AN ANALYSIS OF WAVING BEHAVIOUR: AN ALTERNATIVE MOTOR PROGRAMME FOR THE THORACIC APPENDAGES OF DECAPOD CRUSTACEA

By V. M. PASZTOR

Department of Biology, McGill University, Montreal, Quebec, Canada

AND F. CLARAC

Laboratoire de Neurobiologie Comparée, C.N.R.S. Arcachon, France

SUMMARY

1. Decapod crustacea display a slow metachronal rhythm of the third maxillipeds and pereiopod pairs one to four when undisturbed in the natural habitat. The dactyl tips are lifted off the substrate, and the unweighted limbs promote and remote at a slow frequency (range for all species 9–30 min^{-1}). Movement is limited to the T-C joint.

2. This behaviour, called waving, has been observed in many macrurans and an analogous activity, limited to the third maxillipeds, has been seen in several brachyurans.

3. Analysis of EMGs shows: (a) the promotor activity increases in strength during a burst due to facilitation and motoneurone recruitment; (b) the rhythm period is stable throughout long waving sessions; (c) the promotor and remotor strokes are of equal duration and co-vary with period.

4. Ipsilateral coupling is strong, with equal phases between adjacent limbs of 0·05. The metachronal wave can pass anteriorly or posteriorly along an ipsilateral row.

5. Bilateral coupling is weak. The two sides maintain equal frequency, and antiphasic coordination between the two ipsilateral sets of limbs is favoured.

6. Possible functions for waving include gill grooming and supplementary gill ventilation.

7. Comparisons between waving and other rhythmical motor programmes are discussed. Waving is an alternative programme for the walking legs, and is expressed when proprioceptive feedback is reduced.

INTRODUCTION

The repetitive and stereotyped nature of most arthropod behaviour has encouraged neurobiologists to use these animals extensively in studies on the neurological control of muscular activity and behaviour. Analyses have been made of the rhythmical activities of various parts of the insect and crustacean body, covering most aspects of their behaviour. Much interest has been focussed recently on walking, especially since the success achieved by Pearson and his group in identifying at the cellular level

important components of the neuronal system responsible for pattern generation in walking insects (Pearson, Fourtner & Wong, 1973). Detailed descriptions of crustacean limb coordination are reviewed by Evoy & Ayers (1982). The impression which has emerged from these studies is that crustacean walking is not nearly so rigidly patterned as had earlier been supposed. The system seems to be highly variable and as responsive to environmental contingencies as are the locomotory systems of higher vertebrates. The role of proprioceptive feedback (reviewed by Clarac, 1977, 1981a) is complex and subtle in its interaction with the centrally generated pattern.

This paper describes another, slower and more stereotyped rhythmical activity of the walking legs and third maxillipeds in decapod crustaceans. The animal positions its body so that most of its legs are off the substrate and then oscillates the unweighted limbs. This behaviour, which we have called waving, is readily observed under natural conditions. No description of waving appears in the literature, probably because of its ephemeral and unpredictable occurrence.

This study was undertaken not only to provide a detailed account of waving and to rectify an omission in the literature of crustacean behaviour, but also because it provided an opportunity to compare the activity of a population of motoneurones during two very different types of rhythmical behaviour: walking, in which proprioceptive feedback is of prime importance, and waving, where the flow of feedback is reduced. It is hoped that this may contribute to an understanding of the underlying central mechanisms which generate these patterned activities.

MATERIALS AND METHODS

Behavioural observations were made on 15 species of decapods listed in Table 1. Most animals were collected by divers from the Mediterranean, but some were obtained by commercial suppliers from other waters. These qualitative studies were mostly made on animals kept in large display tanks in a public marine aquarium, which provided a good replica of the natural habitat, with a rapid flow-through of well-oxygenated water directly from the sea. Some individuals had lived in the aquarium for many months.

The *Scyllarus latus, Palinurus elephus* (formerly *P. vulgaris*), *Panulirus argus* and *Jasus lalandii* used for quantitative studies were kept in large aquaria, and were adapted to laboratory conditions for at least a month before use. Only animals feeding regularly on mussels were selected. The aquaria were filled with aerated running sea water, and contained a plentiful supply of sand, pebbles, rocks, clay pots etc. to provide animals with individual shelters. Every effort was made to provide as natural a habitat as possible, even in the smaller experimental tanks used for recording sessions.

Electromyograms (EMGs) were recorded from bipolar electrodes implanted chronically using the method described by Ayers & Davis (1977). Long lengths of 50 μm formvar-coated copper wires or 125 μm Teflon-coated silver wires gave sufficient flexibility for complete freedom of movement by the unrestrained animals. The occurrence of waving is completely unpredictable, and will only occur in unstressed animals. Furthermore, when it does start, it may involve any or all of 10 anterior limbs (the pair of third maxillipeds plus pereiopod pairs one to four).

Consequently, in order to minimise trauma to the animal, while maximising the chances of recording a useful sequence of interlimb coordination, only one electrode pair was implanted into each limb. The more accessible promotor muscles were chosen, and the bared electrode tips were inserted via the apodeme at the antero-dorsal coxal rim.

The success rate was not high as the combination of well placed electrodes with actively participating limbs did not always occur. Animals were observed during periods of up to 48 h after implantation and some never displayed waving behaviour during this time. The best data came from *Scyllarus latus* 18–36 h after implantation. The EMG sequences were stored on tape and subsequently filmed, digitized and analysed with a Minc Digital System.

<div align="center">RESULTS</div>

<div align="center">*Behavioural observations on a variety of decapod species*</div>

Macrurans

Waving behaviour is easily distinguishable from all other activities of the appendages because it has a very slow rhythm and is displayed only by unweighted limbs. Typically, the animal adopts a high stance, with the thorax lifted up off the substrate and the abdomen moderately flexed, so that much of the body weight is supported on what is anatomically the dorsal surface of the curled telson. If space permits, the legs are disposed regularly around the thorax in the resting mode with the meropodites horizontal. Then the weight is shifted onto a minimal number of legs, frequently the fifth pereiopods, and the unweighted legs and the third maxillipeds begin promoting and remoting in a slow, regular waving motion ($10-30\,\mathrm{min}^{-1}$). Where the animal is not on a horizontal substrate, it will cling to the rocks with one group of legs, either the posterior ones or all the legs on one side, and wave the free legs. In such cases the body's orientation is determined by the configuration of the rocks and the ventral thorax is tightly pressed against the substrate. In those limbs participating in waving, movement is only seen at the thoracico-coxal (T-C) joint, and all other articulations are held loosely flexed in the resting posture so that the limbs wave to and fro without configurational change. This behaviour has a metachronal rhythm, and typically a wave of promotion passes forward along one row of legs, then both third maxillipeds sweep to the other side and a wave of remotion continues back along the other row of legs.

