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MOTOR PATTERN ANALYSIS IN THE SHORE CRAB (*CARCINUS MAENAS*) WALKING FREELY IN WATER AND ON LAND*

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SUMMARY

1. Neuromuscular activity underlying lateral walking was studied in the shore crab *Carcinus maenas*. Electromyograms (EMGs) were recorded from legs on both the trailing and leading sides during free walking on land and under water in a pool (Figs 1, 2, 6, 7).

2. In a trailing leg, the levator, flexor and closer muscles were active during the return stroke (RS) in alternation with the depressor, extensor and opener muscles which were responsible for the power stroke (PS). In a leading leg a different pattern of activity was observed. The flexor and closer muscles were active during the PS, and the extensor and opener muscles during the RS. Trailing steps were shorter and less variable in duration than leading steps (Figs 2, 3 for walking under water, Fig. 6 for walking on land, see also Fig. 7).

3. A comparison of the activity patterns of the single common motor neurone innervating the opener and the stretcher muscle during trailing and leading showed a difference in burst length and instantaneous frequency (Fig. 2C,D). The discharge of this motor neurone usually lasted longer in leading steps. The discharge frequency started at a high level and then decreased during a trailing step, whereas in a leading step it was irregular (Fig. 8).

4. In all walking situations the stretcher and opener muscles, which share a common excitatory motor neurone, received identical excitatory input (Fig. 4).

5. The discharge frequencies of motor neurones innervating the extensor, the stretcher/opener and the closer muscles were investigated (Fig. 5). For motor neurones active during the PS, the frequency was high at the onset of the burst and then declined gradually. With the exception of the closer muscle, the discharge of motor neurones during the RS was more or less constant during the burst.

6. A comparison between walking under water and on land showed that the overall EMG patterns were essentially similar (Fig. 7). However, on land the PS lasted longer and involved the activation of additional motor neurones in muscles which are innervated by several motor neurones, e.g. the extensor (Fig. 6). During walking on land maximal discharge frequencies up to 350 Hz were recorded.

* Dedicated to Professor Dr Ernst Florey on the occasion of his 60th birthday.
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Key words: Crustacea, locomotion, myograms, motor pattern.

7. Homologous muscles in the three different walking legs operated similarly during trailing or leading movements without major differences in their EMG patterns. This indicates a similar load distribution on the different legs (Fig. 9).

INTRODUCTION

Walking behaviour in Crustacea has been studied for more than two decades to understand how walking sequences are organized, which types of coordination patterns exist among legs (Barnes, 1975; Sleinis & Silvey, 1980; Clarac & Chasserat, 1983; Chasserat & Clarac, 1983) and how neuronal events control this behaviour (Atwood & Walcott, 1965; Clarac & Coulmance, 1971; MacMillan, 1975; Ayers & Davis, 1977; Ayers & Clarac, 1978). The techniques employed have ranged from cinematographic analysis of stepping patterns (Clarac & Coulmance, 1971; Barnes, 1975) and measurements of joint movements by means of small transducers (Clarac & Cruse, 1982; Klärner & Barnes, 1986) to electrophysiological recordings (electromyograms, EMGs) from muscles involved in locomotion. EMGs are particularly helpful for analysing temporal patterns of muscular activity during leg movements. A precise correlation with neuronal events, however, is only possible under favourable conditions, and in cases where muscles receive innervation through only one or two excitatory motor axons (Ayers & Clarac, 1978). When several motor neurones innervate a muscle, a particular difficulty is the separation of single motor units, because motor neurones can be coupled synaptically within the central nervous system (Wiens, 1976; Wiens & Atwood, 1978). Furthermore, electromyographic recordings cannot yield information about the activity of the different inhibitory neurones innervating the leg muscles. To elucidate the role of these neurones during walking, it is necessary to record directly from the nerves (neurograms).

In only two studies have neurograms been successfully recorded. Barnes, Spirito & Evoy (1972) described neurograms for four distal leg muscles in the crab *Cardisoma* walking on a treadmill. Besides excitatory neuronal activity they could also discriminate the activity of the two specific inhibitory neurones, one innervating the opener muscle and the other the stretcher muscle. Ballantyne & Rathmayer (1981) recorded neuronal activity from the nerves innervating the opener and the closer muscles in the crab *Eriphia*. They showed that during walking bouts the common inhibitory neurone, which innervates all muscles of the crab leg (Rathmayer & Bévengut, 1986) is mainly tonically active in contrast with the phasic activity of all the other motor neurones.

Most of the previous electrophysiological studies of crustacean locomotion have been performed under tethered conditions, with the crab walking on a treadmill or on a motor-driven treadband. The aim of the present investigation was to study walking in unrestrained crabs moving as freely as possible in their natural habitat, both under water and on land. The shore crab, *Carcinus maenas*, was chosen, first because it displays a well-defined lateral locomotor behaviour; this implies that the same leg, depending on the direction of walking, can either push (trailing leg) or pull (leading leg) the animal. Second, living near the seashore, this crab frequently walks on land.

Efferent control must therefore cater for the difference between the loading upon the legs during walking on land and that during walking under water.

