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FURTHER STUDIES OF CRAYFISH ESCAPE BEHAVIOUR

I. THE ROLE OF THE APPENDAGES AND THE STEREOTYPED NATURE OF NON-GIANT ESCAPE SWIMMING

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SUMMARY

1. High-speed cinematography of the escape behaviour of freely-moving crayfish showed that the thoracic and abdominal appendages exhibit stereotyped movements in giant axon-mediated tail flips and in non-giant flips. Three distinct classes of non-giant tail flips were recognized in this study: linear, pitching and twisting flips.

2. In medial giant flips and linear non-giant flips the chelipeds and pereiopods were promoted and extended in a manner which minimized the hydrodynamic resistance of the animal. The exopodites of the uropods were promoted. In lateral giant flips and pitching non-giant flips the thoracic appendages moved only passively. The uropod protopodites were promoted but the exopodites remained remoted.

3. When giant axon-mediated tailflips were elicited with natural stimuli they were followed by sequences of non-giant flips which appeared quite stereotyped.

INTRODUCTION

Crayfish can escape from dangerous situations by swimming away rapidly using repeated tailflips. The general form of this behaviour has been described well and much of the neural circuitry responsible for the initiation and generation of tailflips in escape swimming is now known (reviewed by Wine & Krasne, 1982). In these studies, attention has focused mainly on those tailflips mediated by spike activity in the medial and lateral giant interneurones in the nerve cord; the organization and generation of non-giant tailflips (Schrameck, 1970) in escape behaviour are only now being studied more closely (Reichert & Wine, 1982, 1983; Kramer & Krasne, 1984). Furthermore, the role of the appendages in escape behaviour has not been examined. Descriptions of the escape behaviour of crayfish and lobsters have made reference to the occurrence of characteristic

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movements of the appendages of the head, thorax and abdomen in swimming (Voelkel, 1922; Wiersma, 1938, 1947, 1952; Lindberg, 1955; Larimer, Eggleston, Masukawa & Kennedy, 1971; Wine & Krasne, 1972). Although most of these movements have been assumed to reduce the drag on the animal during escape, the only supporting observation is that the resistance to a water current of an animal in a backward swimming posture is significantly less than that of an animal walking forwards (Pond, 1975). However, these data do not allow one to determine the effects of the appendages alone on drag.

In this paper, we have re-examined the escape behaviour of the crayfish to provide a more detailed description of movements of the appendages during swimming and have extended the experiments of Pond (1975) to measure the effectiveness of these movements in minimizing the resistance of the animal in escape. In addition, we report observations on the sequential organization of non-giant tailflips in escape swimming which suggest that there is considerable stereotypy in this apparently complex behaviour.

MATERIALS AND METHODS

Australian freshwater crayfish (*Cherax destructor*), 8–10 cm in length, were used in these experiments. Animals used in behavioural studies were first cooled in crushed ice and fitted with stimulating and recording electrodes (100 μm stainless steel wires, insulated except at the tips). The medial giant (MG) axons in the nerve cord were stimulated with a pair of electrodes inserted through holes in the epistome to span the circumoesophageal connectives. The lateral giant (LG) axons were stimulated with a pair of electrodes inserted into the fifth segment of the abdomen so that the tips lay adjacent to the lateral surface of the nerve cord. A third pair of electrodes was inserted into the first abdominal segment to monitor activity in the nerve cord. The leads of the electrodes were secured to the exoskeleton with epoxy cement. A short length of rubber tubing was also cemented to the dorsal surface of some animals. This provided an anchor point to tether the crayfish if necessary. The animals were warmed gradually and allowed to recover for a day before experimentation.

The behaviour of freely-swimming and tethered crayfish was filmed with a Hycam rotating prism camera (Redlake Laboratories) operated at 200–500 frame s^{-1}. Escape responses were elicited by tapping the animal with a blunt probe or by direct stimulation of the giant axons in the nerve cord *via* the implanted electrodes. Electrical stimuli were single square pulses (0·5 ms duration) and the intensity was adjusted to be just above the threshold for a behavioural response. Signals from the recording electrodes in the abdomen were amplified conventionally, stored on FM tape and displayed on an oscilloscope. Films were analysed with a stop-frame projector (L. W. International). The timing of a mechanical stimulus was taken as the first frame in which the probe was seen to touch the crayfish. The instant of an electrical stimulus was marked on the film with a monitor light.

Following behavioural experiments, the abdomens of twelve crayfish were separated from the thorax and placed ventral side down on paper. The outline of the ventral surface was traced and the area measured with a Zeiss MOP image analyser. Measurements were made with the uropod exopodites placed in both the promoted and remoted positions.

The effect of leg position on the drag of swimming crayfish was measured in a flume. Three equal-sized crayfish (14·0 cm from rostrum to telson, 29·5–30·9 g in weight) were killed by freezing and the antennae, antennules and chelipeds were removed. The pereiopods of one animal were promoted and extended, those of the other two were arranged in typical stance positions. Each crayfish had its abdomen flexed completely, its uropods remoted and was then covered in a smooth, thin layer of polyester resin to make it rigid. A plastic rod (3 mm diameter) was glued to the cephalothorax and the crayfish suspended in a flume and submerged to a depth of 2·0 cm. A semiconductor strain gauge on the rod measured the drag on the crayfish over a range of water current velocities. These values were corrected for the drag on the supporting rod alone.