Waving has been observed in several large decapods (see Table 1) and the parameters of the activity are sufficiently similar in all cases to suggest that this is a widespread phenomenon. Small species-specific differences can usually be ascribed to anatomical features. For example, *Homarus* was never seen to wave the chelipeds, they stayed immobile on the substrate during waving. *Scyllarus* sometimes propped the broad paddle-like antennae up onto a rock during waving in order to support the considerable weight of the cephalo-thorax. Within one species, body size of individuals was not correlated with waving frequency, but between species, the faster rhythms were displayed by those species with the smaller body sizes (e.g. *Scyllarus arctus* and *Astacus leptodactylus*).

Table 1. *Occurrence of waving in decapod crustaceans*

Species	No. of animals available		Occurrence (− to +++)	Beat frequency cycles/min (range)
MACRURANS				
Palinurus elephans Fabricus	a)	11	+++	14 (9 – 18)
	b)	8	+++	15 (11 – 22)
Panulirus argus Gruvel	a)	8	+++	14 (12 – 15)
Palinurus mauritanicus Latreille	a)	1	+++	15 (11 – 22)
	b)	2	+++	15 (12 – 22)
Jasus lalandii Milne-Edwards	a)	7	++	14 (13 – 14)
Scyllarus latus Latreille	a)	3	++	11 (9 – 14)
	b)	2	++	7 (6 – 9)
Scyllarus arctus Fabricus	a)	1	+	31 (28 – 34)
	b)	6	−	−
Homarus vulgaris Milne-Edwards	a)	2	++	12 (9 – 14)
	b)	1	++	12 (9 – 15)
Astacus leptodactylus Eschscholz	a)	5	+	33 (30 – 36)
Nephrops norvegicus Bell	b)	6	−	−
BRACHYURA				
Maia squinado Latreille	b)	4	++	23 (20 – 26)
Cancer magister Dana	a)	48	++	47 (44 – 56)
Carcinus maenas Peach	a)	26	++	71 (60 – 88)
OTHERS				
Palaemon sp.	b)	8	−	−
Pagurus sp.	b)	30	−	−

a) Laboratory–held animals.
b) Animals in natural habitat or equivalent.

In species which select a hole or crevice as a shelter, waving usually takes place just outside the entrance to the shelter. *Homarus, Scyllarus* and the rock lobsters were all seen to sweep clean an area on the threshold so that, when waving began, the oscillating appendages were not impeded with debris and the dactyl tips could glide freely a few millimeters above the substrate.

Observations were made randomly throughout the day and night but no correlation could be ascertained between the occurrence of waving and a circadian rhythm. The data from the 12 rock lobsters living in the large display aquaria showed only rare periods when no animal was waving. The laboratory-held animals waved less and spent more time crouched low in a corner with the legs tucked under the body or gripping a rock. Adequate space and the availability of individual shelters were necessary pre-requisites for waving. Even then, it sometimes took weeks for an animal to become sufficiently habituated to its tank to show waving. In undisturbed animals, waving behaviour could be of long duration and observed sessions ranged from 10 min to 8 h. Rock lobsters have been observed waving in their natural marine habitat by divers (W. Herrnkind, personal communication).

Participation of the various limbs in waving was variable. The fifth pereiopods never took part and frequently formed a tripod with the telson to support the body, but other legs could also be weight-bearing and stay immobile during a waving

Table 2. *Observations of waving on one individual* Palinurus mauritanicus

	Beat frequency cycles/min	R5	R4	R3	R2	R1	Appendages active RMX	LMX	L1	L2	L3	L4	L5
Bout A	15	−	−	−	+	+	+	−	−	−	−	−	−
Duration: 30 min	14	−	−	−	+	+	+	+	+	+	−	−	−
Observations:	13	−	+	+	+	+	+	+	+	−	−	−	−
1 per 3 min	14	−	22*	+	+	+	+	−	−	−	−	−	−
	15	−	−	−	−	−	−	+	+	−	−	−	−
	16	−	+	+	+	+	+	−	−	−	−	−	−
	15	−	−	−	−	−	−	+	−	+	+	−	−
	18	−	+	+	−	−	−	−	−	−	+	+	−
	17	−	21*	21*	−	+	−	−	−	−	−	−	−
	17	−	−	−	−	−	−	−	+	+	+	−	−
	14	−	−	−	+	+	+	−	−	−	−	−	−
Bout B	14	−	−	−	+	−	+	+	+	+	+	−	−
Duration: 8 h	15	−	−	−	+	−	+	+	+	+	+	−	−
Observations:	14	−	−	+	+	+	+	+	−	−	−	−	−
1 per h	14	−	−	−	−	−	−	−	+	+	−	−	−
	15	−	−	−	+	+	−	−	+	+	−	−	−
	14	−	−	−	+	+	−	−	+	+	+	−	−
	12	−	−	−	15*	+	−	−	+	+	−	−	−
	13	−	−	−	+	+	+	+	+	−	−	−	−
Participation of each appendage in waving. % activity during 29 observations.		0	21	33	66	69	54	39	48	39	33	9	0%

* Occurrence of a faster rhythm in posterior limbs.

session. Table 2 summarizes the patterns of active appendages during two prolonged bouts of waving in *P. mauritanicus*. These are samples of 29 observations made on one individual over a two–month period, and all bouts are characterized by frequent changes in the pattern of actively waving and inactive limbs. In rock lobsters and scyllarids, the whole spectrum of possibilities was observed, from one waving limb to ten (two third maxillipeds plus pereiopod pairs one to four). In the *P. mauritanicus* that provided the data for Table 2, the right side appendages were used more frequently than those on the left, and the maxillipeds and the first two pereiopod pairs were favoured (see the percentages of activity at the bottom of the table).

Usually all participating appendages maintained the same frequency of waving and a metachronal rhythm of burst onsets linked all the legs of one side. Even if a mid-row leg was immobile, the metachronal wave passed over that segment without hindrance. Occasionally, however, spontaneous perturbations of the metachronicity appeared and some legs would drift out of coordination leading to leg collisions. In some sessions of waving it was noted that the posterior limbs had a higher average frequency than the anterior ones (see Table 2); this was especially noticeable if the two groups were separated by an immobile leg.

In animals with autotomized limbs, the stumps participated in waving as would a normal unweighted leg. But participation of stumps was as unpredictable as it was for intact legs, and stump activity was obviously not obligatory in a waving animal.