We have recorded EMGs from the 10 main muscles involved in leg movements (Fig. 1C). The four proximal leg muscles, located within the cephalothorax and the coxopodite, move the thoraco–coxopodite (T–C) joint (the promotor and the remotor) and the coxo–basipodite (C–B) joint (the depressor and the levator). These muscles receive complex innervation from about 35 motor neurones (Bévengut, Simmers & Clarac, 1983). The distal leg muscles move the mero–carpopodite (M–C) joint (the main flexor and the extensor), the carpo–propodite (C–P) joint (the bender and the stretcher) and the pro–dactylopodite (P–D) joint (the opener and the closer). Together they are innervated by 14 motor neurones, of which three are inhibitory (Wiersma & Ripley, 1952; Wiens & Rathmayer, 1985). The flexor muscle receives four excitatory axons; the extensor, bender and closer muscles are each innervated by two excitatory neurones; the opener and stretcher muscles share a common excitatory neurone, but each receives a specific inhibitory neurone. In addition, all muscles are innervated by branches of the common inhibitory neurone (Rathmayer & Bévengut, 1986). In the case of the opener and the stretcher muscles, the EMG is a mirror image of the activity of the single common excitatory neurone. This allows study of locomotor behaviour at the level of an identified motor neurone during different locomotor conditions such as trailing and leading or walking under water or on land.

Some of the results have been presented previously as a short communication (Rathmayer, Pflüger & Clarac, 1985).

MATERIALS AND METHODS

The crabs, *Carcinus maenas*, were obtained from local sources in Arcachon (France). Thirty specimens of large size were used (mass about 60 g, carapace width about 8 cm). A small swimming pool (2·20 m in diameter) served as a walking arena (Fig. 1A). Half of this pool contained sea water (15 cm deep), the other half was covered with sand to serve as a terrain for terrestrial walking. The submerged part of the pool was covered with neoprene and a fine layer of sand to prevent the animal from slipping. Electrical grounding of the elevated part was achieved by covering it with paper moistened with sea water.

In this arena the animals were able to walk as many as 20 steps in any direction. They could also walk directly from land into water and *vice versa*. Walking patterns (trailing and leading) under water or on land were analysed and compared only when crabs displayed a clear lateral walking sequence of more than 10 steps.

Additional data concerning the movement of the different leg joints during walking sequences (trailing and leading) were obtained using a super-8-ciné camera (Beaulieu ZM 4004) or a video camera (Panasonic CCTV model WV-1460/6) filming the crabs from the front and from above either in the laboratory or in their natural habitat on the beach. To study locomotion in freely moving animals two strings (length 50 cm), one on each side, were fixed to the carapace and secured to a wooden

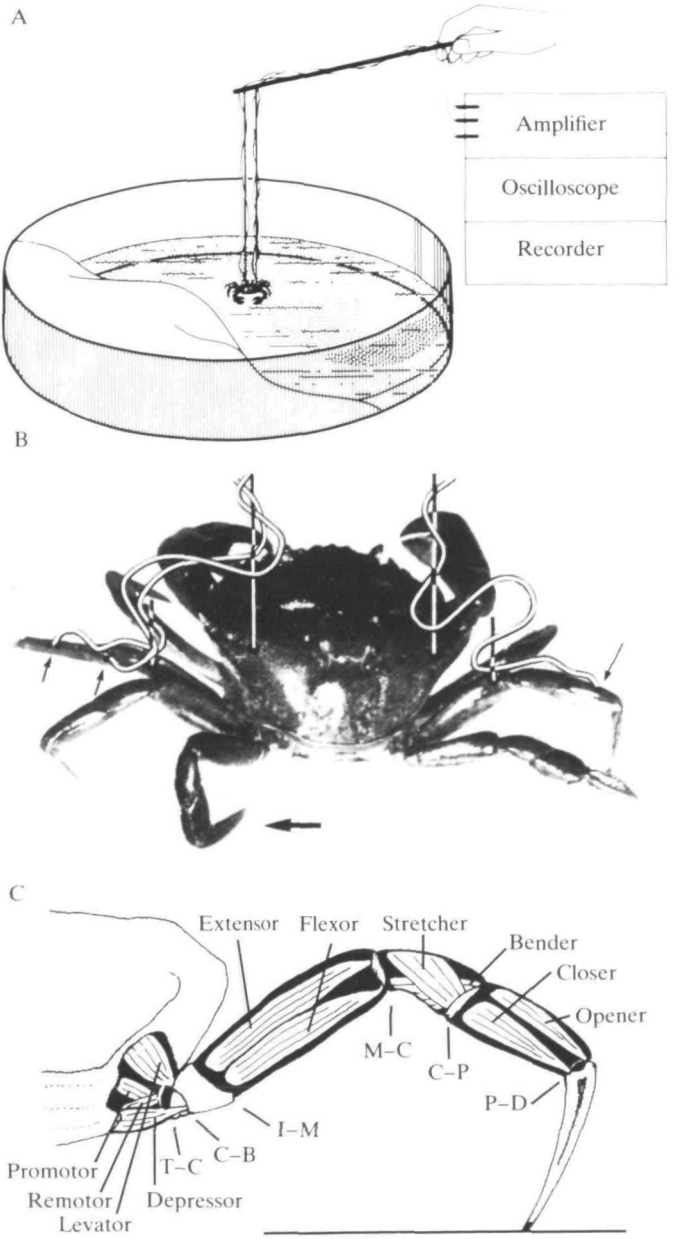

Fig. 1. (A) The pool which served as an artificial environment for studying free walking in the shore crab in water and on land. (B) A photograph of a crab walking laterally to the left. The wire electrodes and their points of insertion in the opener and the stretcher muscles of a leg on the leading side (small arrows left) and in the opener muscle on the trailing side (small arrow right) are shown. (C) Posterior view of a third walking leg showing the joints and, schematically, the muscles responsible for their movements. For abbreviations see text.

bar which was held above the crab by the experimenter (Fig. 1A,B). It was then possible to follow the crab with the bar in whatever direction it moved. Film analyses showed no walking disturbance in crabs loosely tied to this bar compared with crabs walking freely in their natural habitat. In such animals up to three pairs of electrode wires were implanted simultaneously. To investigate differences in activity patterns during leading and trailing in muscles which were innervated by only one excitatory neurone, simultaneous recordings were made from the ipsilateral stretcher and opener muscles as well as from the contralateral opener muscle.