RESULTS

Giant axon-mediated tailflip behaviour

The general features of tailflips mediated by spikes in the giant axons of *C. destructor* were the same as those described for *Procambarus clarkii* by Wine & Krasne (1972). A sharp tap to the rostrum elicited 2–3 spikes in the MG axons at 4- to 8-ms intervals, followed by a stereotyped tailflip (MG flip) in which all segments of the abdomen were flexed, causing the crayfish to first tilt through 30–45° and then move backwards in a linear trajectory, reaching a velocity of up to 1·0 m s^{-1} (Fig. 1). A sharp tap to the dorsal surface of the abdomen elicited a similar burst of spikes in the LG axons and a stereotyped tailflip (LG flip) in which the anterior segments of the abdomen flexed while the posterior segments remained extended, causing the animal to somersault upwards and forwards (Fig. 2). Direct stimulation of the nerve cord to elicit single spikes in the MG or LG axons produced MG and LG flips identical to those elicited with abrupt tactile stimuli.

Different stereotyped movements of the appendages occurred in MG flips and LG flips. These will be described in turn.

MG flips

The pereiopods and chelipeds were promoted and extended in MG flips, being brought close to the body to produce a streamlined body profile (Figs 1,2,3). All five pairs of limbs were promoted simultaneously. The chelipeds and the anterior three pairs of pereiopods were extended simultaneously but extension of the posterior pair of pereiopods was delayed until they had been promoted forward of perpendicular to the body axis (Figs 1,2,3). These movements occurred during the initial tilting phase of the MG flip. Promotion and extension of the chelipeds

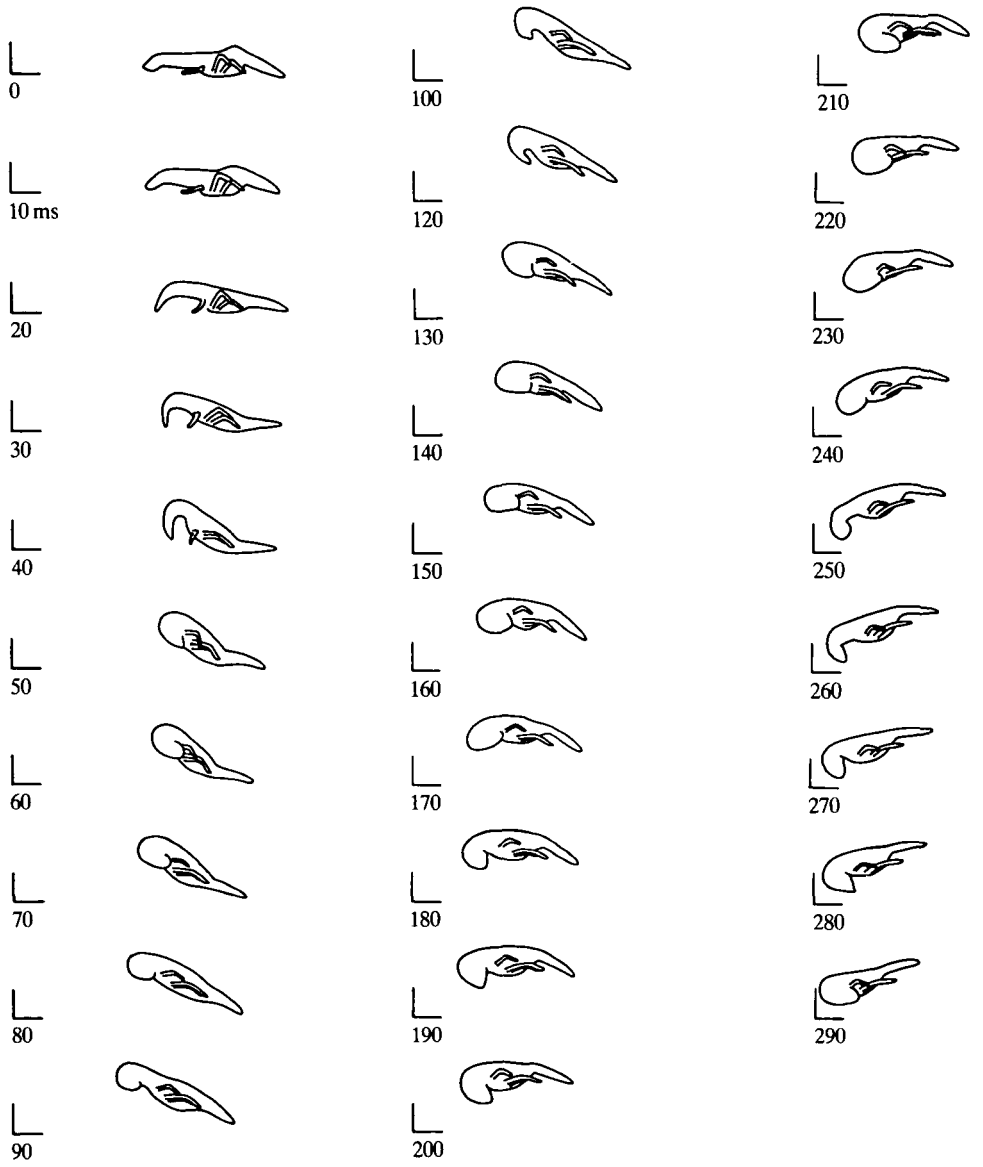

Fig. 1. Tracings from a high speed motion picture of a swimming sequence evoked by a sharp tap to the rostrum. The probe struck the animal in the time between the first and second frames. The axes shown in this and subsequent figures show the position of the bottom and one side wall of the tank. The crayfish executed one MG flip and three linear non-giant flips before it struck the wall of the tank. The MG flip was performed between t = 10 ms and t = 70 ms and the linear flips in the intervals from t = 70 ms to t = 140 ms, t = 140 ms to t = 230 ms and t = 230 ms to t = 290 ms. This animal was missing the third pereiopod on its right hand side.

and the anterior pereiopods were completed within 30–35 ms of the MG axons firing, before the animal began to move backwards rapidly. Extension of the posterior pair of pereiopods continued as the animal moved backwards after tilting.