Crustacea spend much time grooming their appendages, especially their ante‑nules, and it was frequently observed that the maxillipeds could switch from waving to the faster, bilateral cycles of antennule brushing while the legs continued waving uninterruptedly. One rock lobster, with its weight resting on the first pereiopods and the telson, was seen to be brushing the antennules with the maxillipeds, waving the second and third pereiopod pairs, using the fourth pair to groom the telson and flexing the swimmerets rhythmically, involving four concurrent, yet different, rhythms. However, two rhythmical activities which were never seen concurrently were waving and walking.

Brachyurans

Slow waving involving both legs and maxillipeds, characteristic of macrurans, was not seen in crabs. However, one of the types of maxilliped behaviour is so similar that it warrants inclusion in the same category. During feeding and grooming the maxillipeds adduct and abduct together so that they function as a pair of pincers for grasping or thrusting. At other times the two third maxillipeds sweep from side to side as a widely spaced pair, just as the maxillipeds do in macruran waving. The waving rhythms have a higher frequency than in macrurans (see Table 1) but for each crab studied the frequency is slower than during feeding or grooming behaviour. Bouts of brachyuran waving tend to be short (20 s–5 min) but are frequently repeated during an active period. The legs were never seen to participate in waving, with the exception of one isolated observation in *Maia squinado*. These animals, like the rock lobsters, commonly support all the body weight on just two pairs of legs, and on one occasion the unweighted first pereiopods began promoting and remoting with the same rhythm as the ongoing maxilliped waving.

In macrurans, walking and waving are mutually exclusive behaviours. This is not so in brachyurans. In most observations the animal was squatting in its normal resting posture during bouts of waving but, on occasion, waving seemed to initiate a period of locomotion and continued during the first few walking cycles.

Other decapods

A few species of Caridae and Anomura were available for observation but no activities similar to waving were seen. In all cases, bilaterally symmetrical types of maxilliped behaviour dominated.

Myographical analysis in Scyllarus and Palinurus

Large samples of data could only be collected from two species, *Palinurus elephas* and *Scyllarus latus*, but myograms from all the species tested emphasised the essential conformity of the waving behaviour.

Description of the muscular activity

Figs 1A and 2A show typical patterns of promotor activity during waving in *Scyllarus* and *Palinurus*. Although the burst frequency is considerably faster in the latter species, the two sequences of myograms display common characteristics. The cycle lengths are long (6 s in *Scyllarus*, 3 s in *Palinurus*) and regular, and the bursts
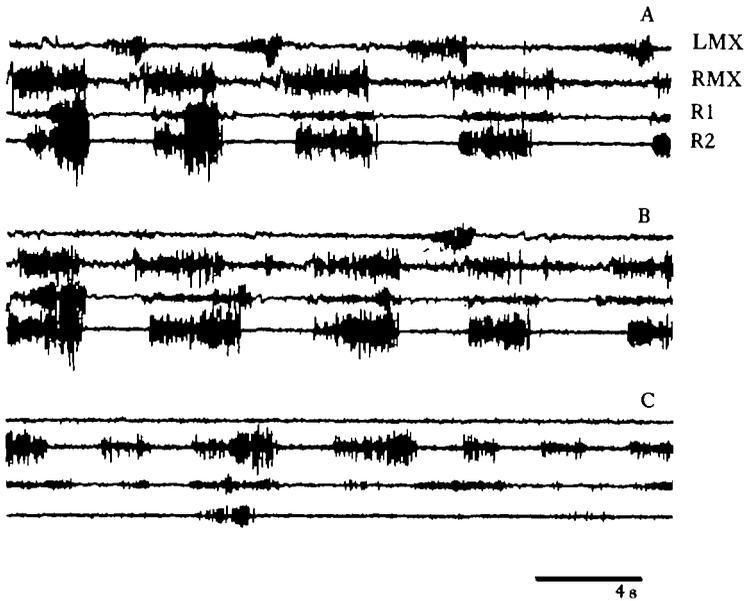
Fig. 1. Normal waving in *Scyllarus latus*. EMGs recorded from promotor muscles of the left and right third maxillipeds (LMX, RMX) and the first and second right pereiopods (R1, R2) during waving. The three samples are taken from a long bout of continuous waving during which the number of active limbs changed frequently. (A) Right and left side appendages all waving (only LMX shown of left group) with the two ipsilateral groups in antiphase. (B) Waving maintained in right group only. (C) Regular pattern restricted to RMX; vestiges of low amplitude bursting still evident in R1 and R2. Calibration: 4 s.
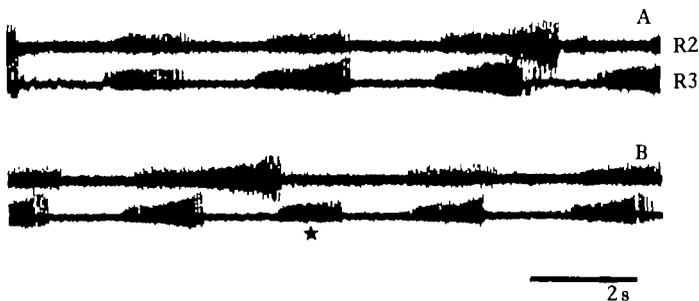
Fig. 2. Waving in *Palinurus elephas*. Burst activity in the promotor muscles of the second and third pereiopods (R2, R3) showing recruitment and facilitation towards the end of most bursts. (A) Regular waving. (B) A momentary perturbation in coordination, R2 performs an 'extra cycle' (black star). Calibration: 2 s.

themselves are also long (3 s and 1·25 s respectively) and occupy approximately half of the total cycle. Promotor bursts of homolateral appendages are almost concurrent with only short interappendage delays (0·1–0·8 s) and the bursts are of similar duration. The top two lines in Fig. 1A show the promotors of the bilateral maxillipeds in antiphase, their most common mode during waving, as they sweep in unison from side to side. Thus the promotor of one is active concurrently with the remotor of the other.

The sequence in Fig. 1A was recorded from an animal in which both rows of legs were waving, and the strict antiphasing of the right and left sides was pronounced for

many cycles. After a while, the appendages of the left side spontaneously stopped waving, leaving those on the right side cycling as before (Fig. 1B), and later, regular bursting persisted solely in the right maxilliped (RMX). It should be noted that when non-cycling appendages displayed spasmodically isolated bursts, they occurred always in appropriate phases with respect to the ongoing rhythm.

In both species it can be seen that the potentials tend to increase in amplitude towards the end of the burst. In *Palinurus* (Fig. 2) this is clearly due to recruitment of a second, higher frequency unit with facilitating synapses. Such bursts produce a forceful promotion which carries the limb through its full range. The smaller, single unit bursts were seen to produce a lesser contraction which only promoted the limb to its resting, mid-point position. No consistent pattern of large and small bursts could be discerned, but adjacent legs tended to co-vary, except for occasions as at the asterisk in Fig. 2B when one leg gave a small extra cycle.

