter) of aerated seawater at 10°C, in a temperature controlled room. This temperature was close to the ambient water temperature at the time of collection and therefore no temperature stress was involved. The tank was covered in order to cut down evaporation and the water changed at regular intervals. The tank was provided with a layer of sand, to reduce stress and allow the shrimps to bury. The shrimps were kept at a density of around 20 to 25 per tank and were fed regularly on minced squid. Animals of both sexes were used, ranging in total body length from 39 to 55 mm.

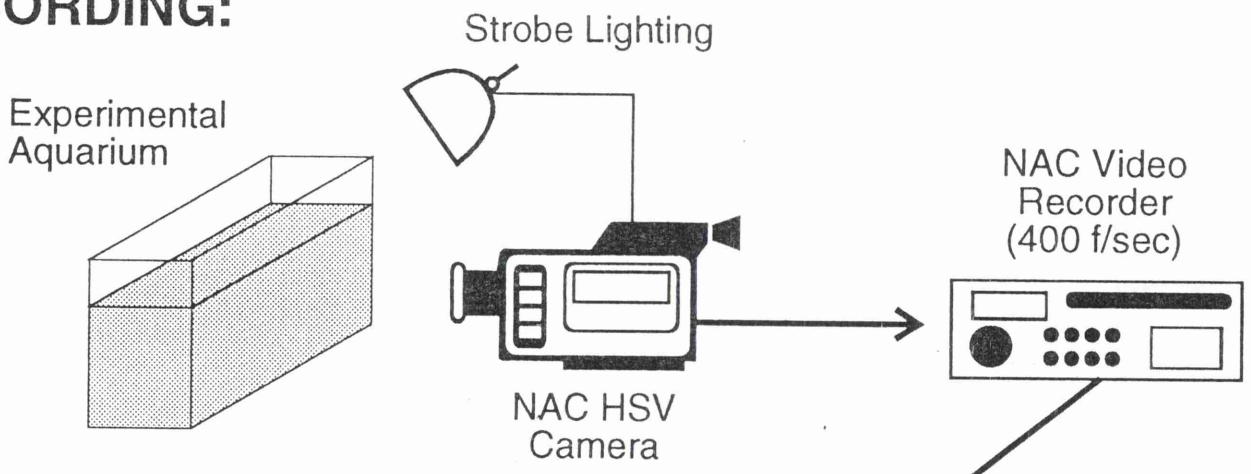
VIDEO RECORDING.

The shrimp were placed in seawater at 10°C, in a small Perspex aquarium measuring approximately 15 × 15 × 20 cm. The size of the tank was such that every tail-flip elicited inside the tank could be filmed in its entirety. A tail-flip was elicited by a mechanical stimulus on either the rostrum or the tail of the shrimp. Escape responses were recorded at a frame rate of 400/s using an NAC high-speed video

recorder (model HSV 400) illuminated by strobe lighting (Fig. 2.1). Recordings were then analyzed using the digitizing tablet and x-y coordinator of the NAC HSV system. Positional coordinates from selected points on the body of the shrimp (rostrum, point of flexion, and tail; Fig. 2.2) were entered from each frame and analyzed using "Movias" software. Key parameters of the tail-flip of *Crangon crangon* which were measured included: velocity, acceleration, degree of flexion, angular velocity during flexion and trajectory and time course of the flip (Fig. 2.3). Because the "Movias" software package is able to handle only two dimensional information, only tail-flips which occurred predominantly within the plane of focus, were analyzed.

Fig. 2.1. Diagram of experimental aquarium and high speed video recording and analysis systems.

RECORDING:



ANALYSIS:

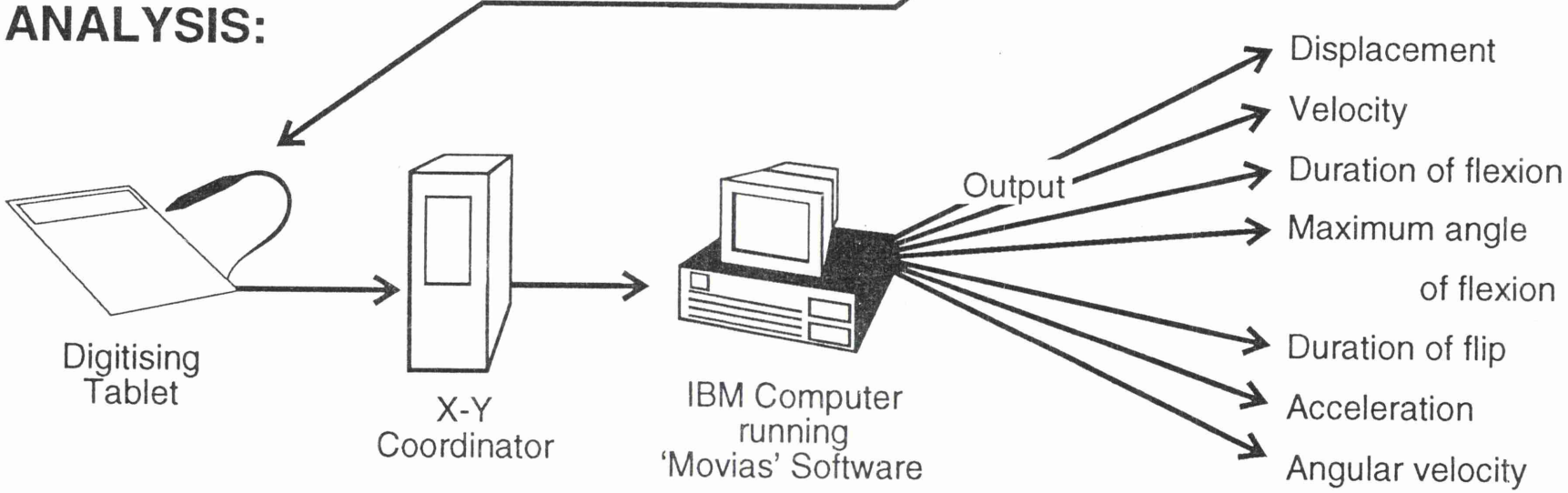


Fig. 2.2. Positional co-ordinates from 1. the head; 2. the point of flexion of the abdomen; and 3. the tail, which were entered into the computer for the analysis of the tail-flip using the digitising tablet.

Fig. 2.3. Diagram of the derivation of motion parameters, velocity (metres per second - $m.s^{-1}$); acceleration ($m.s^{-2}$); angle (°); and angular velocity (degrees per millisecond - $°ms^{-1}$), where θ=angle (°); t=time (ms); s=distance (mm); v=velocity ($m.s^{-1}$). Velocity and acceleration are calculated from point 2 only.

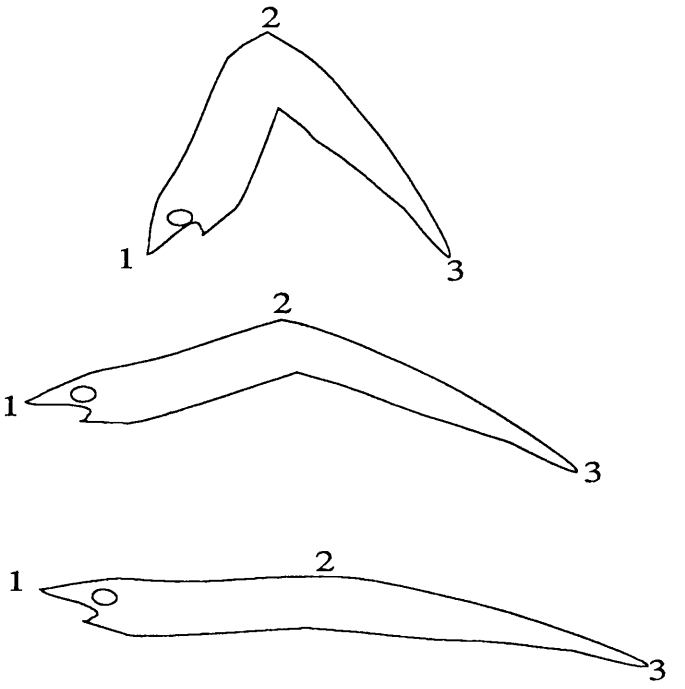
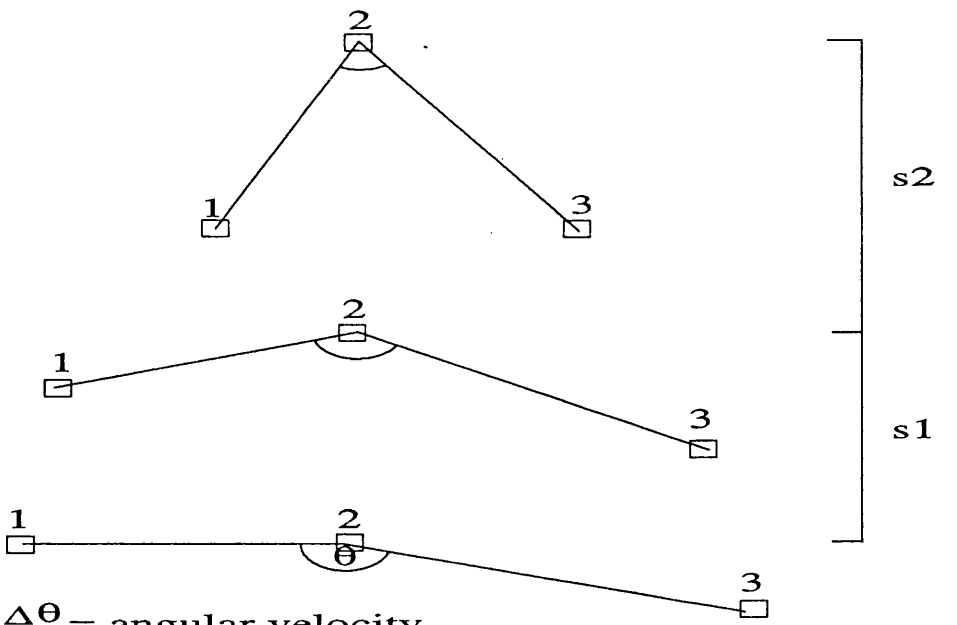


Figure 2.2



$\frac{\Delta\theta}{t}$ = angular velocity

$\frac{\Delta s}{t}$ = velocity

$\frac{\Delta v}{t}$ = acceleration

Figure 2.3

The analysis of video recordings of 28 tail-flips at 10°C, show that the whole tail-flip lasts an average of 211 ms, during which time the shrimp moves at a mean velocity of 0.58 $m.s^{-1}$ and covers a distance of around 63 mm (Table 2.1). The flexion of the abdomen which powers the tail-flip occurs at a mean maximum angular velocity of 6.31°ms^{-1} and this propulsion produces a mean maximum velocity of 1.07 $m.s^{-1}$. As the shrimp moves backwards through the water it accelerates at a mean maximum rate of 48.31 $m.s^{-2}$.

Observation of video recordings of tail-flips shows that during the initial phases of the escape response the tail-fan spreads open, thus increasing the surface area of the tail exposed to the water and therefore also the propulsive force generated by the flexion of the tail. At the end of the flexure phase, the tail-fan can be seen to be closed up, presumably to counter the hydrodynamic forces acting on the body, reducing drag when the abdomen is extended. When multiple tail-flips are performed the tail fan can be seen to open after the extension phase of the previous tail-flip has finished and prior to flexion in the second flip. The antennal scales also appear to open during flexion, further increasing the thrust force produced by the flexion of the abdomen and counterbalancing the opening of the tail fan.

A typical tail-flip, performed at 10°C, can be used to describe the sequence of events and the parameters of motion of the tail-flip of *Crangon crangon.* Figure 2.4

is a 'stick diagram' which links the positions of the head (1); the point of flexion of the abdomen (2); and the tail (3) of the shrimp sequentially during the tail-flip at 10°C. The parameters of movement for the same tail-flip are illustrated in Figs 2.5 - 2.8.

At the time of stimulation the tail is almost fully extended and the angle formed between the head, the midpoint (point of flexion) and the tail is about 145° (Fig. 2.5). This angle decreases rapidly in the first 50 ms of the tail-flip, as the abdomen is flexed, to form an angle of around 10°. The abdomen remains fully flexed for around 30 ms before a more gradual extension occurs. After around 260 ms the abdomen is fully extended. Figure 2.6 shows the velocity of this angular change. The flexion of the abdomen is extremely rapid, the amplitude of angular velocity rising to a maximum of around 4.1°ms^{-1} (degrees per millisecond) within the initial 10-20 ms. (Where the angle between the tail and cephalothorax is decreasing, angular velocity is expressed as a negative in Fig. 2). After 25 ms the velocity of the angular change decreases again as the flexed abdomen reaches its minimum angle at around 60 ms. A more gradual extension of the abdomen then occurs, which reaches a peak angular velocity of 1.2 °ms^{-1}.

The velocity of the midpoint of the shrimp, which represents the movement of the whole animal in the course of the tail-flip, is plotted in Figure 2.7, and the acceleration produced is shown in Figure 2.8. The velocity of the shrimp increases rapidly from 0 to around 0.8 $m.s^{-1}$ in the initial 25 ms of the tail-flip. During this

time acceleration of the body of the shrimp reaches its peak at 23.53 $m.s^{-2}$. After reaching a peak of velocity of 0.8 $m.s^{-1}$ at 25 ms, the shrimp begins to decelerate, initially quite rapidly and then more gradually after 100 ms until velocity drops to almost zero at 210 ms.

The trajectory followed by the body of the shrimp, in the course of a tail-flip, has important implications for the success of the escape reaction and therefore the survival of the shrimp. Tail-flip trajectories recorded from tail-flips performed in response to stimuli either to the rostrum or to the tail, over a range of temperature (5°C to 25°C) are shown in Fig. 2.9. The different patterns of trajectories do not provide sufficient evidence to suggest that a difference between the trajectories performed following rostral or caudal stimulation exists.

Other trajectories were also observed in the tail-flip of *C. crangon*, which involved a rotation of the body during the tail-flip, however, these were not included in the analysis as this would require 3-dimensional analysis techniques.

During the analysis only single tail-flips were examined. Observations suggest that in most cases a single mechanical stimulus elicits a single tail-flip in response.

Parameter	Value	Standard deviation
Mean size	50.7 mm	± 6.7
Mean duration	211.5 m.s	± 87.3
Mean displacement	63.3 mm	± 22.5
Mean velocity	0.58 $m.s^{-1}$	± 0.14
Mean maximum velocity	1.07 $m.s^{-1}$	± 0.16
Mean maximum acceleration	48.31 $m.s^{-2}$	± 20.18
Mean maximum angular velocity	6.31 $°ms^{-1}$	± 1.06

Table 2.1: Measures of the parameters of movement of a typical tail-flip at 10°C (calculated from 28 tail-flips).

Fig. 2.4. 'Stick' diagram linking the sequential positions of the head (1), point of flexion (2) and tail (3) of *Crangon crangon* in the course of a typical tail-flip at 10°C. Arrow indicates direction of motion. Scale bar = 40mm. Tail-flip last 260ms. This diagram summarises Figs 2.5 - 2.8.

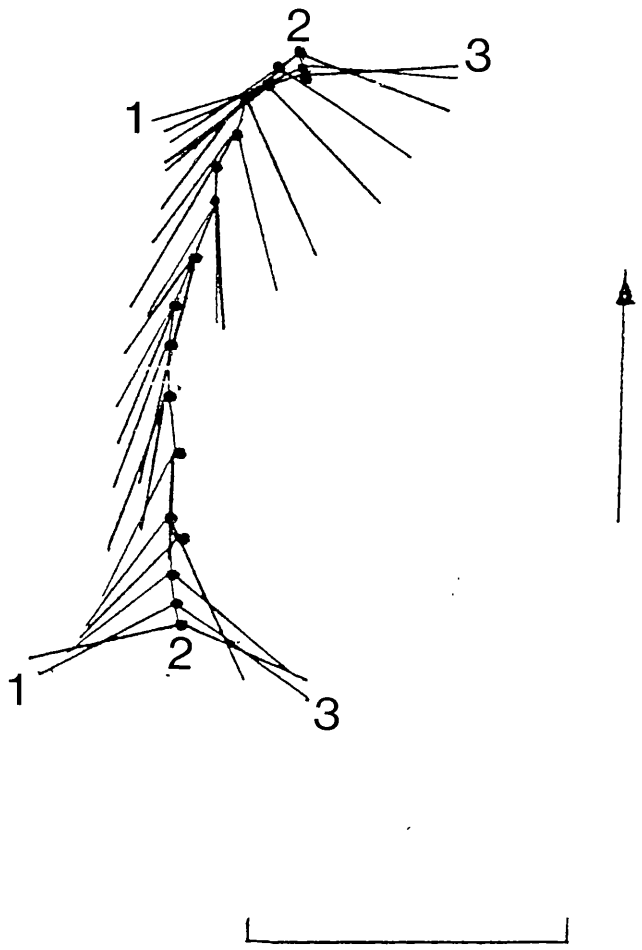
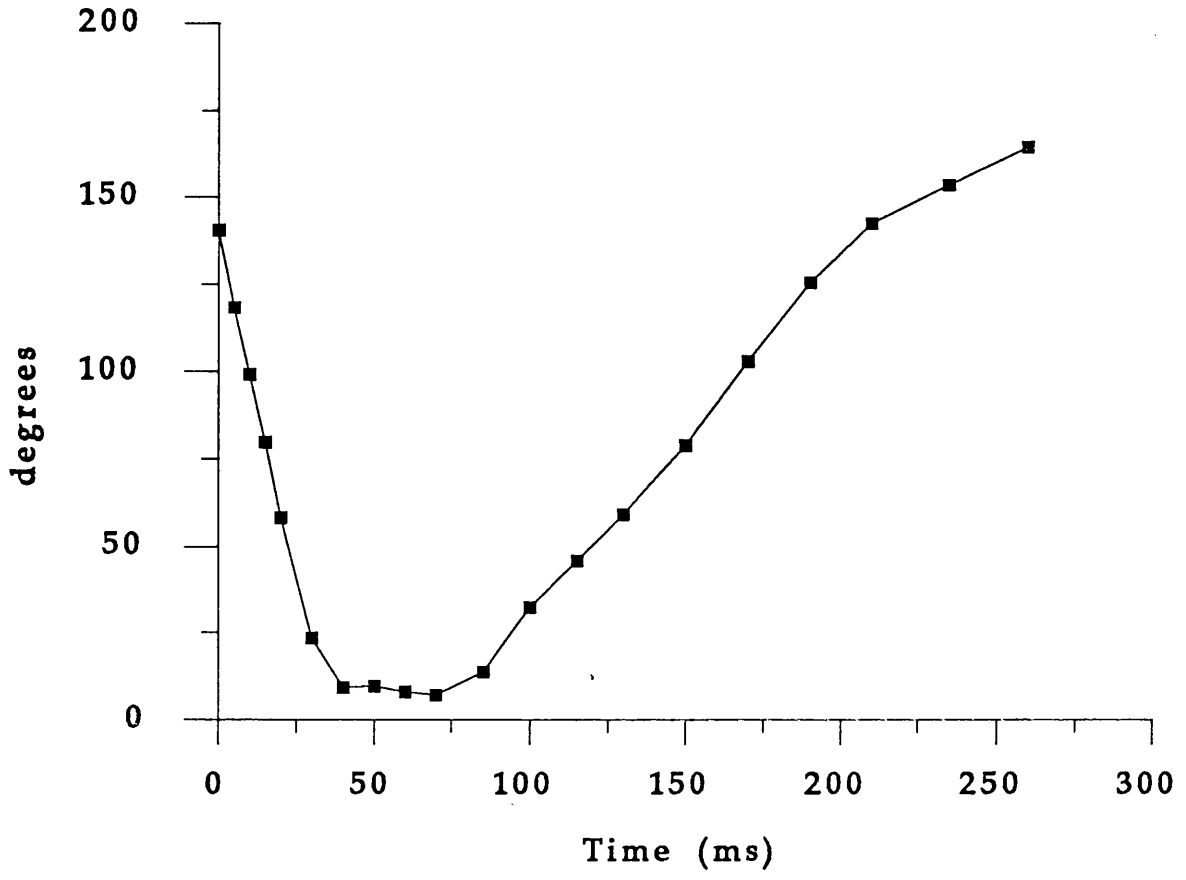

2
3
1
1
2
3

Fig. 2.5. The change in the angle (°) formed between the cephalothorax and the abdomen of *Crangon crangon* in the course of a typical tail-flip at 10°C.

Fig. 2.6. The angular velocity (degrees per millisecond - $°ms^{-1}$) recorded in the course of a typical tail-flip at 10°C. A negative value is recorded when the angle between the cephalothorax and abdomen is decreasing.


200
150
100
50
0
degrees
0
50
100
150
200
250
300
Time (ms)

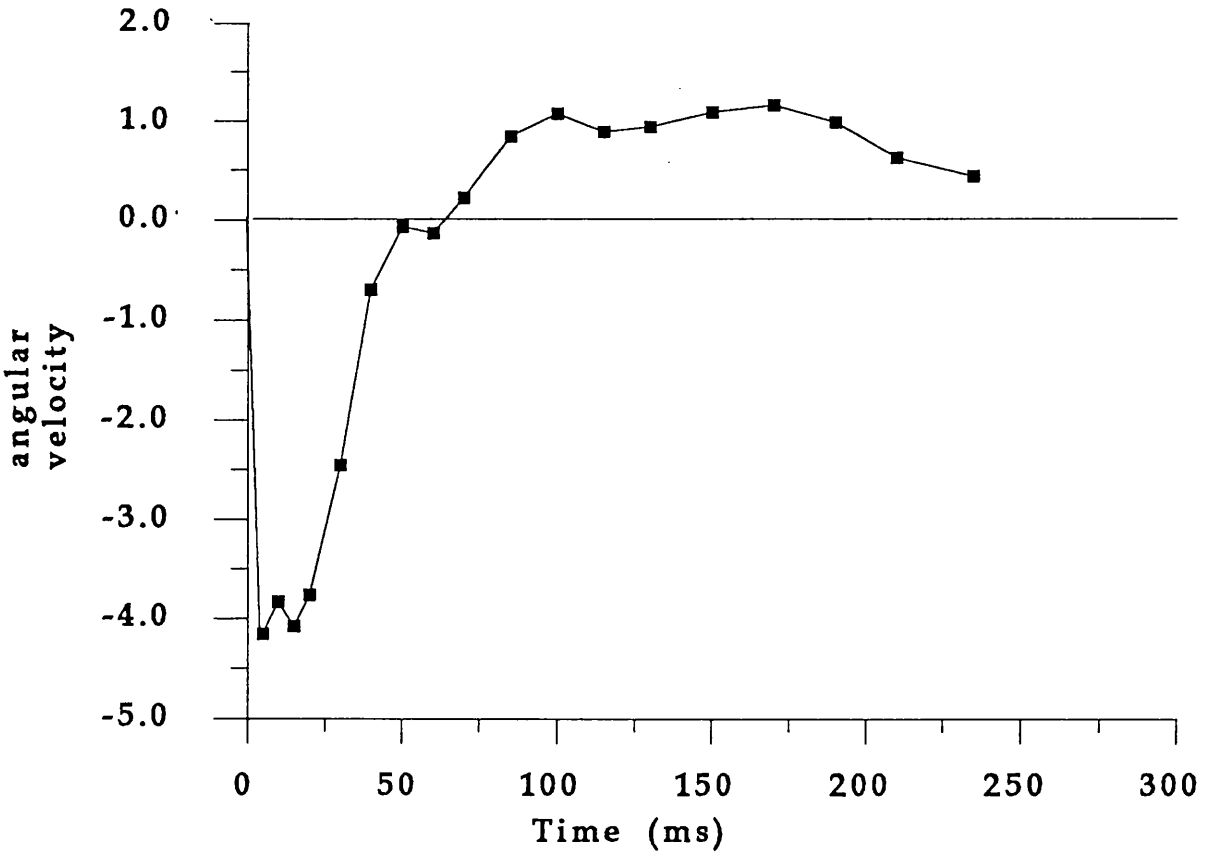
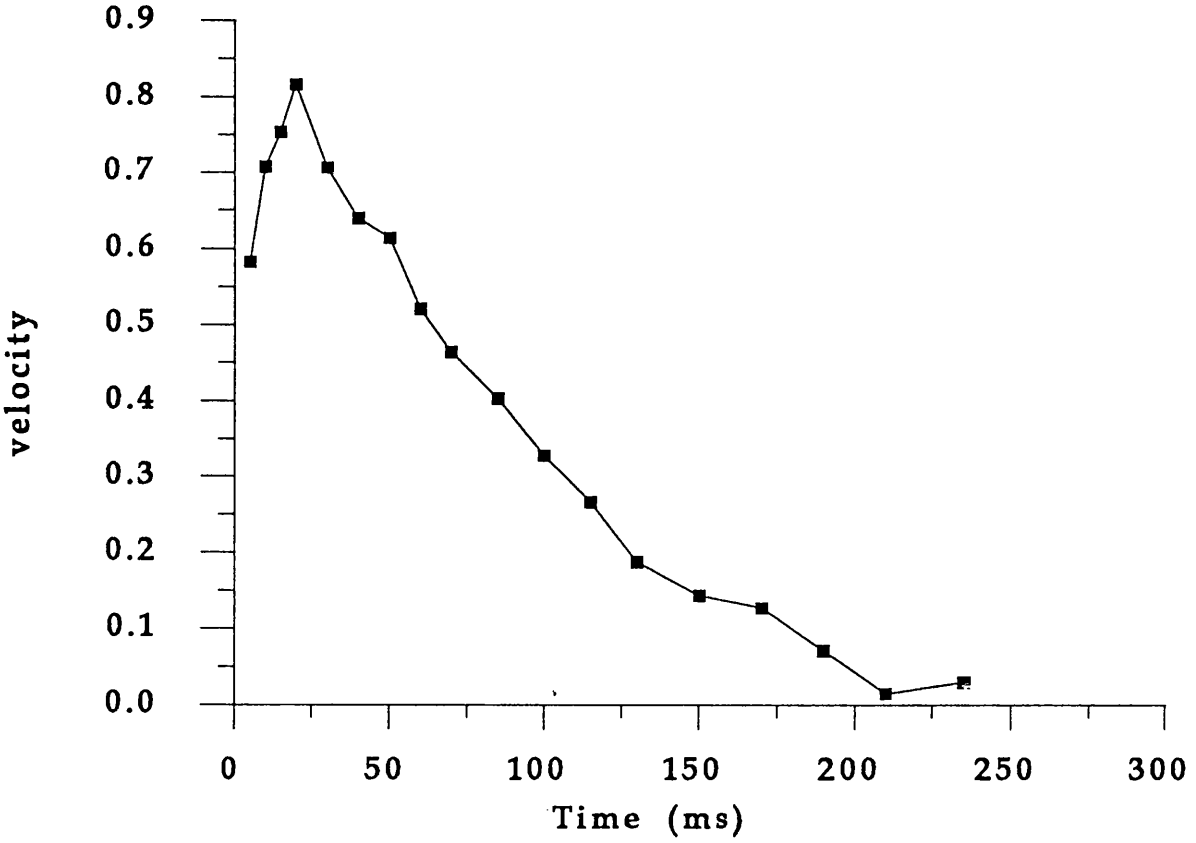

2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
angular velocity
0
50
100
150
200
250
300
Time (ms)

Fig. 2.7. The velocity ($m.s^{-1}$) of the body midpoint recorded in the course of a typical tail-flip at 10°C. Velocity is measured between frames 10-20ms apart, the entire tail-flip lasting 260ms.

Fig. 2.8. The acceleration ($m.s^{-2}$) of the body midpoint recorded in the course of a typical tail-flip at 10°C.


velocity
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
250
300
Time (ms)

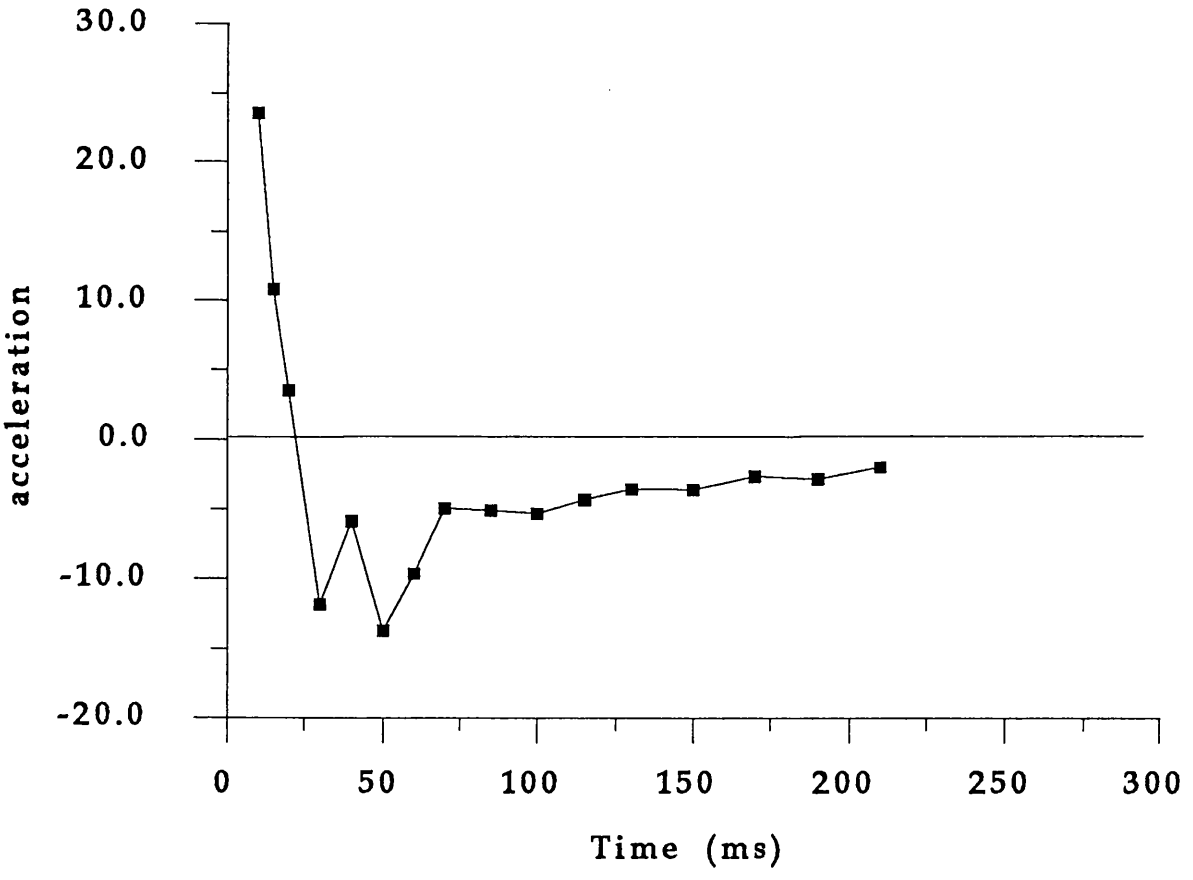
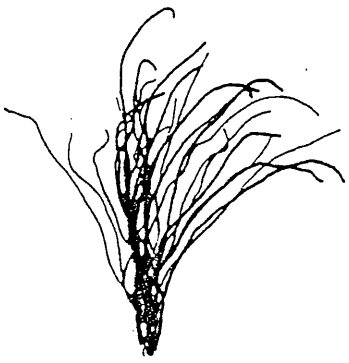

acceleration
30.0
20.0
10.0
0.0
-10.0
-20.0
0
50
100
150
200
250
300
Time (ms)

Fig. 2.9. Plot of trajectories of 40 tail-flips from video recordings. A = tail-flips elicited by rostral stimulus; B = tail-flips elicited by caudal stimulus. Scale bar = 40 mm. Direction of view is perpendicular to plane of tail-flip.