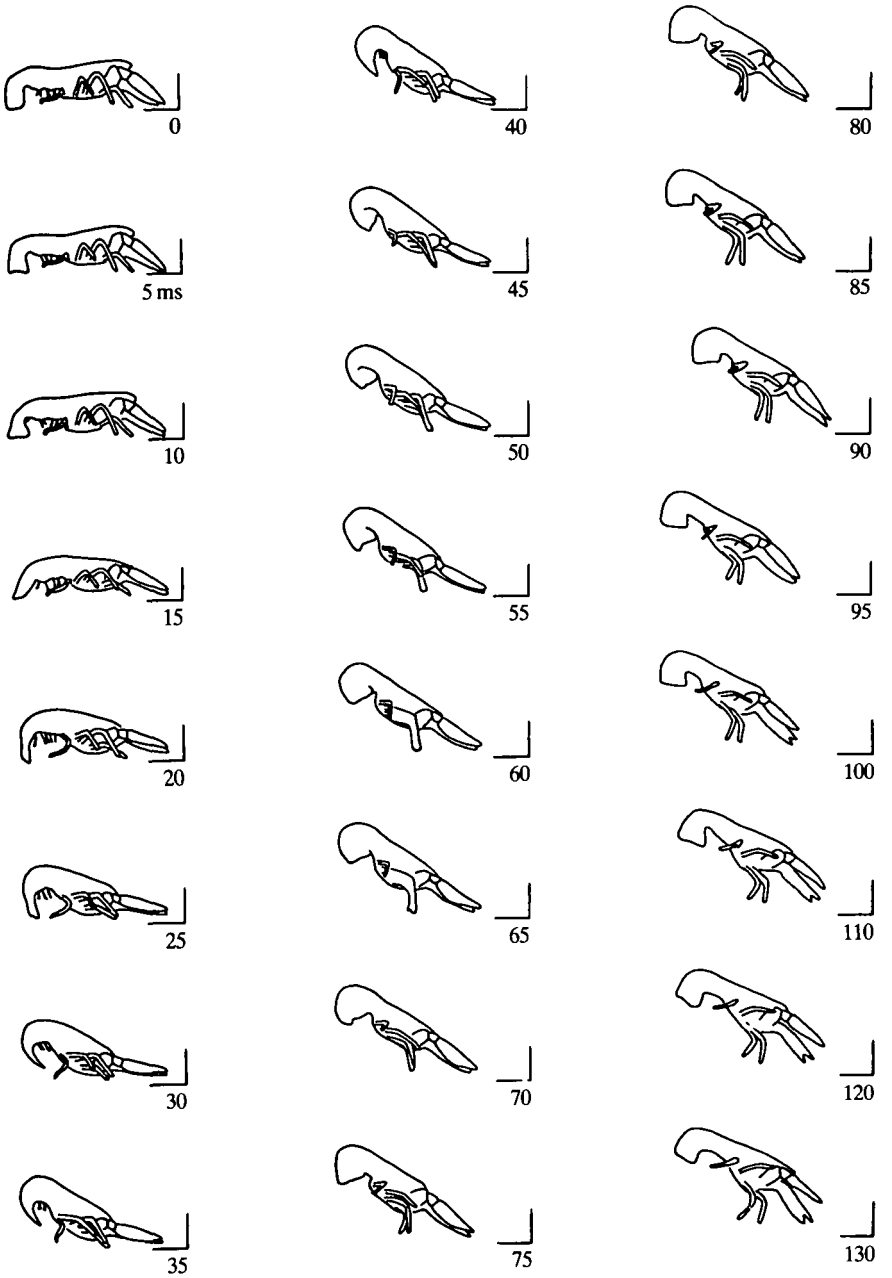

Fig. 2. MG flip evoked by electrical stimulation of the MG axons in the circumoesophageal connectives. Stimulation occurred at the time of the first frame shown. The three anterior pereiopods visible clearly reached the streamlined position well before the posterior pereiopod. Note that the chelae remained elevated throughout the MG flip. The position of the uropods cannot be resolved clearly from this lateral perspective. The MG flip was completed within 60–65 ms.