Extra cycles and other perturbations of the pattern are commonly observed, especially in the myograms of the maxillipeds as can be seen in Fig. 3. In sample 3A, the bursting of LMX, R1 and R2 maintained normal phasing (similar to Fig. 1A), whereas RMX bursting became aberrant. The second RMX burst shown was unusually early, the antiphase relationship to LMX was lost and the two maxillipeds collided. Subsequently RMX displayed two cycles in which burst length and cycle length were reduced by 50 % before the rhythm came back to normal. Thus RMX underwent an extra cycle (indicated with a black star) during this adjustment. Fig. 3B shows a sequence where LMX has an increasingly faster rhythm than the right side. At first RMX waved in time with its ipsilateral legs (see dashed enclosures) while, by giving extra bursts (white arrow heads), it also waved in antiphase with its contralateral LMX. Finally an extra cycle in LMX (black arrow) corrected the
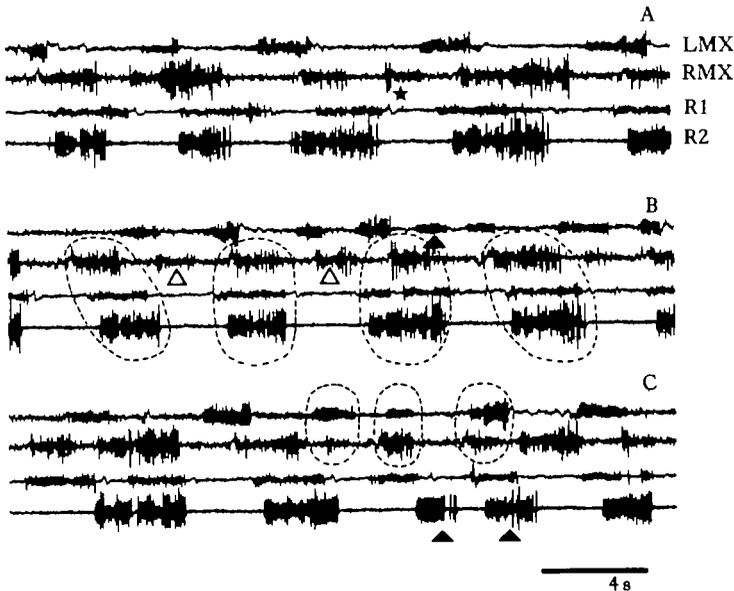

Fig. 3. (A–C) Examples of rhythm perturbation in *Scyllarus* (explanations in text). Calibration: 4 s.

rhythm which subsequently settled into a normal pattern. In Fig. 3C, the perturbation was due to the interpolation of a sequence of a different maxilliped behaviour: antennule cleaning. The three short cycles of bilateral maxilliped promotion, when the maxillipeds adducted concurrently (dashed enclosures) are typical of the grasping, cleaning movements as the antennules are drawn through the setous maxillipeds. This temporary pattern was accompanied by a perturbation of leg waving. R1 promoted 1·5 s early, concurrently with RMX, so that when it remoted it collided with the forward moving R2. This caused an attenuated promotor burst in R2, a shortened interburst interval and a second brief R2 burst during the subsequent promotion of R1 (see black arrowheads). Thus the system, by interpolating brief bursts and shorter cycle periods, is capable of cycle to cycle adjustments and can compensate for transitory disturbances in coordination.

Quantification of the waving pattern

Visual observations of a waving animal and inspection of the myograms suggested that, though complex sequences of wave interaction and adjustment were possible, waving is basically a stable, predictable pattern. The analysis presented in Fig. 4 confirms this supposition. Period lengths were measured in three appendages during a 13·5 min sample of uninterrupted waving in *Scyllarus*. As noted earlier, the maxillipeds have the most irregular rhythm, and the RMX histogram shows a wider dispersion around its mean (5·12s; s.d.±1·60) than does either R1 (5·34s; s.d.±0·97) or R2 (5·48s; s.d.±1·06). RMX has many more short periods between
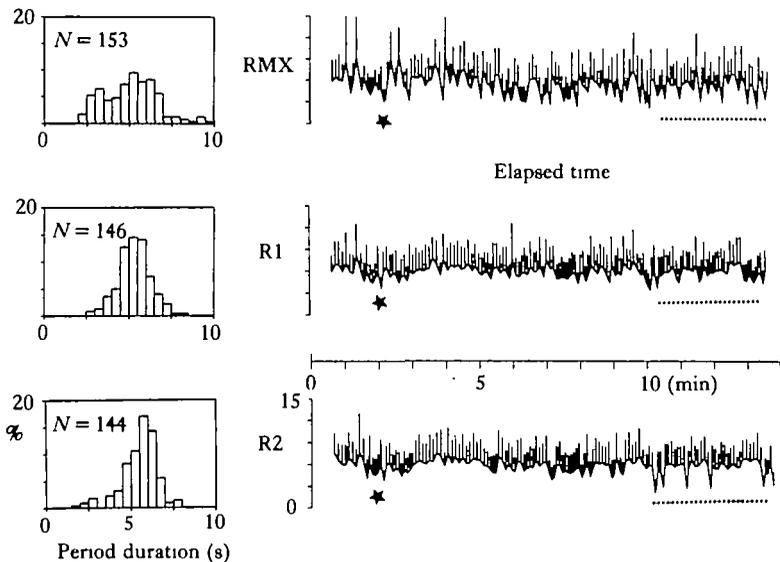