A

B



DISCUSSION

High speed video analysis provides a comprehensive view of the tail-flip of *Crangon crangon* and the interactions of the parameters of movement which govern it. *C. crangon*, like other crustaceans (Webb; 1979), is unable to produce thrust during both flexions and extensions of the abdomen, and is limited by its body plan to using an intermittent thrust system of a power and recovery phase. The thrust force of the tail-flip is exerted only in the flexion part of the flip, and the greatest part of the thrust force is produced in the very early stages of the flexion, (the greatest accelerations being produced in the first 10 ms). The initial burst of acceleration is due to the reactive force from the down beat of the open tail-fan. As velocity of the body of the shrimp increases the amount of drag and added mass force generated will also increase, and these forces, when they begin to counter-balance the body's inertial force, cause the deceleration of the motion of the shrimp. The process of deceleration starts before complete flexion is achieved. During the extension recovery phase, the tail-fan closes up to reduce drag forces.

Daniel & Meyhöfer (1989) developed predictions for the body movement and thrust forces associated with this type of escape locomotion in the prawn, *Pandalus danae*, using a system of differential equations. The predicted body movements were found to be within 10% of those produced during a tail-flip recorded using high speed ciné-photography. An important factor in these calculations was the thrust force generated by the squeeze of fluid from the closure of the abdomen against the

cephalothorax at the end of the flexion phase. Daniel & Meyhöfer (1989) found this squeeze force to be considerable enough to overwhelm any propulsive drag forces arising from the tail-flip. This does not appear to be the case in the tail-flip of *Crangon crangon.* It must be assumed that in order to produce a significant amount of thrust and achieve a jet propulsion effect from the squeeze force, a substantial quantity of water would have to be expelled from between the head and tail. This would lead us to expect the main squeeze force to be generated at the end of the closure of the abdomen against the cephalothorax, when water is forced out. However, reference to the graphs of velocity and acceleration show that this is not the case, deceleration beginning before the closure of the cephalothorax and abdomen or before the minimum angle is reached. Although there is a decrease in the rate of deceleration after 50 ms, the balance between thrust and drag is never offset to such an extent that acceleration is achieved. The decrease in the rate of deceleration may simply be due to a decrease in both the pressure drag (the amount of disturbance a body creates when moving through a fluid) and friction drag (the work done against the viscosity of the fluid) (see Alexander, 1968, for definitions) at the time the shrimp reaches its minimum angle and its most streamlined shape; almost ellipsoid.

Resolving the fine detail of the tail-flip and obtaining measures of the motion parameters, allows comparison with other studies of crustacean tail-flipping. The results of some of these studies suggest that a large linear acceleration is common to most tail-flippers (Webb, 1979; Newland *et al,* 1988; Daniel & Meyhöfer, 1989)

and that the maximum velocities reached by crustaceans during tail-flipping fall within a relatively narrow range. At the upper end of the range is the prawn, *Pandalus danae* which can reach maximum velocities of $3.1 m.s^{-1}$ (43 body lengths s^{-1}, calculated from Daniel & Meyhöfer, 1989). Krill (*Euphausia superba*) were found to reach velocities of around $1 m.s^{-1}$, (11 body lengths s^{-1}) during the tail-flip (Kils, 1982), though this measure was recorded at 1°C, and the mysid, *Praunus flexuosus* (O.F.Muller) attains a maximum velocity of approximately $0.66 m.s^{-1}$ (34 body lengths s^{-1}, from Ansell & Neil, 1991). *N.norvegicus* can reach a mean velocity over a bout of tail-flip swimming, of around $0.46 m.s^{-1}$ (5.4 body lengths s^{-1}, from Newland *et al*, 1988). *Crangon crangon* appears to fit into the middle of this range, attaining a mean maximum velocity of $1.07 m.s^{-1}$ (21 body lengths s^{-1}) at 10°C. Clearly these crustaceans are very different and are found in very different habitats, but the reason for the variations in velocity of tail-flipping is not immediately apparent and whether it is a reflection of predator-prey relationships or the physical restrictions of these habitats remains to be seen.

At a fundamental level, the method of tail-flipping is almost universal in macrurous crustaceans, involving a rapid flexion of the abdomen followed by a more gradual extension. In the larger nephropid crustaceans such as *Nephrops norvegicus* it appears that the flexion during the tail-flip is largely due to movement of the tail (Newland & Neil, 1990, I). This differs somewhat from the tail-flips of *Crangon crangon* (see Fig. 2.1) and the mysid, *Praunus flexuosus* (Ansell & Neil, 1991) which appear to involve more of a "jack-knife" action. It is clearly possible that this

difference is due primarily to morphological and size differences in the animals. However, a similar pattern of tail flexion to that of *N. norvegicus* is also found in krill, *Euphausia superba* which are of a similar size range to *C. crangon* (Kils, 1982) and therefore it appears unlikely that size is the important factor.

Both *Nephrops norvegicus* and *Homarus* spp. have heavy claws which must alter the mechanics of the tail-flip, as moving the mass of the claws and cephalothorax in the course of an escape response would presumably be more energetically costly than a simple tail movement. However, *Euphausia superba*, which shows a similar pattern of tail flexion, is not impeded by heavy claws. *N. norvegicus* is known to perform multiple tail-flips and after the initial flip the point of flexion is thought to move from anterior segments of the abdomen to posterior segments (Newland & Neil, 1990, I). It is likely that in changing the point of flexion, the mass of the cephalothorax is balanced and kept horizontal, the animal moving along its longitudinal axis, with only the tail moving in subsequent abdominal flexions. It is probable that this is the most efficient method for producing multiple flips, and allows more directional control. It is thought that the flexion of more posterior abdominal segments is also used to produce the more horizontal trajectories of rostral elicited tail- flips in *N. norvegicus* (Newland & Neil, 1990, I). This method of altering the point of flexion may also used by *E. superba*, which is known to perform multiple tail-flips in tail-swimming (Kils, 1982).

In addition to morphological differences, differences in tail-flip method may also reflect the different predator-prey interactions that the animals encounter in their own ecological niche. Both *Nephrops norvegicus* and *Homarus spp.* rely on some sort of permanent shelter near their foraging areas for refuge. In *N. norvegicus* this is a permanent burrow in mud, whereas *Homarus spp.* rely for shelter on excavations near rocks, boulders or vegetation. Both animals must be able to produce fast, efficient tail-flip swimming which enables them to find their refuge successfully. *Euphausia superba* on the other hand is more likely to be found in a school or swarm and as such will aim to escape capture by a predator by swimming rapidly and efficiently, changing direction erratically with the swarm until the predator gives up or catches a less able animal. It seems likely that *Crangon crangon* has a different set of constraints, living as it does on virtually featureless sand without a permanent burrow. There being no advantage in being able to escape swim for any considerable distance in these conditions, it may be more important to produce an extremely rapid tail-flip at the expense of efficiency, hence the jackknife type tail-flip. This possibility will be considered in greater depth in a later chapter.

Although able to resolve a great deal of detail of the tail-flip, the results reported here were not able to prove the use or absence of use of steering by *Crangon crangon* during the tail-flip. Hessler (1983) mentions the use of the tail fan and the scale-like antennal exopods in the control of direction during normal swimming in caridoids. However there is little evidence of this kind of steering during the tail-flip escape response of *C. crangon.* Studies have reported the opening

of the tail-fan at the onset of the tail-flip (Daniel & Meyhöfer, 1989) and this is also the case in *C. crangon.* Some video recordings of *C. crangon* suggest that the antennal plates also open in conjunction with the uropod fan. However, whether this is purely for symmetry, which would appear to be important to reduce uncontrolled rotational movement, or whether the scales are used for steering is unknown. *Praunus flexuosus* has been shown to use asymmetrical spreading of the uropods and the antennal scales to produce rolling of the body in the initial stages of the escape response, which acts to determine the direction of travel during the flip (Ansell & Neil, 1991) and Krill, *Euphausia superba,* show a definite ability to control the direction of travel during tail-flip swimming, to the extent of actively avoiding collision with other animals (Kils, 1982). It may be that these species have the ability to steer during tail-flipping because they are pelagic and have no other way of determining the direction they take in an escape response. Benthic species, such as *C. crangon,* tail-flip from the substratum into the water column, and may be able to gain some control of direction from pushing off the substratum. Further investigation into the 'take-off' of the tail-flip of *C. crangon* would be useful to examine the effects of pushing off the substratum on the direction the tail-flip takes and to determine if any thrust is generated from it. Paul (1990) suggests that the tail-flip developed from a backwards jump escape response in ancestral malacostracans, and indeed leg promotion is associated with medial giant-type flexions in crayfish (Cooke, 1985). Webb (1979) reports that the crayfish *Orconectes virilis* push off the substratum during the lift-off of a lateral giant type tail-flip. It is possible therefore that there is a leg jump aspect

to the *C. crangon* tail-flip, though no evidence was found to support or refute this in the video recordings.

The direction the animal takes in the tail-flip is, to varying degrees, affected by the location of the stimulus on the body. The correct direction of response would appear to be essential to the success of the tail-flip and therefore this finding is not unexpected. However the speed at which these movements are made is extremely high. Both *Nephrops norvegicus* and *Procambarus clarkii* show tail-flips directed almost directly backwards following rostral stimulation, and a more elevated trajectory following a stimulus to the tail (Wine & Krasne, 1972; Newland & Neil, 1990). These trajectory differences are due to the flips being produced via different neuronal routes. Two pairs of giant axons in the ventral cord, the medial giants (MGs) and the lateral giants (LGs) are responsible for this separation of response, the medial giants being activated following rostral stimulation and the lateral giants following stimuli to the tail. There is not enough evidence found in the present analysis of tail-flips of *Crangon crangon* of distinct trajectory differences between the rostrum stimulated flips and the tail stimulated flips. Also there is no evidence to suggest that *C. crangon* alters the point of flexion during tail-flipping to produce more horizontal trajectories in the way that *N. norvegicus* does, and it may be that these low trajectories are achieved in a different way. As has already been mentioned a number of tail-flips were performed which involved a rotation outwith the X-Y plane (plane of focus), and as such these could not be analysed as they would require 3-dimensional analysis. It is possible that this method of rotation is used primarily to

produce low trajectories in a similar way to the mysid, *Praunus flexuosus* (Ansell & Neil, 1991). Whether these rotational movements are elicited more often by rostral stimulation in *C. crangon* is unknown.

CHAPTER 3 : TEMPERATURE EFFECTS ON ESCAPE SWIMMING

There is a large body of work examining the effects of temperature on a variety of species, many studies focussing on marine invertebrates (for reviews see Newell & Bayne, 1973; Wieser, 1973; Cossins & Bowler, 1987). The level of stress due to temperature change is proportional to the magnitude and rapidity of change and in the case of marine animals, the most rapid changes in temperature are those accompanying tide and weather cycles (Somero & Hochachka, 1976).

Crangon crangon is found in brackish estuarine areas with strong tidal currents and on tidal mud flats. Both situations are subject to relatively rapid changes in temperature, either through tidal movement in estuaries or the drying or inundation of tidal pools. *C. crangon* is reported to survive temperatures of between 0 and 30 °C (Lloyd & Yonge, 1947; Tiews, 1970), and the tidal flats in the Wadden Sea where *C. crangon* is abundant undergo temperature increases to around 30°C during periods of tidal drying.

Most studies of temperature effects have concentrated on changes in metabolic rate and on physiological effects (Newell & Bayne, 1973; Wieser, 1973), and there is little information about the effects of temperature on behavioural events. Although *Crangon crangon* is known to tolerate large temperature changes, it is not known how temperature affects the behaviour of *C. crangon*, and in particular the escape response of the shrimp. Some studies examining the effects of temperature on

the nerve-muscle systems of crustaceans (Florey & Hoyle, 1976; Harri & Florey, 1977; Fischer & Florey, 1981) may provide some insight into the implications of temperature change for the tail-flip of *C. crangon.*

The experiments in Chapter 2 allowed the measurement of parameters of the tail-flip of *Crangon crangon.* These can be used as a gauge of the performance of an escape response and are used in the experiments presented here to compare the effects of temperature change on escape behaviour.

Animals were exposed to sudden or acute temperature change, and were allowed to become acclimated to different temperatures. It appears likely that both of these types of temperature change may be encountered by *Crangon crangon* in the field.

MATERIALS AND METHODS

Two experiments examining the effects of temperature on tail-flip escape behaviour were carried out using the high speed video recording system. The first involved exposing the shrimps to acute changes in temperature and examining the effects of these changes on escape behaviour. The second experiment examined the escape behaviour of animals which were acclimated to different temperatures. The results of these two experiments were then compared.

EXPERIMENTAL ANIMALS - ACUTE TEMPERATURE CHANGES.

Crangon crangon were taken from Dunstaffnage Bay by trawl and kept in an aquarium, with aerated, running seawater at temperatures of between 8°C and 12°C, until required. They were fed at regular intervals on minced squid. *C. crangon* of both sexes were used in the experiment, ranging in total body length from 47 to 55mm.

EXPERIMENTAL ANIMALS - TEMPERATURE ACCLIMATED.

Shrimps were obtained by trawl from Kames Bay, Millport and transported to the Dunstaffnage Marine Laboratory (D.M.L.), where they were divided between 4 acclimation tanks. These tanks were around 0.5m deep, with a diameter of 0.5m,

and contained aerated seawater and a layer of sand, to reduce stress and allow the shrimps to bury. The aquarium tanks were maintained at 4 different temperatures: 5°C, 10°C, 15°C and 20°C temperature in temperature controlled rooms. They were covered in order to cut down evaporation and the water was changed at regular intervals. The shrimps were kept at a density of around 20 to 25 shrimps per tank and were fed regularly on minced squid. Animals of both sexes were used, ranging in body length from 39 to 55mm. The acclimation tanks were set up for a period of six weeks before filming began.

As the animals which were exposed to an "acute" change in temperature had been taken from water at a temperature of around 12°C on the day of video recording, those encountering temperatures of between 10 and 15°C under the acute regime were essentially acclimated and should therefore perform in a similar way to acclimated animals.

VIDEO RECORDING.

The tail-flips of shrimps were recorded in the same way as is described in Chapter 1 (Fig. 2.1). The shrimps were placed in seawater at the temperature of acclimation, in the Perspex aquarium. Again, tail-flips were elicited by a mechanical stimulus on the rostrum or on the tail of the shrimp and recorded at a frame rate of 400/s using an NAC high-speed video recorder (model HSV 400) illuminated by strobe lighting. Recordings were then analyzed using the digitizing tablet and x-y

coordinator of the NAC HSV system. Positional coordinates of the rostrum, the point of flexion and the tail were entered from each frame and analyzed using the "Movias" software package. Velocity, acceleration, degree of flexion, angular velocity during flexion and trajectory and time course of the flip were measured. As before only tail-flips which occurred predominantly in the plane of focus, could be analyzed due to the limitations of the single direction of filming and the two dimensional analysis of the "Movias" software package. In the course of recording the tail-flips of animals exposed to an acute change in temperature, some tail-flips continued beyond the field of view and as a result the final stages of the tail-flip were not recorded. In these cases the duration of the entire tail-flip and the total displacement of the shrimp could not be determined. However, incomplete tail-flips contributed to analyses of the flexion part of the tail-flip.

When animals are exposed to an acute change in temperature, the maximum velocity achieved during the tail-flip shows a direct relationship to temperature. This relationship is shown in Figure 3.1 which shows the mean maximum velocity recorded at each temperature plotted against temperature. The slope of the line of increase is found to be highly significant ($t = 5.14$, $p < 0.01$, at 131 degrees of freedom), (Table 3.1). Similar increases in both the maximum acceleration (Fig. 3.2) achieved and the maximum angular velocity (Fig. 3.3) achieved in the course of a tail-flip, are seen after an acute temperature increase, the slope of the increases in both parameters being highly significant ($t = 41.49$, $p < 0.01$, at 111 degrees of freedom, and $t = 12.12$, $p < 0.01$, at 115 degrees of freedom, respectively, Table 3.1). It is interesting to note the occurrence of high values at 10°C, in both of these cases. As all the animals tested came from an ambient water temperature of 12°C, it may be that animals tested at 10°C suffered less temperature stress than those animals tested at other temperatures after an acute change in temperature.

When exposed to an acute change in temperature the duration of the flexion phase of the tail-flip decreases with increasing temperature (Fig. 3.4). This decrease is found to be highly significant ($t = 25.89$, $p < 0.01$, at 115 degrees of freedom, Table 3.1). A similar, though steeper, decrease is recorded in the total duration of the tail-flip with increasing temperature (Fig. 3.5), which is again found to be highly significant ($t = 15.18$, $p < 0.01$, at 46 degrees of freedom, Table 3.1). Over the

range of increased temperature, the total displacement of the shrimp during the tail-flip shows a slight rise (Fig. 3.6). The slope of regression of this line is found to be highly significant ($t = 4.32$, $p > 0.01$, at 46 degrees of freedom, Table 3.1). The slope of both the lines of total duration and total displacement during the tail-flip were calculated only from complete tail-flips that included an entire extension of the abdomen.

After an acclimation period, the effect of temperature on the parameters of motion which govern the tail-flip is reduced. The results are summarised in Table 3.1. Increases in the acclimated temperature result in only very slight increases in the maximum velocities which are recorded (Fig. 3.1), the slope of which is found to be not significant ($t = 1.15$, $p > 0.1$, at 114 degrees of freedom). Over the range of temperature, a shallow slope of increase is found in the maximum acceleration achieved in the course of the tail-flip, as well as in the maximum angular velocity achieved (Figs 3.2 and 3.3). The slopes of regression of these increases were found to be highly significant ($t = 14.39$, $p < 0.01$, at 114 degrees of freedom and $t = 5.14$, $p < 0.01$, at 113 degrees of freedom, respectively).

After acclimation, a more gradual slope of decrease in the duration of the flexion phase of the tail-flip is recorded than that recorded after an acute increase in temperature (Figure 3.4). The slope of the line is still highly significant ($t = 12.7$, $p < 0.01$, at 113 degrees of freedom, Table 3.1). Similarly the slope of decrease of total duration of a tail-flip at increasing acclimation temperature is less than after an

acute change, though still highly significant ($t = 21.96$, $p < 0.01$, at 114 degrees of freedom, Table 3.1), (Fig. 3.5). Lastly an increase in acclimation temperature produces a significant decrease in the total displacement of the shrimp in the course of an escape response ($t = 2.06$, $p < 0.05$, at 114 degrees of freedom, Table 3.1), (Fig. 3.6).

Similar values are recorded at 15°C for maximum velocity, maximum acceleration, maximum angular velocity and duration of flexion. This is presumably due to the acclimation effect of animals coming from a similar temperature before an acute temperature change.

By calculating the 95% confidence limits of the slope of the regression lines for each graph and comparing the results of tail-flips performed after acclimation with those recorded after an acute temperature change, the difference between the two treatments, in respect of escape behaviour, can be estimated. These confidence limits are summarised in Table 3.2

Using this method a significant difference was found between the slopes of maximum velocity achieved after an acclimated temperature increase and an acute increase. The slope of increase of the maximum tail-flip velocities recorded was found to be steeper after an acute change in temperature than after acclimation ($p < 0.05$). Similarly a significant difference was also found between the slopes of maximum acceleration recorded at increasing temperature after an acute change and

after acclimation. The slope of maximum angular velocity achieved at increased temperature after an acute change in water temperature and after acclimation was also found to differ significantly ($P < 0.05$, in both cases). In both cases an acute change in water temperature causes a more steeply sloping line of increase in the maximum values achieved over a range of increasing temperature.

There was no significant difference between the slopes of values recorded after an acute temperature change and after acclimation to temperature, in the total duration of tail-flips ($p > 0.05$). However, a significant difference was found in the degree of slope between acclimated animals and those exposed to an acute temperature change with regard to duration of flexion and the total displacement of the body in the course of a tail-flip ($p < 0.05$, in both cases).

As no significant difference was found between the slopes of acclimated and acute temperature change in relation to the total duration of the tail-flip, the 95% confidence limits of the points of intercept were examined for the two lines (acute change and acclimation). These were found to be significantly different ($p < 0.05$), (Table 3.3). The total duration of the tail-flip being greater after acclimation to temperature.

The relationships between the maximum velocity achieved during the tail-flip, the maximum angular velocity reached, and the total displacement are examined in Figures 3.7 - 3.10. Figure 3.7 illustrates the close relationship between maximum

angular velocity and the maximum velocity reached after an acute change in temperature, with the maximum velocity of the body of the shrimp increasing with increased velocity of body flexion during the tail-flip. This relationship is reflected by the significant correlation coefficient ($r = 0.525$; $t = 4.2$; $p < 0.01$). Interestingly, as Figure 3.8 shows, the initial increase in the maximum velocity recorded is not reflected in a greater total displacement of the shrimp in the course of the tail-flip. The correlation coefficient of this relationship is not found to be significant ($r = 0.168$; $t = 1.16$; $p > 0.1$).

After acclimation the points of maximum angular velocity and maximum velocity becoming more clumped. However a significant correlation is found to exist ($r = 0.515$; $t = 6.36$; $p < 0.01$). Similarly a significant correlation is also found between the maximum velocity recorded and the total displacement of the animal in the course of a tail-flip, after acclimation ($r = 0.196$; $t = 2.13$; $p < 0.05$). (Figures 3.9 and 3.10).

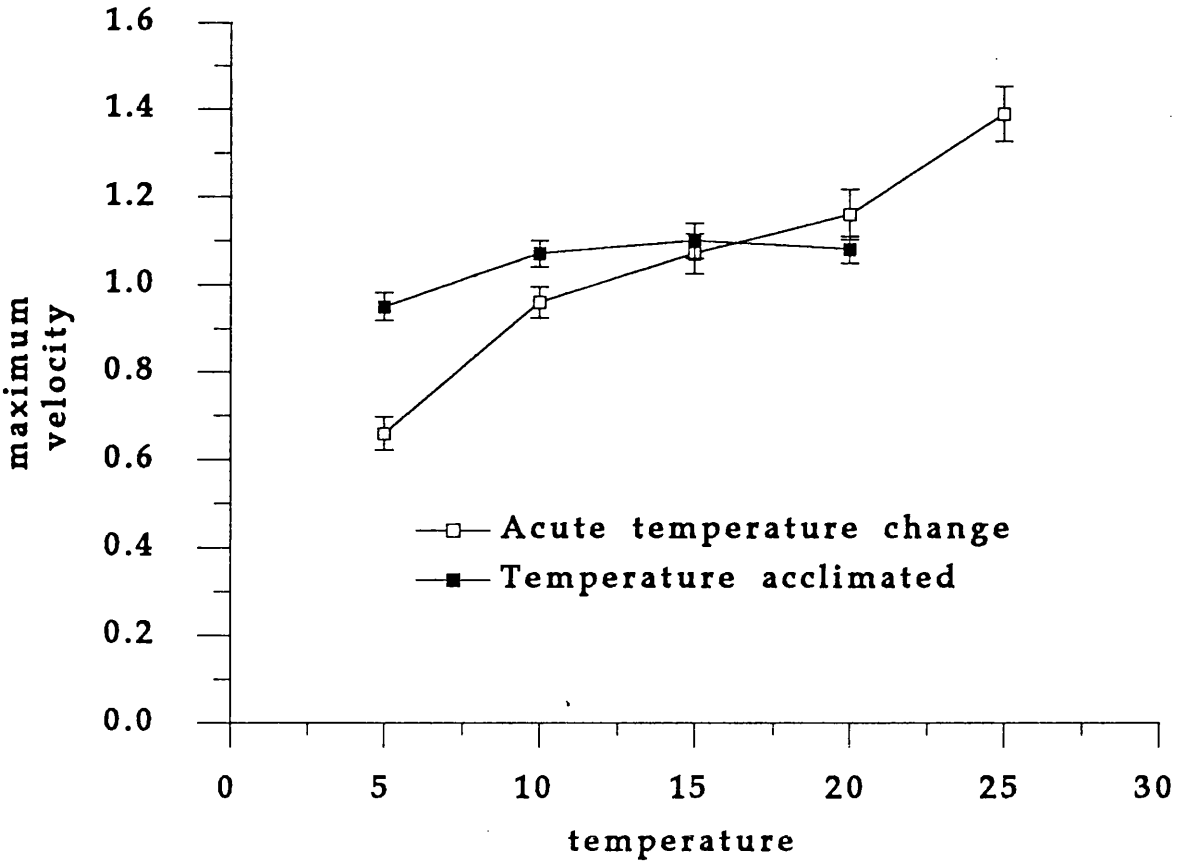
		n	Slope	T value of slope	P
Velocity	Acute	133	0.033	5.14	< 0.01
	Acclimated	116	0.008	1.15	NS
Acceleration	Acute	113	3.34	41.49	< 0.01
	Acclimated	116	1.04	14.39	< 0.01
Angular Velocity	Acute	117	0.226	12.12	< 0.01
	Acclimated	115	0.100	5.14	< 0.01
Duration of flexion	Acute	117	-1.35	25.89	< 0.01
	Acclimated	115	-0.68	12.7	< 0.01
Total duration	Acute	48	-3.30	15.18	< 0.01
	Acclimated	116	-3.22	21.96	< 0.01
Total displacement	Acute	48	0.47	4.32	< 0.01
	Acclimated	116	-0.14	2.06	< 0.05

NS = Not significant

Table 3.1: Relationship between increasing temperature and the parameters of the tail-flip.

Fig. 3.1. Mean maximum velocities ($m.s^{-1}$), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 23; 27; 33; 24; 24, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).

Fig. 3.2. Mean maximum acceleration ($m.s^{-2}$), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 23; 27; 33; 24; 24, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).


1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
maximum velocity
Acute temperature change
Temperature acclimated
0
5
10
15
20
25
30
temperature

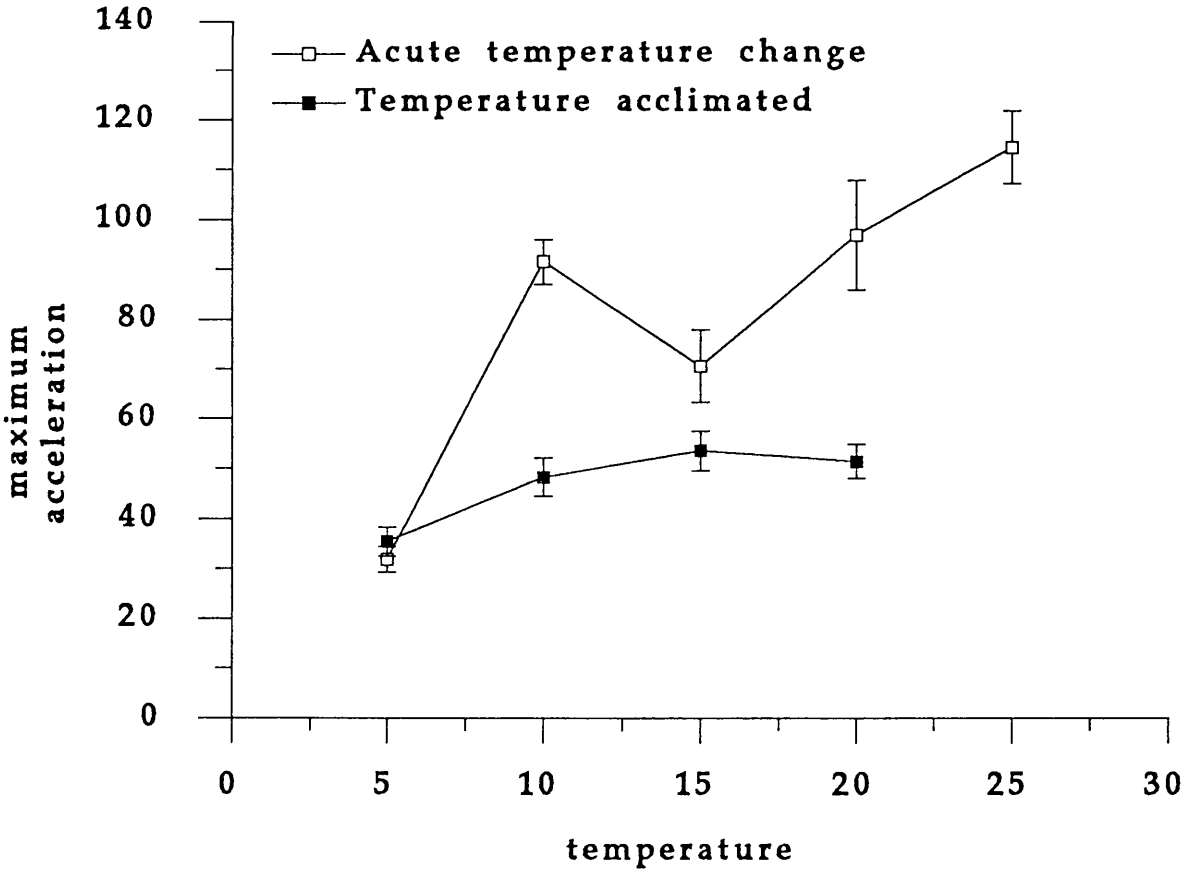
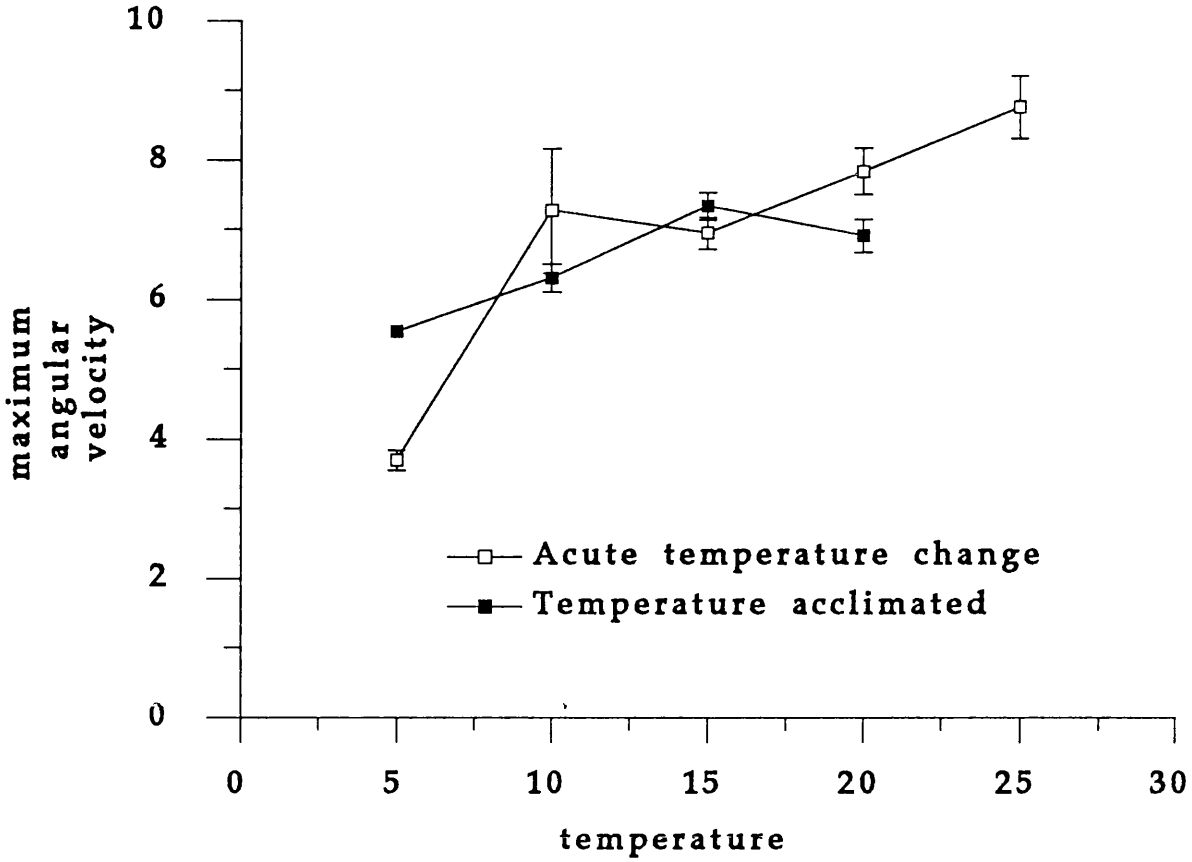

140
120
100
80
60
40
20
0
maximum acceleration
Acute temperature change
Temperature acclimated
0
5
10
15
20
25
30
temperature

Fig. 3.3. Mean maximum angular velocity ($°ms^{-1}$), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 23; 27; 33; 24; 24, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).