The muscular activity of the distal muscles was recorded differentially by silver or nickel–chrome wire electrodes (diameter 100 μm) of about 70 cm in length. To insert the electrodes, the crab was rigidly fixed and a small area of cuticle on the leg was carefully removed without damaging the underlying hypodermis. Small holes were punctured in the hypodermis with a fine insect pin (preferably at points of insertion of muscle fibres to the cuticle) and the electrode wires, insulated to the tips, were carefully inserted about 1 mm into the muscle bundles more or less parallel to the course of muscle fibres to limit fibre damage and movement artefacts. The electrodes were fixed to the hypodermis with tissue adhesive glue (histoacryl) and then covered with wax. A thin nylon rod, about 4 cm long, was glued to the dorsal cuticle of the meropodite and served as a support for the electrodes which were loosely twisted around it (Fig. 1B). The electrode wires were then glued to the dorsal carapace, leaving enough length to permit undisturbed leg movements. They were guided along the strings to the bar held above the animal and finally connected to small plugs at the end of the wooden bar. Shielded cables led from the plugs to the preamplifiers (Grass P15).

In our experiments we analysed muscle activity of the first, second and third walking legs (legs 1, 2 and 3 equivalent to pereiopods P2, P3 and P4). The first pair of pereiopods, the chelae, are not used in walking, although when walking on land they can be used for support. The last pair of pereiopods, enlarged and modified as paddles, are efficient in swimming but less so in walking.

To record the activity of the proximal muscles we usually monitored the activity of both antagonistic muscles with a pair of electrodes in a particular joint. Each electrode wire was inserted along the apodeme of one muscle, but recorded the muscular activity of both antagonistic muscles. The potentials recorded from the different motor units of a given muscle were distinguishable from those of the antagonist on the basis of the number of inflections and the relative amplitude of positive and negative components (Ayers & Clarac, 1978). The animals tolerated the use of up to three pairs of electrode wires without impairment of walking. Due to the better electrical insulation in the air, the EMG recordings from crabs walking on land were usually of larger amplitude and with more cross-talk.

Simultaneous recordings were made either from the main leg muscles of one leg (T–C joint, the remotor and the promotor; C–B joint, the levator and the depressor; M–C joint, the extensor and the main flexor; C–P joint, the bender and the stretcher; P–D joint, the opener and the closer, see also Fig. 1C), or from selected muscles in one leg and a 'control' muscle in the leg on the contralateral side, or from

homologous muscles of several different walking legs. The contact of the dactyl with the ground, which corresponds to the powerstroke (PS) duration in the step cycle, can be defined by the sensory activity of the mechanoreceptors at the tip of the dactyl (see Libersat, Clarac & Zill, 1987; it is indicated by the thick horizontal bars in Figs 2A,B, 6). Recordings were stored on tape (Racal 4 DS) and later displayed on a chart recorder (Gould ES 1000) or statistically analysed by a computer program.

RESULTS

In the pool, animals moved freely in and out of the water. Walking sequences were either spontaneous or induced by tactile stimulation over the dorsal carapace (usually gently pressing the crab down for a short while). Locomotory bouts were variable in speed and duration but induced walking was always faster than spontaneous bouts.

Motor patterns during walking under water

Simultaneous EMG recordings of antagonistic pairs of muscles of leg 3 were performed to compare muscular activity in legs of the leading and the trailing side (Fig. 2). All muscles which move joints in a vertical plane were rhythmically active during walking (levator–depressor and flexor–extensor in Fig. 2A, closer–opener in Fig. 2B). In contrast, muscles such as the promotor and remotor (Fig. 2A), which cause leg movements in a horizontal plane, lacked a clear rhythmic activity during lateral walking. They began to display alternate bursting only when the animal turned. However, the bender and the stretcher muscles, which move the carpo–propodite joint horizontally, were activated rhythmically during lateral walking (Fig. 2C,D for the stretcher). In Fig. 2C,D the difference in the discharge patterns of trailing and leading is also obvious: in trailing the burst duration was shorter with the exception of the closer; in the levator and depressor it was about the same in trailing and leading. The instantaneous discharge frequency distribution in trailing was different from that in leading.

The activity of the major leg muscles participating in lateral trailing and leading steps in water was analysed for 70 steps in each situation (Fig. 3). The time from the onset of one extensor muscle burst to the next was measured and taken as the step period. The step periods were normalized for determining the phase relationships (Fig. 3).

In trailing a clear pattern emerged: the depressor, extensor and opener muscles were active together during the PS (marked with an asterisk in Fig. 3A). The PS was initiated by the activity in the depressor and opener muscles, but movements of the joint first occurred with the onset of extensor activity. The RS started with the activity of the levator muscle, which was already active at the end of the PS. It was followed by closer muscle activity during the RS, and a short burst in the flexor muscle towards the end of the RS. In the trailing situation, muscle activities associated with either the PS or the RS were clearly separated.