Fig. 4. Analysis of waving period in three ipsilateral appendages in *Scyllarus* (RMX, R1, R2). Left. Histograms showing the percentage distribution of period durations for each appendage. Abscissae in seconds. Means: RMX 5·12; s.d. ± 1·60; R1 5·34; s.d. ± 0·97; R2 5·48; s.d. ± 1·06. Right. Curves for the three appendages showing the evolution of the waving period versus the real time scale (in min). Vertical bars represent the promotor burst durations. Ordinates in seconds. The sequences indicated by stars and dotted lines emphasize the co-varying patterns displayed by adjacent appendages.
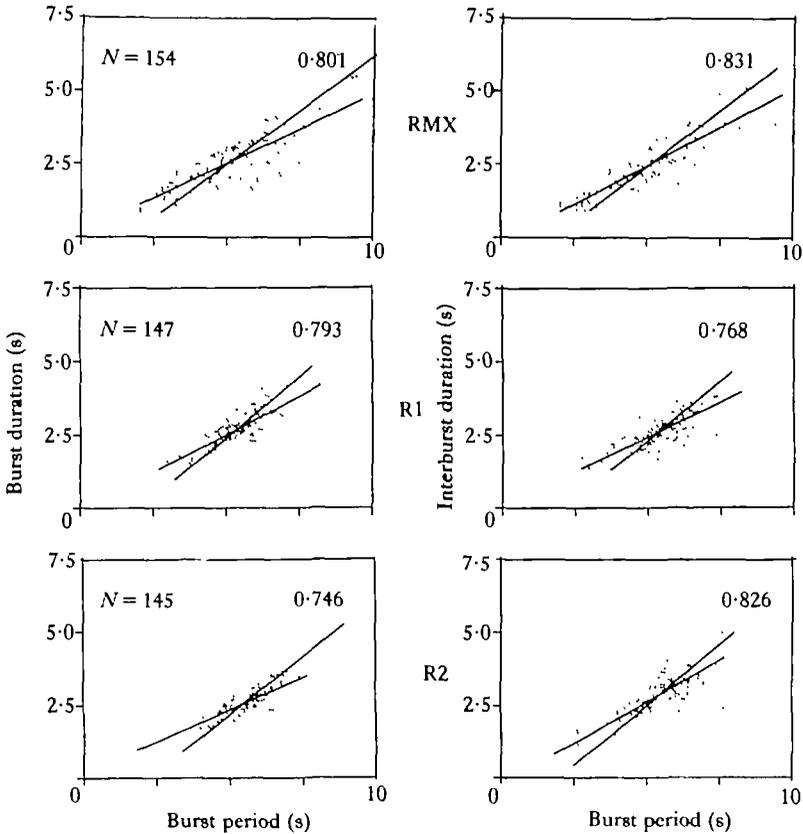
Fig. 5. Correlation of the two strokes of the waving cycle with period (*Scyllarus*). Left. Promotor burst duration plotted against promotor burst period. Right. Interburst interval duration plotted against promotor burst period. For each graph, the two regression lines have been drawn. Correlation coefficients shown in top RH corner of each plot. Axes in seconds.

2·5 and 5·0 s and makes nine extra cycles during the sample, due to its interactions with LMX (not shown). The plots of successive periods and burst lengths versus real time show no discernible overall drift in frequency and, when compared one with another, show that the periods of the promotors tend to co-vary. For example, the short periods indicated (black stars) in the RMX, R1 and R2 plots are preceded and followed by patterns of increasing and decreasing periods common to all three appendages. Similarly, the seven troughs which form prominent features of the last part of the R1 plot (dotted lines) appear also in the R2 and RMX plots.

The correlation that is apparent in Fig. 4 between promotor burst duration and cycle period is shown to be highly significant in the plots of Fig. 5. Those on the left display the same promotor parameters of RMX, R1 and R2 as are used for Fig. 4, while those on the right display, for comparison, approximations of the remotor bursts for the same appendages. EMGs were not recorded from remotor muscles, but, since the waving limbs are continually in motion without perceptible pauses in muscular activity, it seems reasonable to assume that the promotor interburst interval gives a reasonable measure of remotor burst duration. Plots of this parameter versus promotor cycle period gave similar regression lines and high correlation coefficients.

From this we can conclude that the two phases of the stroke, promotion and remotion, show a strong symmetry. The bursts are of similar duration and co-vary with cycle period in a linear manner.

The promotor myograms showed that whereas the bursts of ipsilateral appendages were almost concurrent, the burst onsets were not synchronous but staggered as a wave of activation passed either anteriorly or posteriorly along a row. The direction of the metachronal wave changed frequently, and occasionally two distinct waves were visible in one row. In order to display the interappendage coordination, and in particular the delays in promotor onsets of adjacent limbs, phase histograms were drawn for three appendages of promotor onset in the promotor cycle of a neighbour (Fig. 6, Table 3). From the plot of R2 onset within the cycle of R1, we see a high concentration of phase values (concentration coefficient $r = 0.85$) around a mean of 0.05 (C.S.D. ± 0.08). This was typical for pairs of ipsilateral adjacent legs in both *Scyllarus* and *Palinurus*. The relationship between the legs and maxillipeds was a looser one, as seen in the histogram (B) of R1 in RMX. Although the mean is similar (0.07 C.S.D. ± 0.13) the dispersion is broader ($r = 0.63$). The LMX in RMX
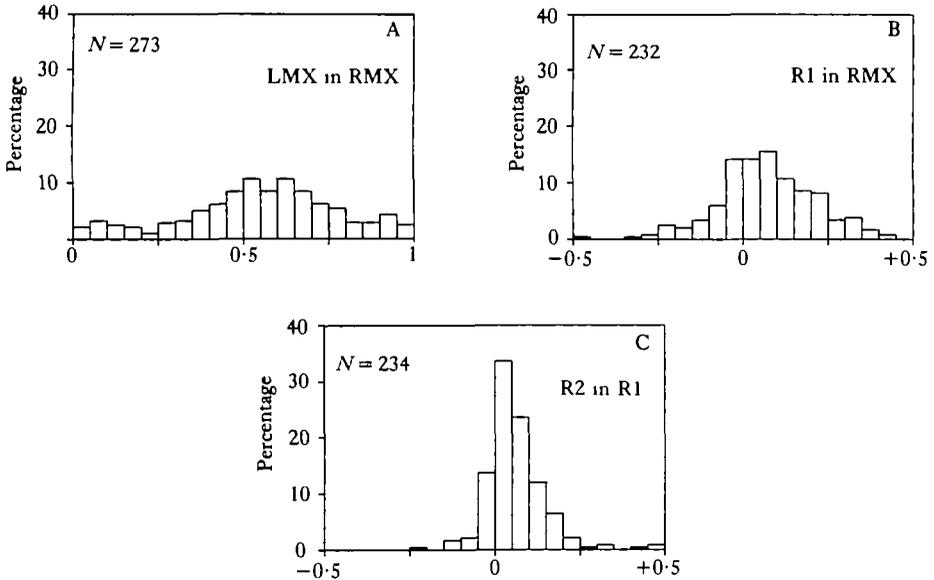
Fig. 6. Interappendage phase values during waving in *Scyllarus*. Histograms of percentage distributions of phase values plotted for RMX promotor burst onsets within the promotor cycle of LMX (A), R1 onsets within the RMX cycle (B) and R2 onsets within the R1 cycle (C).