Fig. 3.4. Mean duration of flexion (ms), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 23; 27; 33; 24; 24, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).


maximum angular velocity
10
8
6
4
2
0
0
5
10
15
20
25
30
temperature
Acute temperature change
Temperature acclimated

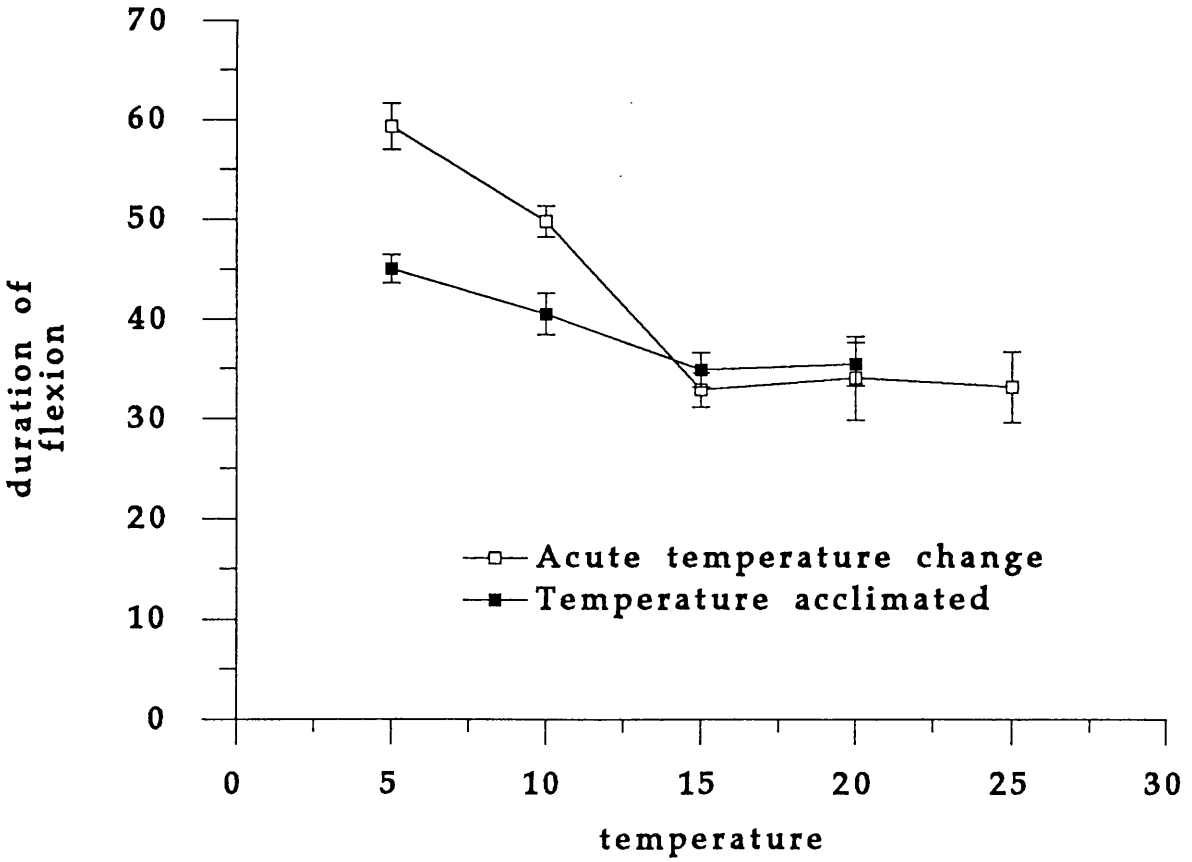
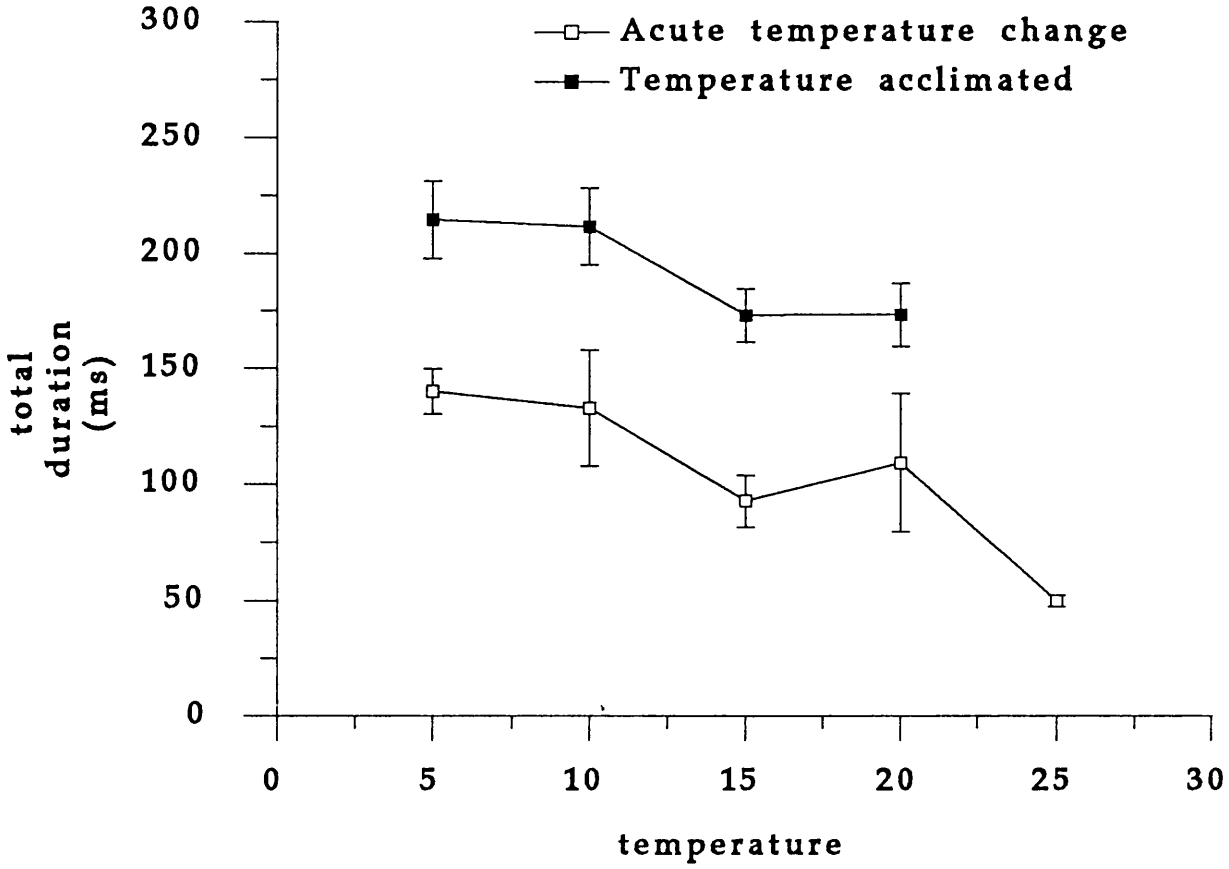
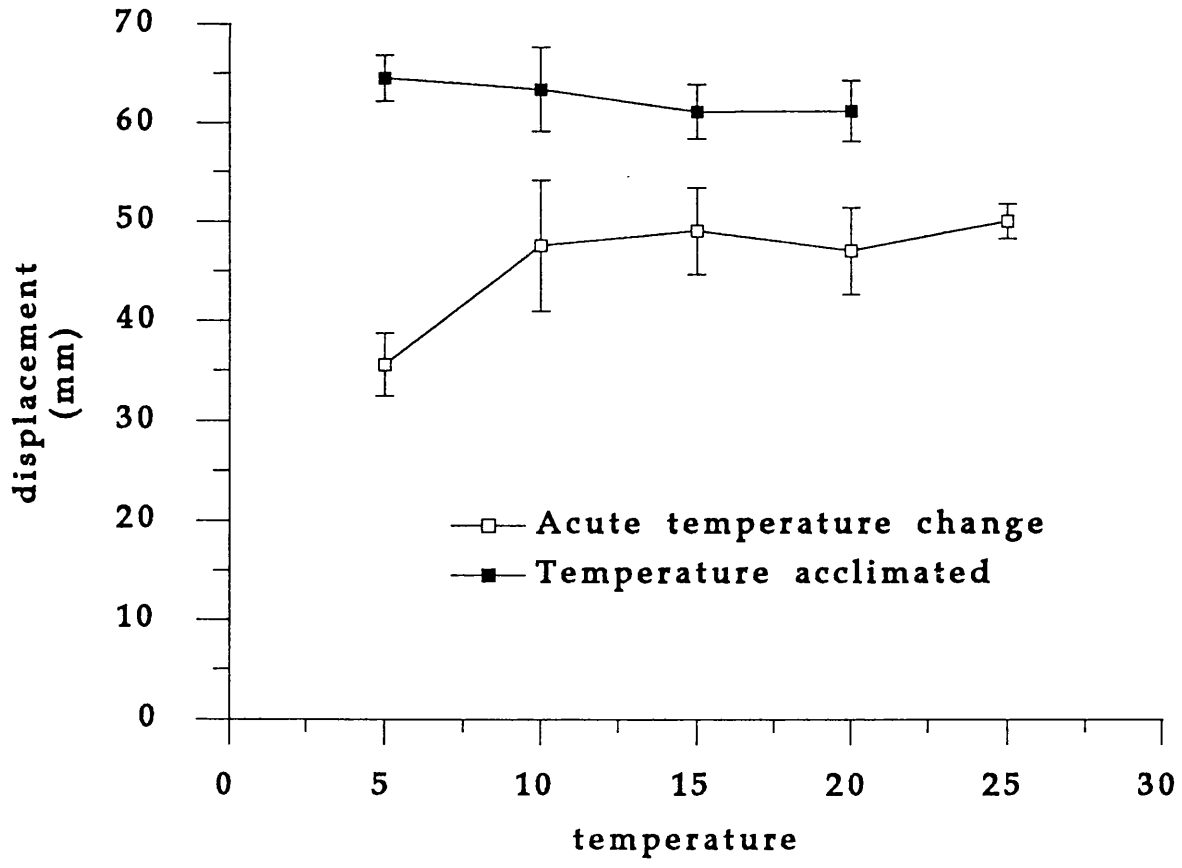

duration of flexion
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
temperature
Acute temperature change
Temperature acclimated

Fig. 3.5. Mean total duration (mm), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 6; 11; 18; 10; 3, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).

Fig. 3.6. Mean total displacement (mm), (± S.D.) recorded during tail-flips performed: 1. After an acute change of temperature to 5°, 10°, 15°, 20° or 25°C (n = 6; 11; 18; 10; 3, respectively) and 2. At 5°, 10°, 15° or 20°C after an acclimated change in temperature (n = 28; 28; 29; 31, respectively).


Acute temperature change
Temperature acclimated
total duration (ms)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
temperature


displacement (mm)
70
60
50
40
30
20
10
0
Acute temperature change
Temperature acclimated
0
5
10
15
20
25
30
temperature

		Slope	Standard deviation	95% confidence interval	P
Velocity	Acute	0.329	0.0032	(0.335, 0.323)	< 0.05
	Acclimated	0.008	0.0030	(0.014, 0.002)	
Acceleration	Acute	3.34	0.508	(4.336, 2.344)	< 0.05
	Acclimated	1.04	0.317	(1.66, 0.42)	
Angular velocity	Acute	0.226	0.0259	(0.277, 0.175)	< 0.05
	Acclimated	0.100	0.0227	(0.145, 0.055)	
Duration of flexion	Acute	-1.35	0.2022	(-0.954,-1.746)	< 0.05
	Acclimated	-0.68	0.1716	(-0.343,-1.015)	
Total duration	Acute	-3.3	1.767	(0.16,-6.76)	NS
	Acclimated	-3.22	1.303	(-0.67, -5.77)	
Total displacement	Acute	0.475	0.452	(1.36,-0.41)	< 0.05
	Acclimated	-0.14	0.285	(0.418,-0.70)	

NS = Not significant

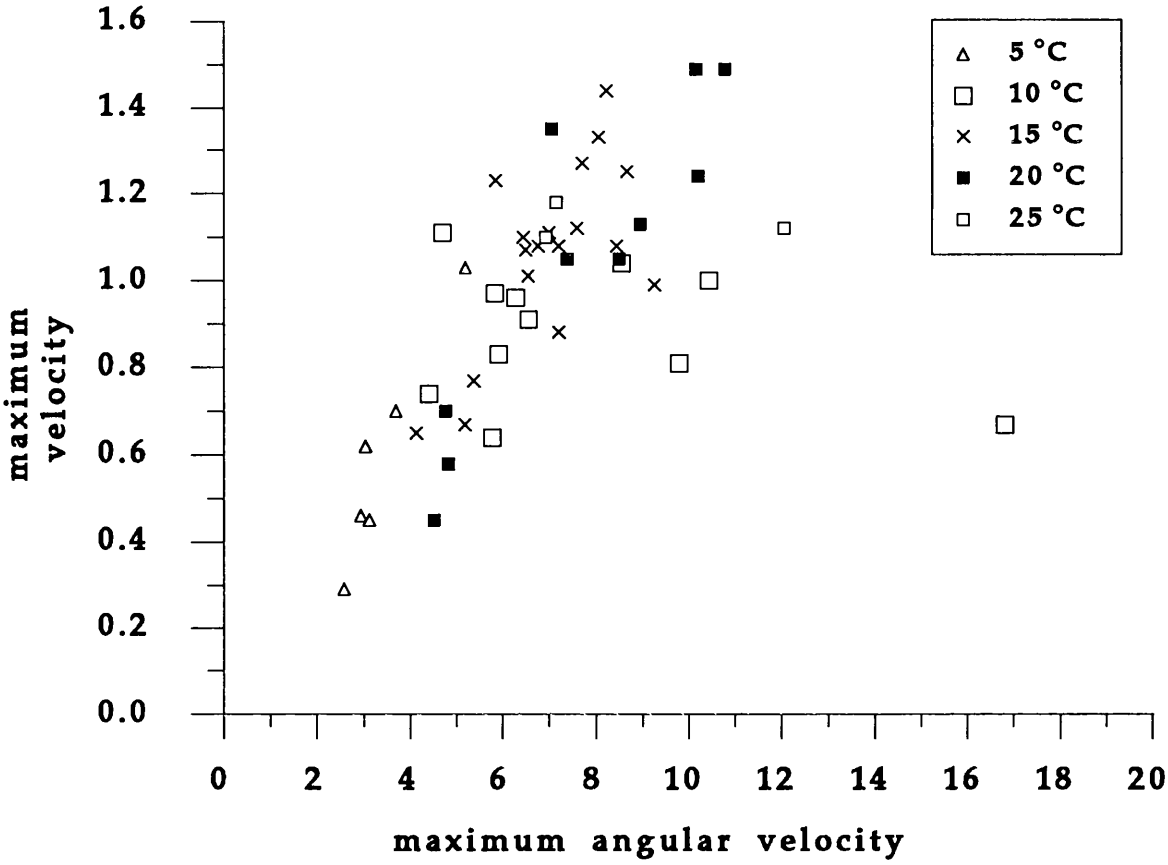
Table 3.2: 95% confidence limits of slopes of regression of the parameters of the tail-flip under two temperature regimes.

		Point of Intercept	Standard deviation	95% confidence interval	P
Total duration	Acute	155	26.96	207.8 - 102.2	< 0.05
	Acclimation	233	18.11	268.5 - 197.5	

Table 3.3: 95% confidence limits of points of intercept of regression slopes of the total duration of tail-flips under two temperature regimes.

Fig. 3.7. Relationship between the maximum angular velocity reached during the flexion of the body and the maximum velocity of the body after an acute change in temperature to between 5° and 25°C. ($r = 0.525$; $t = 4.2$; $p < 0.01$). (Both graphs consist of a constricted data set analysing full tail-flips only).

Fig. 3.8. Relationship between the maximum velocity achieved during tail-flips and the displacement of the shrimp after an acute change in temperature to between 5° and 25°C. ($r = 0.168$; $t = 1.16$; $p > 0.1$).


maximum velocity
maximum angular velocity
5 °C
10 °C
15 °C
20 °C
25 °C
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
2
4
6
8
10
12
14
16
18
20

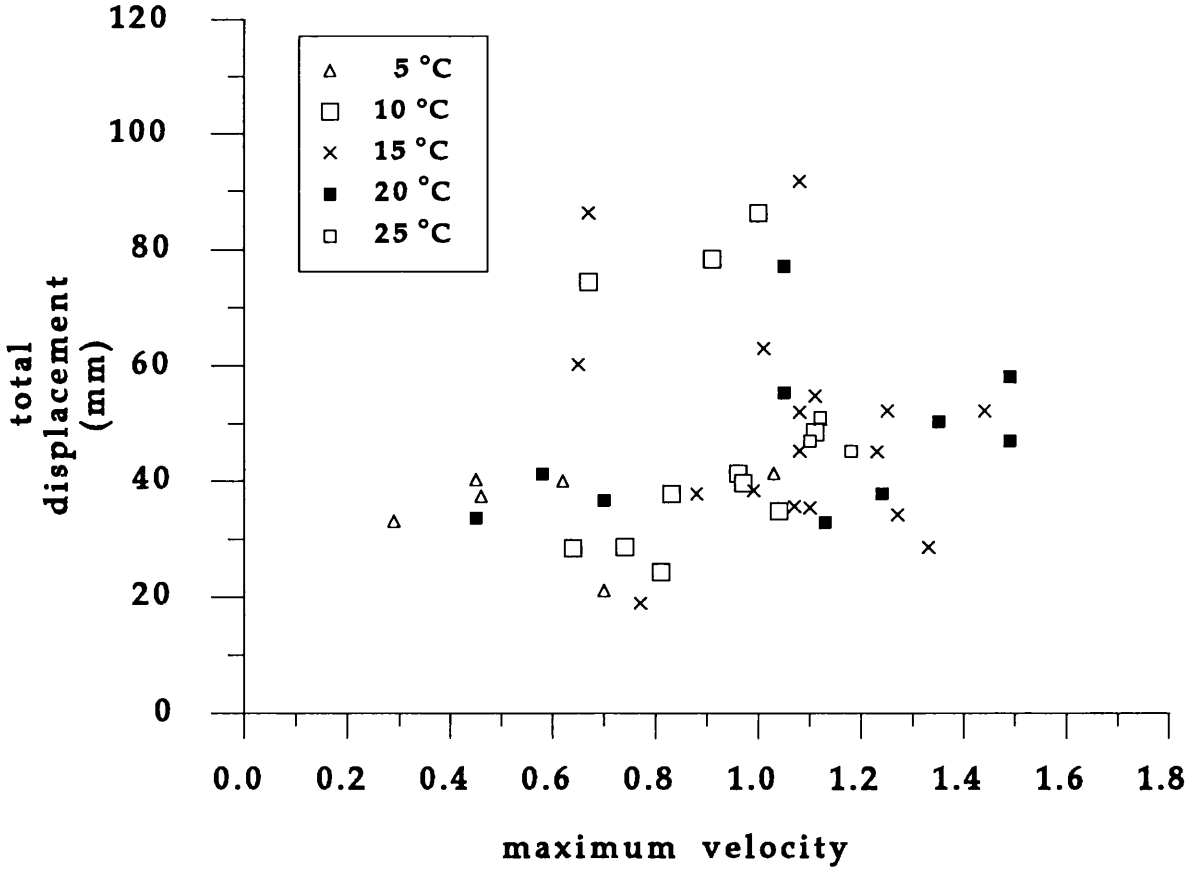
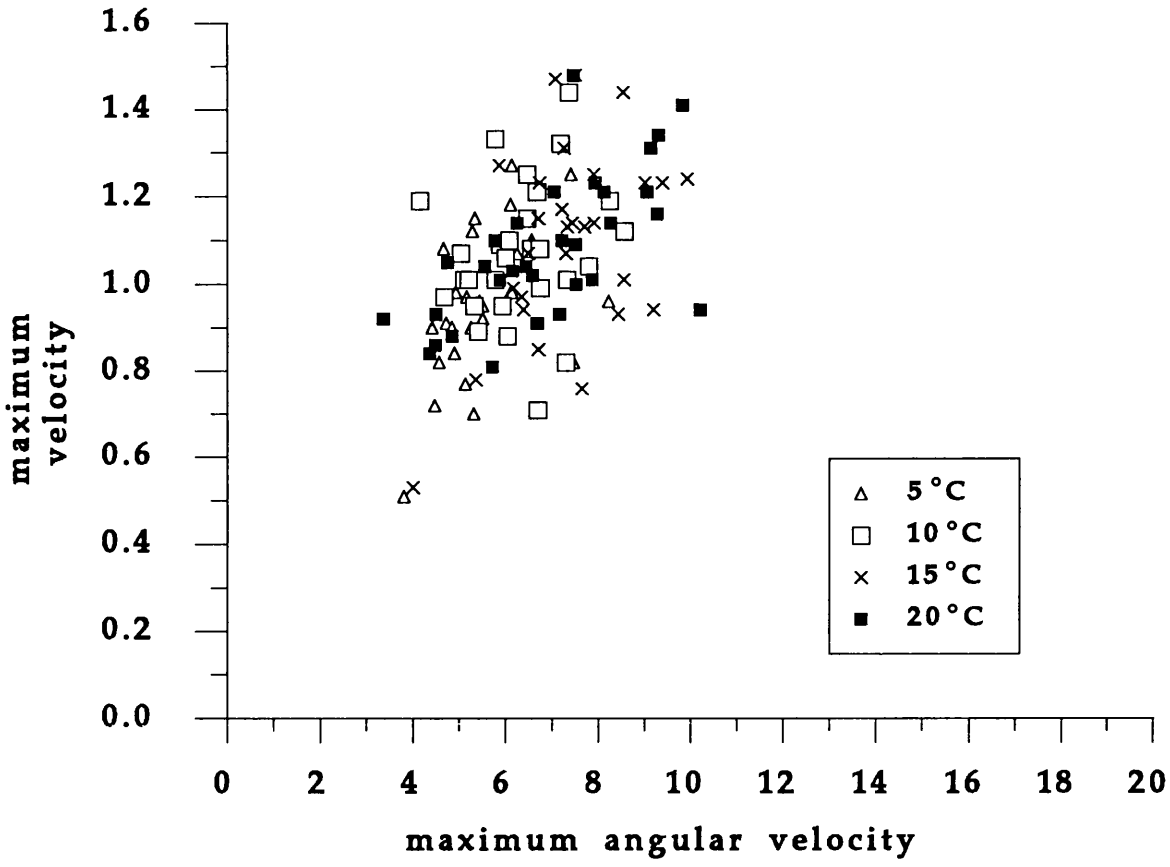
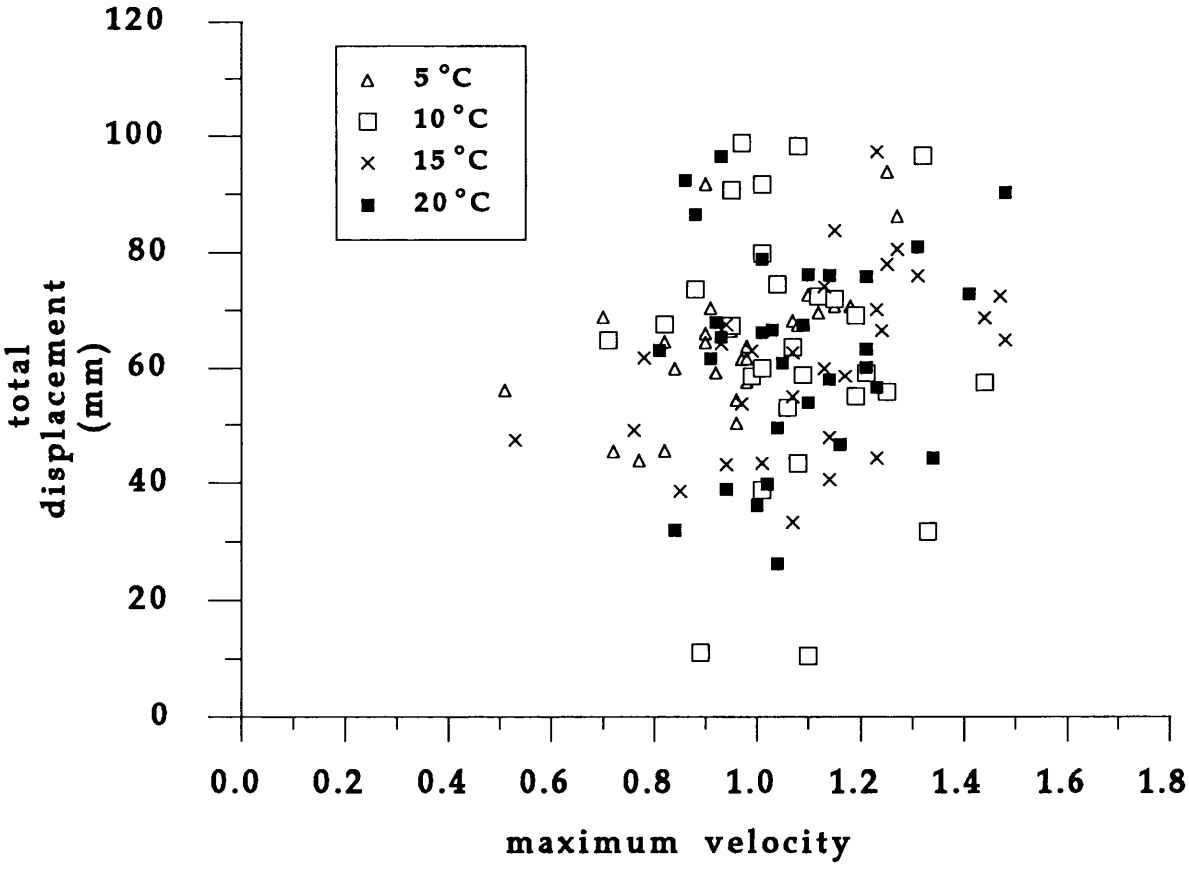

total displacement (mm)
maximum velocity
5 °C
10 °C
15 °C
20 °C
25 °C
0
20
40
60
80
100
120
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8

Fig. 3.9. Relationship between the maximum angular velocity reached during the flexion of the body and the maximum velocity of the body after an accclimated change in temperature to between 5° and 20°C. ($r = 0.515$; $t = 6.36$; $p < 0.01$).

Fig. 3.10. Relationship between the maximum velocity achieved during tail-flips and the displacement of the shrimp after an acclimated change in temperature to between 5° and 20°C. ($r = 0.196$; $t = 2.13$; $p < 0.05$).


maximum velocity
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
maximum angular velocity
5 °C
10 °C
15 °C
20 °C


total displacement (mm)
120
100
80
60
40
20
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
maximum velocity
5 °C
10 °C
15 °C
20 °C

DISCUSSION

There are a great many studies examining the effects of temperature on biological systems and often these effects are extremely complex (for reviews see Newell & Bayne, 1973; Wieser, 1973; Cossins & Bowler, 1987). The relationship between temperature and the parameters of motion during a tail-flip are apparently no less complex. Results obtained in the present study show a significant increase in the maximum angular velocity, maximum velocity, and maximum acceleration achieved during the course of a tail-flip, with an acute increase in temperature. After acclimation, animals perform similarly across the range of temperature. The graphs of both acute and acclimated temperature change show similar values at 15°C in all cases apart from the graphs of total displacement and total duration. The similar values are due to the fact that animals exposed to 15°C as an "acute" change in temperature had been kept previously at a similar temperature and they were therefore essentially acclimated. It is possible that the separation which is seen at 15°C between the acute and acclimated graphs of total duration and total displacement is not due only to the effects of acclimation. As is mentioned in the Material & Methods section, some tail-flips recorded after an acute change in temperature were not filmed in their entirety and only complete tail-flips were analysed. This may have led to selection of small tail-flips which may be reflected in the graphs of total duration and total displacement.

An increase in animal activity is generally expected with an increase in temperature, the increase in the kinetic energy of constituent molecules of an animal being tranlated into increases in metabolic rates and rates of other processes (Cossins & Bowler, 1987). However, the process by which the increase in the parameters of motion of a behavioural mechanism occurs is not immediately apparent. Some possible suggestions as to why the escape response would be faster at higher temperature are: at elevated temperatures motoneurons may fire at higher frequencies therefore promoting rapid muscle contractions; conduction along axons is enhanced at increased temperature; transmitter output at neuromuscular synapses may by greater at higher temperatures resulting in larger junction potentials and concomitantly larger and faster contractions.

There is a sizable body of work examining temperature effects on synaptic events in nerve-muscle systems of crustaceans (Florey & Hoyle, 1976; Harri & Florey, 1977, 1979; Fischer & Florey, 1981). In all cases the resting membrane potential of muscle fibres was found to increase with temperature in an almost linear fashion. Fischer & Florey (1981) found that with direct excitation of muscle fibres, the tension reached increased with increasing temperature. This was found to be due to greater suprathreshold depolarisation which resulted in a more rapid increase in tension and a higher level of tension being reached. However, in the case of nerve evoked tension development, depolarisation is affected by four factors: the amplitude of the first, unfacilitated excitatory post synaptic potential (e.p.s.p.); the facilitation ratio (the quotient of amplitude of the last, or nth e.p.s.p. and that of the first

e.p.s.p.); the time course of facilitation; and the time constant of the e.p.s.p. decay (which determines the degree of summation of e.p.s.ps). In previous studies all of these factors have been found to be temperature dependant.

According to Fischer & Florey (1981), unfacilitated e.p.s.p. amplitude increases with increasing temperature and as a result so does the amount of facilitation, for although the facilitation ratio itself is interval dependant and not temperature dependant, the increase in the amplitude of the first, unfacilitated e.p.s.p. due to temperature has a bearing on the ratio. When the interval between successive e.p.s.ps is shorter than the decay time of individual e.p.s.ps, summation occurs, and can reach a constant level where facilitation reaches its maximum and the facilitation rate drops to zero, resulting in the plateau depolarisation described by Harri & Florey (1977). Harri & Florey (1977) also report an increase in the amplitude of unfacilitated e.p.s.ps, resulting from stimulation of the fast axon, at increased temperature, but also find that there is a decrease in the rate of decay of e.p.s.ps at decreased temperature which is important in the summation of e.p.s.ps to produce plateau depolarisations. This results in an increase in tension achieved at decreased temperature. Despite this increased efficiency of contraction of fibres via a nerve-muscle synapse, at lower temperatures, Harri & Florey (1977) and Fischer & Florey (1981) argue that there must be some positive temperature coefficient in the region of the central nervous system which compensates this negative temperature coefficient and allows the crayfish to sustain normal behavioural activity across the wide range of temperatures in which it is found.