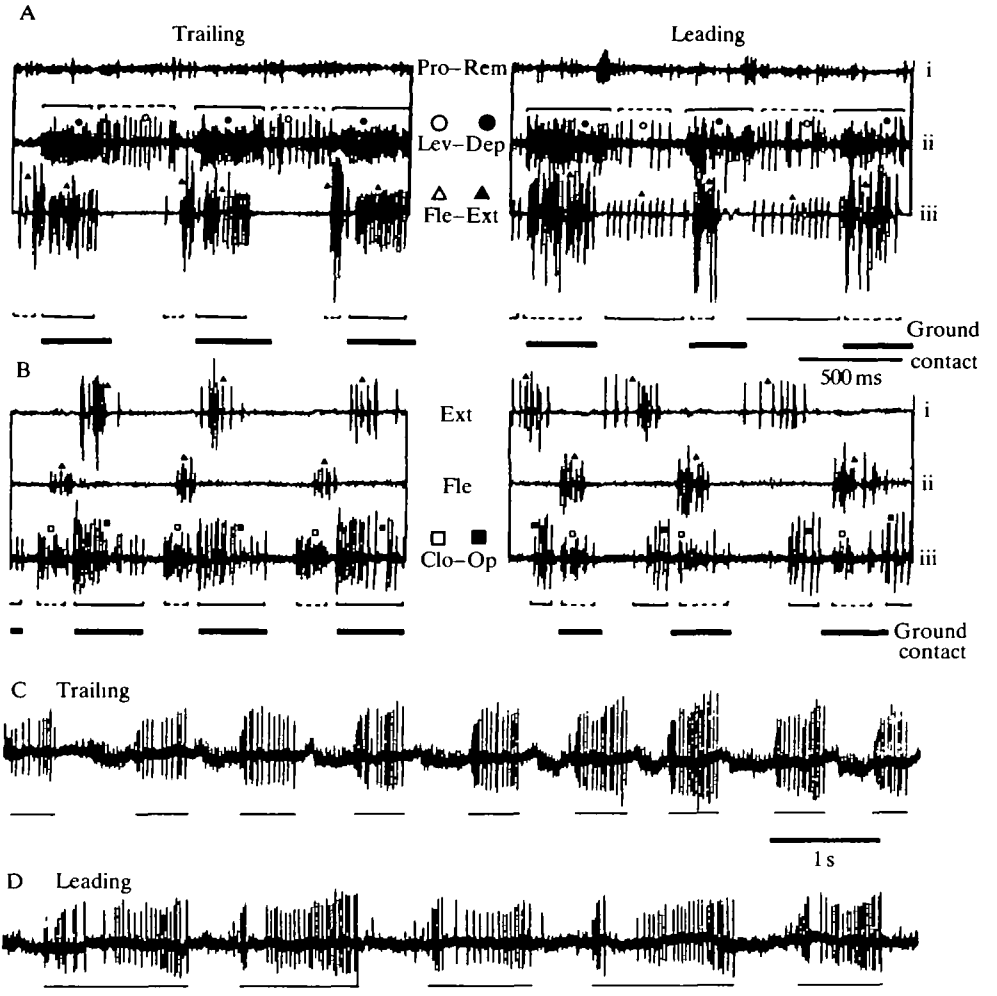

Fig. 2. Extracellular recordings of the activity of the main muscles involved during lateral walking under water in the second leg of the crab *Carcinus maenas* during trailing (left) and leading (right). (A) Simultaneous recordings from antagonistic pairs of muscles at three joints: the promotor (Pro) and remotor muscles (Rem) at the T–C joint (trace i); the levator (Lev) and depressor muscles (Dep) at the C–B joint (trace ii), and the flexor (Fle) and extensor muscles (Ext) at the M–C joint (trace iii). (B) Simultaneous recordings from selected muscles of the M–C joint (trace i, extensor activity; trace ii, flexor activity) and of the P–D joint (trace iii, closer and opener activities). The recordings from the depressor, extensor and opener muscles, the powerstroke (PS) muscles during trailing, are marked with a black circle, a black triangle and a black square, respectively, and the recordings from the levator, flexor and closer muscles, the RS muscles during trailing, with a white circle, a white triangle and a white square, respectively, in each record. The duration of PS corresponding to ground contact is indicated by a thick black horizontal bar. For clarification in each trace the duration of individual bursts is indicated by a solid or dashed fine line. (C) Activity of the stretcher muscle during nine trailing steps; (D) activity of the stretcher muscle during five leading steps.

During leading, a different pattern occurred. The depressor muscle still initiated the PS, but in contrast to trailing, the flexor and closer muscles became active during the PS. The levator muscle usually started its activity in the middle of the PS and continued to be active in the first half of the RS. In addition to these differences, the burst duration of the muscles was more variable in leading than in trailing. This can be seen from the greater standard deviation in leading. For the opener and the stretcher muscles, the burst length was longer in most leading steps (see also Fig. 2D and below).