Table 3. *Ipsilateral interappendage phase mean values during waving in* Scyllarus latus

	No. of cycles N	Mean phase	Circular standard deviation C.S.D.	Concentration parameter r
LMX in RMS	273	0·59	0·17	0·36
R1 in RMX	232	0·07	0·13	0·63
R2 in R1	234	0·05	0·08	0·85

histogram shows even wider dispersion ($r = 0.36$), reflecting the complex interaction between the two maxillipeds, their frequent collisions, adjustments and interpolated bouts of grooming. Phase values cover the whole range from zero (or one) when the two maxillipeds adduct synchronously (for example in grooming) to 0·5 when they sweep together across the midline with their promotors in antiphase.

In these three histograms the array of phase values is almost symmetrical about the mean as a result of the frequent changes in direction of the metachronal waves. In samples of *Scyllarus* leg recordings there was a slight asymmetry to the right of zero, indicating that a posteriorly directed wave is slightly favoured. In the *Palinurus* samples (where the mean phase value was 0·99 c.s.d. ±0·13) there was an asymmetry to the left of zero, indicating dominance of an anteriorly directed metachronal wave.

Experimental leg perturbation

Occasionally, waving activity was sufficiently prolonged and stable to permit a cursory study of experimental interference. A vertical glass rod was lowered into position in front of a waving leg so that it prevented promotion past the rest position of that leg. The range of the wave cycle possible was thus reduced by 50 %. In the *Palinurus* sample shown in Fig. 7 only two legs were waving: R2 and R3. When R2 was prevented from completing its promotion, the effect of the block was expressed in both legs. The cycles became shorter and the bursts briefer and of lesser amplitude. The amplitude reduction was most marked in R2 (the blocked leg) where the number of potentials declined to such an extent that movement was scarcely visible. When R3 was blocked, its promotor firing became feeble and almost tonic, but the stimulus had little effect upon R2 bursting. Thus the descending distribution of the reflex is much stronger than the ascending pathway.

DISCUSSION

The function of waving

Waving has a widespread distribution and individuals, especially from the Scyllaridae, spend considerable periods of time waving, yet it is difficult to ascribe a

Fig. 7. Effects of blocking leg promotions during waving in *Palinurus*. (A) Block of R2 (black bar) modulates rhythm in R2 and R3. (B) R2 and R3 discharge without stimulation. (C) Block of R3 (black bar) inhibits bursting in R3; R2 shows minimal modulation. Calibration: 2 s.

ecise function to this activity. As has been emphasized above, waving only involves the T-C joint and the rest of the limb moves passively, precluding waving from contributing to most of the familiar functions of legs: locomotion, food gathering etc. However, in macrurans the coxa articulates with a pair of gill structures, the epipodite and the podobranch, by means of a common podomere. When a limb is promoted this podomere lies horizontally, pressed closely against the margin of the carapace. As the limb remotes, the podomere pivots dorsalwards, thrusting the epipodite and gill further up into the branchial cavity. Thus the moveable gills brush across the immoveable ones attached to the thoracic wall. It is significant that these animals never wave the fifth pair of legs which lack the podobranch and epipodite. Brachyurans do not wave any of their legs and these all lack articulated gills, but they do wave the third maxillipeds which bear extensive epipodites. These are well equipped with setae, and as the maxillipeds abduct they brush the whole inner surface of the gill array. It is obvious that the epipodites have an important grooming function. These anatomical features suggest that, in both macrurans and brachyurans, waving has a significant role in keeping the gills free of detritus, and in helping to avoid the attachment of the planktonic young stages of gill ectoparasites.

Under natural conditions, waving occurs in unstressed animals living in well-oxygenated water. However, in the laboratory, waving could sometimes be induced by keeping macrurans for prolonged periods under low oxygen tensions. This suggests that waving may have an accessory ventilatory role. Not only might the dorsal thrusting of the articulated podobranchs facilitate mixing of the water around the gills, but as the coxa remotes and the podomere pivots, the Milne-Edwards opening becomes wider. The slight lag between the movements of adjacent limbs ensures the widest possible opening and closing of these inhalant channels.

Thus gill grooming and ventilation are two possible functions for waving, but the amount of time devoted to waving in situations where the respiratory intake is clean and well-oxygenated suggests that waving may have as yet undetected additional roles.

The distinctive characteristics of waving

Waving involves appendages which show many other types of rhythmical behaviour, but the parameters of the rhythm, the selection of active muscles, the particular combination of appendages involved and interappendage coordination make waving distinct from all other activities. It might be considered that waving is a special gait, a mere variant, of walking. However, a detailed comparison of walking and waving based on rock lobster studies by Clarac (1981b) will show that this is not likely.

Waving is one of the slowest, most stereotyped appendage rhythms in rock lobsters, whose restricted frequency range (9–18 min^{-1}) does not overlap the variable stepping frequencies observed during walking (30–60 min^{-1}). The stability of the waving rhythm is probably correlated with the fact that the waving legs make no contact with the substrate, and at no phase in the cycle bear any load. Thus waving, unlike walking, does not have to adapt to the exigencies of the terrain nor respond to variations in body weight.

The unhindered motion of the waving limbs certainly underlies the observation

that the promotor and remotor strokes are of similar duration. In both strokes, the only force to be overcome is the drag of water viscosity on the slowly moving leg, so the muscle power needed is equal in both halves of the cycle. It was therefore of no surprise to find that both strokes contribute proportionate variations to changes in waving period. In all the walking patterns (forwards, backwards and sideways) during one phase of the cycle, the power stroke, the leg is touching the ground, it is bearing part of the body weight and it is contributing to the propulsion of the body in the direction of the walk. In the other phase, the return stroke, the leg is lifted up off the ground, unweighted and flexed, and is swinging into readiness for a new power stroke. Thus the forces acting upon the two strokes are entirely different. In rock lobster forward walking, for example, the return stroke (promotion) is of relatively constant duration and variation in stepping frequency derives from variation in the power stroke (remotion) (Clarac, 1981b).

The lobster can walk in all directions and uses 16 leg muscles in various programmed combinations to achieve the appropriate movements at six joints (Ayers & Davis, 1977). In all modes, the power stroke involves downward thrust and the return stroke leg lifting, that means depression and levation about the C-B joint. This component is combined with prominent promotion and remotion about the T-C pivot during forward and backward walking, or flexion and extension about the M-C joint during sideways walking. Thus the pattern of strongly bursting motoneurones changes with direction of walking. However, as a conservative estimate, more than 75 % of the total motoneurone population of each leg is receiving rhythmical drive during an average walking bout. By contrast, during waving, C-B, M-C and all the distal joints are immobile, and only the muscles activating the T-C joint show rhythmical contractions. Thus a much smaller set of motoneurones is active during waving than during walking.