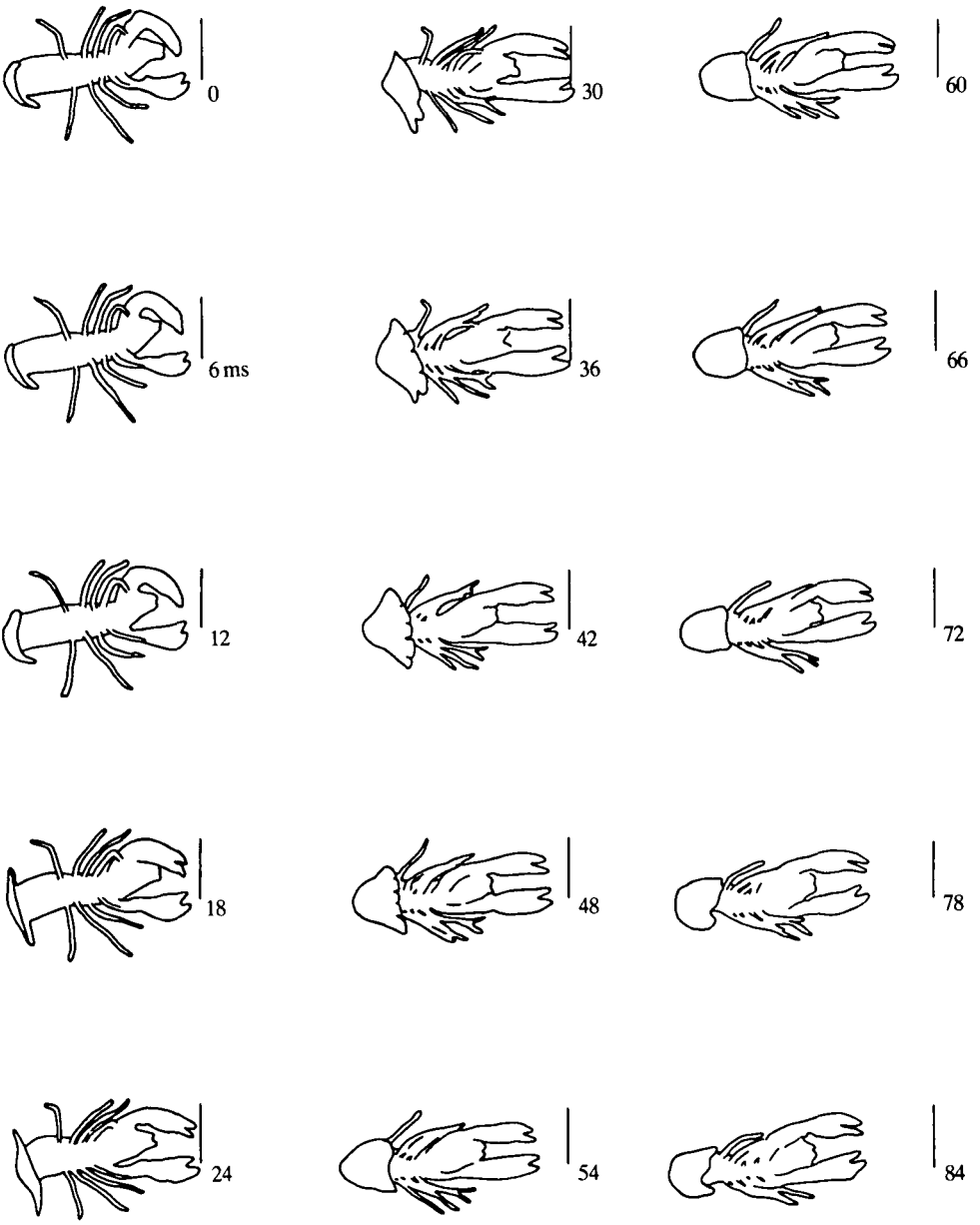

Fig. 3. Ventral view of an MG flip evoked by electrical stimulation of the MG axons in the circumoesophageal connectives. Stimulation occurred at the time of the first frame shown. The position of the left-hand wall of the tank is shown by the line in each frame. The movements of the chelipeds, pereiopods and uropods are clear in this sequence. Note that the left posterior pereiopod was already extended at the start of this sequence. The uropod exopodites were promoted fully 24 ms after the stimulus and they were remoted again about 30 ms later. The exopodites were kept in the remoted position during the subsequent extension of the abdomen.