The difference in muscle activity between legs on the trailing and the leading side is illustrated in the step period histograms for the extensor muscle (Fig. 3C,D). The mean duration of a step period on the trailing side was 522 ms (Fig. 3C) and 688 ms for leading (significantly different, $P \leq 0{\cdot}0001$, Fig. 3D). Trailing steps were shorter and, as can be seen from the steeper curve in Fig. 3D, less variable in duration than

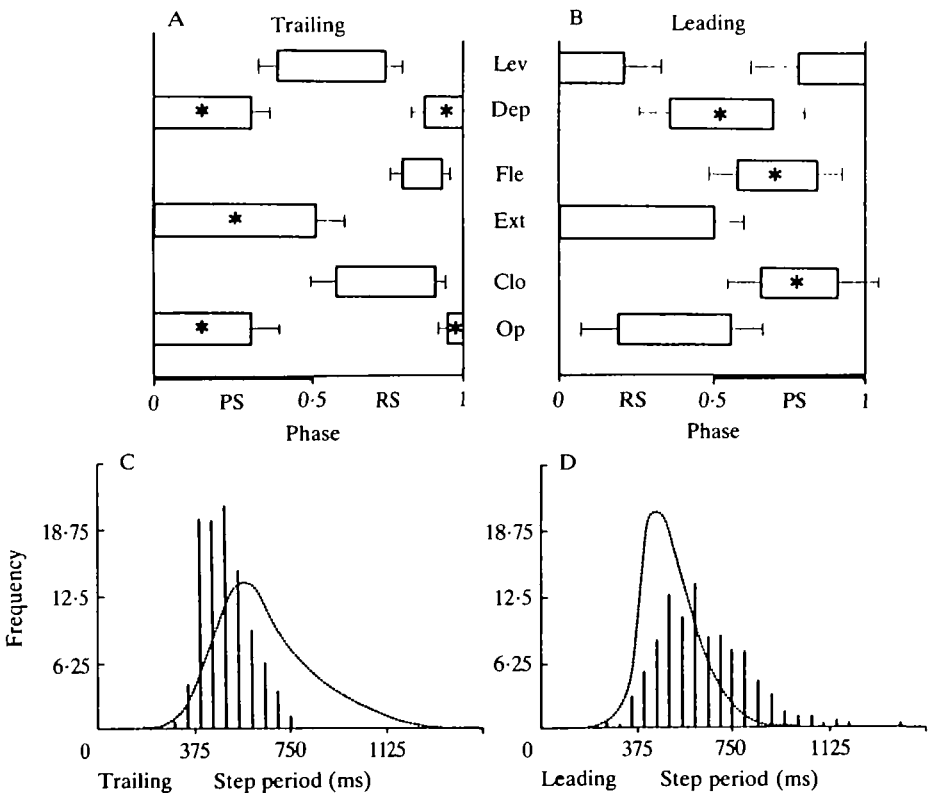

Fig. 3. (A,B) Diagram showing the burst duration and phase relationships of muscles active during trailing (A) and leading (B) sequences when the crab walks under water (70 steps analysed for each situation). Mean values and standard deviations of burst durations are shown. The onset of the extensor activity was taken as a reference point for the step period. The powerstroke (PS) muscles are indicated by asterisks. (C,D) Histogram of the step duration period during trailing (C) and leading (D) gained from 275 steps. For clarity, the outline of the histogram of the step period of the opposite side is indicated (dashed line) in each histogram.

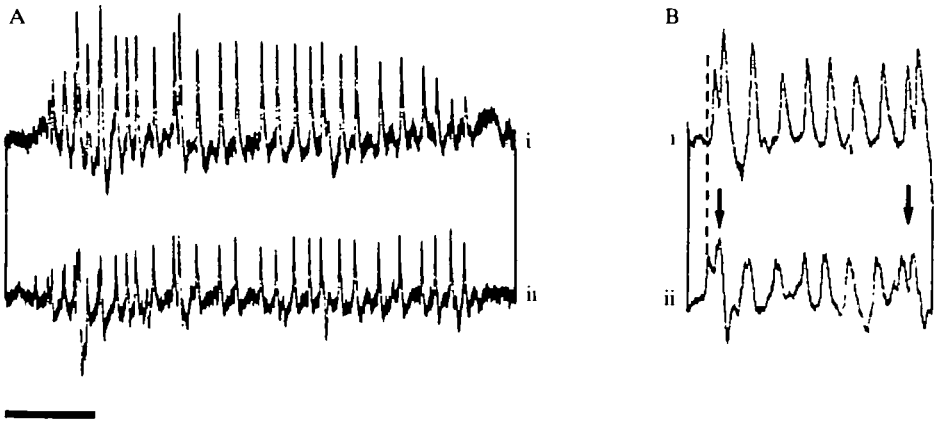

Fig. 4. EMG activity in the opener (trace i) and the stretcher muscles (trace ii). (A) Trailing step in water. (B) Expanded portion of a recording from a trailing step on land. The arrows point to doublet discharges at high frequency. The dashed line shows the delay in the onset of the opener activity. Calibration: 100 ms for A, 40 ms for B.

leading steps. The consequence for walking is that on the leading side fewer and more irregular steps occurred, a finding supported by the EMG recordings presented in Figs 2, 6 and 7.

Discharge of identified motor neurones during trailing and leading under water

When the activities of the opener (Fig. 4 trace i) and the stretcher (Fig. 4 trace ii) muscles were recorded together, the discharge patterns were identical during walking under water or on land, for trailing or leading sides. This 1:1 correlation is preserved over the entire frequency range encountered during walking; even up to 350 Hz, when doublets of potentials occurred (see arrows in Fig. 4B). This contrasts with results obtained in isolated legs (Hatt & Smith, 1975). The potentials recorded from the opener muscle (trace i) follow those of the stretcher muscle with a constant delay of 3 ms (trace ii), which is explained by the more distal location of the opener muscle in the leg.