Walking in rock lobster usually involves the three most posterior pairs of legs, although, when necessary, the anterior two pairs can also participate. In waving, the anterior legs predominate and the fifth pair act as stationary postural struts. Furthermore, the third maxillipeds, which are far too short to reach the substrate and assist in propulsion, play an important role in waving (see Fig. 8). It is quite common for only a subset of the limbs, either the most anterior ones or an ipsilateral group, to be active at any one time, and participation shifts during a prolonged waving bout, although the maxillipeds are invariably active. So we can conclude that the segmental distribution of the two patterns is rather different.

Finally, interlimb coordination is totally different during walking and waving. Although walking can vary on a cycle to cycle basis, and the individual legs show a high degree of independence (Chasserat & Clarac, 1980), the basic pattern can best be described as a tetrapod alternating gait. Adjacent limbs, whether ipsilateral or members of a contralateral pair, are usually in antiphase. Waving tends to be an 'in phase' pattern with bursts in adjacent homologues almost concurrent, separated only by short onset latencies. It is thus a typical metachronal rhythm (see Fig. 8D, E).

Table 4 compares waving with other metachronal rhythms. Functionally, waving bears no similarity to these rhythms because the legs oscillate so slowly that neither propulsion (as in swimmeret and expopodite beating) nor water movement (as in flagellar beating) is achieved. This large difference in cycle length occurs because the
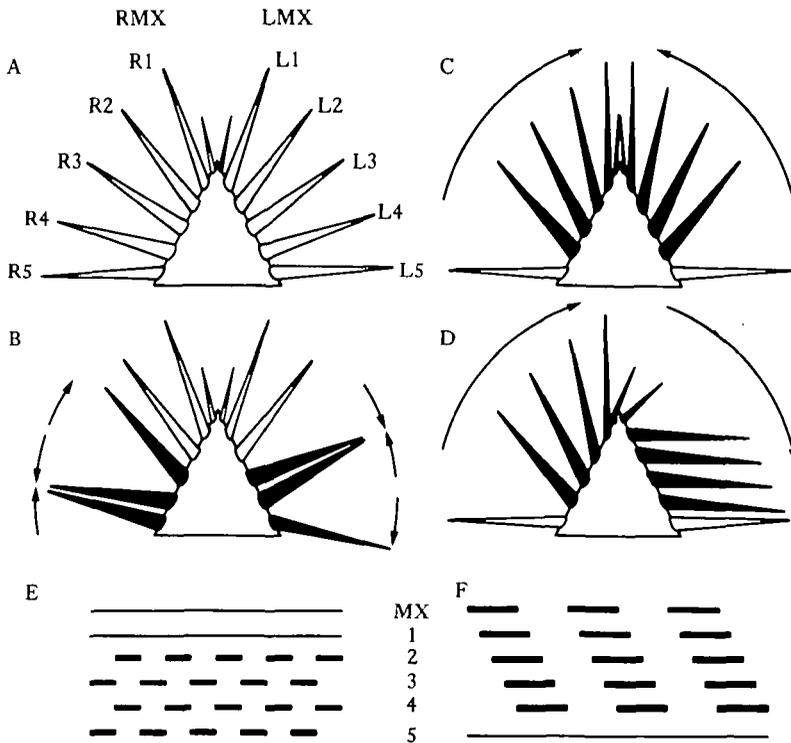
Fig. 8. Diagrams to illustrate the differences between walking and waving. (A) Resting position of the limbs (sticks) around the plastron of the ventral thorax in panulirids. (B) Alternating movements of active limbs (black sticks) during walking. (C) and (D) Metachronal movements of active limbs during waving. In (C) right and left series of appendages both display concurrent waves of promotion (least favoured mode). In (D) the two ipsilateral series are in antiphase (most favoured mode). A wave of promotion is passing along the right series and a wave of remotion is passing along the left series. (E) and (F) Patterns of ipsilateral promotor bursts during walking and waving. In (E) is shown the alternating tetrapod gait typical of walking. (F) shows the metachronal sequence of burst onsets typifying waving.

walking legs are more massive than the small appendages involved in the other rhythms. On many other criteria however, these four rhythms appear similar. In each case, the onsets of homologous bursts proceed in a regularly phased sequence along the appendages of one side, a type of ipsilateral interappendage coordination which fits very well the metachronal model described by Wilson (1966). In waving, swimmeret beating and flagellar beating, one wave of burst onsets passes along the whole row before the next sequence starts, while in expopodite beating, the cycle length is shorter than the total of all onset delays, so that the sequences overlap. This results in expopodites four segments apart having the same phase. Such a possibility was predicted by the model.

Wilson, in his metachronal model for insect walking, predicted that the return stroke would be of constant duration while the power stroke would vary with stepping frequency. None of the four rhythms described in Table 4 fulfil this prediction, but all show variations in both parts of the cycle, such that the phase of the power stroke in the return stroke cycle (the power stroke/return stroke ratio as

Table 4. *A comparison between waving and other metachronal rhythms*

	Maxilliped flagella beating (1)	Thoracic exopodites beating (2)	Swimmerets beating (3)	Waving
Appendages involved	1st, 2nd and 3rd maxillipeds	3rd maxillipeds & 5 pereiopod pairs	4 pairs swimmerets	3rd maxillipeds & 4 pereiopod pairs
Beat frequency	12 – 16 Hz	8 – 10 Hz	40 – 120/min	9 – 18/min
Beat variability	Stable	Stable	Variable	Stable
Power stroke/Return stroke ratio	0·5	0·5	0·5	0·5
Ipsilateral coupling	Strong latency locking	Strong phase locking	Strong phase locking	Strong phase locking
Phase of adjacent appendages	0·3 – 0·4 (Fixed latency 19 ms)	0·4 – 0·6	0·2	0·05
Direction of metachronal wave	Ascending	Ascending	Ascending	Ascending or descending
Bilateral coupling	Tight[+]	Loose	Tight	Loose

(1) *Cancer*. ([+]*Munida*). Burrows & Willows, 1969.
(2) Larval *Homarus*. MacMillan *et al.* 1976.
(3) *Homarus*. Davis, 1969; Laverack, MacMillan & Neil, 1976.

defined by MacMillan, Neil & Laverack, 1976) remains near 0·5 for all frequencies.

The Wilson model was also constrained by the necessity for the legs to support the weight of the insect while walking on land. Thus contralateral legs of the same segment had to alternate in phase. Waving, like the other three rhythms considered in Table 4, has no such constraints and bilateral coordination during waving is very weak. The two sides appear to function essentially as independent units. They retain, for the most part, the same beat frequencies and, although no quantitative analysis was possible, qualitative observations suggest that a loose gliding coordination does exist. As with exopopodite beating, certain preferred phases can be seen. Fig. 8D shows a commonly observed configuration of the appendages during waving. Here a sequence of promotor onsets is passing forwards along one side, while a remotor sequence is passing back along the other. Thus phase histograms for contralateral pairs would show a low modal peak around 0·5 (see Fig. 6A). Waving differs from the other metachronal systems in that the sequences frequently reverse and descending metachronal waves are as common as ascending ones.