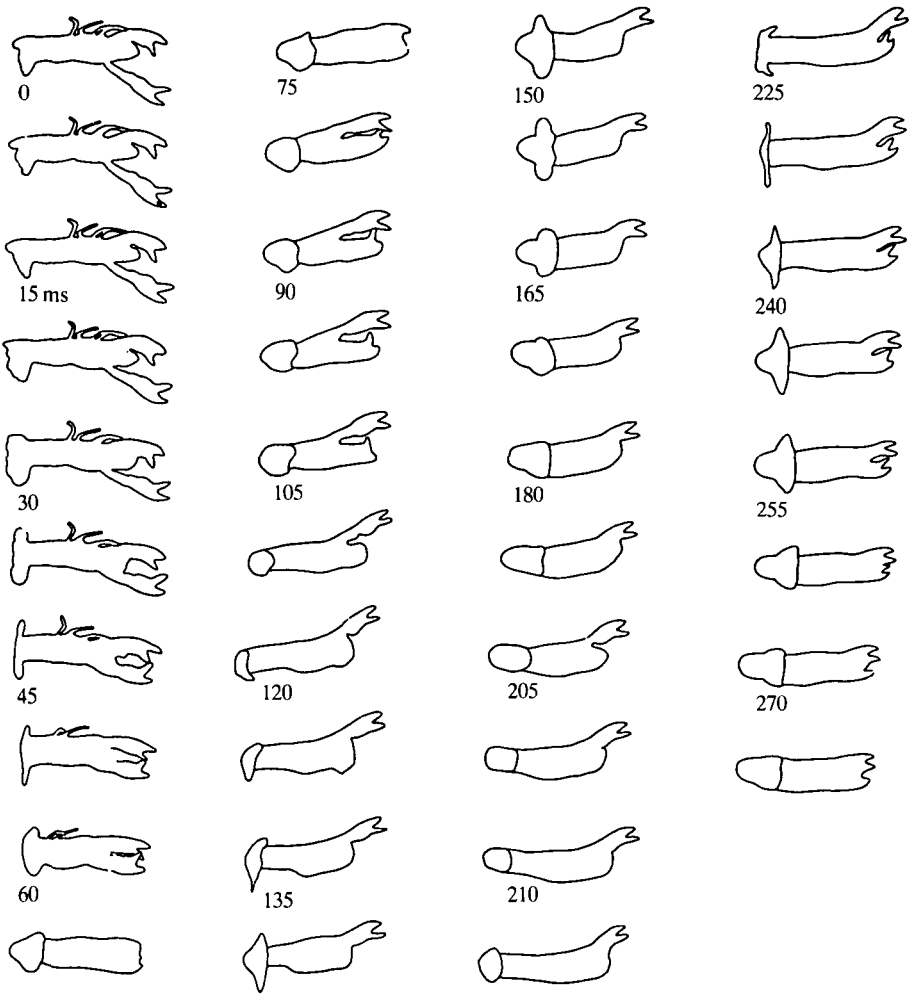

Fig. 4. Ventral view of a swimming sequence elicited from a tethered crayfish by a sharp tap to the rostrum in the time between the first two frames shown. The animal performed one MG flip (from t=0 to t=90 ms) and two linear non-giant flips (t=90 ms to t=180 ms and t=180 ms to t=277 ms). Note that the legs were held in the streamlined position throughout the sequence of non-giant flips. The uropod exopodites were promoted as the abdomen was flexed and remoted as it was extended in this sequence.

The swimmerets appeared to flow together passively as the abdomen was flexed but no active movements were detected.

The exopodites of the uropods were promoted as the abdomen started to flex in an MG flip (Figs 3,4). They remained promoted for most of the powerstroke but were remoted again as flexion was completed (Figs 3,4). Promotion of the exopodites during flexion increased the area of the abdomen acting against the medium in the powerstroke by almost 60 % over the initial remoted orientation (59 ± 8 %, mean $\pm$ S.D., $N=12$). No stereotyped movements of the uropod protopodites were detected.

LG flips

The thoracic limbs and the swimmerets appeared to move only passively in LG flips. Stereotyped, active movements were confined to the uropods. The uropod protopodites were promoted as the abdomen flexed. This motion was difficult to detect from a lateral aspect but was evident from a posterior view (Fig. 6). The exopodites did not move actively in LG flips.

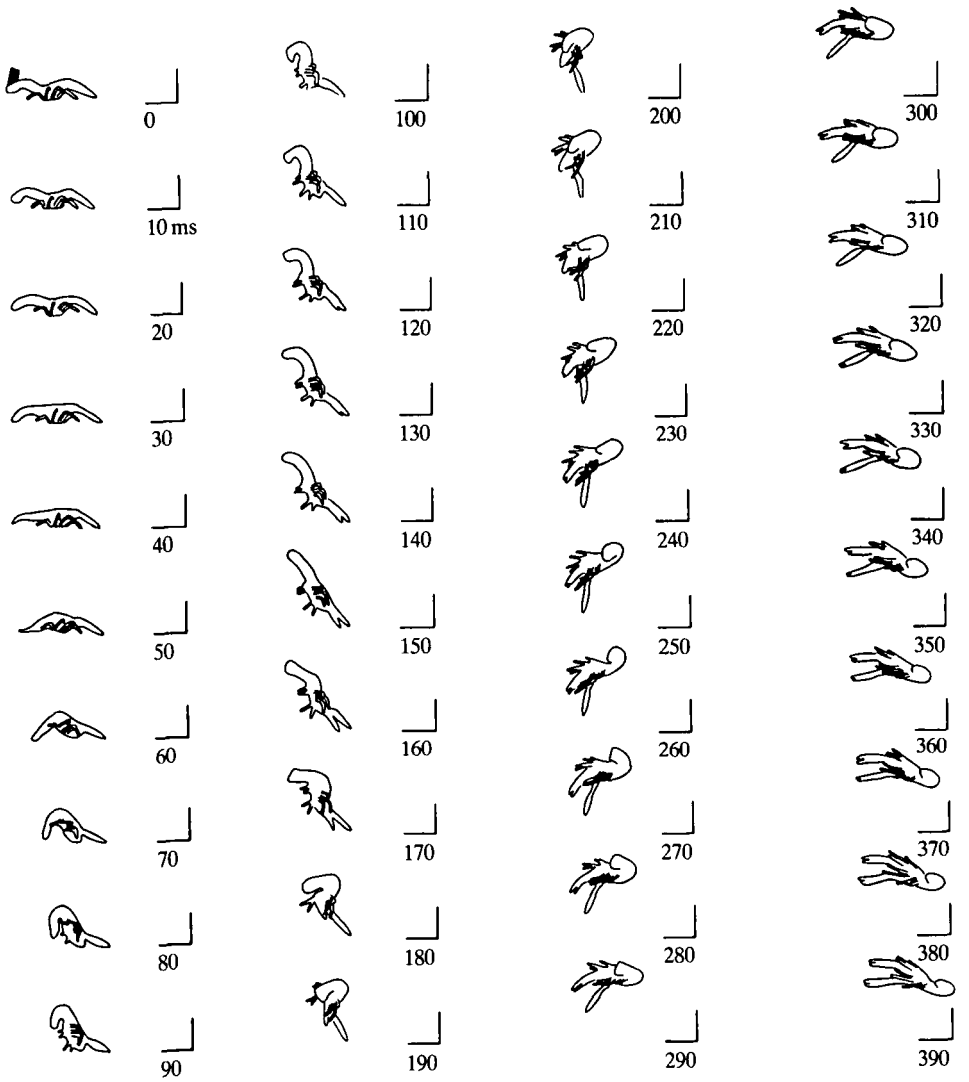

Fig. 5. Swimming sequence evoked by a sharp tap to the abdomen. The position of the stimulus probe is shown in the first frame; contact was made in the time between the first and second frames. The animal performed an LG flip (t=0 to t=80 ms), a pitching flip (t=80 ms to t=210 ms) and a twisting flip (t=210 ms to t=310 ms) in this sequence. Note that the legs did not become streamlined in either the LG flip or the pitching flip.