Muscles other than the stretcher and the opener receive a more complex innervation through two (a fast and a slow) or more excitatory motor neurones. It was easy, however, to identify in the EMG recordings at least one motor unit according to the size and the shape of the muscle potentials generated. In some favourable recordings (e.g. from the extensor muscle) it was even possible to discriminate between muscle potentials generated by activity of the fast and the slow motor neurone (see Fig. 5Ai). Analysis of the discharge frequency of single identified motor neurones showed for the extensor muscle (Fig. 5A) that both motor neurones (fast and slow) were active during trailing whereas only the slow motor neurone was active during leading. In trailing, when this muscle acted as a PS muscle, both motor units discharged at a high frequency (150 Hz for the slow, 100 Hz for the fast) at the beginning of the burst. The discharge frequency then decreased rapidly for the fast and less rapidly for the slow motor neurone in the trailing situation. In the leading

situation, when the extensor acted as an RS muscle, the discharge frequency of the slow motor neurone remained high during the burst and dropped only towards the end (Fig. 5Aii). The absolute value of the discharge frequency, however, varied in both units from animal to animal, and even in the same animal in different walking bouts, even though the time course of the discharge frequency remained the same.

In the trailing situation, the excitatory motor neurone innervating the opener muscle, then acting as a PS muscle, started at a high discharge frequency, up to 190 Hz, at the onset of the burst (Fig. 5Bi). The frequency then decreased rapidly

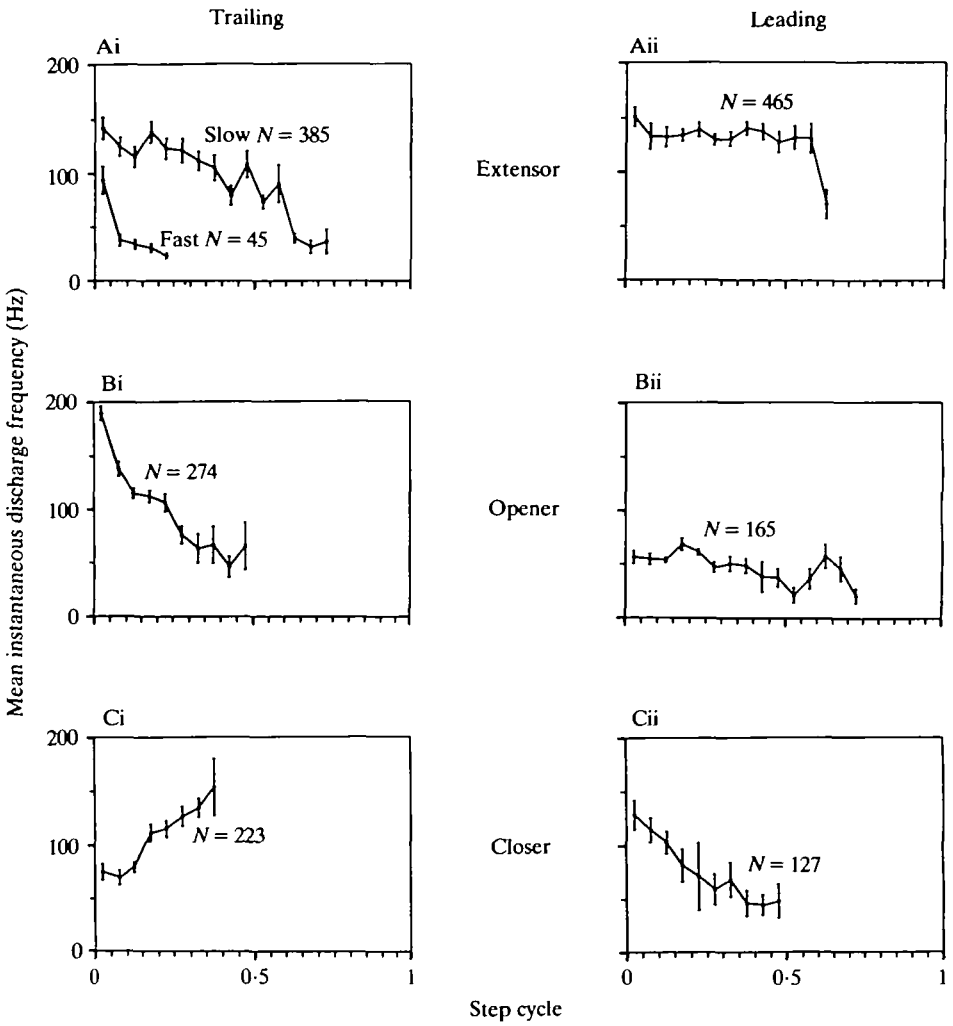

Fig. 5. Mean discharge frequency of different motor neurones calculated from trailing (left) and leading (right) walking sequences (extensor, opener and closer muscles) under water. For each bin (20 bins per step cycle, abscissa) the mean instantaneous frequency with its standard error was calculated (ordinate). Each step cycle is calculated from the onset of a given burst to the onset of the same muscle burst in the consecutive step (15 steps analysed).

reaching approx. 30 Hz at the end of the burst. In leading, when the opener acted as an RS muscle, the discharge frequency was much lower and stayed around 60 Hz throughout the entire burst (Fig. 5Bii).