The generation of the waving pattern

The two features which best distinguish waving are the extremely long cycle periods and the limited set of motoneurones which receive the output of the waving central pattern generator (CPG). Possibly both have as an underlying cause the low level of sensory input during waving. Since waving does not move the body, visual and statocyst inputs are stable throughout a bout of waving. Furthermore, the active legs are not mechanically coupled to the substrate during any part of the cycle, and never bear any load. Ayers & Davis (1978) found that joint receptors which produced

effective reflex responses in leg motoneurones were tuned to the range of velocites commonly observed during walking. Examination of their published data suggests that the slow movements displayed during waving would be unlikely to provide effective reflex drive. Each waving limb moves as a single unit with almost no movement of any joint other than the T-C; thus position and velocity receptors in the distal segments are unlikely to be stimulated at all, which accords with the observation that autotomized stumps wave in a manner indistinguishable from that of intact legs. This leaves only the hydrostatic receptors described by Vedel & Clarac (1976), innervated coxal setae (M. Berengut & F. Clarac, in preparation), and the T-C MRO (Bush, 1976; Cannone & Bush, 1980) as possible sources of feedback during waving.

In other systems where it has been possible to remove normal sensory feedback, either by sectioning the nerves or by applying a blocking agent to prevent actual muscle contraction (fictive locomotion, Grillner, 1977), the result has been a significant increase in the cycle length of the CPG output. The very slow rhythm of waving is therefore consistent with the idea that waving is an expression of an oscillator system cycling alone without the feedback provided by sensory input. It is also not unreasonable to assume that a low amplitude oscillation will not be distributed as widely to the leg motoneurone pool as would a feedback-boosted oscillation. Thus we find that the waving programme is expressed only by the promotors and remotors.

Of course, the waving CPG is not entirely isolated from sensory input as the experiments described in Fig. 7 show. When the waving R2 met a stationary block, the resulting reflex response was not that of a resistance reflex (which would have resulted in a prolonged promotor firing) but a modulation of the CPG to produce briefer, more frequent bursts, a response which was distributed over more than one segment. Similarly, when legs or maxillipeds collide, the contact between them results in temporary perturbation of the rhythm with subsequent compensatory adjustments in burst duration, cycling and phasing.

These collisions, which are not infrequent, arise anteriorly due to loose bilateral coordination and the gliding of the phase into values around zero (or one). Then the maxillipeds collide and the disturbance is transmitted back to the adjacent legs. But collisions can also arise within a row, as some segments drift out of coordination, reverse the metachronal wave, initiate a second, inappropriate, wave or adopt a new frequency. Such 'errors' indicate noise in the coupling system which does not receive the stabilizing reinforcement normally provided, in a system like walking, by sensory feedback.

Several authors have speculated on the occurrence of multifunctional CPGs where a single oscillator (or oscillatory circuit) can be modulated to drive more than one distinct motor programme (Sherman, Novotny & Camhi, 1977; Paul, 1976; Ayers & Clarac, 1978). Since waving involves a sub-set of the motoneurones used for walking, might it not be the case that waving is the motor programme expressed by the walking CPG when the central oscillator is cycling independently of sensory modulation? MacMillan *et al.* (1976), in postulating that the same CPG which programmes expopodite beating in larval *Homarus* is also responsible for walking in the adult, emphasize that the two programmes never function simultaneously in the same developmental stage. In the adult macruran walking and waving are alternative programmes but they are never expressed at the same time. (In brachyurans, waving

is restricted to the non-locomotory maxillipeds, and walking and waving frequently occur simultaneously. Here the CPGs are obviously different and segmentally isolated from one another.) Clarac & Chasserat (1979) describe the changes in inter-limb coordination that occur as the legs are successively autotomised during treadmill-driven walking. The autotomized stumps lost their normal alternating coordination and moved into phase with their nearest anterior neighbour. When all the legs were autotomized, the stump muscles took on the slow rhythms, prolonged bursts and interappendage phase relations that we now recognize as waving. We found that when an intact, walking animal was lifted up off the substrate, if the limbs showed any coordinated pattern (as opposed to unpatterned struggling) the activity was waving. These observations suggest that of the two alternative programmes, walking is selected in the presence of feedback (e.g. contact with the moving treadmill) while waving is selected in the absence or diminution of feedback (e.g. the unweighting of the legs). The role of the feedback in walking would be three-fold: firstly to provide a higher background of generalized excitation to the oscillators to increase the cycle frequency and recruit a wider motoneurone pool, secondly to change the interappendage coupling systems to the walking pattern, and thirdly to modulate CPG activity on a cycle-to-cycle basis to meet the power requirements of the loaded legs on a possibly uneven substrate.

Since we lack any evidence about the CPG at the cellular level, such theorizing is highly speculative. It is important for researchers engaged in intracellular probing of the macruran thoracic ganglia to realize that the waving motor programme exists, and is the one likely to be expressed in the semi-isolated preparations used in such experiments. A somewhat analogous situation is found in the leech (W. B. Kristan Jr., personal communication). The dorsal and ventral flexor motoneurones participate in two alternative motor programmes, one subserving swimming and the other ventilation of the body surface. During ventilation the body is fixed to the substrate by the posterior sucker, and the dorso-ventral body undulations have a much slower periodicity and larger amplitude than during locomotion. The slower programme is frequently elicited in the semi-intact preparation used for experimentation.

REFERENCES

Ayers, J. & Clarac, F. (1978). Neuromuscular strategies underlying different behavioural acts in a multi-functional crustacean leg joint. J. comp. Physiol. 128A, 81–94.

Ayers, J. & Davis, W. J. (1977). Neuronal control of locomotion in the lobster, Homarus americanus. I. Motor programs for forward and backward walking. J. comp. Physiol. 115A, 1–27.

Ayers, J. & Davis, W. J. (1978). Neuronal control of locomotion in the lobster, Homarus americanus. III. Dynamic organization of walking leg reflexes. J. comp. Physiol. 123A, 289–298.

Bush, B. M. H. (1976). Non-impulsive thoracic-coxal receptors in crustaceans. In Structure and Function of Proprioceptors in the Invertebrates, (ed. P. J. Mill), pp. 115–151. London: Chapman and Hall.

Burrows, M. & Willows, A. O. D. (1969). Neuronal coordination of rhythmic maxilliped beating in brachyuran and anomuran crustacea. Comp. Biochem. Physiol. 31, 121–135.