This proves confusing in the case of *Crangon crangon* which is also classed as a eurytherm, being found at a broad range of temperatures (Tiews, 1970), as an acute change in temperature (like that undergone by the nerve-muscle preparations in the experiments of Harri & Florey, 1977) clearly increases the maximum velocity reached in the course of the tail-flip. It is arguable that this sort of increase in the maximum velocity reached is achieved either by an increase in the force of contraction of the muscles powering the tail-flip, or by an increase in the speed of contraction of the muscles in reducing the angle between the tail and cephalothorax, in other words the angular velocity. According to the results shown previously, there is clearly a significant increase in the angular velocity with an increase in temperature, and as the graph of maximum velocity and maximum angular velocity show, a strong link exists between the two across the course of the experiment, with an increase in maximum velocity achieved at increased angular velocities. Neither an increase in the speed of contraction or an increase in the force of contraction was found to occur in crayfish in the course of an increase in temperature (Harri & Florey, 1977; Fischer & Florey, 1981). Harri & Florey failed to find any change in the behaviour exhibited by crayfish after exposure to an increase in temperature, stating that normal behavioural patterns were maintained over the range of temperature. Although there is an increase in the maximum velocity achieved and an increase in the maximum angular velocity attained in the course of the tail-flip of *C. crangon*, there is no evidence of a change in behaviour. *C. crangon* is still able to affect normal escape response behaviour across a broad range of temperature and to this extent there is consistency with the work of Harri & Florey. However if the

increase in the maximum velocity attained during tail-flipping cannot be explained in terms of the contraction of muscle fibres, as appears to be the case, it is difficult to see what might control this aspect of the tail-flip. It is possible of course that the system of *C. crangon* differs from that of *Astacus leptodactylus. C. crangon*, as a marine species, may have a system geared to coping with a combination of temperature and salinity which is not examined here at all. Indeed, Weber & Spaargaren, (1970) report that the effect of temperature change on the concentration of the blood depends on ambient salinity, with uptake and loss rates of ions changing in relation to temperature depending on salinity.

Following acclimation, shrimps between 5° and 20°C show the same escape performance in terms of the parameters of motion of the tail-flip, suggesting that some degree of rate compensation has occurred. Somero & Hochachka (1976) list potential mechanisms for rate compensation at a metabolic level. Rate compensation may involve a change in enzyme concentration or substrate and co-factor concentration, for example decreasing enzyme concentration or decreasing substrate availability during warm adaptation. Other possible mechanisms are the modulation of enzyme activity or the formation of new enzyme variants with different catalytic efficiencies. These mechanisms are understood to come into affect over different timescales and therefore some would appear in the early stages of acclimation and others later. How these processes are involved in acclimation and subsequent tail-flipping in *C. crangon* is unknown, but whatever method is used to compensate for changing rates of activity due to temperature change, it is likely to involve and affect

a large array of enzymatic processes amongst which temperature effects are highly diverse.

A number of animals are known to show improved locomotory performance following physiological compensation for temperature, in comparison to animals acclimated to different temperatures (for review see Cossins & Bowler, 1987). Cossins & Bowler (1987) review possible mechanisms of compensation, in terms of locomotion, in response to temperature change. One of the possible mechanisms of adaptation is to vary the pattern or number of muscle fibres active as temperature changes. Another involves alteration of the contractile properties of individual muscle fibres, either through energy metabolism or by alterations of the myofibrillar apparatus itself. Cossins & Bowler (1987) also discuss the possibility of alteration to the functional properties of neurones and axons during acclimation. It is suggested that there may be changes in conduction properties of axons and changes in the synapses and neuromuscular junctions.

Whatever the mechanism, is appears that acclimation should act to shift this optimum peak towards the acclimation temperature so that the animal performs as efficiently as possible at the temperature it is subject to. Harri & Florey (1979) found this to be the case in experiments on crayfish, *Astacus leptodactylus*, with the electrophysiological parameters of the nerve-muscle system showing a shift in efficiency towards the temperature of acclimation.

The work presented here has only enabled the identification of acclimation to temperature change and the behavioural consequences in regard to tail-flipping. However, the timecourse of this acclimation is still unclear and is likely to be of significant survival importance for *Crangon crangon*, and as such may merit further study.

CHAPTER 4 : EXTINCTION AND HABITUATION OF ESCAPE BEHAVIOUR

One of the most important aspects of tail-flipping, with regard to its success as an evasive mechanism is its endurance. Depending on the success of an escape tail-flip *Crangon crangon* may be subjected to subsequent attacks by the same predator, and the ability to sustain repeated tail-flips would be of great advantage. With energetic and ecological costs of its own, the predator is limited in the amount of time it can afford to pursue one animal. Therefore one strategy the shrimp may employ is to keep a predator at bay until the costs of pursuit become too high for the predator to continue that pursuit.

This may entail not only an ability to sustain prolonged escape behaviour, but also an ability to recover rapidly after extreme exertion, since the inability to recover from this stress may reduce the shrimp's subsequent survival in predator-prey interactions. These considerations also have practical consequences, since shrimps will perform escape tail-flips in response to commercial fishing gear. Animals which escape the nets will be subjected to the same deficits in subsequent tail-flip ability as those which confront predators. Rapid recovery might improve the prospects of survival for shrimps.

Any factors which reduce the capacity of the tail-flip are of significant importance to the survival of *Crangon crangon* and are therefore of interest. Behavioural habituation or "the decrement of a response as a result of repeated or

continuous stimulation" as defined by Hinde (1970) may be caused by a number of factors. Such habituation may be due to neuronal habituation, other neuronal factors or metabolic constraints. Metabolic limitations to tail-flipping in *Crangon crangon* and subsequent recovery have been studied in some detail by a number of studies (Onnen & Zebe, 1983; Kamp & Juretschke, 1987; Kamp, 1989; Gruschczyk & Kamp, 1990). These studies confirm that a dramatic degradation of phosphoarginine fuels the tail-flip until reserves are depleted and ATP concentration consequently drops. During recovery L-lactate builds up but subsequently reduces to normal levels.

Although these metabolic processes of extinction and recovery during exhaustive work have been examined in some detail, less is known about the effects of metabolic changes during recovery on the ability to produce subsequent escape responses. This chapter aims to look more closely at the behavioural consequences of muscle fatigue. The experiments also examine the possibility of neuronal habituation which has been identified in other studies of crustacean escape swimming (Krasne & Woodsmall, 1969; Zucker, 1972; Wine et al, 1975).

Crangon crangon were collected from Kames Bay, Millport, either by trawl or using a push net. They were maintained in an aquarium provided with aerated, running seawater, at temperatures between 9° and 11°C. The aquarium was provided with a layer of sand, 2 to 3 cm deep in order to reduce stress and to allow the shrimps to bury. Shrimps were fed on alternate days on chopped *Mytilus edulis.* Providing sand for burying and a regular and frequent supply of food was particularly important as the animals were kept at relatively high densities and were therefore more likely to become cannibalistic. Experiments were conducted on both male and female *C. crangon,* ranging in total body length from 35 to 74 mm.

Experiments were carried out in small circular aquaria measuring 22 cm in diameter and 25 cm deep, with a thin layer of sand on the bottom. The sand was again to reduce stress, but was not deep enough to allow the animals to bury themselves completely. The experimental tanks were provided with aerated seawater at temperatures between 9° and 11°C. During long experiments these were covered to reduce evaporation.

Individual *Crangon crangon* were placed in the tanks and allowed to settle for approximately 30 minutes. The animal was then exposed to a mechanical stimulus, in the form of a tap on the tail with a metal rod, to elicit an escape response. The response was noted. The stimulus was repeated until the shrimp failed to produce

a response on three consecutive occasions. The shrimp was then allowed to recover for an allotted time, ranging from 2 to 90 minutes, and the process repeated. In the final trial, when the shrimp had failed to respond to the stimulus on three consecutive occasions, instead of a recovery phase the animal was exposed to further stimuli to the rostrum and again the response noted. The experiment was carried out on three animals at each recovery time.

Four measures were noted from each trial. The number of stimuli delivered before extinction occurred (as defined by three consecutive failures to respond); the total number of tail-flips performed; the mean number of tail-flips performed in response to each stimulus (derived from total number of tail-flips divided by the number of stimuli delivered); and the proportion of response (derived from the total number of responses scored, regardless of number of tail-flips in each response, divided by the number of stimuli delivered). Any disparity between the mean number of tail-flips and the proportion of response is therefore due to the occurrence of multiple tail-flips. The three occasions where no response is scored defining extinction are included in all four cases.

Due to the experimental protocol, the number of tail-flips were in most cases less than the number of stimuli and therefore the mean number of tail-flips recorded for each stimulus is commonly less than one.

SWIMMING PERFORMANCE.

When induced to perform tail-flips by mechanical stimulus to the tail, *Crangon crangon* performed a mean number of 0.89 tail-flips in response to a single stimulus (as defined by the total number of tail-flips divided by the number of stimuli). The shrimps responded to a mean total number of 49.1 stimuli before the escape behaviour was extinguished and in response performed a mean total of 42.7 tail-flips before failing to respond to three consecutive stimuli. This represents a proportion of response to mechanical stimulus of 0.71 (See Table 4.1). These figures take into account the three occasions on which no response was produced, which defined the point of extinction of the behaviour. Therefore, although one tail-flip was usually performed in response to each stimulus, the protocol of the experiment produced a mean number of tail-flips per stimulus of less than one. Before extinction was reached, a waning in the strength of the tail-flips performed in response to stimuli was apparent in many animals.

RECOVERY.

The measures noted above were recorded over the course of six trials consisting of stimulation until extinction of behaviour and a recovery interval, to chart the time course of recovery of escape behaviour. Trends observed over the six trials were tested

using a Spearman's rank correlation. For the purposes of the statistical analysis, the results recorded in each three repeat experiments were pooled, although results are presented as mean values in graphs for simplicity.

The number of stimuli delivered and the number of tail-flips performed before tail-flipping behaviour ceased in each of the six trials, are plotted in Figures 4.1 - 4.18 (the results of the statistical analysis are summarised in Table 4.2). Figure 4.1 shows the change in the number of stimuli and the number of tail-flips over the six trials, with a two minute recovery interval between each trial. There was a steady reduction in both measures with each successive trial. In both cases the decrease is found to be highly significant (number of tail-flips performed, $p < 0.01$; number of stimuli, $p < 0.01$). At a recovery interval of five minutes, more tail-flips were recorded in successive trials and more stimuli were applied before the animal ceased to respond (Fig. 4.2). In both cases the decrease is found to be highly significant ($p < 0.01$ in both cases). A similar pattern of decrease in successive trials, but less well defined, is shown with recovery intervals ranging from 10 to 30 minutes between trials (Figs 4.3 - 4.6), all the decreases recorded being found to be significant at the 5% probability level or better. When the interval between trials was increased to 40 minutes, the rate of decrease in the number of stimuli received and in the number of tail-flips performed, levels out across the series of trials, as is shown in Figure 4.7. The reduction in both measures was found to be significant (number of stimuli, $p < 0.05$; number of tail-flips, $p < 0.01$). At a recovery interval of 60 minutes the reduction of response measures between trials was not evident at all, and no

significant decrease was found in the number of stimuli responded to ($p > 0.05$). With a recovery interval of 60 minutes the number of tail-flips recorded during a trial was found to increase significantly with successive trials ($p < 0.05$). A highly significant slope of decrease is apparent again at an interval of 90 minutes in the cases of both stimuli number and the number of tail-flips ($p < 0.01$ in both cases).

Figures 4.10 - 4.18 plot the mean number of tail-flips performed and the proportion of response achieved by shrimps during each trial. These measures take into account the occasions throughout the trial when a failure to respond occurs (in addition to the three consecutive occasions at the end of a trial defining extinction). Differences between these two measures reflect the frequency of multiple tail-flip responses (see Materials and Methods section).

At a recovery interval of two minutes, a sharp decrease occurred in the mean number of tail-flips performed and in the proportion of response over the second trial compared with the first (Fig. 4.10). These measures showed a slight decrease over the remaining trials, however, no overall significant decrease was found in either case ($p > 0.05$, in both cases). At a recovery interval of five minutes the decrease in both of the measures was more steady (see Fig. 4.11) and was found to be significant in both cases (proportion of response, $p < 0.01$; mean number of tail-flips, $p < 0.05$). At a recovery interval of 10 minutes there was a decrease in the proportion of response of shrimps over the six trials, recorded at a level of significance of $p < 0.05$. No significant decrease was recorded in the mean number of tail-flips recorded over

the six trials ($p < 0.05$). Figure 4.13 (15 minute recovery interval) shows a steady decrease in both measures over the six trials, both of which were found to be highly significant ($p < 0.01$, in both cases). At a 20 minute recovery interval between trials (Fig. 4.14), no significant decrease was found in either the proportion of response or in the mean number of tail-flips performed, over the six trials ($p > 0.05$, in both cases). A significant decrease was found in the mean number of tail-flips performed in successive trials at recovery intervals of 30 and 40 minutes, however at the same intervals, the proportion of response showed no significant decrease across the six trials ($p < 0.05$ and $p > 0.05$, respectively at both recovery intervals) (Figures 4.15 - 4.16). Both measures ceased to decrease over the six trials, at a recovery interval of 60 minutes (Figure 4.17), with the mean number of tail-flips performed appearing to increase over the trials. No significant relationship was found to exist in either case between the response measure and successive trials ($p > 0.05$, in both cases). In Figure 4.18, the recovery interval has been extended to 90 minutes and the mean number of tail-flips performed over each trial and the proportion response of the shrimp over each trial appears almost uniform with no significant relationship being identified in either case ($p > 0.05$).

The effect of the varying rest intervals on the performance of escape responses over the series of trials was considered using regression analysis (Table 4.3). The lines of the number of stimuli applied before the extinction of tail-flip behaviour at intervals from 2 to 90 minutes, have a positive gradient in all trials. In each case the gradient of the slope of regression is found to be highly significant ($p < 0.01$), and

a steady increase in the gradients of the slopes is apparent between trial 1 and trial 6.

Between trial 1 and trial 6, an increasing gradient is found for the lines of the total number of tail-flips performed in each trial across the range of recovery intervals (see Table 4.3). All the regression slopes are found to be highly significant ($p < 0.01$).

The slope of the graph of the proportion of response recorded in trial one across the range of rest intervals, was not found to be significant ($p > 0.1$), as expected. However the slopes of the graphs of the other trials from 2 to 6 were all found to be highly significant ($p < 0.01$), and an increase in the gradients calculated for each trial is evident across the trials from 1 to 6.

Lastly, the mean number of tail-flips performed in each trial remained almost uniform across the range of intervals in all trials, the gradient of slopes being very slight. The slopes of regression were not found to be significant in any of the trials ($p > 0.1$).

Switching the point of stimulus from the tail to the rostrum to investigate the possibility of sensory habituation, resulted in some escape responses being recorded. After escape behaviour had been extinguished in response to a tap on the tail, 12 of the 27 animals tested responded to a stimulus to the rostrum.

	Value	Standard Deviation
Mean no. of flips	0.89	± 0.21
Total no. of stimuli delivered	49.1	± 16.6
Total no. flips performed	42.7	± 14.4
Proportion of response	0.71	± 0.59

Table 4.1 : Measures of tail-flip performance in initial trials (27 experiments).

	No. of stimuli		No. of tail-flips		Mean no. of tail-flips		Proportion of response	
Recovery interval (mins)	r_s	p	r_s	p	r_s	p	r_s	p
2	-1.0	< 0.01	-0.943	< 0.01	-0.600	NS	-6.00	NS
5	-0.943	< 0.01	-0.943	< 0.01	-0.886	< 0.05	-0.943	< 0.01
10	-0.899	< 0.05	-0.943	< 0.01	-0.771	NS	-0.829	< 0.05
15	-1.000	< 0.01	-1.000	< 0.01	-0.943	< 0.01	-1.000	< 0.01
20	-0.829	< 0.05	-0.829	< 0.05	-0.543	NS	-0.600	NS
30	-1.000	< 0.01	-0.886	< 0.05	-0.886	< 0.05	-0.771	NS
40	-0.900	< 0.05	-0.972	< 0.01	-0.900	< 0.05	-0.700	NS
60	0.600	NS	0.900	< 0.05	0.700	NS	-0.100	NS
90	-1.000	< 0.01	-1.000	< 0.01	0.000	NS	-0.800	NS

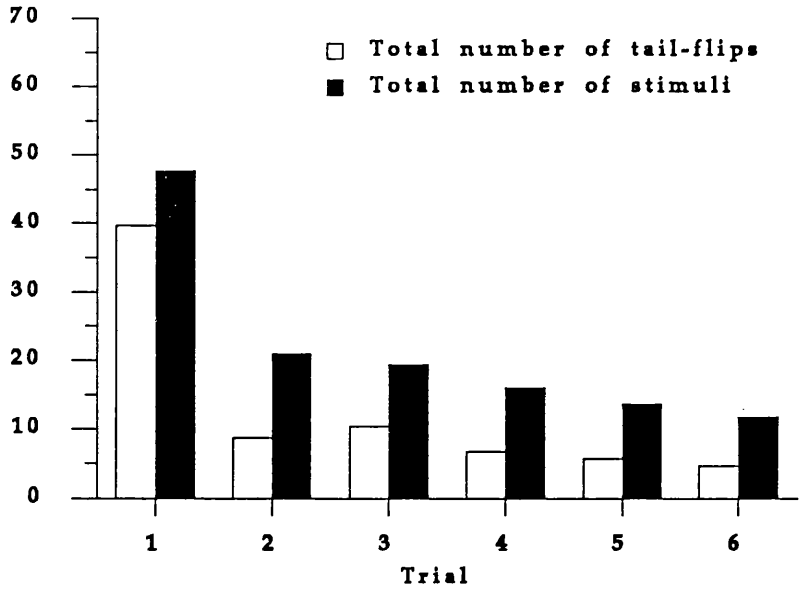
NS = Not significant

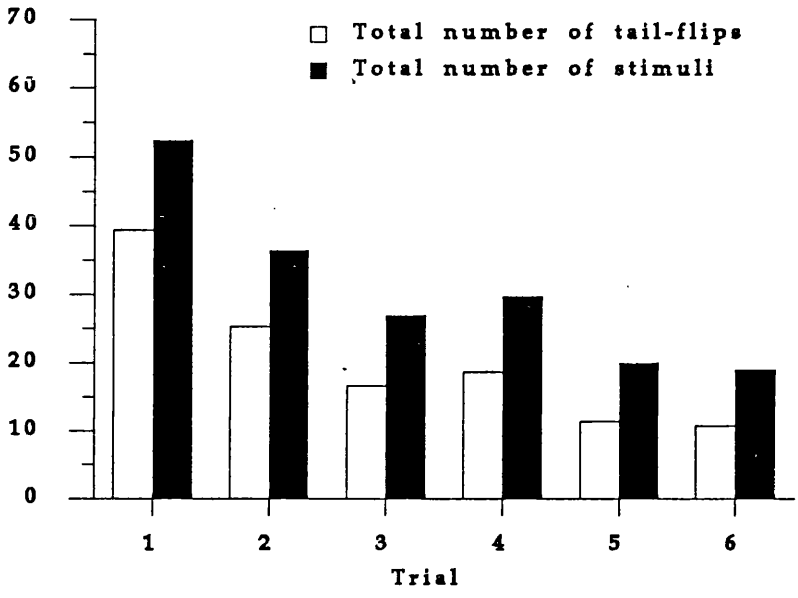
Table 4.2: Results of Spearman's rank correlation of performance measures with number of trials.

Fig. 4.1. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 2 minutes between trial.

Fig. 4.2. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 5 minutes between trials.

Fig. 4.3. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 10 minutes between trials.


70
60
50
40
30
20
10
0
Total number of tail-flips
Total number of stimuli
1
2
3
4
5
6
Trial


70
60
50
40
30
20
10
0
Total number of tail-flips
Total number of stimuli
1
2
3
4
5
6
Trial

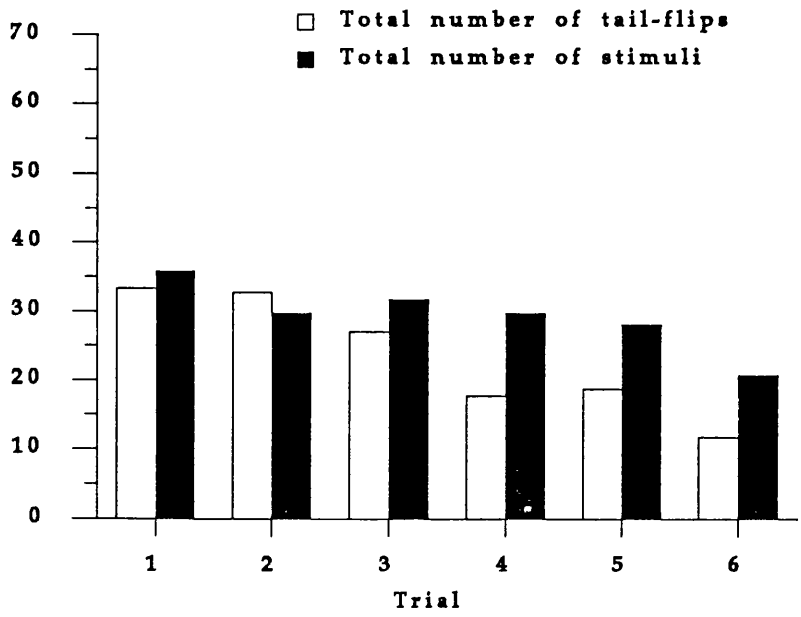
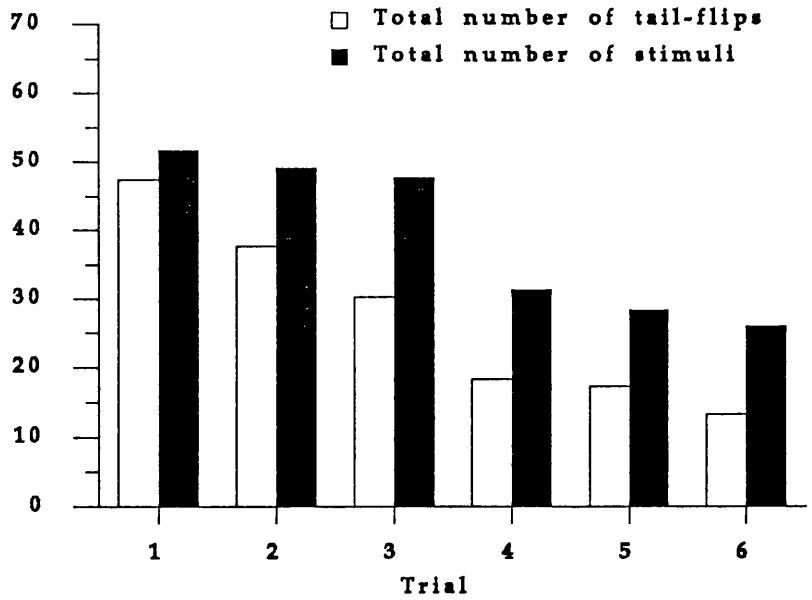
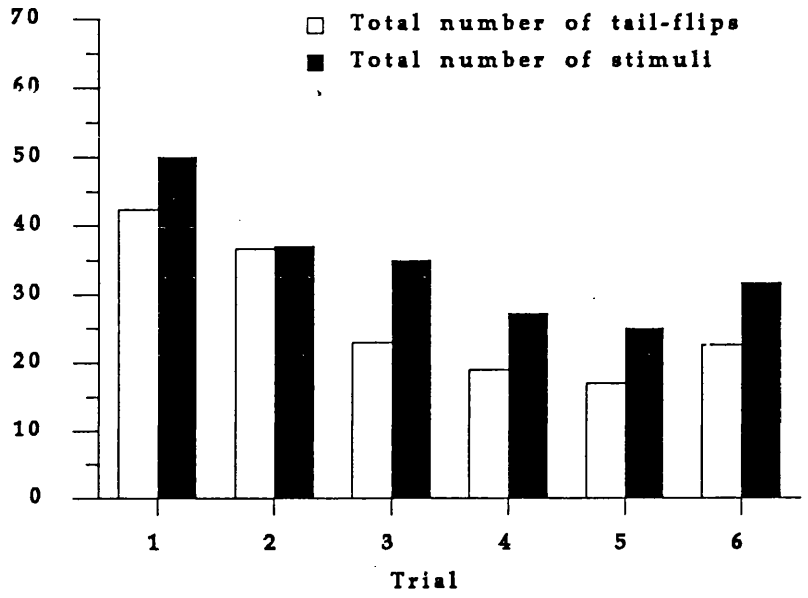

70
60
50
40
30
20
10
0
Total number of tail-flips
Total number of stimuli
1
2
3
4
5
6
Trial

Fig. 4.4. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 15 minutes between trials.

Fig. 4.5. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 20 minutes between trials.

Fig. 4.6. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 6 trials, with a recovery interval of 30 minutes between trials.


Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Trial


Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Trial

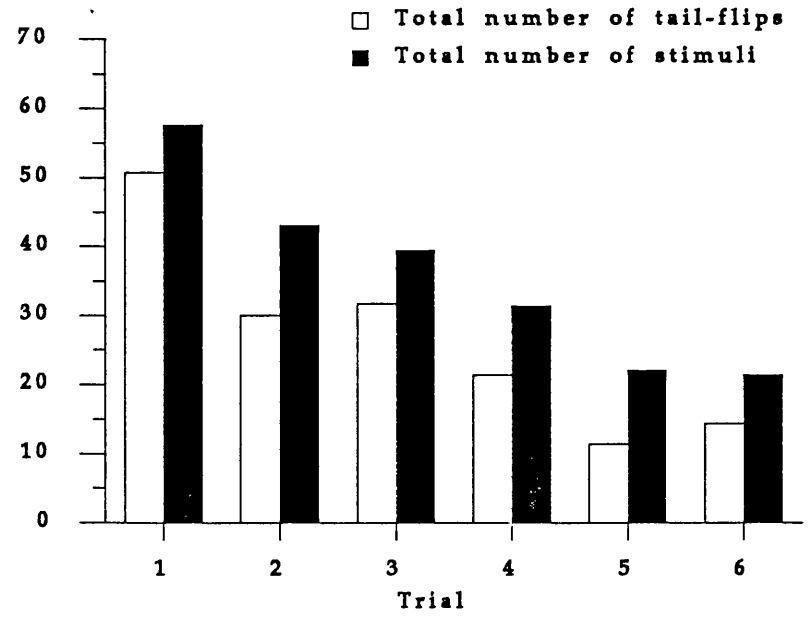
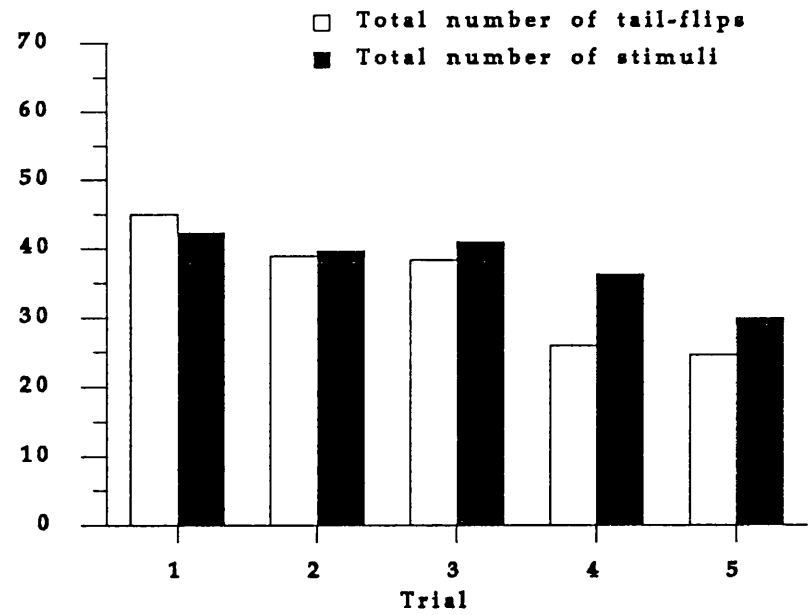
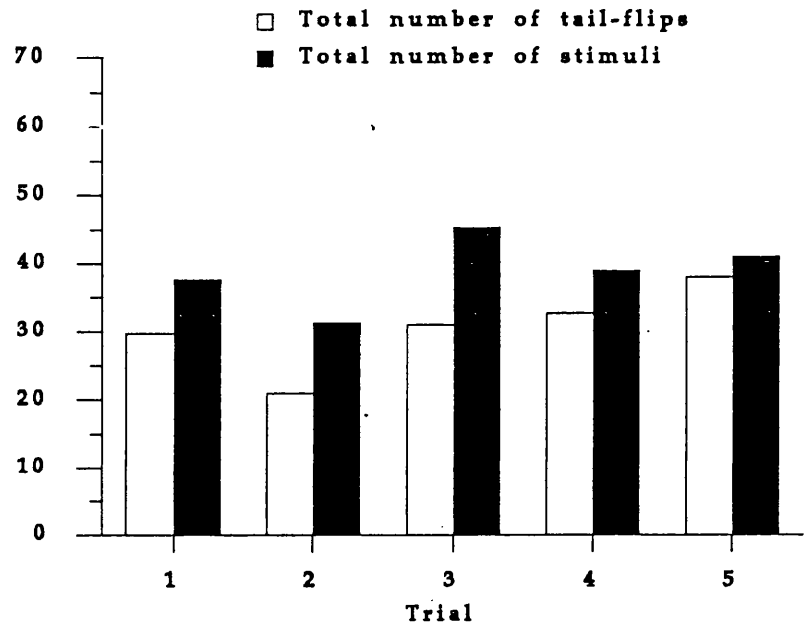

Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Trial

Fig. 4.7. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 5 trials, with a recovery interval of 40 minutes between trials.

Fig. 4.8. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 5 trials, with a recovery interval of 60 minutes between trials.

Fig. 4.9. The number of stimuli and the number of tail-flips performed before extinction of escape behaviour over the series of 4 trials, with a recovery interval of 90 minutes between trials.


Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
5
Trial


Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
5
Trial

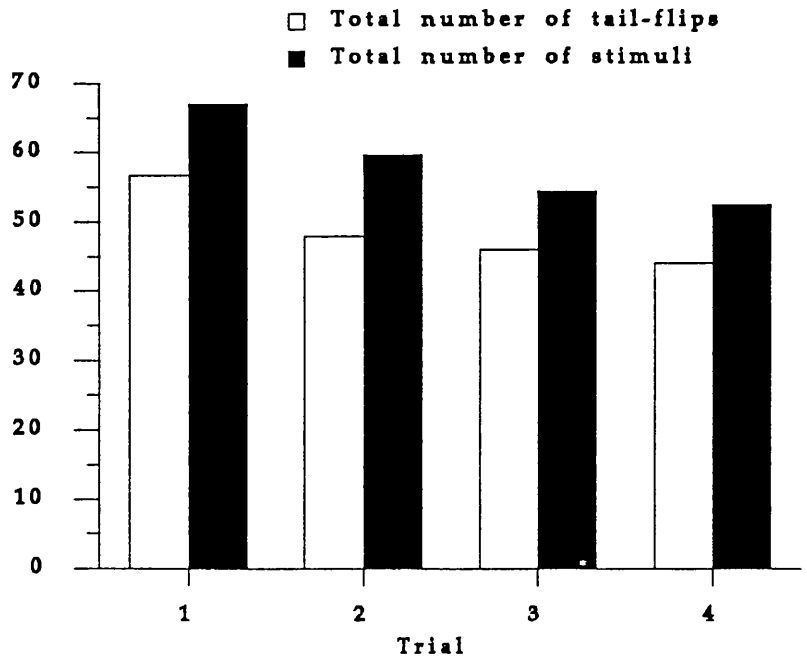
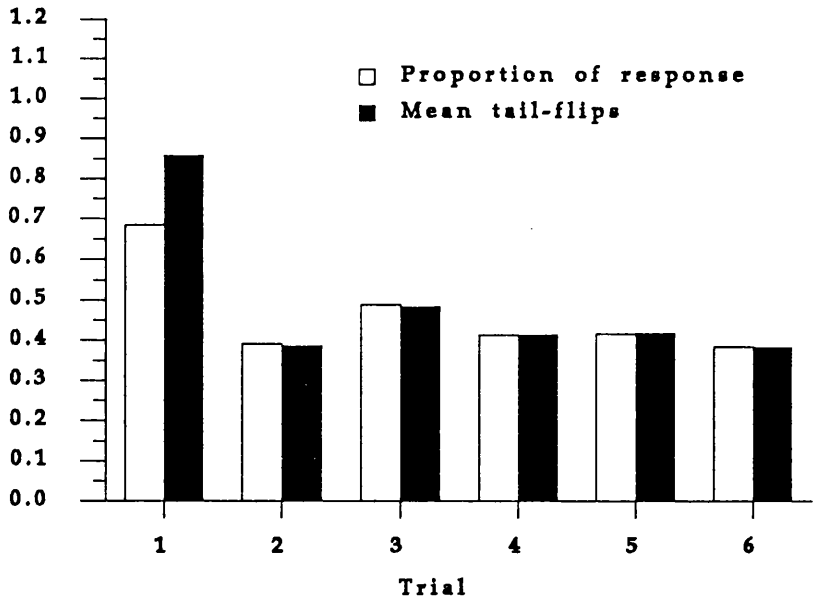
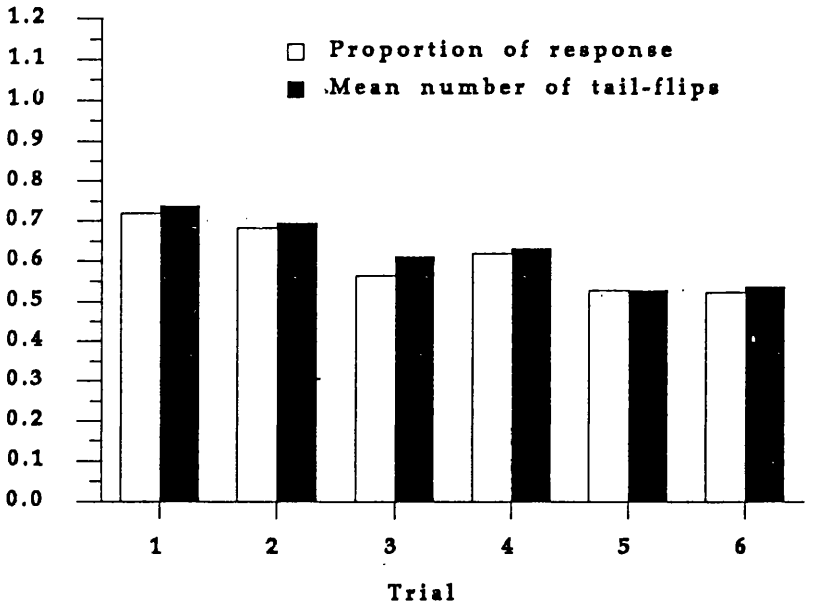

Total number of tail-flips
Total number of stimuli
70
60
50
40
30
20
10
0
1
2
3
4
Trial

Fig. 4.10. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 2 minutes between trials.

Fig. 4.11. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 5 minutes between trials.

Fig. 4.12. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 10 minutes between trials.


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of response
Mean tail-flips
1
2
3
4
5
6
Trial


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of response
Mean number of tail-flips
1
2
3
4
5
6
Trial

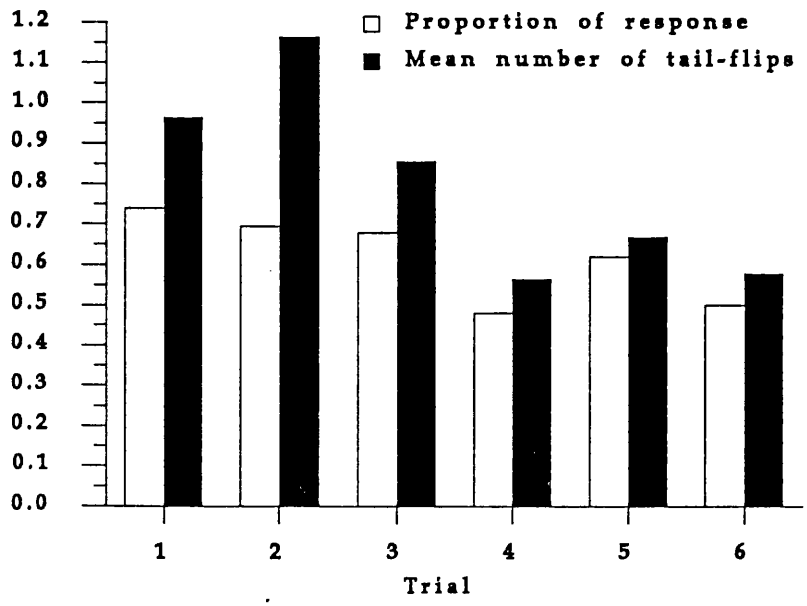
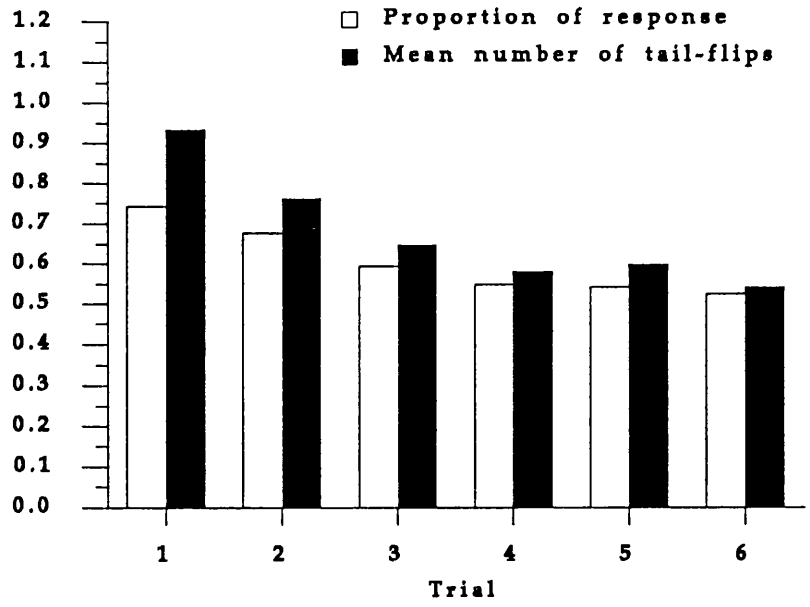
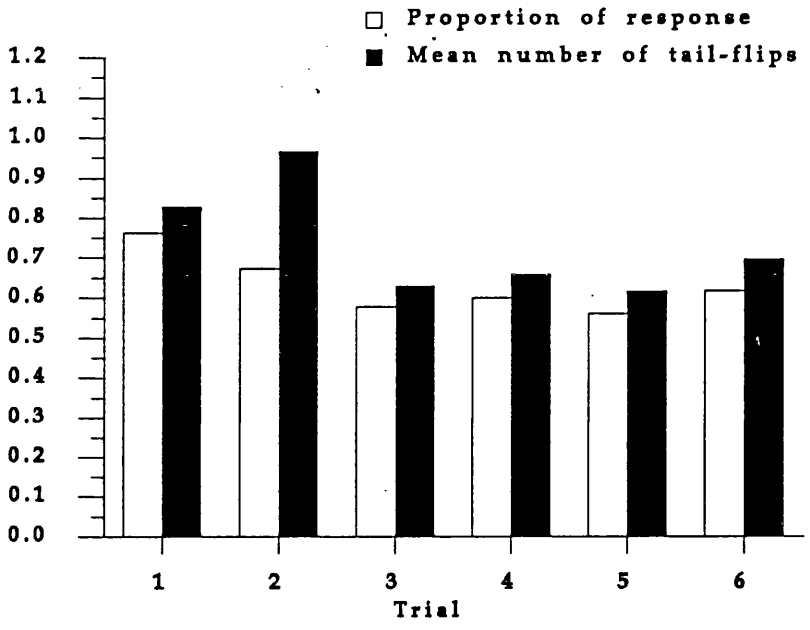

1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of response
Mean number of tail-flips
1
2
3
4
5
6
Trial

Fig. 4.13. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 15 minutes between trials.

Fig. 4.14. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 20 minutes between trials.

Fig. 4.15. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 6 trials, with a recovery interval of 30 minutes between trials.


Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
Trial


Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
Trial

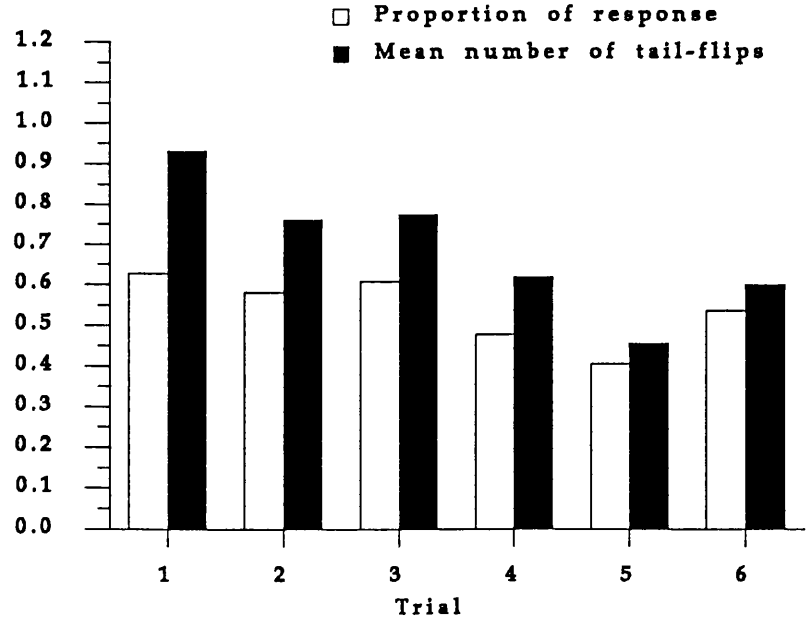
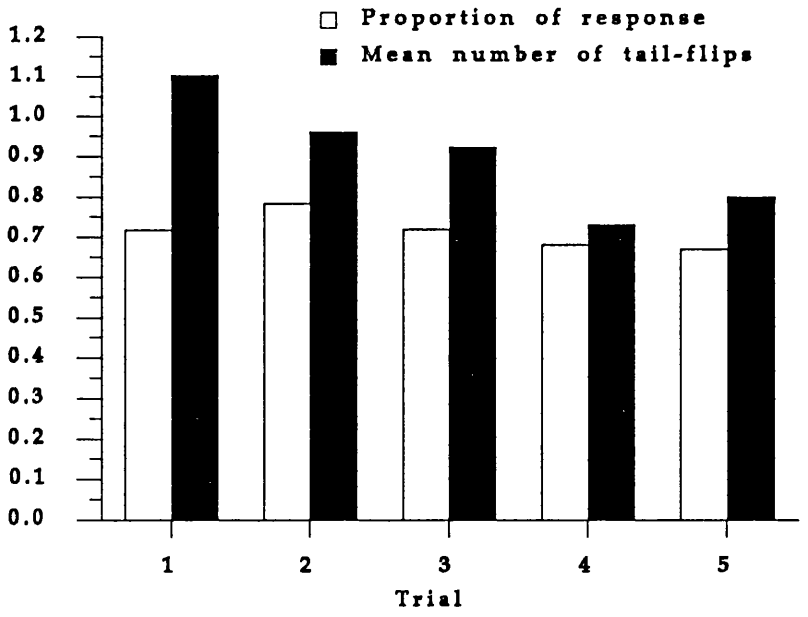
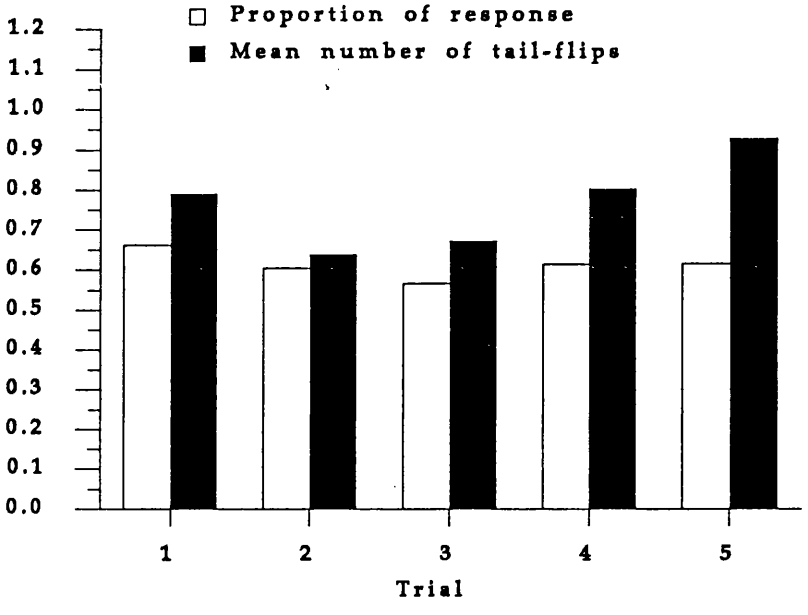

Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
Trial

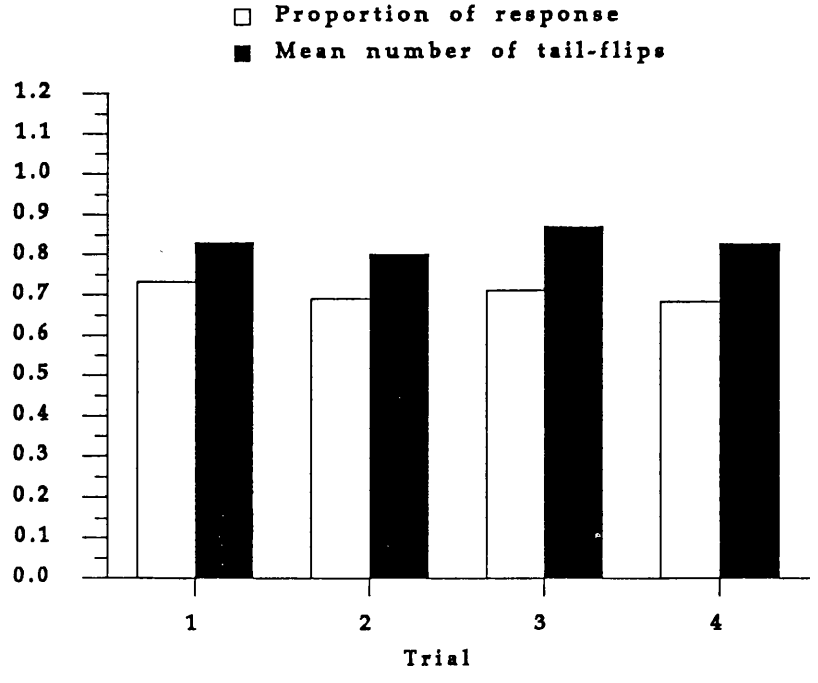
Fig. 4.16. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 5 trials, with a recovery interval of 40 minutes between trials.

Fig. 4.17. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 5 trials, with a recovery interval of 60 minutes between trials.

Fig. 4.18. Graph of the mean number of tail-flips performed in response to each stimulus and the proportion of response in each of the 4 trials, with a recovery interval of 90 minutes between trials.


Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
Trial


Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
Trial


Proportion of response
Mean number of tail-flips
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
Trial

		No. of tail-flips			No. of stimuli			Mean no. of tail-flips			Proportion of response		
Trial	n	slope	t	p	slope	t	p	slope	t	p	slope	t	p
1	27	0.11	4.2	< 0.01	0.13	4.4	< 0.01	-0.0002	0.6	NS	-0.02	1.0	NS
2	27	0.20	7.4	< 0.01	0.24	9.1	< 0.01	0.0004	0.09	NS	0.11	4.4	< 0.01
3	27	0.31	12.8	< 0.01	0.30	12.7	< 0.01	0.003	0.8	NS	0.14	6.2	< 0.01
4	27	0.35	15.5	< 0.01	0.31	13.8	< 0.01	0.004	1.4	NS	0.21	9.2	< 0.01
5	24	0.44	12.1	< 0.01	0.34	9.7	< 0.01	0.007	1.3	NS	0.22	5.2	< 0.01
6	18	0.39	5.7	< 0.01	0.39	5.5	< 0.01	0.007	0.7	NS	0.46	5.4	< 0.01

NS= Not significant

Table 4.3: Regression analysis of relationship between performance measures and recovery interval in each trial.

A number of studies have examined in detail the biochemical processes underlying energy utilisation during tail-flipping and recovery of *Crangon crangon* (Onnen & Zebe, 1983; Kamp & Juretschke, 1987; Kamp, 1989, Gruschczyk & Kamp, 1990). The study of Onnen & Zebe, (1983) is probably most appropriate for comparison with the present study and although using a different technique for eliciting tail-flips from shrimps (an electrical stimulus), it provides relevant information about the endurance and recovery of *C. crangon* which complements the behavioural material presented here.

The study of Onnen & Zebe (1983), found that after only ten tail-flips the concentration of phosphoarginine in the muscle tissue of *Crangon crangon* has decreased to one half of the resting value, and that after 50 seconds of work the phosphoarginine was almost depleted. Onnen & Zebe (1983) also found that when the phosphoarginine was almost completely depleted, the energy charge started to drop, with the rapid depletion of remaining ATP. Reserves of ATP were found to be replenished within as little as five minutes of recovery time and phosphagen within 30 minutes.

In the experiments reported here the most dramatic reduction between trials, in the number of stimuli responded to and the number of tail-flips performed before the extinction of escape behaviour, occurs is in the group of animals allowed a

recovery interval of only two minutes, with the greatest drop found between the first and second trial. According to the findings of Onnen & Zebe (1983) we could assume that the phosphoarginine reserves of the shrimp are exhausted on completion of around 40 tail-flips and therefore it is likely that in the first trial both the phosphoarginine reserves and the energy charge have reduced significantly to produce fatigue and therefore an inability to respond. The fact that the two minute recovery interval is too short for the replenishment of these reserves is reflected in the drop in the escape response in the second trial. The continuing decrease in the escape response of shrimps in subsequent trials, after only a two minute recovery interval between each, is probably an indication that these stores are not replenished fully after each trial and may form a backlog of depleted reserves to renew. It is interesting to note that although the escape responses of the shrimps are reduced drastically from a proportion of response of 0.7 - 0.4 between the first and second trials, there is no further dramatic decrease, the subsequent reductions in results remaining small.

With a recovery interval of five minutes, it might be expected according to the results of Onnen & Zebe (1983) and Kamp & Juretschke (1987), that the ATP reserves will have been replenished, and the graphs of stimuli number and total tail-flip number as well as those of mean number of tail-flips performed in a trial and of the proportion of response during that trial appear to reflect this. There is no rapid reduction in responsiveness between trials one and two as occurs with the 2 minute interval, and the reduction seen in the measures recorded subsequently being

more gradual. This pattern is repeated with increasing recovery interval up to 40 minutes, when the reduction seen between trials in the results scored levels out with no apparent reduction in the proportion of response and the mean number of tail-flips performed in the course of a trial. Again this would appear to be consistent with the findings of Onnen & Zebe (1983), that after 30 minutes the phosphagen reserves are replenished and ATP reserves are restored within five minutes. This suggests that a full phosphoarginine store is important for the performance of consecutive tail-flips.

It is interesting to note that although a levelling out of the graphs of stimuli number and total number of tail-flips performed in the course of a trial is evident after recovery intervals of 40 to 60 minutes, there is still a slight decrease in the response of animals allowed a 90 minute recovery interval between trials. It is possible that this is due to the effects of lactate accumulation which is reported to reach a peak 30 minutes into recovery and only returns to resting levels after 10 hours (Onnen & Zebe, 1983). Field (1992) found no correlation between the recovery of tail-flipping ability and the recovery of resting levels of L-lactate in *Nephrops norvegicus.* If, as evidence suggests, lactate levels do not return to normal for considerable time (10 hours) a link between recovery of lactate resting levels and recovery of escape behaviour which would rule out tail-flipping for this length of time would prove critical in terms of survival.

Another possible explanation for the decrease in response occurring after a 90 minute recovery interval (which is long enough to allow replenishment of energy reserves) is neuronal habituation. Studies on crayfish (Wine *et al*, 1975) have shown that the excitability of pathways involved in the tail-flip is reduced by 50% after as few as 10 tactile stimuli have been applied at five minute intervals. The results from the experiments reported here provide little information about the possible role of habituation on the tail-flipping of *Crangon crangon.* After a switch in the site of stimulation, some responses to stimuli do occur after extinction of escape behaviour has been reached, which indicates that some form of habituation has occurred. Also, the occurrence of some responses applied immediately after extinction (i.e. to head stimuli after tail stimuli) shows that dishabituation can occur by the activation of different neuronal pathways, although it is occurring here against a background of energy depletion. From a simple behavioural experiment it is not possible to determine what form this habituation takes, but it is known that habituation occurs in the sensory pathway. Zucker (1972) was able to show that behavioural habituation observed in crayfish escape swimming is the result of a failure of synaptic transmission between the tactile afferent and sensory interneurons. Observations by Krasne & Woodsmall (1969) and Newland (1985) also support the idea of an afferent site of habituation.

The use of rapid degradation of phosphagen to provide the energy necessary for an escape response is not confined to *Crangon crangon,* it is also found in molluscs, which are specialised in performing work in bursts. Similar mechanisms are also

found in the mantle muscle of cephalopods and also in the adductor muscle in *Pecten* (for review see Gäde, 1983), which are involved in escape responses entailing work in bursts.

Although initial examination of data obtained from these experiments suggests a trade-off between the occurrence of occasional multiple flips and the total number of stimuli delivered before the extinction of escape behaviour, no significant information is found. Clearly, if such a trade-off exists and is frequently involved in the tail-flip reaction, there is added complexity to the decision to tail-flip. The elucidation of this particular aspect of tail-flip endurance may merit further study.

The type of "attack" the shrimps were exposed to in the course of these experiments are probably unrealistic in that shrimps would undergo attacks until escape behaviour ceased. This resulted in a mean of number of 49.1 stimuli before extinction occurred. In the course of a normal attack this degree of perseverance on the part of a predator is unlikely unless prey items are very rare. It must be assumed that the energetic costs of pursuing one prey item for around 50 attacks would be far too great for the catch to be worthwhile, the energy costs far outweighing the energy gains. In terms of recovery, responses were recorded from animals after only a two minute recovery interval following exhaustive work, which suggest that a partial recovery may be adequate for subsequent response. Above all, it appears likely that *Crangon crangon* has adequate abilities of endurance and recovery to sustain repeated predatory attacks. This suggests that the success of the initial tail-flips is

most important for the survival of shrimps.

CHAPTER 5 : HISTOCHEMICAL AND BIOCHEMICAL ANALYSIS

INTRODUCTION

The swimming performance of *Crangon crangon* must be determined to a large degree by the contractile properties of the abdominal muscles. An attempt has been made to define these properties in *C. crangon* by using histochemical and biochemical techniques, which have been used extensively in studies of other crustacean muscles (Mykles, 1985a, 1985b; Silverman *et al*, 1987). When used in combination with physiological and morphological measures, these techniques have allowed two main types of crustacean muscle fibres, fast and slow, to be identified. Fast muscle fibres have characteristic short sarcomeres and low ratios of thin to thick myofilaments. They also show high myofibrillar ATPase activities, which indicate high contraction rates, and low oxidative capacities, which give an indication of poor fatigue resistance. In contrast slow fibres have longer sarcomeres, high ratios of thin to thick myofilaments, low myofibrillar ATPase activities, indicative of low contraction rates, and high oxidative capacities, which suggest increased fatigue resistance (Jahromi & Atwood, 1969; Lang *et al*, 1977; Govind & Atwood, 1982; Mykles, 1985). There is great variation in the proportion and distribution of each fibre type in different muscles, with a correspondingly wide diversity of contractile abilities (Maier et. al., 1984). In the abdominal muscles of macrurous decapods this extends to a distinct division of labour, with the thin sheets of superficial muscles containing slow fibres and large blocks of deep muscles containing fast fibres (Kennedy & Takeda, 1965). These two muscle groups produce different abdominal

movements: slow postural movements by the superficials and the characteristic rapid tail flipping by the deep muscles.

The most extensively used histochemical techniques to distinguish between fast and slow muscle test for oxidative capacity and myofibrillar ATPase activity. These techniques have been used on transverse sections of whole *Crangon crangon* abdomens, in order to determine the distribution of muscle fibre types in the different muscle bundles of the abdomen. A derivative of the ATPase test, involving a pre-incubation in an acid or alkali solution, is also used which reliably distinguishes fibres containing either a labile or stable form of myosin ATPase. This technique, in combination with the other methods, allows the convenient discrimination of fibre types.

More detailed information can be gained by separating out myofibrillar proteins using gel electrophoresis (Mykles, 1985a). This technique has established that certain proteins, most often regulatory, can be expressed in multiple isoforms and that the presence of some of these can be used as a reliable indicator of fibre type. Thus fast fibres can be identified on the basis of the presence of a particular isoform of paramyosin (P1, Mr = 110000 (Mr= Migration ratio)) and a 75kD protein. Similarly electrophoresis has revealed that there are two subtypes of slow fibres. These can be distinguished by reference to the T_1 isoform of troponin, which is absent in the S_1 fibre type, but present in the S_2 fibre type.

Information about the proportion and location of these different fibre type bundles is useful, not only as it relates to the functional morphology of the abdominal locomotory system of *Crangon crangon*, but also in the wider context of the behavioural ecology of the animal. These histochemical and biochemical analyses have been employed with the aim of providing more information about the locomotory abilities of *C. crangon*. Detailed knowledge of the histochemical properties of the main fast flexor and extensor muscles provides a basis for interpreting the observed rates of flexion and extension of the *C. crangon* tail-flip. These properties will also be reflected in the precise combination of regulatory proteins which are expressed.

MATERIALS AND METHODS

Crangon crangon were obtained from Kames Bay, Millport, either by trawl or using a push net. They were kept in a large aquarium tank with running seawater until required.

HISTOCHEMISTRY

Whole abdomens were frozen in liquid nitrogen and then mounted on a cork disc in OCT mounting medium (Miles Scientific, Naperville, Illinois, USA). The preparation was then allowed to equilibrate to -20°C., the block trimmed and sections cut at 20μm on a cryostat (Bright Starlet, 2210). The sections were then lifted onto coverslips and air-dried at room temperature. They were then stained for one of the following:

SUCCINIC DEHYDROGENASE

An incubation medium of 1M sodium succinate (at pH 7.5), 0.1M sodium phosphate and 1mg/ml Nitro blue tetrazolium (Sigma N6876) was used to stain for succinic dehydrogenase activity. This gives a measure of the oxidative capacity of muscle fibres which is in turn indicative of fatigue resistance. The cryosections were placed in a petri dish on moist filter paper with a drop of incubation medium

applied to each coverslip and allowed to incubate at room temperature for two hours. The sections were then dehydrated in an alcohol series, cleared in Histoclear (National Diagnostics, New Jersey, USA) and mounted in Histomount.

MYOFIBRILLAR ATPASE ACTIVITY

A reaction medium was prepared by mixing stock solutions of 0.05M N-glycylglycine (amino acid Sigma G1002), 1M calcium chloride, 0.1M magnesium chloride and 0.1M ATP (disodium salt) and adjusting the pH to 8.0. The sections were then immersed in the reaction medium at 4°C for 30 minutes, washed, immersed in 1% calcium chloride for three minutes, washed, immersed in 2% cobalt chloride for three minutes, washed and immersed in 1% $(NH4)_2S$ for one minute. The sections were then dehydrated in an alcohol series, cleared in Histoclear and mounted in Histomount.

PH LABILITY OF MYOSIN ATPASE ACTIVITY

The lability of myosin ATPase activity at low and high pH values was determined using a technique modified by Fowler (1990) from Maier et al. (1984). Two pre-incubation media, one alkali, one acid were prepared. The acid pre-incubation medium contained 50mM sodium acetate and 50mM KCl and was adjusted for pH to 4.6 using 100% acetic acid. The alkali pre-incubation medium

consisted of 100mM glycine, 100mM NaCl and 50mM CaCl2 which was adjusted to a pH of 10.2 with NaOH. The sections were pre-incubated for 20 minutes, washed and incubated in a reaction medium of 0.05M glycine, 0.05M NaCl, 0.03M CaCl2 and 0.0018g/ml of ATP for 30 minutes. The sections were then washed and processed as described in the test for myofibrillar ATPase activity.

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

Samples of muscle tissue were separated out from the deep muscles of the abdomen of *Crangon crangon* and transferred to 100µl of SDS sample buffer (62.5mM Tris-HCl (pH 6.8), 12.5% glycerol, 1.25% SDS, 1.25% B-mercaptoethanol). These samples were immediately boiled for three minutes and then stored at -20°C until required.

Discontinuous SDS-PAGE was carried out using the technique described by Laemmli (1970). A 12.5% acrylamide stacking gel and a 10% separating gel were prepared from a 30% (w/v) acrylamide and a 0.8% (w/v) N,N'-methylene bisacrylamide stock solution. Standards of known molecular weights (Sigma Dalton Mark VII-L) and the samples of muscle tissue were then applied to the wells in the stacking gel. The gels were mounted in a chamber containing a reservoir buffer of 0.2M glycine, 22mM Tris-HCl and 3.5mM SDS, and run with applied currents of around 40mA across the gels.

The gels were fixed in 10% (w/v) trichloroacetic acid and stained in 0.2% (w/v) Coomassie blue in 45% (v/v) methanol and 10% (v/v) acetic acid for up to eight hours. The gels were then destained in a methanol/acetic acid mixture.

HISTOCHEMISTRY

In section, the main abdominal muscles of *Crangon crangon* can be identified. The superficial flexor muscles form a thin sheet of fibres along the medial ventral surface of the abdomen with the superficial extensor muscles laid out more laterally at the dorsal surface. The superficial extensor muscle appears to be made up of three blocks with subtle differences in properties. The largest proportion of the abdomen is taken up by the deep extensor and flexor muscles. These lie adjacent to their corresponding superficial muscles. Additionally the swimmeret muscles, which power the movement of the pleopods, are found at either side of the abdomen (see Figures 5.1 - 5.3).

MYOFIBRILLAR ATPASE ACTIVITY.

The superficial extensors and superficial flexors show a low intensity of stain in comparison to the dark staining of the deep extensors and deep flexors. This indicates lower levels of total myofibrillar ATPase activity in the superficial muscles (see Fig. 5.1). The deep flexor muscles appear slightly darker than the deep extensors, suggesting a higher level of myofibrillar ATPase activity in the deep flexors (see Fig. 5.1). The swimmerets have two areas of different staining: one dark and one pale. This indicates the presence of two types of muscle fibre, one with a high

level of myofibrillar ATPase activity, the other low (see Fig. 5.1). The section used for this particular staining technique also shows remnants of the cuticle (see Fig. 5.1).

PH LABILITY OF MYOSIN ATPASE ACTIVITY

Both the superficial extensor muscles and the superficial flexor muscles show dark staining, indicating a stable isoform of myosin ATPase. In addition, both the superficial extensor muscles and the superficial flexor muscles also have areas where no staining has occurred, indicating the presence of the labile isoform of myosin ATPase (see Fig. 5.2). In the case of the superficial extensors, the stable isoform of myosin is manifested in only one of the three muscle blocks (see Fig. 5.2). The large deep muscles are uniformly colourless, indicating the labile form of myosin ATPase (see Fig. 5.2). The swimmeret muscles display areas of dark staining as well as a colourless area (see Fig. 5.2), indicating the presence of the labile and the stable forms of myosin ATPase in different fibres.

SUCCINIC DEHYDROGENASE.

The superficial flexor muscles and the superficial extensor muscles show concentrations of dark blue staining (black in prints), mostly around the edges of the muscle blocks (see Fig. 5.3). Staining is absent in one of the three blocks of the

superficial extensors. The blue stain indicates areas of high succinic dehydrogenase activity. The deep flexor muscles and the deep extensor muscles are stained with only occasional dark grains or lines, indicating that these muscles have less succinic dehydrogenase activity (see Fig. 5.3). Lastly the swimmeret muscle shows areas of intense dark stain and two unstained areas which appear to correspond with the areas which showed the stable form myosin ATPase (see Fig. 5.3 and 5.2).