The fast motor neurone of the closer muscle was never seen to be active during any walking sequence. The EMG was produced by the slow motor neurone only. This is in accordance with earlier observations for the crab *Eriphia* walking on a treadmill (Ballantyne & Rathmayer, 1981). The time course of the discharge frequency in the trailing situation (Fig. 5Ci) was opposite to the one observed in the leading situation (Fig. 5Cii) and different from that of the opener muscle. In trailing, the frequency started around 75 Hz at the beginning of the burst and increased up to 150 Hz. In leading the discharge frequency started at a high frequency (120 Hz) and steadily decreased to around 40 Hz, with a slight rise at the end of the burst.

Motor patterns during walking on land

In sea water, the crab's weight is about seven times less than in air (mean mass for 30 crabs: 60 g in the air, 8·5 g in sea water). The step period lasted longer in walking bouts on land than under water. Two explanations can be given: either the stepping speed slows down or the animal changes its posture to compensate for the load acting on its legs (see below).

A comparison (Fig. 6) between trailing and leading on land shows that in trailing the depressor and extensor muscles were active during the PS of the step and the flexor muscle was active during the RS. In leading the depressor muscle was still active during the PS but the flexor muscle had become a PS muscle, whereas the extensor muscle had changed to become active during the RS. Similar changes were observed when the crab walked under water (compare Fig. 6 with Fig. 3).

In all trailing and leading steps the bursts of the different muscles were well separated but differences occurred with respect to the number of active motor units. In the flexor muscle the different amplitudes suggest the activation of more than two of the four excitatory motor neurones innervating this muscle. For most steps this is in contrast to recordings from the flexor muscle obtained when the crab walked under water (compare Fig. 6A,B with Fig. 2A,B). Also, in contrast to walking under water, both excitatory motor neurones of the extensor muscle, the fast and the slow, were active during trailing and leading on land (compare Fig. 6A,B with Fig. 2A,B).

Nevertheless, the overall EMG patterns recorded on land resembled those obtained when the crab walked under water (compare Fig. 6 with Fig. 2) even though the animal often used its chelae for support while walking on land.

A study of the muscle potentials generated by the common excitatory motor neurone in the stretcher and the opener muscles was used to compare neuronal activity in different walking situations. During walking on land (Fig. 7A) and under water (Fig. 7B), simultaneous recordings were made from ipsilateral muscles: the stretcher (trace ii) and the opener (underlined, trace iii) of a trailing leg 2 and of the contralateral opener muscle (trace i) of a leading leg 2. In these recordings the activity of the antagonistic muscles (the bender, trace ii) and the closer (trace iii) can be seen from the cross-talk. A measurement of burst duration of the opener/stretcher

muscles revealed differences between trailing and leading steps in water and on land (mean burst durations for trailing on land, 704 ± 182 ms, $N = 35$; for trailing under water, 405 ± 65 ms, $N = 32$; for leading on land, 732 ± 161 ms, $N = 36$; for leading under water, 649 ± 162 ms, $N = 42$). In general, burst duration during walking under water was shorter than during walking on land. In addition, burst duration during trailing steps was shorter than during leading steps under water (see also Fig. 2C,D). On land, burst durations during both trailing and leading steps were

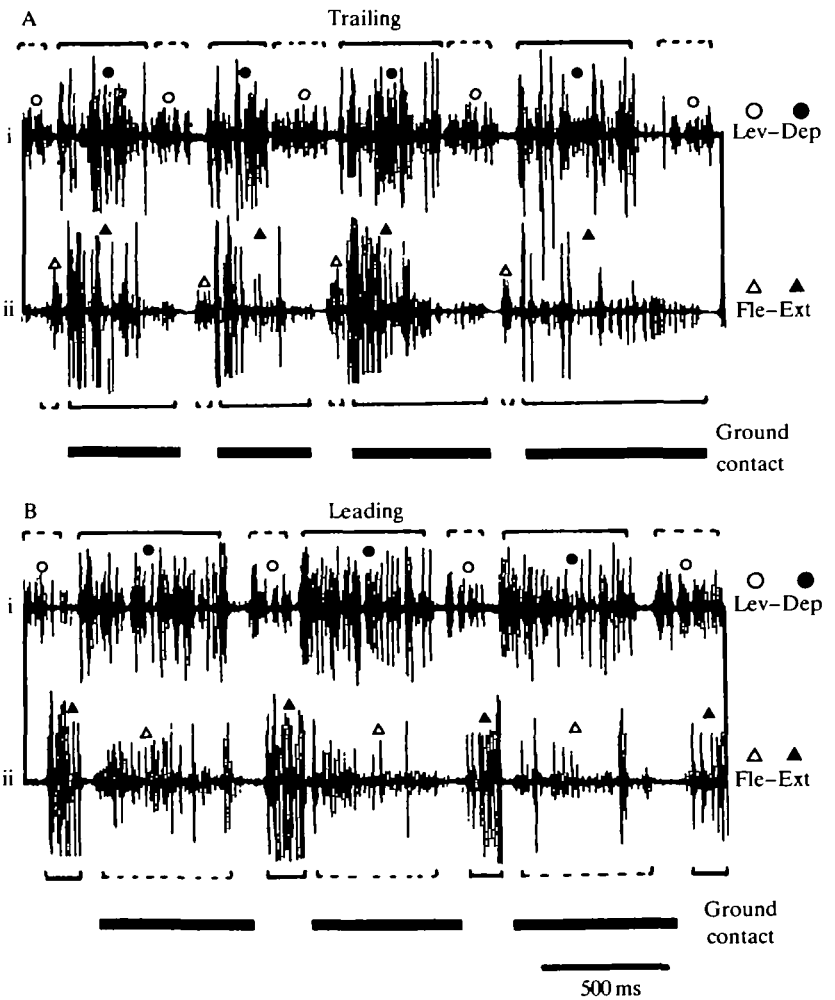

Fig. 6. Comparison of the EMG activity for a crab walking on land when the leg is involved in either a trailing (A) or a leading (B) sequence. Trace i: activity of the levator (Lev) and depressor (Dep) muscles, C–B joint; trace ii: activity of the flexor (Fle) and extensor (Ext) muscles, M–C joint. As in Fig. 2, the activity in the depressor and extensor muscles has been marked with a black circle and a black triangle and that in the levator and flexor muscle with a white circle and white triangle, respectively. Powerstroke duration corresponds to ground contact and is indicated by a thick black bar. For clarification, in each trace the burst duration is indicated by a solid or dashed fine line.