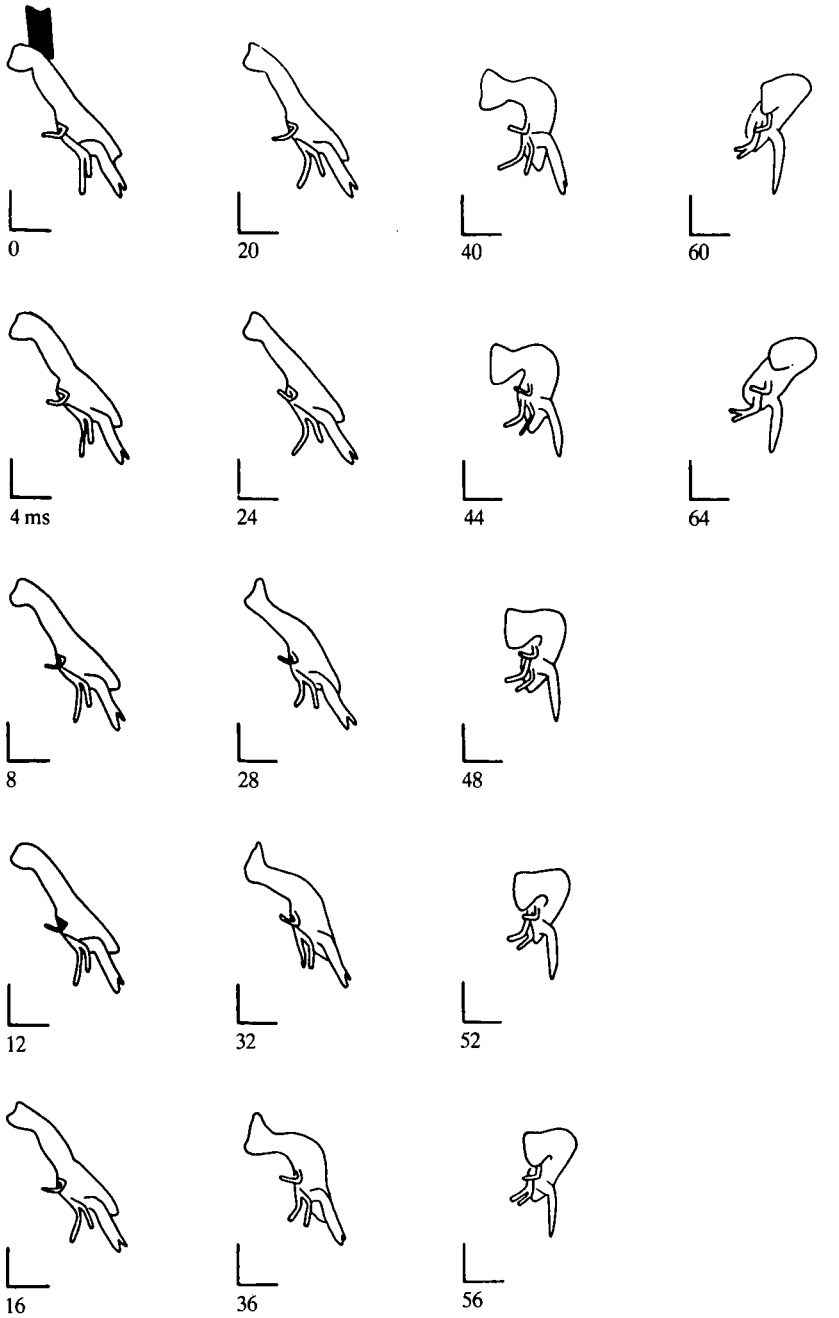

Fig. 6. LG flip elicited with a sharp tap to the abdomen while the crayfish was righting itself after being lifted from the substrate. Stimulus contact was made in the time between the first and second frames shown. The stimulating probe deflected the abdomen toward the camera and the position of the uropods can be seen. Some spreading of the uropods is evident; this is due to promotion of the protopodites.

Non-giant tailflip behaviour

Direct stimulation of the giant axons elicited only single giant axon-mediated tailflips. By contrast, giant axon-mediated tailflips elicited with abrupt taps to the rostrum or abdomen were always followed by sequences of non-giant tailflips. Such sequences were composed of three types of stereotyped tailflips, termed linear, pitching and twisting flips, which were performed in a predictable order in escape.

In an escape sequence initiated with an MG flip, all subsequent non-giant flips had the same general form (Figs 1,4). These were linear flips and propelled the crayfish backwards without pitching or twisting. Each linear flip consisted of abdominal extension followed by flexion. Extension appeared to occur sequentially from the anterior to the posterior segments (Fig. 1). The fifth abdominal segment and the telson both remained flexed throughout the movement. The first linear flip in the sequence was often especially truncated, with extension being restricted to only the anterior two or three segments. All the segments became flexed in the subsequent flexion movement in linear flips, generating a thrust with only a small vertical component so that the trajectory of escape was a straight line or gentle parabola in the vertical plane. Continuous swimming sequences initiated with an MG flip were always straight lines in the horizontal plane.

The exopodites of the uropods were held tightly remoted during abdominal extension in linear flips. They were promoted fully at the start of the subsequent flexion and then remoted again as flexion was completed (Fig. 4). The chelipeds and the pereiopods were held promoted and extended in the position they reached in the initial MG flip.

In escape sequences initiated with LG flips, the first non-giant flip was a pitching flip (Fig. 5). This served to continue the forward and upward somersaulting movement begun by the LG flip. In the pitching flip, the abdomen was extended completely prior to flexion. Flexion was similar to that in LG flips, being restricted to the anterior segments of the abdomen with the posterior segments remaining extended. The exopodites of the uropods were held remoted during the extension and flexion movements. The uropod protopodites could not be resolved clearly in the records obtained. The chelipeds and pereiopods did not exhibit any stereotyped movements in the pitching flip.