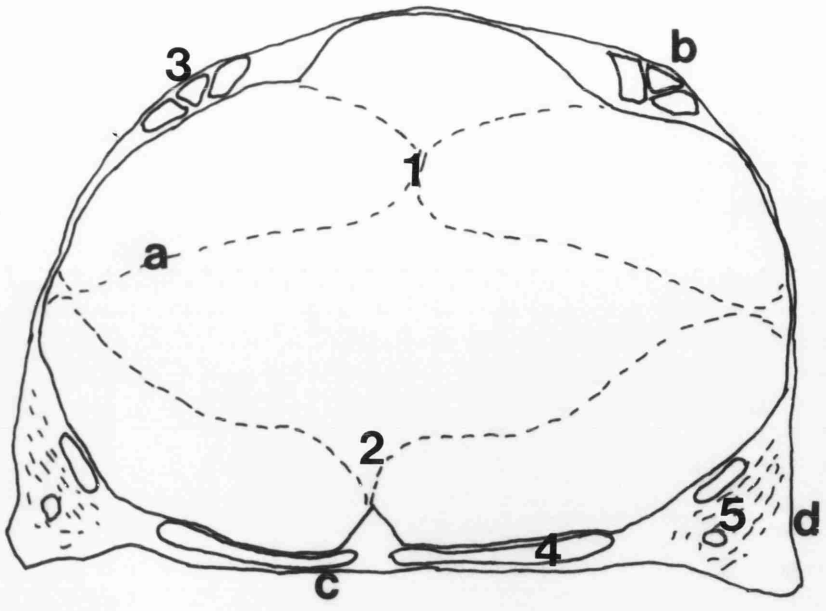
SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

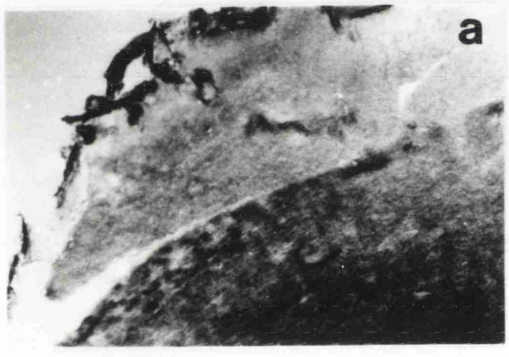
SDS-PAGE shows the deep fast muscles of *Crangon crangon* to comprise of myosin heavy chain (Mr = 200,000); two isoforms of paramyosin (P_1, Mr = 110,000 and P_2, Mr = 105,000); three isoforms of troponin H (previously called the 75kD protein, Mr ~ 84,000, 74,000 and 70,000); actin (Mr = 45,000); tropomyosin (Mr = 36,000) and an isoform of myosin beta light chain (Mr ~ 18,500). Both troponin-C and troponin-I were poorly resolved by Coomassie blue.

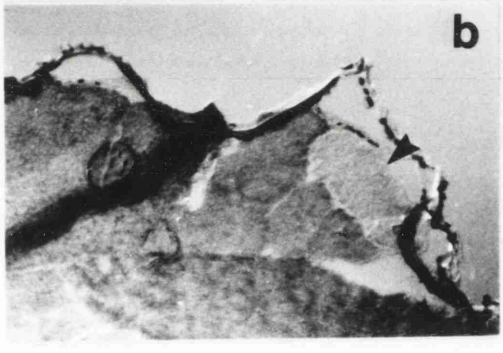
For comparison, fibres from the deep abdominal flexor muscle and medial bundle of the superficial flexor muscle, of *Nephrops norvegicus* were also electrophoresed. Clear differences between the fast muscles exist in the amount of the P_1 isoform of paramyosin, and in the isoform of Troponin H expressed.

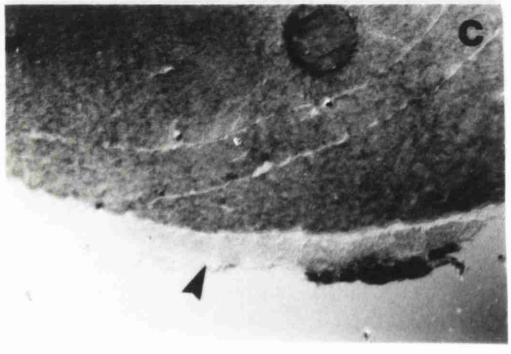
The slow (S_2) phenotype of *Nephrops norvegicus* lacks the P_1 isoform, and expresses the T_1 variant of Troponin T. No slow fibres of *Crangon crangon* were analysed in this study.

Fig. 5.1. Section of *Crangon crangon* abdomen showing muscle blocks stained for myofibrillar ATPase activity. 1. Deep fast extensor muscles; 2. Deep fast flexor muscles; 3. Superficial extensor muscles; 4. Superficial flexor muscles; 5. Swimmeret muscles. a) Darker staining of deep fast flexor compared to deep fast extensor. b) Superficial extensor muscles, arrow shows pale staining of one of the superficial extensors. (Fig. 5.3. shows all three superficial extensors). c) Superficial flexor muscle next to deep flexor, arrow shows pale staining of superficial flexor. d) Swimmeret muscles showing areas of pale and dark staining.


3
b
1
a
2
5
d
4
c


a


b


c

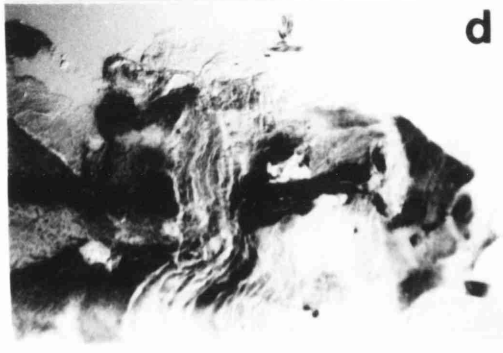
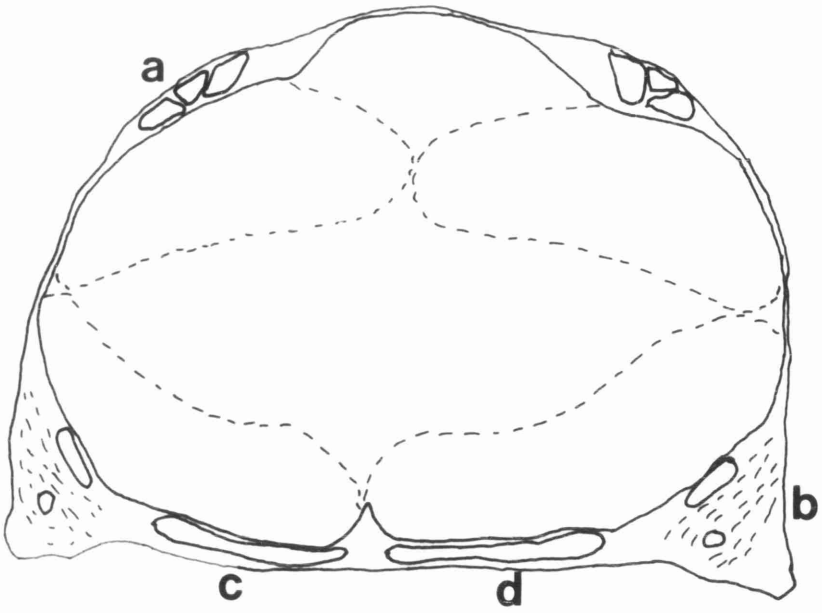

d

Fig. 5.2. Section of *Crangon crangon* abdomen showing muscle blocks stained for pH lability of myosin ATPase activity. a) Superficial extensors with arrow showing staining for stable myosin ATPase on one extensor. b) Swimmeret muscles showing areas of pale and dark staining. Arrows show two muscles staining for stable myosin ATPase. c) Superficial flexor muscle with both stable and labile myosin ATPase. d) Other superficial flexor.


a
b
c
d

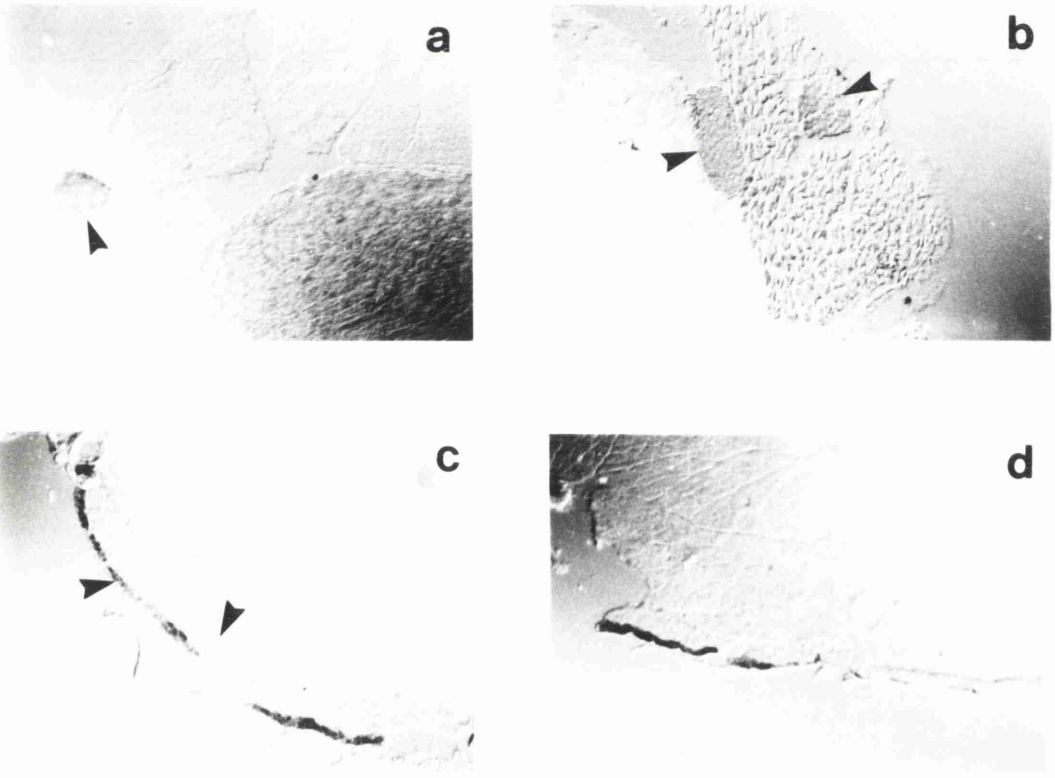
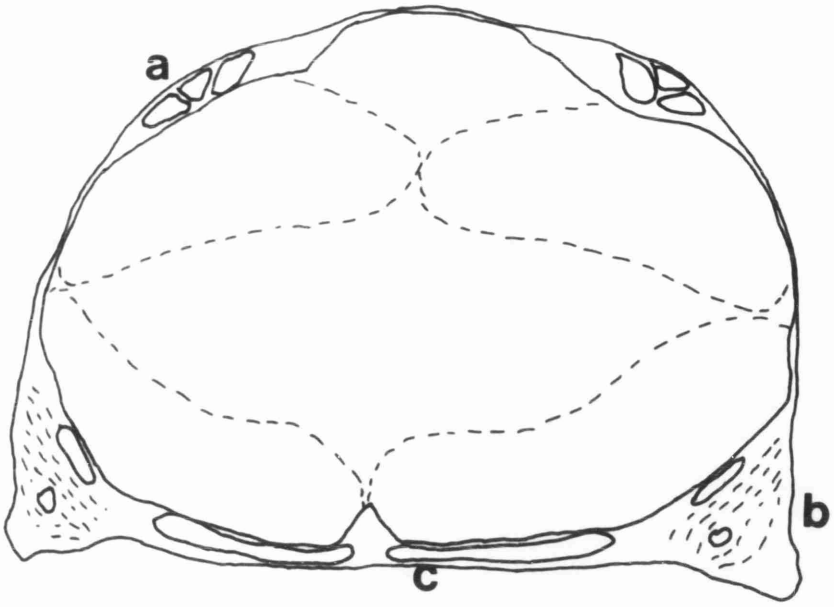
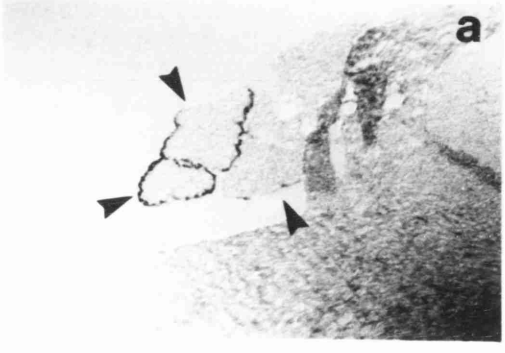
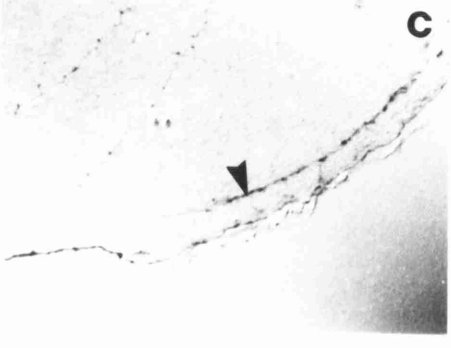

a
b
c
d

Fig. 5.3. Section of *Crangon crangon* abdomen showing muscle blocks stained for succinic dehydrogenase. a) Superficial flexor muscles with arrows showing all three muscles. Staining for succininc dehydrogenase occurs in only two. b) Swimmeret muscle showing large area of staining for succinic dehydrogenase. Arrows show two areas without staining. c) Superficial flexor muscle with arrow showing line of intense staining for succinic dehydrogenase. Grains of dark staining can also be seen in the deep flexor muscle.


a
b
c


a


c

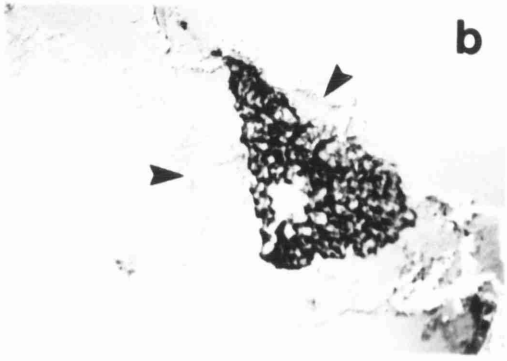
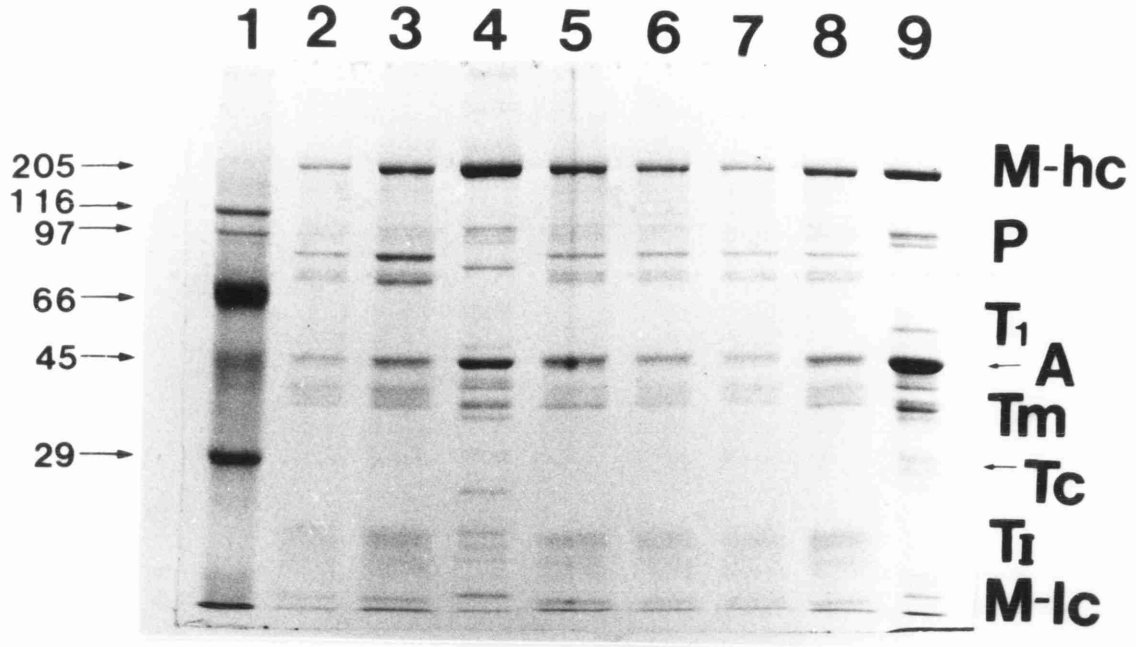
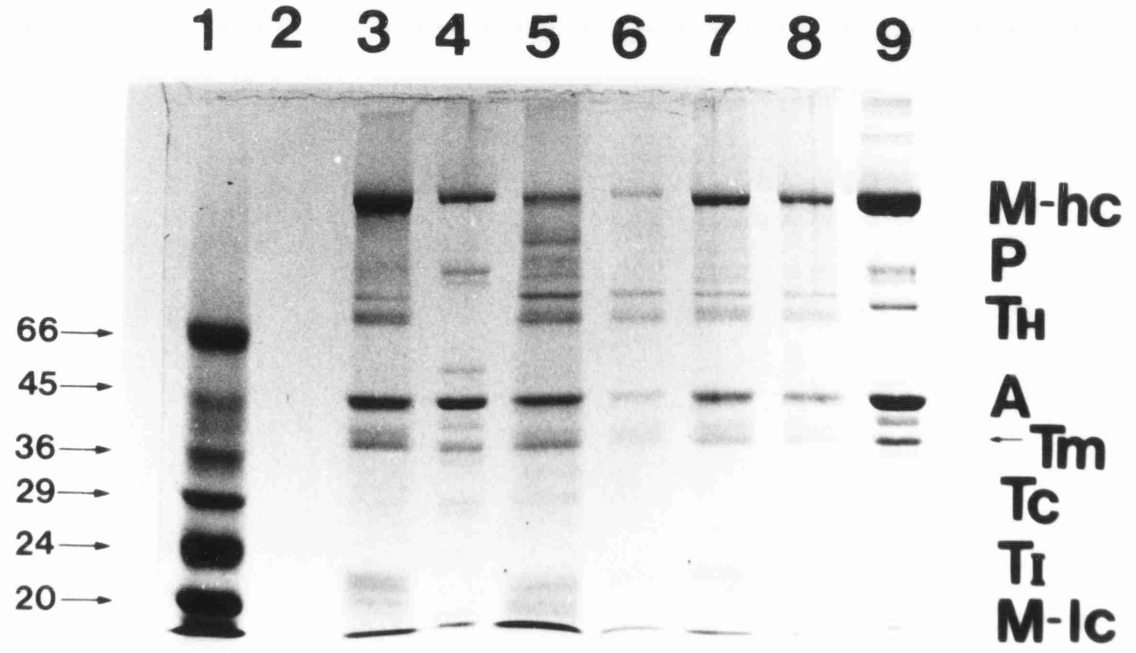

b

Fig. 5.4. Regulatory proteins from shrimp abdominal flexor muscles, electrophoresed on 10% SDS-polyacrylamide gels, and stained with Coomassie blue. Upper gel: 1 = high molecular weight markers; 2 & 3 = *C. crangon* fast muscle; 4 = *N. norvegicus* fast muscle; 5 - 8 = *C. crangon* fast muscle; 9 = *N. norvegicus* slow muscle (S_1 phenotype). Lower gel: 1 = low molecular weight markers; 2 = blank lane; 3 = *C. crangon* fast muscle; 4 = N. norvegicus slow muscle (S_1 phenotype); 5 - 8 = *C. crangon* fast muscle; 9 = *N. norvegicus* fast muscle. Numbers on left of gels represent Migration ratios.

M-hc	-Myosin heavy chain
P	-Paramyosin
T_1	-Troponin T_1
T_H	-Troponin H
A	-Actin
Tm	-Tropomyosin
T_C	-Troponin C
T_I	-Troponin I
M-lc-	-Myosin light chain


1 2 3 4 5 6 7 8 9
205
116
97
66
45
29
M-hc
P
T1
A
Tm
Tc
TI
M-Ic


1 2 3 4 5 6 7 8 9
66
45
36
29
24
20
M-hc
P
TH
A
Tm
Tc
TI
M-Ic

The layout of muscle in the abdomen of *Crangon crangon* conforms to the basic body-plan of the macrurous decapods, having superficial muscles at the dorsal and ventral surfaces of the abdomen and large blocks of deep muscles taking up the remainder of the abdomen. These different muscle blocks have different characteristics which make them specialised for their particular function. This specialisation in *C. crangon* takes a similar form to that described in other decapod crustaceans (eg. crayfish, *Procambarus clarkii* Kennedy & Takeda, 1965; Wine & Krasne, 1982: and the Norway lobster, *Nephrops norvegicus*, Neil & Fowler, 1990; Fowler & Neil, 1992).

Both the superficial flexor muscles and superficial extensor muscles have low levels of myofibrillar ATPase and a high oxidative capacity, which indicate muscle fibres with low contraction rates and high resistance to fatigue. Both of these characteristics are typical of slow muscle, adapted to produce slow postural movements. The superficial muscles also contain the stable form of myosin ATPase, which has been used as a label for slow muscle fibres (Silverman & Charlton, 1980). Interestingly, both the superficial extensor and superficial flexor muscles in *Crangon crangon* also contain the labile form of myosin ATPase, which suggests a second type of slow fibre present in the superficial extensor. The existence of two types of slow muscle fibre has been demonstrated in the superficial flexor and extensor muscles of *Nephrops norvegicus* (Neil & Fowler, 1990; Fowler & Neil, 1992). In *N. norvegicus*,

the superficial flexor slow muscle and one of the superficial extensor muscles are composed of S_1 and S_2 fibres which have labile and stable forms of myosin respectively. The S_1 fibres are known as slow twich fibres, responsible for slow movements and the S_2 or tonic fibres sustain tension for long periods of time without tiring and are used for postural, anti-gravity motion. The results of this study suggest that the superficial abdominal muscles of *C. crangon* are similar in this respect, with a similar pattern being found. This finding challenges the previously held view (Kennedy & Takeda, 1965) that these blocks represent homogeneous muscles, adapted to do one specific task. It is more likely that the heterogeneous muscle block has a broader function, especially if these two fibre types prove to have different contractile properties. Further extensive SDS-PAGE would be necessary to confirm the possibility that the superficial muscles of *C. crangon* are also heterogeneous muscles.

The swimmeret muscles also show similarities with those of *Nephrops norvegicus.* There are clearly two types of muscle fibre present in the swimmeret muscles of *Crangon crangon,* one with high levels of myofibrillar ATPase and one with low levels. Similarly, one has the stable form of myosin ATPase and the other has the labile form (these sections represent reversed images and illustrate nicely the inverse relationship between contraction rate and fatigue resistance). The swimmeret muscles also appear to be highly oxidative and thus resistant to fatigue. These results follow the same pattern as *N. norvegicus* (W. Fowler, pers comm) and suggests that the swimmeret muscles of *C. crangon,* like those of *N. norvegicus,* are composed of

fast muscle fibres and a variant of normal fast fibres which are highly oxidative. It is interesting to note that the areas of dark staining which denote highly oxidative tissue, correspond to the distribution of mitochondria, which are concentrated just under the sarcolemma.

The deep extensor and deep flexor muscles exhibit the typical histochemistry of fast muscle fibres. They have high levels of myofibrillar ATPase, indicating high contraction rates, and a low oxidative capacity which reflects low resistance to fatigue. The muscle masses uniformly comprise the labile form of myosin ATPase, and show all the characteristics of muscle fibres adapted to produce the rapid movements of the tail-flip. There is an indication in histochemistry results of the deep fast muscles that there is greater intensity of staining for myofibrillar ATPase in the deep flexor muscle, compared with that of the deep extensor muscle (Fig. 5.1). This would appear to suggest faster contraction rates in the flexor muscle. This would be consistent with the kinematic findings of Chapter 2, which show that the flexion part of the tail-flip is consistently faster than the extension, and that the rate of angular change of the abdomen during flexion is higher than that of the extension. This is not particularly surprising, as the rapid flexion of the abdomen quite obviously provides the power for the backwards thrust of the animal during the tail-flip. If the extension of the abdomen was carried out as rapidly as the flexion it would tend to slow the backwards movement of the shrimp considerably. Previous studies of macrurous decapod abdominal musculature have not found this histochemical difference between the deep fast muscles, although the same

considerations must also apply. Further experimentation on this particular aspect of the muscle histochemistry of *Crangon crangon* is obviously necessary both to clarify and to examine the ramifications of this finding.

It seems reasonable to assume that the relative size of each of the abdominal muscles found in *Crangon crangon*, may give an indication of the relative importance of that muscle to the behavioural ecology of the animal. The superficial extensor muscles of *C. crangon* are of similar relative size to those of *Nephrops norvegicus* (Neil & Fowler, 1990). Both animals must use their superficial extensors in postural adjustments and for stability when walking, but it is likely that in *C. crangon* the superficial extensors are also used frequently in the process of burying in the substratum. As has already been described by Lloyd & Yonge (1947) and Pinn & Ansell (1992), *C. crangon* makes a hollow in the sand which it then sinks into. By forcing water through its gills it ejects more sand from the hollow, allowing the shrimp to sink further into the sand. In sinking and shuffling into the hollow it has made, *C. crangon* appears to use small flexions and extensions of its abdomen which act to flick or shovel sand over its back where it is then smoothed into place by the antennae (personal observations). *N. norvegicus*, on the other hand, flexes its abdomen more frequently in burrowing. There is some evidence to suggest that the abdomen is flexed and the tail-fan used as a scoop in moving substrate from the burrow (Rice & Chapman, 1971). *N. norvegicus* has also been seen to flex its abdomen and tuck the tail-fan underneath its body to ease turning around. *C. crangon* would appear to use the superficial flexors less for postural adjustments

because a high proportion of its time is spent either buried in the sand or lying flat against the surface of the sand where it is best camouflaged. These differences in behavioural ecology do not appear to affect muscle morphology, however. Clearly, more detailed study into this aspect of the functional morphology of muscle tissues would be necessary to obtain a true picture of the relative importance of these muscles to each species.

The swimmeret muscles of *Crangon crangon* are also relatively large, which reflects the fact that *C. crangon* uses its pleopods for normal swimming when feeding, both in the water column and along the bottom, as well as in the process of burying. The superficial extensor muscles may also be used in pleopod swimming, to keep the abdomen fully extended (especially when swimming vertically).

The size of the deep fast muscles of *Crangon crangon*, which take up almost the entire abdominal region, is perhaps more a reflection of the power they must produce rather than of their ecological importance. However it is interesting to postulate whether the malacostracan tail evolved because of the tail-flip or whether tail-flip became the best solution to the problem of escape behaviour with a long tail. Hessler (1983) suggests that the escape reaction was probably not the sole reason for the emergence of the abdomen and that it was the tail-fan which emerged first in the ancestral malacostracan, in order to stabilise the body during forward walking over the ocean floor. This tail would become an impediment during retreat from predators or competitors and was probably flexed to keep it out of the way. It

subsequently added power to the escape jump and the abdomen filled out to accommodate the large fast muscles (Paul, 1990). The tail-flip appears to have been successful in subserving the escape, as it is now widespread.

It is evident that the deep fast abdominal muscles must be large in order to power the very rapid tail-flip which can propel the shrimp, from a complete standstill, to velocities of more than 1 ms^{-1}, in a few milliseconds at accelerations of 48 ms^{-2}. As has already been noted, in flexion the tail-fan is opened to force water between the fan and the head, while in extension it is closed up, to reduce drag during the extension of the abdomen. Thus the extensor muscles do not need to provide the large amount of power that the deep flexor muscles produce and are therefore smaller.

The deep fast abdominal muscles are examined more closely in the biochemical analysis of the myofibrillar proteins of the muscle tissue. Once again there are strong similarities between the muscle of *Crangon crangon* and that of *Nephrops norvegicus*, both animals sharing the same myosin heavy chain, actin and tropomyosin. Previous studies have found these proteins to be common to many muscles and species (Mykles, 1985a,1985b). Like *N. norvegicus*, the deep abdominal muscle of *C. crangon* has two types of paramyosin: P_1, which is only found in fast muscle, and P_2, which is found in both fast and slow fibre types, though usually in smaller amounts in fast muscle. In the case of *C. crangon* it appears that P_1 and P_2 are present in equal amounts in the fast muscle. In fact the staining for paramyosin

is very weak, which suggests that paramyosin occurs in lower amounts in *C. crangon* than it does in *N. norvegicus.* Mykles (1985) suggests that these two paramyosin variants may reflect structural, rather than contractile differences, which may be expressed in the dimensions of the thick filament. This possibility could be investigated ultrastructurally.

Another myofibrillar protein which can be used as a label for fast fibres is troponin H, previously referred to as the 75kD protein. *Crangon crangon* has 3 different isoforms of troponin H, none of which correspond to those found in *Nephrops norvegicus.* Although interesting, this is not a particularly unexpected result, since isoforms of troponin H vary even between the different fast muscles of lobster, such as those of the claw and the abdomen (Neil & Tobasnick, unpublished data). Crayfish have yet another variant of Troponin H. It is interesting to speculate how the differences between these isoforms are manifested in the performance of the muscle, but it is reasonable to conjecture that differences in troponin H may relate to the speed of contraction in these different muscles. Relevant findings in this context are those of Bullard *et al* (1992) who have established the presence of a 75-80 kD protein to be associated with the stretch activity properties of insect muscles. It will be necessary to apply techniques such as immunoblotting to determine whether the crustacean and insect proteins are in fact the same.

Electrophoresis bands of both troponin I and C were poorly resolved, as were the bands of the myosin alpha light chains. This is probably due to a low intensity

of staining with Coomassie blue (Mykles, 1985), so that at low protein loadings these proteins were hardly visible, in the case of troponin C, or not visible at all, as is the case of troponin I and the alpha light chains. The experimental gels also failed to produce a band of troponin T. There have been 3 variants of this protein found in the lobster muscles, one of which is used as a label for slow fibres and as such would not be expected in *Crangon crangon* fast muscle, however Mykles (1985) found that the three isoforms of troponin T were poorly resolved on 10% gels which would explain their absence from these gels. On the whole the physiological significance of these variations in the myosin light chains and the troponin subunits remains unclear but it is possible that the troponin subunits anyway affect the binding of calcium to the binding sites and thus contraction rates.

CHAPTER 6 : PREDATOR-PREY INTERACTIONS

INTRODUCTION

Having studied the tail-flip of *Crangon crangon* in detail in terms of its physical parameters, its endurance and the functional morphology of the muscle tissues which produce the movements involved, it is appropriate to examine the escape response in terms of predator-prey interactions. Although several studies have examined the tail-flip response, which is so characteristic of macrurous decapods (Wine & Krasne, 1972; Newland *et al*, 1988; Daniel & Meyhöfer, 1989; Newland & Neil, 1990 I & II), few have considered the tail-flip in the context of predator-prey interactions and analyzed the success of the strategy employed in the execution of the escape response.

The sandy and sparsely-vegetated substrates inhabited by *Crangon crangon* provide very little protection in the form of shelter for the shrimp and as such should increase the risk of predation. It is also apparent from a previous experiment (Chapter 4), that the tail-flip is costly in terms of energy and cannot be sustained over long distances. However *C. crangon* is a common species in sparsely vegetated environments and appears to be successful. One must therefore also assume that an escape strategy which is employed by such a widespread organism must be optimal in terms of survival. The way in which the tail-flip is employed is therefore of great interest.

An experimental series was designed to examine the strategy used by *Crangon crangon* in an escape reaction and to assess its success with reference to well documented behavioural strategies. Probably the simplest way to examine the behaviour is to use an artificial predator as has been used in many behavioural experiments. This allows the observation of escape behaviour without the loss of significant numbers of experimental animals and also allows some control over the movements of the predator. The experiments aim to describe the strategy used by *C. crangon*, and to identify some of the cues which trigger the escape reaction that follows. Of particular interest in identifying an escape strategy is the timing of an escape response in terms of the approach of the predator. Measuring the distance travelled in the course of the tail-flip is also important not only as part of the escape strategy, but also to compare with the values recorded in Chapters 2 & 3, under somewhat more artificial conditions. As many studies have reported the use of burial in the substratum as an important line of defence (Fuss & Ogren, 1966; Stein & Magnuson, 1976) the importance of burial in the escape strategy has been investigated as has the effect on the strategy of different degrees of burial. The events which follow the tail-flip have also been examined.

Crangon crangon were obtained from Kames Bay, Millport, either using a push net or by trawling. In an attempt to discourage cannibalism, which becomes more prevalent at increased animal density, the shrimps were kept in an aquarium with a layer of sand on the bottom. This allowed the shrimps to bury, increased the carrying capacity of the aquarium and served to reduce stress. The shrimps were fed on *Mytilus edulis* every two days. The animals were maintained in running, aerated seawater at temperatures of between 9° and 11°C. Both male and female *C. crangon*, ranging in size from 33 to 60 mm (from rostrum to telson), were used in the experiments.

The escape responses of *Crangon crangon* were observed in a glass aquarium tank, with a layer of sand, 3 to 4 cm deep. The experiments were carried out in aerated seawater at a temperature of between 9° and 11°C. Escape responses were elicited using a plaster-of-paris model fish (25cm) which was attached to, and moved by, a length of fishing line. Rulers along the front and the sides of the tank allowed the position of both the shrimp and the fish to be noted throughout the experiment (see Figure 6.1). Shrimps were placed individually in the tank and allowed 20 to 30 minutes 'settling time' before the 'fish' was introduced. The initial position of the shrimp in the tank was noted. It was also given a burial score on an arbitrary scale of 1-3 (1: the shrimp had made no attempt to bury itself and remained on the surface of the substratum, 2: the shrimp was partially buried, and 3: the shrimp was

fully buried, almost completely concealed but for its eyes and antennae). In the course of each experiment the model fish was moved towards the shrimp across the bottom of the tank with its vertical position kept uniform, at a height above the sand so that the model fish was just able to pass across its surface without disturbing the substratum. The approach of the model fish was continued until an escape response was triggered and, if the shrimp had not responded with an escape response prior to its arrival, the model fish was used to produce a tactile stimulus at the end of the approach. When an escape response was elicited, a number of things were noted; the distance between the shrimp and the model fish when the shrimp responded (reaction distance); the horizontal distance travelled by the shrimp in its escape; the time taken for the shrimp to re-bury itself in the sand, and the degree to which it re-buried itself. Distances measured from the markers were only accurate to ± 0.5 cm. Two or 3 repeat experiments were performed on each shrimp.

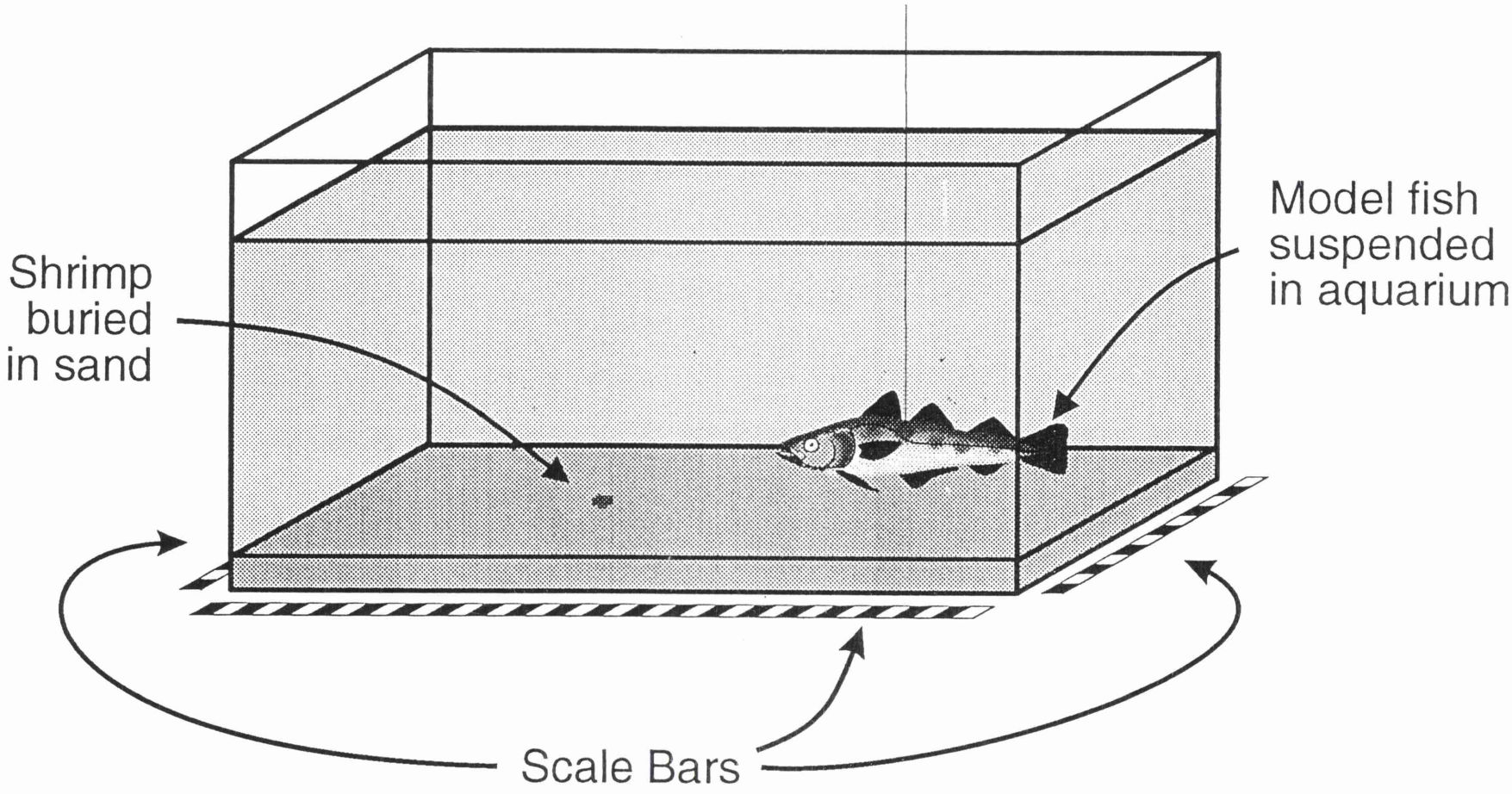
In order to examine the cues received by the shrimp which trigger the decision to tail-flip, the experiments were repeated with another set of animals, using a glass bottle of a similar size and shape to the model fish as the predator. This 'glass fish' was employed in an attempt to minimize visual stimulus, either from the shape and markings or from the shadow cast by the fish. It was assumed, therefore, that the only significant stimulus the shrimp was able to detect from the glass fish was the change in near field water displacement occurring when it was moved in towards the shrimp. The glass fish was manoeuvred in the same way as the model fish and the same measures were noted in the course of the experiment. By comparing the

results of the two experiments the possible involvement of visual cues in escape behaviour could be identified.

As some differences were observed in the measures of escape behaviour between animals at different initial burial depths, all measures of response were considered separately in the three burial categories: surface; partially buried; and fully buried.

Fig. 6.1. Diagram showing experimental aquarium.

Experimental Aquarium



RESULTS

OBSERVATIONS WITH MODEL FISH

Crangon crangon which were placed in the observation tank, in most cases, immediately buried in the sand and often did not move from this initial position over the course of the settling time. Some shrimps were seen to bury deeper after the introduction of the model fish.

REACTION DISTANCE

It was assumed that if horizontal reaction distance was not important in terms of escape strategy, the frequency distribution of the reaction distances recorded from experimental animals would be spread uniformly across the range of distances recorded. Therefore the frequency distributions recorded were tested against a uniform distribution using a Kolmogorov-Smirnov one sample test of goodness of fit. Shrimps responding to the model fish did so over a range of reaction distances from 0 to 12 cm.

Animals responding from a position on the surface of the sand showed the highest frequency of response when the fish was 1 - 2 cm away from the shrimp (see Figure 6.2) and this distribution was found to differ significantly from a uniform distribution ($p < 0.0009$). Shrimps which were partially buried or fully buried,

responded most frequently at a horizontal reaction distance of 0 cm (i.e. when the fish was directly above the shrimp). The frequency distributions in each case were also found to differ significantly from a uniform distribution ($p < 0.0009$). Using a Kruskal-Wallis one-way analysis of variance, a highly significant difference was found to exist between animals buried to these three degrees of burial, in the reaction distances of their response ($p < 0.0002$).

Of the model fish predator 'attacks,' some resulted in a tail-flip only after a tactile stimulus had been applied. A tactile stimulus accounted for 16% of the tail-flips elicited from animals on the surface of the substratum, 54% of tail-flips performed by partially buried animals, and 62% of tail-flips performed by fully buried animals. These results suggest that an increasing proportion of animals respond only to a tactile stimulus as the degree of burial increases. Approaches by the model fish which elicited no response (even after tactile contact) represented 5% of cases for animals on the surface, 14% for animals which were partially buried, and 5% for fully buried animals.

The number of tail-flip responses elicited by tactile stimuli and the number of negative responses recorded in each of the three burial groups were also compared using a Kruskal-Wallis one-way analysis of variance. In both cases significant differences were found between burial categories ($p < 0.00009$ and $p < 0.005$, respectively).

These results clearly show that at an increased level of burial a higher proportion of responses are elicited at a reaction distance of 0 cm, more responses are as a result of a tactile stimuli and shrimps are less likely to respond with a tail-flip. Because the reaction distance proved to be significantly different at different levels of burial, the other response measures were also considered in relation to burial level.

DISTANCE TRAVELLED.

Shrimps responding to the model fish predator tail-flipped over horizontal distances ranging from 0 to 60 cm, with mean values of 9.12 (± SD = 7.24) for those on the surface, 10.95 (± SD = 14.66) for those partially buried and 17.21 (± SD = 19.33) for completely buried animals (Fig. 6.3).

The effect of burial depth on the tail-flip distance was considered, by comparing the distances travelled by animals in the different burial categories using the Kruskal-Wallis one way analysis of variance. The distance travelled in the course of a tail-flip was found to be unaffected by the initial burial depth, there being no significant difference between the distances recorded in the three burial categories ($p > 0.35$).

TIME TO REBURY.

The time taken between the completion of the tail-flip and the onset of re-burying in the substratum was also measured for the three burial categories (Fig. 6.4). Mean values of 33.05 s (± SD = 51.11), 31.82 s (± SD = 39.19) and 32.62 s (± SD = 40.55) were obtained for animals on the surface, partially buried and completely buried, respectively.

Using a Kruskal-Wallis one-way analysis of variance no significant difference was found between the three groups of burial in respect of the time taken to re-bury after completion of a tail-flip escape response ($p > 0.6$).

OBSERVATIONS WITH GLASS FISH

REACTION DISTANCE

Shrimps responding to the glass predator from a position on the surface of the substratum, were most frequently found not to respond until a reaction distance of 0 cm. The frequency distribution of the reaction distances recorded was found to differ significantly from a uniform distribution ($p < 0.0009$) (using a Kolmogorov-Smirnov test of goodness of fit). Animals which were partially buried and those which were fully buried in the substratum were all found to respond to the glass fish at a reaction distance of 0 cm. In both cases, the frequency distribution of the

reaction distances recorded was therefore assumed to be significantly different from a uniform distribution. Figure 6.5 summarises these results. The reaction distance results from interaction with the glass fish were then compared with those recorded in response to the model fish, using a Kruskal-Wallis one-way analysis of variance. The pattern of frequency distributions of reaction distances recorded in response to the model fish were found to differ significantly from those recorded in response to the glass fish at all three burial categories ($p < 0.05$, for tail-flips performed by shrimps on the surface; $p < 0.005$, for both partially buried and fully buried shrimps). Tail-flips performed by shrimps in response to the glass fish almost always occurred at a reaction distance of 0 cm, whereas those elicited by the model fish, although most frequently occurring in the last few centimetres of the approach of the predator, were seen to be elicited during the approach from a distance of 12 cm. These results suggest, firstly that the tail-flip is performed at the last moment in terms of predator approach, presumably as this is the optimal time to employ it in order to evade capture, and secondly that there is an important visual component in the decision to tail-flip.

As was the case in response to the model fish, a proportion of the tail-flips elicited by the glass fish, resulted from a tactile stimulus delivered at the end of the approach of the fish. A tactile stimulus accounted for 58% of the flips performed by animals remaining unburied on the surface of the sand, 97% by partially buried animals, and 98% by fully buried shrimps. In all three burial categories a significant difference was found to exist between the proportion of tail-flips which resulted from

a tactile stimulus in the different experimental groups (Kruskal-Wallis, one-way analysis of variance, $p < 0.0001$). Animals failed to respond to the stimulus in 8%, 15% and 48% of cases of animals on the surface, partially buried and fully buried respectively.

A comparison of the proportion of approaches by the model and glass fishes that failed to elicit a response found no significant difference for shrimps on the surface of the substratum (Fisher's one-tailed exact test, $p > 0.5$), or for partially buried shrimps (Fisher's one-tailed exact test, $p > 0.4$). However for shrimps that were fully buried, a highly significant difference was found between the responses to glass and model fish (X^2, corrected for continuity, $p < 0.0006$, at 1 degree of freedom). It seems that the absence of a visual stimulus has a greater effect on the responsiveness of shrimps when the animal is fully buried in the sand than when partially or not buried.

DISTANCE TRAVELLED

The effect of varying the stimulus received by the shrimp, on the distance travelled in the course of the tail-flip was examined by comparing the results obtained from the model fish and glass fish, using a Kruskal-Wallis one-way analysis of variance (Fig. 6.6 shows the distances travelled during tail-flips elicited by the glass fish). No significant differences were found between the distances travelled by shrimps on the surface or partially buried at the onset of the response ($p > 0.5$ and

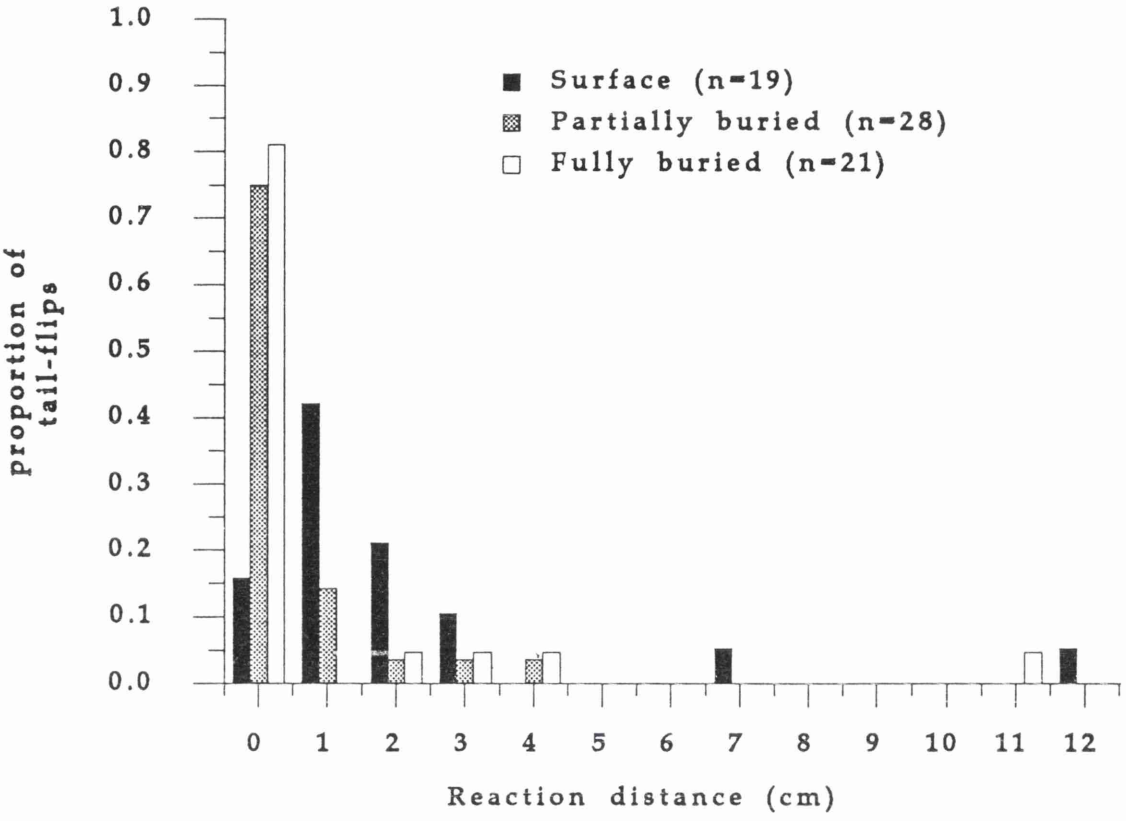
0.9, respectively). However in the group of animals which were fully buried highly significant differences were found between the escape distances recorded by shrimps in response to the model and glass fish ($p < 0.0001$).

REBURIAL

To determine if differences exist between the time taken to rebury after tail-flips elicited by the model and glass fish, results were compared using a Kruskal-Wallis one-way analysis of variance (Fig. 6.7 shows the reburial times achieved after tail-flips elicited by the glass predator). No significant differences were found in any of the three burial groups between the two stimulus types ($p > 0.5$, in all cases).

Fig. 6.2. Frequency histogram showing the proportion of tail-flips elicited at each reaction distance in the range of 0 - 12 cm, by a model fish.

Fig. 6.3. Frequency histogram showing the proportion of tail-flips travelling over each distance (from 0 - 60 cm) in response to a model fish.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
proportion of tail-flips
Surface (n=19)
Partially buried (n=28)
Fully buried (n=21)
0 1 2 3 4 5 6 7 8 9 10 11 12
Reaction distance (cm)

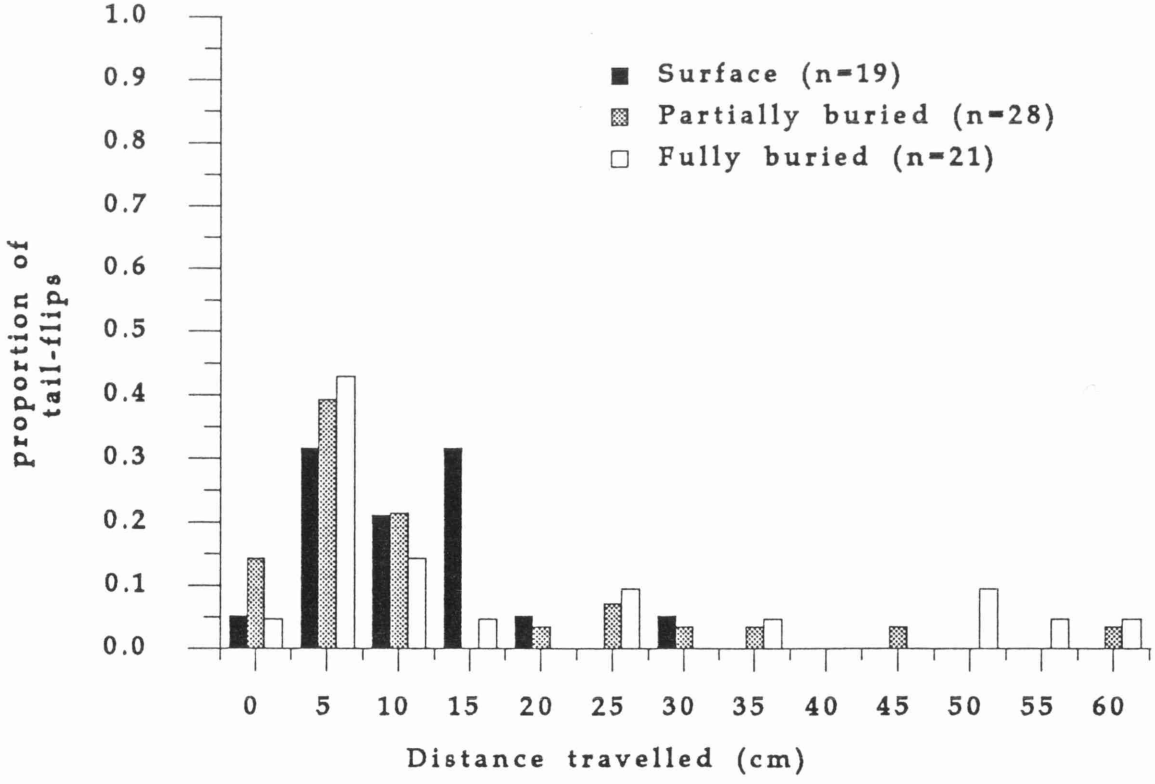

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
proportion of tail-flips
Surface (n=19)
Partially buried (n=28)
Fully buried (n=21)
0 5 10 15 20 25 30 35 40 45 50 55 60
Distance travelled (cm)

Fig. 6.4. Frequency histogram showing the proportion of escape responses elicited by a model fish, resulting in re-burial within each time band (from 0 - 160 sec).

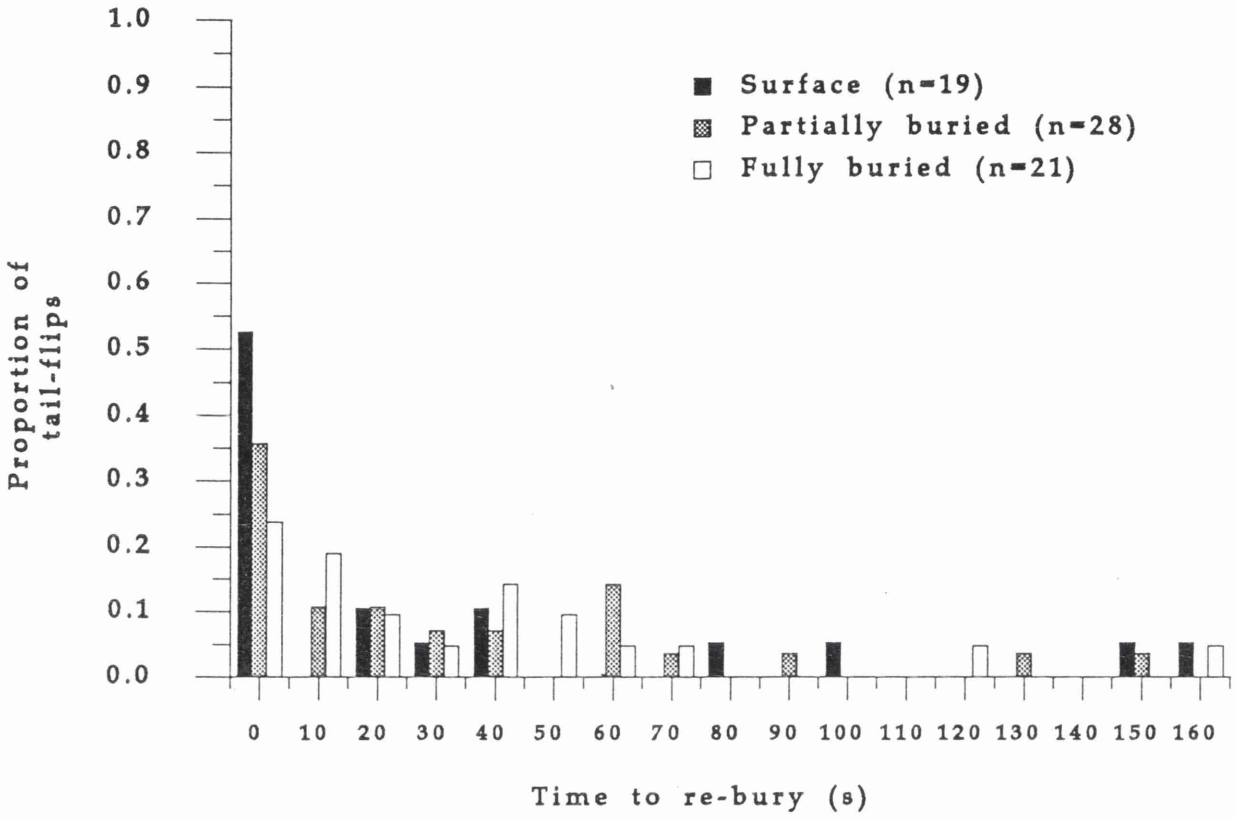
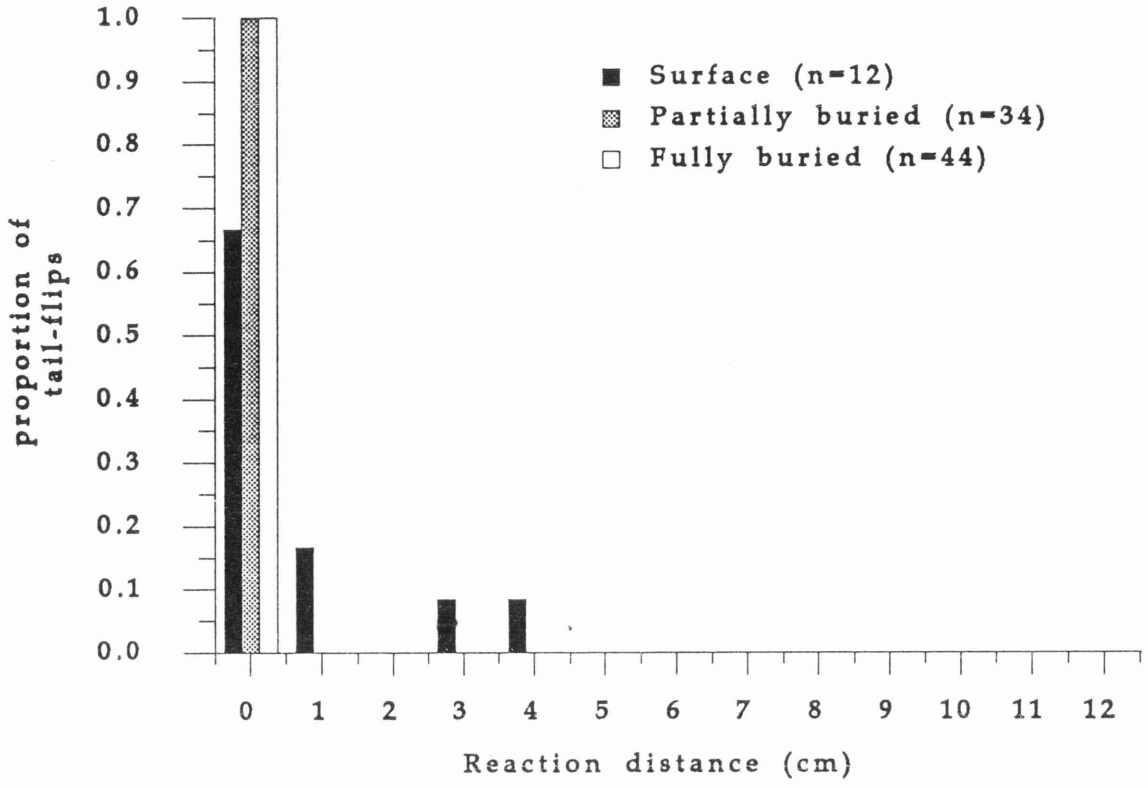

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of tail-flips
Surface (n=19)
Partially buried (n=28)
Fully buried (n=21)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
Time to re-bury (s)

Fig. 6.5. Frequency histogram showing the proportion of tail-flips elicited at each reaction distance in the range of 0 - 12 cm, by a 'glass' fish.

Fig. 6.6. Frequency histogram showing the proportion of tail-flips travelling over each distance (from 0 - 60 cm) in response to a 'glass' fish.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
proportion of tail-flips
Surface (n=12)
Partially buried (n=34)
Fully buried (n=44)
0 1 2 3 4 5 6 7 8 9 10 11 12
Reaction distance (cm)

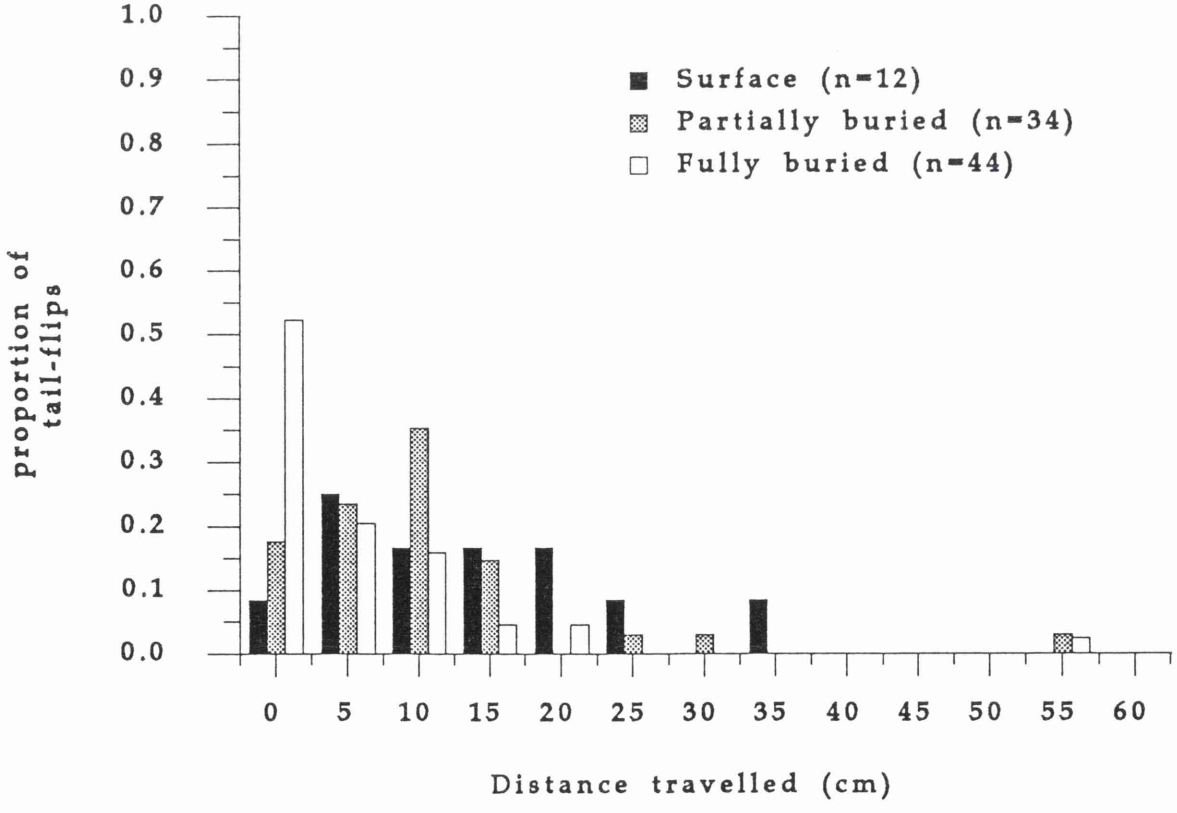
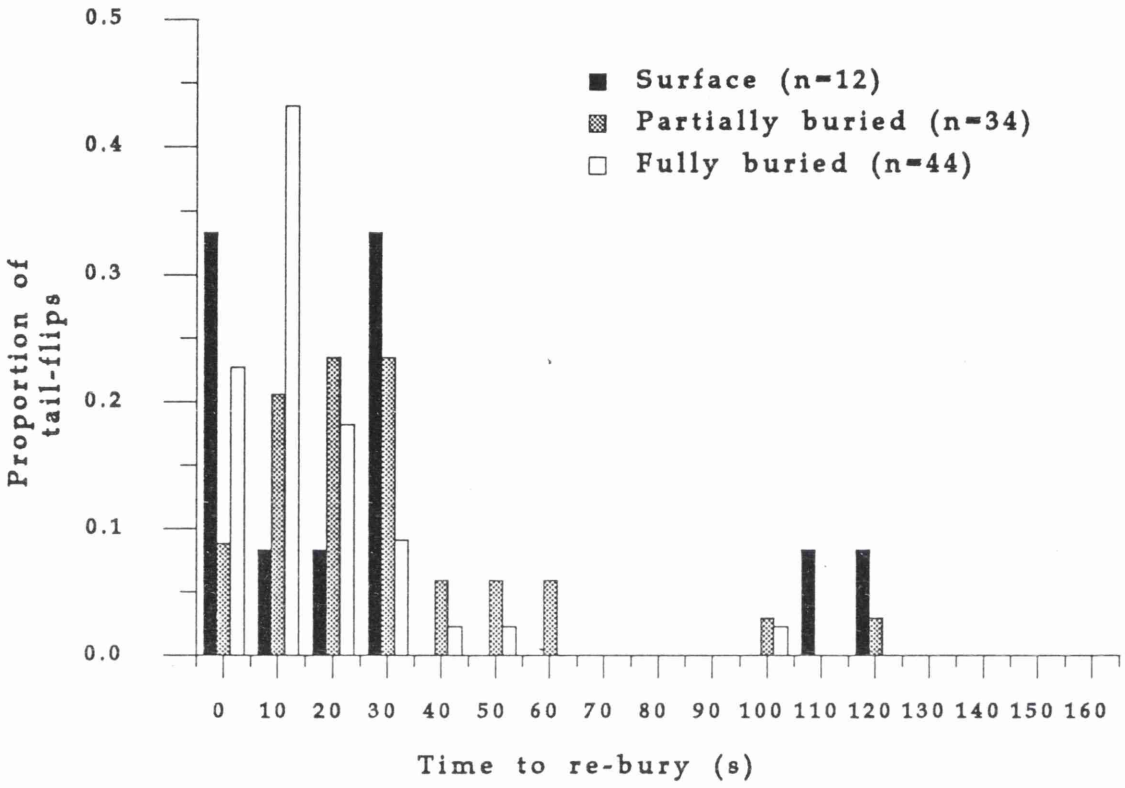

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
proportion of tail-flips
Surface (n=12)
Partially buried (n=34)
Fully buried (n=44)
0 5 10 15 20 25 30 35 40 45 50 55 60
Distance travelled (cm)

Fig. 6.7. Frequency histogram showing the proportion of escape responses elicited by a 'glass' fish, resulting in re-burial within each time band (from 0 - 160 sec).