An LG flip followed by a pitching flip left the crayfish ventral-side-up above the substrate and facing in the opposite direction. The pitching flip was followed by two or three twisting flips which rotated the animal to an upright position and accelerated it along a horizontal trajectory. In some of these sequences of non-giant flips there did appear to be some steering of the crayfish away from the direction of the initial stimulus. This phenomenon has been described previously (Reichert & Wine, 1983) and we did not examine it in detail. We were not able to resolve the movements of the abdomen and appendages clearly enough to determine the means by which the crayfish twisted on its axis. The twisting flips were followed by a variable number of linear flips.

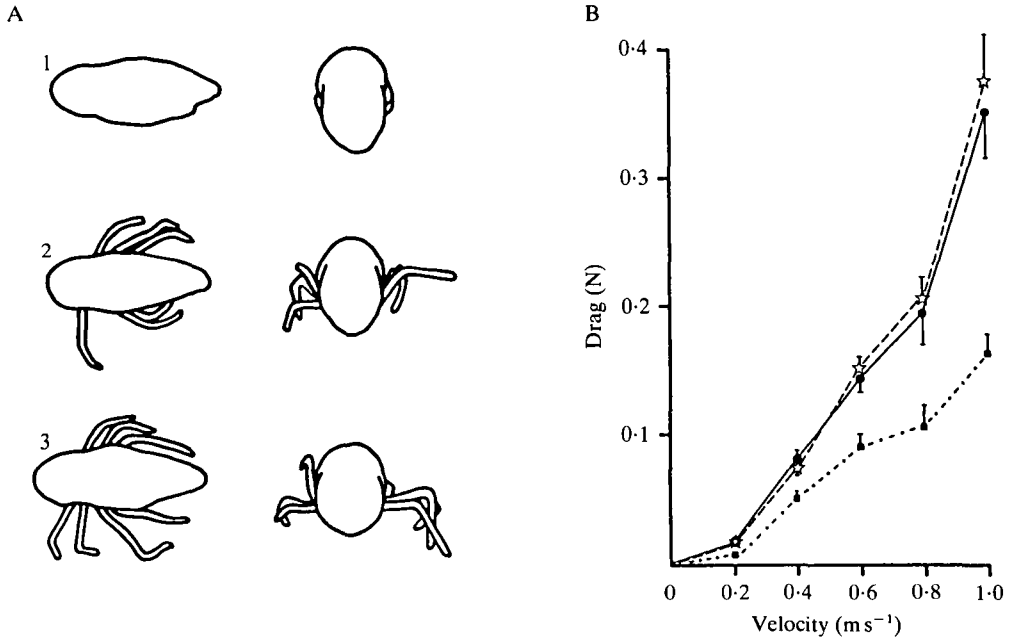

Fig. 7. (A) Tracings of photographs of the three resin-coated crayfish used in drag determinations. Each specimen is shown from dorsal and posterior aspects. (B) Values of hydrodynamic drag measured for each of the three specimens (■1, ●2 and ☆3), plotted against the velocity of the incident water current. Each point is the mean of nine measurements. Bars show standard deviations.

Drag measurements

Fig. 7 shows the relationship between drag and velocity in the medium for the three specimens tested. At typical swimming speeds (0·5–1·0 m s^{-1}) the drag on the animal with its legs held promoted and extended, as in MG flips and linear non-giant flips, was significantly less than that on the animals with legs held in stance positions.

DISCUSSION

The general features of the escape behaviour of *Cherax destructor*, a parastacid astacuran, were essentially as previously found for other crayfish. These have all been astacid species, mainly *Procambarus clarkii* (Wine & Krasne, 1982).

Our results confirm earlier reports that the appendages of crayfish execute stereotyped movements in escape behaviour. The drag measurements show that the thoracic appendages can potentially contribute greatly to the resistance of swimming crayfish. Extension and promotion of the chelipeds and pereiopods in MG flips and linear non-giant flips clearly serve a streamlining function. In MG flips, these movements minimize the hydrodynamic resistance of the crayfish before the flexing abdomen produces a large backward propulsive force, thereby maximizing the acceleration of the animal in escape.

Delayed extension of the fourth pair of pereiopods was a stereotyped feature of MG flips. These legs are used as pushing limbs in walking locomotion and are normally not promoted forward of perpendicular to the body axis (Pond, 1975). The arc through which they must promote to reach a streamlined position in an MG flip is much greater than that for the anterior limbs, which are usually promoted well forward to begin with. This promotion must be carried out against the resistance of the medium. It can be shown that delaying extension with respect to promotion minimizes the torque required to promote the limb (see Appendix). It is likely that the promotor muscles of the fourth pair of pereiopods are not capable of promoting these legs well forward early in an MG flip if extension occurs simultaneously; thus exension is delayed until promotion is well advanced. Simultaneous promotion and extension of the anterior limbs is possible because of the much smaller arc through which they are promoted.

The complete absence of active movements of the legs in LG flips was also noted by Wine & Krasne (1972) for *P. clarkii*. Evidence suggesting a physiological mechanism to promote passive streamlining of these limbs in LG flips is presented in the following paper (Cooke, 1985).