0.5
0.4
0.3
0.2
0.1
0.0
Proportion of tail-flips
Surface (n=12)
Partially buried (n=34)
Fully buried (n=44)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
Time to re-bury (s)

In terms of an escape strategy, the results of the experiment clearly indicate that tail-flips are elicited at very short reaction distances, with shrimps remaining motionless for a long time when approached by a predator, and responding with a tail-flip only in the last few seconds of the predator's approach. There are a number of factors which make this functionally appropriate.

Firstly, *Crangon crangon* is extremely well camouflaged on or in sand and this is obviously very important for the survival of the shrimp, if only because camouflage provides the only concealment available on the sparsely vegetated bare sand that *C. crangon* inhabits. Green (1961) reports that *C. crangon* shrimps have very elaborate chromatophores formed from several cells and having numerous branches. *C.crangon* has chromatophores with four different pigment branches; red, black, white and yellow. This enables the shrimp to change its colour to mimic its surroundings relatively rapidly, and, as many of the important predators of *C. crangon* are visual predators, is crucial in explaining why *C. crangon* leaves the safety of the substratum only at the last moment, as was established in this study.

Many predators rely on prey movement in order to detect prey (Brawn, 1969; Ware, 1973; Tallmark & Evans, 1986), and therefore the shrimp may rely on the ability to conceal itself as the first line of defence. Once buried in the substratum the shrimp is concealed sufficiently well that it may be passed over completely by a

predator when completely motionless. It has been suggested that cod, an important predator of crangonid species in the Clyde (D. Henning, pers. comm.), are unable to detect and retrieve food covered by sand (Brawn, 1969). If this is the case, and predators are likely to fail to detect the shrimp when it is buried in the sand, an early tail-flip escape response would be a dangerous survival strategy to adopt. This would suggest that buried animals are safest if they remain buried in the substratum and it is perhaps for this reason that buried *Crangon crangon* are most frequently found to wait until the predator is directly above them before tail-flipping. Animals which are fully buried in the sand are more likely to respond at a shorter reaction distance than animals which fail to bury, or are only partially buried, as the deeper the shrimp is buried the better it is protected. This would also explain why such a high proportion of shrimps will only produce an escape tail-flip in response to a tactile stimuli, the proportion rising in animals which are buried to a greater degree.

Furthermore, although the tail-flip escape response can be executed at very high speed, *Crangon crangon* is unlikely to tail-flip over sufficient distance to outswim a predator. Results from previous experiments (Chapter 4) suggest that *C. crangon* produces one tail-flip in response to a stimulus. It seems therefore that *C. crangon* does not produce bouts of tail-flip swimming in the way reported for lobsters such as *Nephrops norvegicus* (Newland *et al*, 1988). The cuttlefish is a predator which relies heavily on its visual acuity and on the accuracy and rapidity of its attack, and is known to prey on shrimps and prawns. Having caught sight of the potential prey item the cuttlefish attacks extremely rapidly, ejecting its tentacles

and seizing its prey within 30 ms. However if the shrimp tail-flips away from the substratum after the attack of the cuttlefish is underway, the cuttlefish is unable to change from its original course of attack and as a result fails to capture the prey (Messenger, 1968). The greatest asset then of the tail-flip is probably the very rapid acceleration it produces, and it is this which provides the best means of escape from predators. These constraints favour the strategy of lying still in the sand until the very last moment and then executing a rapid tail-flip, by which time the predator is committed by its momentum to its initial target area.

It appears that the timing of the tail-flip is all important to the success of the escape response and therefore the mechanism by which the correct timing of a response is accomplished is of importance. It is likely that the shrimp uses external cues to make the correct decision.

Comparison of responses elicited by the glass and model fish suggest that a scale of cues is important in the decision to tail-flip. It is apparent that shrimps are exhibiting escape responses to different stimuli and different levels of stimuli. For example, some tail-flips are produced in response to water movement and vibrational changes only (e.g. in response to the approach of the glass predator within 4 cm). Others are clearly visual responses (e.g. to the model fish at distances greater than 4 cm), while many are only elicited by tactile stimuli, either alone or in conjunction with the other modalities. Why do some tail-flips result from low level cues and in other cases an animal will have to experience a range of cues before a response is

elicited? Also, as the escape response is so rapid, how are these complex cues assimilated and processed fast enough for the response to be viable?

It may be the case that *Crangon crangon* relies on a series of cues of increasing intensity to trigger an escape response and that different animals are differentially responsive (or are in different motivational states). For example a rapidly approaching visual stimulus may itself be sufficient for a few animals to respond and a visual stimulus followed by changes in near field water movement at the approach of a predator, may produce a response in a few more animals. A tactile stimulus coming after the previous two cues is likely to produce a response in almost all shrimps. This sort of motivational staircase is found in the reproductive behaviour of the three-spined stickleback, where mating behaviour in the female is initiated by cues from the male, for example a red belly and zig-zag swimming behaviour. Depending on the motivational state of the female, mating behaviour will be elicited in the female by one or both of these cues (Tinbergen, 1951). In the situation of *C. crangon*, this sort of releaser mechanism, working at increasing threshold levels depending on the motivational state of the shrimp, may allow complex survival decisions to be made at a reaction speed similar to that of a reflex. Each stimulus received produces a certain level of arousal which must cross a threshold to produce a response. The motivational state of the shrimp, affected by many different factors, alters the position of this threshold and therefore the critical level of stimuli necessary to produce a response. As has already been noted, the degree to which an animal is buried affects the timing of the tail-flips in respect of reaction distance, and

at a high degree of burial the shrimp is more likely to respond only to tactile stimuli or to fail to respond at all. This is the sort of factor which would affect the motivational state of the potential prey shrimp. Other possible factors which may affect the motivational state of the animal, and therefore the decision making process, are the size and state of moult of the shrimp. For example, Stein & Magnuson (1976) found that smaller, more vulnerable crayfish (*Orconectes propinquus*) responded more quickly than larger animals to predators, and Cromarty *et al*, (1991) found differences in escape reactions between lobsters (*Homarus americanus*) at different stages of the moult cycle.

This chapter has considered only the most basic of cues received from the predator and has indicated that visual cues are very important. However chemical cues may also play an important part in triggering an escape response and may form part of a very much more complex set of so called releaser cues. Specific visual cues may be involved which give the shrimp more detailed information about the predator, but it appears likely that whatever form these cues take they will act in this releaser fashion which trigger reflex reactions necessary to produce the rapid tail-flip at the appropriate time. More detailed research into this aspect of the escape response of *Crangon crangon* is obviously necessary to determine whether this is in fact the sort of process used by the shrimp in the decision to tail-flips and to what level of detail visual and other cues are perceived.

The horizontal distances travelled in the course of a tail-flip, were found to be similar to the displacements recorded in Chapter One, at between 0 and 10 cm. A visual cue appeared to have no effect on the distances travelled during tail-flip unless shrimps were fully buried, although no difference was established previously between the distances travelled by animals in different burial categories in response to either predators. In fully buried animals a visual stimulus not only alters the timing of the escape response, but also increases the distance of escape. Why a visual stimulus should affect the escape distance only in fully buried animals is unclear.

After an escape response which carries the shrimp out of the substratum and into the water column, most shrimps rebury in the substratum, and as the experiment shows, shrimps tend to rebury within a relatively short period of time after a tail-flip escape response. This reflects the fact that the animal is less vulnerable when buried in the sand and the need to evade capture by the predator encourages the shrimp to bury again as soon as possible. It is likely that reburial also presents a dilemma for the shrimp in that although the sooner it reburies in the substratum, the sooner it is safely concealed, the movement generated in order to rebury may draw the attention of the predator. Quite how the shrimp resolves this problem is unclear but it may be that other factors of the tail-flip escape response mask the reburial of the shrimp and therefore aid its escape.

Certain features of the tail-flip probably aid the shrimp in eluding a predator by producing confusion. Because the tail-flip is so rapid and involves a fairly violent

motion, it may well act to surprise the predator and momentarily disorient it, thus reducing the effectiveness of any subsequent attack. In the course of the tail-flip the shrimp may also produce a flurry of fine sediment as it takes off which again adds to the confusion of the potential predator and allows the shrimp to resettle without the predator being able to follow its escape path. This would lead us to expect that, if the shrimp was going to rebury itself, it would do so very quickly to exploit any momentary distraction of the predator. With re-burial occurring between 0 and 30 seconds, this would appear to be the case. However whether slow re-burial jeopardises the escape is not known. It is an established fact that a dash and subsequent cessation of motion is an effective escape strategy against predation among fish (M^{c}Phail, 1969) and it may be that *Crangon crangon* can wait motionless until a predator has ceased to show interest in it before re-burying.

The other benefit gained from this particular escape response is due to the erratic nature of the tail-flip which makes it difficult for a predator to follow a prey animal by producing a corresponding path of pursuit and this therefore makes a second attack unlikely. This type of protean escape display has been shown to reduce the success of a predator (Humphries & Driver, 1967,1970).

The effectiveness of the tail-flip in allowing the shrimp to evade capture by a predator can only be established by more direct measures in the field and a more detailed knowledge of the prey-capture behaviour of the predator itself. The behaviour of a predator in interactions with its prey will be governed by factors such

as optimal foraging strategies, the effects of prey density, capture success, handling time and the value of the prey item in terms of nutrients.

In conclusion, these experiment indicates that the escape response of *Crangon crangon* is employed in a specific strategy of escape which makes optimum use of the different parameters of the tail-flip which have been investigated in part in previous chapters. It seems likely that the escape response is governed by a complex series of cues, the mechanism of which is unknown. Clearly in order to gain a more detailed picture of the complex series of events which lead up to a predator induced escape tail-flip, a more detailed study must be carried out.

CHAPTER 7 : CONCLUSIONS AND GENERAL DISCUSSION

CONCLUSIONS AND GENERAL DISCUSSION

The aim of the present study was to provide information on the escape behaviour of the brown shrimp, *Crangon crangon* and to examine the role of this behaviour within the behavioural ecology of the animals. The results of the study provide a positive insight into the role of the tail-flip escape response in the behavioural ecology of *C. crangon.*

The use of high speed video analysis in conjunction with the "Movias" software package, proved to be an important tool, not only in clarifying the detail of the rapid escape response of the shrimp and in examining the kinematics of the propulsion, but also in calculating measures of performance such as velocity. The measures of performance of the tail-flip of *Crangon crangon* were found to fall within the range of other tail-flipping crustaceans. However, high speed video analysis allowed the determination of a difference in the style of the tail-flip of *C. crangon* in comparison with other documented tail-flips. Species such as *Nephrops norvegicus,* and krill (*Euphasia superba*) which perform multiple flips in swimming bouts, appear to move only the tail in the course of the flexion of the abdomen, (Newland *et al,* 1988, Newland & Neil, 1990 I and Kils, 1982) whereas *C. crangon* uses more of a "jack-knife" movement with both the abdomen and the cephalothorax moving during flexion, in a similar technique to the mysid, *Praunus flexuosus* (Ansell & Neil, 1991). Whether this difference in tail-flip style is due to morphological differences between species or to the circumstances of escape episodes is not known, but it is

postulated that in the sparsely-vegetated, soft bottomed habitat of *C. crangon*, an escape response based on swimming speed may have to cover some considerable distance and it is unlikely that the shrimp would be able to outswim its larger predators in these circumstances. For example, among the gadoids, common predators of *C. crangon*, Blaxter & Dickson (1958) report swimming speeds of between 75-210cm.s^{-1} for cod (*Gadus morhua*), 75-160cm.s^{-1} for whiting (*Gadus merlangus*) and 6-129cm.s^{-1} for plaice (*Pleuronectes platessa*). Although the shrimp may not outswim a predator, it may be able to out-manoeuvre it, and it is probable that the shrimp exploits the rapid acceleration of a "jack-knife" tail-flip to reduce the likelihood of predator pursuit.

The power of the tail-flip of *Crangon crangon* is provided by contraction of the fast abdominal flexor muscles which propels the animal backwards through the water at high speed. These fast flexor muscles were found to resemble closely those of *Nephrops norvegicus* (Neil & Fowler, 1990; Fowler & Neil, 1992) in that they showed high levels of myofibrillar ATPase activity, indicative of fast contraction rates, and a low oxidative capacity, suggesting low fatigue resistance. At the molecular level some slight differences between *C. crangon* and *N. norvegicus* are evident in the muscle myofibrillar proteins, such as the appearance of different troponin H bands. It is postulated that these differences may be linked with differences in contraction rates in muscles, though more detailed study is necessary to provide more conclusive information.

Despite the evidence of low oxidative capacity in the deep fast muscles, which is indicative of low fatigue resistance, experiments examining the endurance of *Crangon crangon* in sustaining tail-flip responses found that the shrimp is remarkably robust in the face of repeated stimuli and response. The timing of behavioural events associated with the extinction and recovery of tail-flip behaviour were found to be similar to the figures recorded by researchers working on the biochemical events of the extinction and recovery of escape tail-flipping (Onnen & Zebe, 1983; Kamp & Juretschke, 1987), recovery of full tail-flip potential appearing to start after some 60 minutes. With shrimps responding to stimulus after a recovery interval of only 2 minutes after apparently exhaustive work, it is possible that the animal rarely encounters the situation of being unable to respond due to fatigue, partial recovery being sufficient to power subsequent flips.

In the experiments presented here which involved repeated mechanical stimulus immediately after a response, and in the experiments in other studies examining the biochemistry of extinction and recovery of behaviour, which used an electrical impulse as a stimulus (Onnen & Zebe, 1983; Kamp & Juretschke, 1987; Kamp, 1989; Gruschczyk & Kamp, 1990), it is probable that the regime of stimulus was far more rigorous than that encountered in the field during natural predator interactions. In the present study animals were found to respond to almost 50 stimuli before the extinction of escape behaviour. Optimal foraging theory states that the overall profitability of a prey item is a function of its nutritional and energy value, and the costs of prey capture and its consumption. In a natural predator-prey

interaction it is unlikely that a predator will make 50 repeated attacks on a single shrimp as the costs of catching the shrimp will become greater than the energy benefits of the food. This suggests that the shrimps' endurance and ability to recover will allow them to sustain stimulation both by trawl gear and in the face of most predator-prey interactions. Probably more important to the survival of *Crangon crangon* is the success of the tail-flip in evading a predator in initial attacks.

In the course of all of the experiments carried out in the present study, the majority of stimuli resulted in a single tail-flip. This is consistent with the hypothesis presented earlier, that the tail-flip of *Crangon crangon* is adapted for speed of acceleration rather than efficiency in terms of multiple flips. Experiments throughout the study also found that shrimps were not propelled great distances in the course of the tail-flip, moving distances of between 0-10cm. Again this suggests that the escape response is not an attempt to outswim a predator but more an attempt to elude the pursuit of the predator with a sudden, rapid and erratic movement.

If the shrimp is relying on a single tail-flip to remove it from a potential predator it is probable that the timing of that tail-flip is extremely important. This proves to be the case, as the final set of experiments show. The strategy of escape of *Crangon crangon* is to execute a tail-flip in the final stages of the approach of the predator, often after the predator has begun to strike. It is suggested that the decision to tail-flip is triggered by a series of cues of increasing intensity, which will cause the execution of a tail-flip, depending on factors which affect the motivational

state of the shrimp such as burial depth. Some other factors which it is suggested may affect the motivational state of an animal are state of moult or body size.

In conclusion, the mechanism of the tail-flip of *Crangon crangon* the strategy employed in its execution, appear to be finely tuned to meet the demands of the animal and its surrounding habitat so that it is best able to evade capture by its predators.

REFERENCES

ALLEN, J.A. (1960). On the biology of *Crangon allmani* Kinahan in Northumbrian waters. *J. Mar. Biol. Ass. U.K.* **39**, 481-508.

ANSELL, A.D. & NEIL, D.M. (1991). Analysis of escape swimming in the mysid *Praunus flexuosus* by using high-speed video recording. *J. Mar. Biol. Ass. U.K.* **71**, pp 731.

BLAXTER, J.H.S., & DICKSON, W. (1958). Observations on the swimming speeds of fish. *J. du Cons.* **24**, 472-479.

BRAWN, V.M. (1969). Feeding behaviour of Cod (*Gadus morhua*). *J. Fish. Res. Bd. Can.* **26** (3), 583-596.

BULLARD, B., LEONARD, K., LARKINS, A., BUTCHER, G., KARLIK, C. & FYRBERG, B. (1988). Troponin of asynchronous flight muscle. *J. Mol. Biol.* **204**, 621-637.

COOKE, I.R.C. (1985). Further studies of crayfish escape behaviour II. Giant axon-mediated neural activity in the appendages. *J. Exp. Biol.* **118**, 367- 377.

COSSINS, A.R. & BOWLER, K. (1987). *Temperature of Biology Animals.* New York: Chapman & Hall.

CROMARTY, S.I., COBB, J.S. & KASS-SIMON, G. (1991). Behavioural analysis of the escape response in the juvenile lobster *Homarus americanus* over the molt cycle. *J. Exp. Biol.* **158**, 565-581.

DANIEL, T.L. & MEYHÖFER, E. (1989). Size limits in escape locomotion of Carridean shrimp. *J. Exp. Biol.* **143**, 245-265.

ELLIOTT, M., O'REILLY, M.G. & TAYLOR, C.J.L. (1990). The Forth estuary: a nursery and overwintering area for the North Sea fishes. *Hydrobiologia.* **195,** 89-103.

FIELD, R.H. (1992). The control of escape behaviour in, and the histopathology of, the Norway lobster, *Nephrops norvegicus* (L.). PhD. thesis. University of Glasgow.

FISCHER, L. & FLOREY, E. (1981). Temperature effects of neuromuscular transmission (opener muscle of crayfish, *Astacus leptodactylus*). *J. Exp. Biol.* **94,** 251-268.

FLOREY, E. & HOYLE, G. (1976). The effects of temperature on a nerve-muscle system of the Hawaiian Ghost Crab, *Ocypode ceratophthyalma* (Pallas). *J. Comp. Physiol.* **110,** 51-64.

FOWLER, W.S. (1990). Histochemical staining of lobster muscle demonstrating no ATPase pH reversal. *J. Physiol. 420,* 177P.

FOWLER, W.S. & NEIL, D.M. (1992). Histochemical heterogeniety of fibres in the abdominal superficial flexor muscles of the Norway lobster, *N. norvegicus* (L.). *J. Exp. Zool.* **264,** 406-418.

FUSS, C.M. & OGREN, L.H. (1966). Factors affecting activity and burrowing habits of the pink shrimp, *Penaeus duorarum* Burkenroad. *Biol. Bull.* **130,** 170-191.

GADE, G. (1983). Energy metabolism of arthropods and molluscs during environmental and functional anaerobiosis. *J. Exp. Zool.* **228,** 415-429.

GOVIND, C.K. & ATWOOD, H.L. (1982). Organisation of neuromuscular systems. In *The Biology of Crustacea, Vol. 3.* (ed. D.E. Bliss) pp 63-103. New York: Academic Press.

GREEN, J. (1961). In *Aspects of Zoology: A Biology of Crustacea.* London: H.F. & G. Witherby Ltd.

GRUSCHCZYK, B. & KAMP, G. (1990). The shift from glycogenolysis to glycogen resynthesis after escape swimming : studies on the abdominal muscle of the shrimp, *Crangon crangon. J. Comp. Physiol. B.* **159**, 753-760.

HARRI, M. & FLOREY, E. (1977). The effects of temperature on a neuromuscular system of the crayfish, *Astacus leplodactylus. J. Comp. Physiol.* **117**, 47-61.

HENDERSON, P.A. & HOLMES, R.H.A. (1987). On the population biology of the common shrimp Crangon crangon (L.) (Crustacea: Caridea) in the Severn Estuary and Bristol Channel. *J. Mar. Biol. Ass. U.K.* **67**, 825-847.

HESSLER, R.R. (1983). A defense of the Caridoid facies; wherin the early evolution of the Eumalacostraca is discussed. In *Crustacean Issues I. Crustacean Phylogeny.* (ed. F. R. Schram) pp 145-164. Rotterdam: A.A. Balkema.

HINDE, (1970). *Animal Behaviour* (2nd edition). New York: McGraw-Hill.

HUGHES, D.A. (1968). Factors controlling emergence of the pink shrimp (*Penaeus duorarum*) from the substrate. *Biol. Bull.* **134**, 48-59.

HUMPHRIES, D.A. & DRIVER, P.M. (1970). Protean defence by prey animals. *Oecologia* (Berl.) **5**, 285-302.

JAHROMI, S.S. & ATWOOD, H.L. (1969). Correlation of structure, speed of contraction, and total tension in fast and slow muscle fibres of the lobster (*Homarus americanus*). *J. Exp. Zool.* **117**, 25-38.

KAMP, G. (1989). Glycogenolysis during recovery from muscular work. *Biol. Chem. Hoppe-Seyler.* **370**, 565-573.

KAMP, G. & JURETSCHKE, H.P. (1987). An in vivo ^{31}P-NMR study of the possible regulation of glycogen phosphorylase (a) by phosphagen via phosphate in the abdominal muscle of the shrimp, *Crangon crangon. Biochimica et Biophysica Acta.* **929**, 121-127.

KENNEDY, D. & TAKEDA, K. (1965). Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. *J. Exp. Biol.* **43**, 211-227.

KILS, U. (1982). The swimming behaviour, swimming performance and energy balance of the Antarctic krill, *Euphasia superba. Biomass Sci. Ser.* **3**, 1-121.

KRASNE, F.B. & WOODSMALL, K.S. (1969). Waning of the crayfish escape response as a result of repeated stimulation. *Anim. Behav.* **17**, 416-424.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* (Lond.) **227**, 680-685.

LANG, F., COSTELLO, W.J. & GOVIND, C.K. (1977). Development of the dimorphic claw muscles of the lobster *Homarus americanus* : I. Regional distribution of muscle fibre types in adults. *Biol. Bull.* **152**, 75-83.

LLOYD, A.J. & YONGE, C.M. (1947). The biology of Crangon vulgaris (L.) in the Bristol Channel and Severn Estuary. *J. Mar. Biol. Ass. U.K.* **26**, 626-661.

MACFARLAND, D. (1985). *Animal Behaviour: Psychology, Ethology and Evolution.* London: Pitman.

MAIER, L., RATHMAYER, W. & PETTE, D. (1984). pH lability of myosin ATPase activity permits discrimination of different muscle fibre types in crustanceans. *Histochemistry.* **81**, 75-77.

MCPHAIL, J.D. (1969). Predation and the evolution of a stickleback. *J. Fish. Res. Bd. Can.* **26**, 3183-3208.

MESSENGER, J.B. (1968). The visual attack of the cuttlefish, *Sepia officinalis. Anim. Behav.* **16**, 342-357.

MINELLO, T.J. & ZIMMERMAN, R.J. (1983). Fish predation of juvenile brown shrimp, *Penaeus aztecus* (Ives) : The effect of simulated *Spartina* structure on predation rates. *J. Exp. Mar. Biol. Ecol.* **72**, 211-231.

MOLLER, T.H. & JONES, D.A. (1975). Locomotory rhythms and burrowing habits of *Penaeus semisulcatus* (de Haan) and *P. monodon* (Fabricius) (Crustacea: Penaeidae). *J. Exp. Mar. Biol. Ecol.* 18, 61-77.

MYKLES, D.L. (1985) (a). Heterogeniety of myofibrillar proteins in lobster fast and slow muscles : Variants of troponin, paramyosin, and myosin light chains comprise four distinct protein assemblages. *J. Exp. Zool.* **234**, 23-32.

MYKLES, D.L. (1985) (b). Multiple variants of myofibrillar proteins in single fibres of lobster claw muscles : Evidence for two types of slow fibres in cutter closer muscle. *Biol. Bull.* **169**, 476-483.

NEIL, D.M. & FOWLER, W.S. (1990). Histochemical and biochemical heterogeniety in a crustacean postural muscle. In *Crustacean Pioneer Systems in Neurobiology.* (ed. K. Wiese). Basel: Birkhäuser Verlag.

NEWELL, R.C. & BAYNE, B.L. (1973). A review on temperature and metabolic acclimation in intertidal marine invertebrates. *Neth. J. Sea. Res.* 7, 421-433.

NEWLAND, P.L., CHAPMAN, C.J. & NEIL, D.M. (1988). Swimming performance and endurance of the Norway lobster, *Nephrops norvegicus. Mar. Biol.* **98**, 345-350.

NEWLAND, P.L. & NEIL, D.M. (1990). The tail flip of the Norway lobster, *Nephrops norvegicus.* I. Giant fibre activation in relation to swimming trajectories. *J. Comp. Physiol. A.* **166**, 517-527.

NEWLAND, P.L. & NEIL, D.M. (1990). The tail flip of the Norway lobster, *Nephrops norvegicus.* II. Dynamic righting reactions induced by body tilt. *J. Comp. Physiol. A.* **166**, 529-536.

ONNEN, T. & ZEBE, E. (1983). Energy metabolism in the tail muscles of the shrimp, *Crangon crangon,* during work and subsequent recovery. *Comp. Biochem. Physiol.* **74**(a) (No. 4), 833-838.

PAUL, D.H. (1990). Neural phylogeny - Its use in studying neural circuits. In *Frontiers in Crustacean Neurobiology.* (eds. K. Wiese, W-D. Krenz, J. Tautz, H. Reichert and B. Mulloney) pp 537-546. Basel: Birkhäuser Verlag.

PIHL, L. (1985). Food selection and consumption of mobile epibenthic fauna in shallow marine areas. *Mar. Ecol. Prog. Ser.* **22**, 169-179.

PINN, E. & ANSELL, A.D. (1992). The effect of particle size on the burying ability of the brown shrimp, *Crangon crangon. J. Mar. Biol. Ass. U.K.* **73**, 365-377

RICE, A. & CHAPMAN, C.J. (1971). Observations on the burrows and burrowing behaviour of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides. Mar. Biol., Berlin.* **10**, 332-342.

SCOTTISH OFFICE OF AGRICULTURE AND FISHERIES. (1990). Scottish Sea Fisheries Statistical Tables.

SILVERMAN, H. & CHARLTON, M.P. (1980). A fast oxidative crustacean muscle: Histochemical comparison with other crustacean muscle. *J. Exp. Zool.* **211**, 267-273.

SILVERMAN, H., COSTELLO, W.J. & MYKLES, D.L. (1987). Morphological fibre type correlates of physiological and biochemical proporties in crustacean muscle. *Amer. Zool.* **27**, 1011-1019.

SMALDON, G. (1979). British coastal shrimps and prawns. Keys and notes for the identification of the species. *Synopses of the British Fauna, New Series, Linnean Society.* London, no.15, 102.

SOMERO, G.N. & HOCHACHKA, P.W. (1976). Biochemical adaptations to temperature. In *Adaptation to Environment: Essays on the Physiology of Marine Animals.* (ed R.C. Newell) pp 125-190. London: Butterworths.

STEIN, R.A. (1977). Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. *Ecology.* **58**, 1237-1253.

STEIN, R.A. & MAGNUSON, J. J. (1976). Behavioural response of crayfish to a fish predator. *Ecology.* **57**, 751-761.

TALLMARK, B. & EVANS, S. (1986). Substrate related differences in antipredator behaviour of two gobiid fish species and the brown shrimp, and their adaptive value. *Mar. Ecol. Prog. Ser.* **29**, 217-222.

TIEWS, K. (1970). Synopsis of biological data on the common shrimp, *Crangon crangon* (Linnaeus, 1758). *F.A.O. Fisheries Synopsis No.91. F.A.O. Fisheries Report,* **57**, 1167-1224.

TINBERGEN, N. (1951). *The Study of Instinct.* pp 38-40. Oxford: Clarendon Press.

WARE, D.M. (1973). Risk of epibenthic prey to predation by rainbow trout, (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* **30**, 787-797.

WEBB, P.W. (1979). Mechanics of escape responses in crayfish (*Orconectes virilis*). *J. Exp. Biol.* **79**, 245-263.

WEBER, R.E. & SPAARGAREN, D.H. (1968). On the influence of temperature on the osmoregulation of *Crangon crangon* and its significance under estuarine conditions. *Neth. J. Sea Res.* **5**, 108-120.

WIESER, W. (1973). Temperature Relation of Ectotherms: A Speculative Review. In *Effects of temperature on Ectothermic Organisms.* (Ed. W. Wieser). pp 1-23 Heidelberg: Springer-Verlag.

WINE, J.J. & KRASNE, F.B. (1972). The organisation of escape behaviour in the crayfish. *J. Exp. Biol.* **56**, 1-18.

WINE, J.J. & KRASNE, F.B. (1982). The cellular organisation of crayfish escape behaviour. In *The biology of Crustacea, Vol. 4.* (ed. D.E. Bliss). pp 241-292. New York: Academic Press.

WINE, J.J., KRASNE, F.B. & CHEN, L. (1975). Habituation and inhibition of the crayfish lateral giant fibre escape response. *J. Exp. Biol.* **62**, 771-782.

ZUCKER, R.S. (1972). Crayfish escape behaviour and central synapses. II. Physiological mechanisms underlying behavioural habituation. *J. Neurophysiol.* **35**, 621-637.