Previous reports (Wiersma, 1947, 1952) have referred to the occurrence of movements of the swimmerets of crayfish when the giant axons fired. In addition, Lindberg (1955) reported that the swimmerets of the spiny lobster, *Panulirus interruptus*, were expanded as the abdomen was flexed in swimming and folded again as it extended. It is possible that the large swimmerets of *P. interruptus* can be used to increase the effectiveness of the flexion powerstroke in swimming. While a twitch remotion of the swimmerets of *C. destructor* can be observed if the abdomen is held extended when the giant axons fire (Cooke, 1985), the flimsy nature of the swimmerets in this species make it unlikely that such movements could contribute significantly to the behaviour.

Promotion of the uropod exopodites during abdominal flexion in MG flips greatly increases the area of the abdomen acting on the medium and should maximize the effectiveness of the flexion movement in accelerating the body in a backward trajectory. Retaining the exopodites in the remoted position in LG flips and pitching non-giant flips should contribute to the rigidity of the posterior end of the abdomen, thus maximizing the tilting moment generated when the abdomen flexes. Remotion of the exopodites as the abdomen is re-extended after flexing should minimize the resistance of the crayfish in the returnstroke phase of swimming.

The uropod protopodites were promoted in LG flips and pitching non-giant flips but not in MG flips or linear non-giant flips. Promotion of the protopodites in *C. destructor* involves the contraction of muscles which also cause extension of the tail fan (Cooke, 1985), an action which will contribute to the effectiveness of the abdominal flexion movement in LG and pitching flips but would hamper it in MG and linear flips.

The observed actions of the uropods in the escape behaviour of *C. destructor* contrast with the report of Larimer *et al.* (1971) that the uropods of *P. clarkii* are promoted in LG flips but not in MG flips. These movements are not clear in the lateral views of the abdomen in their published data. They are also inconsistent

Table 1. *Stereotypy in the type and order of non-giant tailflips*

Tap to head ➡ 1 MG flip ➡ sequence of linear flips.
Tap to telson or abdomen ➡ 1 LG flip ➡ 1 pitching flip ➡ 2 or 3 twisting flips ➡ sequence of linear flips.

with the earlier observation that the productor exopodite muscle (a uropod exopodite remoter muscle) of *P. clarkii* is excited when the LG axons fire (Larimer & Kennedy, 1969). It may be that *P. clarkii* shows the same uropod movements in escape behaviour as *C. destructor*.

Our observations suggest that there is considerable stereotypy in the type and order of non-giant tailflips in swimming sequences which follow MG and LG flips in escape. These results are summarized in Table 1. The tailflips we have termed linear and pitching flips are probably the same behavioural units as the rostral and caudal tailflips identified by Kramer & Krasne (1984) in their experiments with semi-intact crayfish preparations. Recent evidence suggests that the neural circuitry responsible for generating non-giant flips in these sequences includes a central pattern generator and is activated by the initial stimulus in parallel with the giant axon circuitry (Reichert & Wine, 1982; Kramer & Krasne, 1984). The extent to which these complex sequences of behaviour are centrally pre-programmed and their susceptibility to modification by environmental stimuli remain subjects for future research.

APPENDIX

The effect of mero-carpal joint angles on the torque required for promotion of the fourth pereiopod

Fig. 8 shows a simplified diagram of a pereiopod of length 2L and width W, free to rotate about the y-axis at the proximal end of the coxa and able to flex and

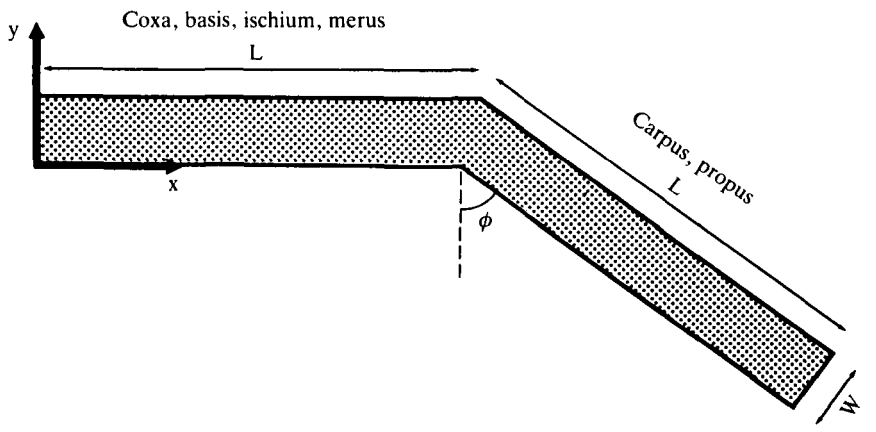

Fig. 8. Schematic representation of a pereiopod showing the parameters used in the calculations in the Appendix.

extend at the mero-carpal (m-c) joint. Assume that movement about the m-c joint is limited so that the leg is fully flexed when the m-c angle $\phi = 0°$ and fully extended when $\phi = 90°$.

Assume $L >> W$. Then the torque, τ, which must be exerted to overcome the resistance of the medium to rotation of the leg about the y-axis at a constant angular velocity ω is given by:

$$\tau = Q\left(L^2 \cos \phi + \int_0^{L + L \sin \phi} x dx\right),$$

where Q is a function of ω, of the surface area of the leg and of the viscosity of the medium. Q is constant for constant ω in this treatment.

When the leg is held in a fully flexed position, $\phi = 0°$ and

$$\tau = Q\left(L^2 + \frac{x^2}{2}\Big|_0^L\right)$$

$$= \frac{3}{2} QL^2.$$

When the leg is fully extended, $\phi = 90°$ and

$$\tau = Q\frac{x^2}{2}\Big|_0^{2L}$$

$$= 2QL^2.$$

In this case then, holding the leg in a fully flexed position reduces the resistance to promotion by 25 %, compared with the fully extended orientation.

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