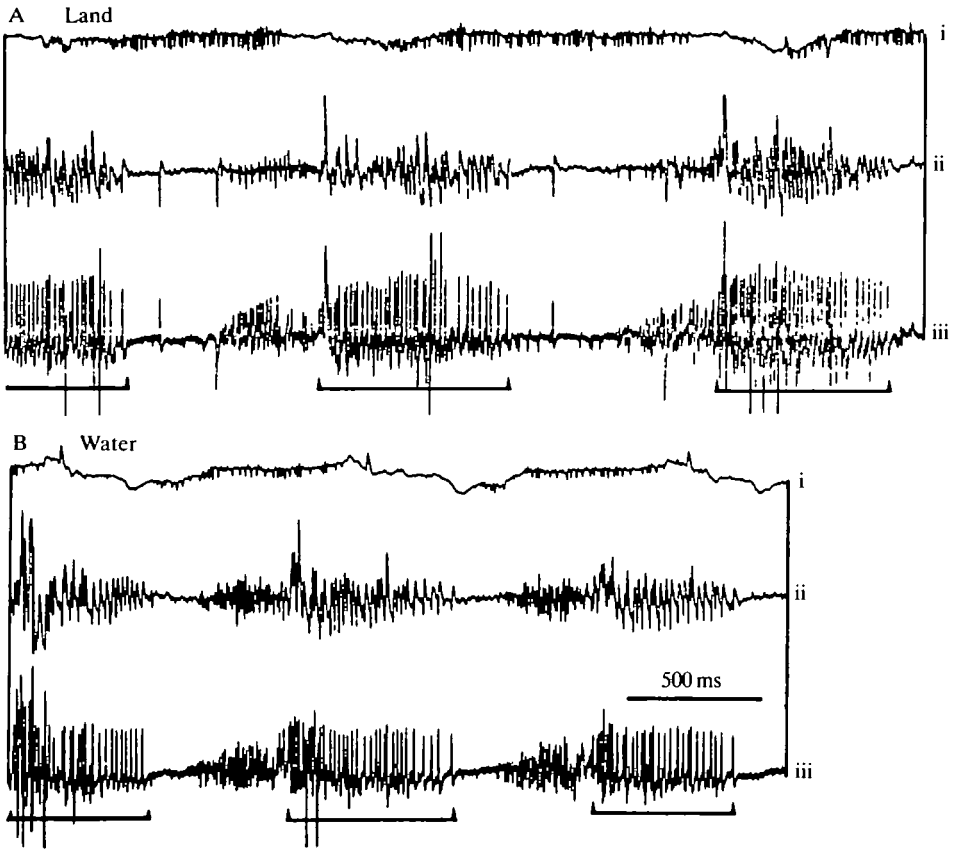

Fig. 7. Simultaneous EMG recordings from the opener and stretcher muscles of a second walking leg on the trailing side (traces ii and iii, respectively) and from the opener muscle of the contralateral leg of the leading side (trace i) during walking on land (A) and under water (B). The bars indicate the powerstroke on the trailing side.

similar. This is consistent with the observation that stepping frequency on land is lower than under water. In Fig. 8 the instantaneous discharge frequency for the motor neurone innervating the opener/stretcher muscles is plotted for eight consecutive steps on land and for six consecutive steps under water. All data are from the same animal. Due to the recording situation from opposite legs (see Fig. 7) activity from legs on the trailing and leading sides was monitored simultaneously. A great similarity can be seen in the time course of the instantaneous discharge frequency during trailing or leading steps under water and on land. In the trailing situation (Fig. 8A,B), the maximal instantaneous discharge frequency, around 90 Hz (85 Hz under water, 95 Hz on land), occurred at the onset of the burst and then decreased at the end of the muscle burst to 25 Hz under water or 30 Hz on land. In the leading situation under water (Fig. 8C) the instantaneous discharge frequency started at about 55 Hz and then decreased to 45 Hz. During leading steps on land (Fig. 8D) a slight increase in the instantaneous frequency of about 5 Hz was observed during the burst (it started at 55 Hz and ended at 50 Hz). When data from

120 steps from several animals were pooled, similar time courses for the instantaneous discharge frequency were obtained for trailing and leading steps. The difference in frequency at the onset of the opener burst between data presented in Figs 5B and 8A is due to a different experimental situation. Sequences analysed for Fig. 5B were faster, being induced by tactile stimulation of the dorsal carapace. Those presented in Fig. 8 are from spontaneous walking sequences.

Comparison of muscle activity in different walking legs

In Fig. 9 the instantaneous discharge frequency of the excitatory motor neurone to the opener muscle (solid line, Fig. 9A) is shown for walking legs 1, 2 and 3 of the

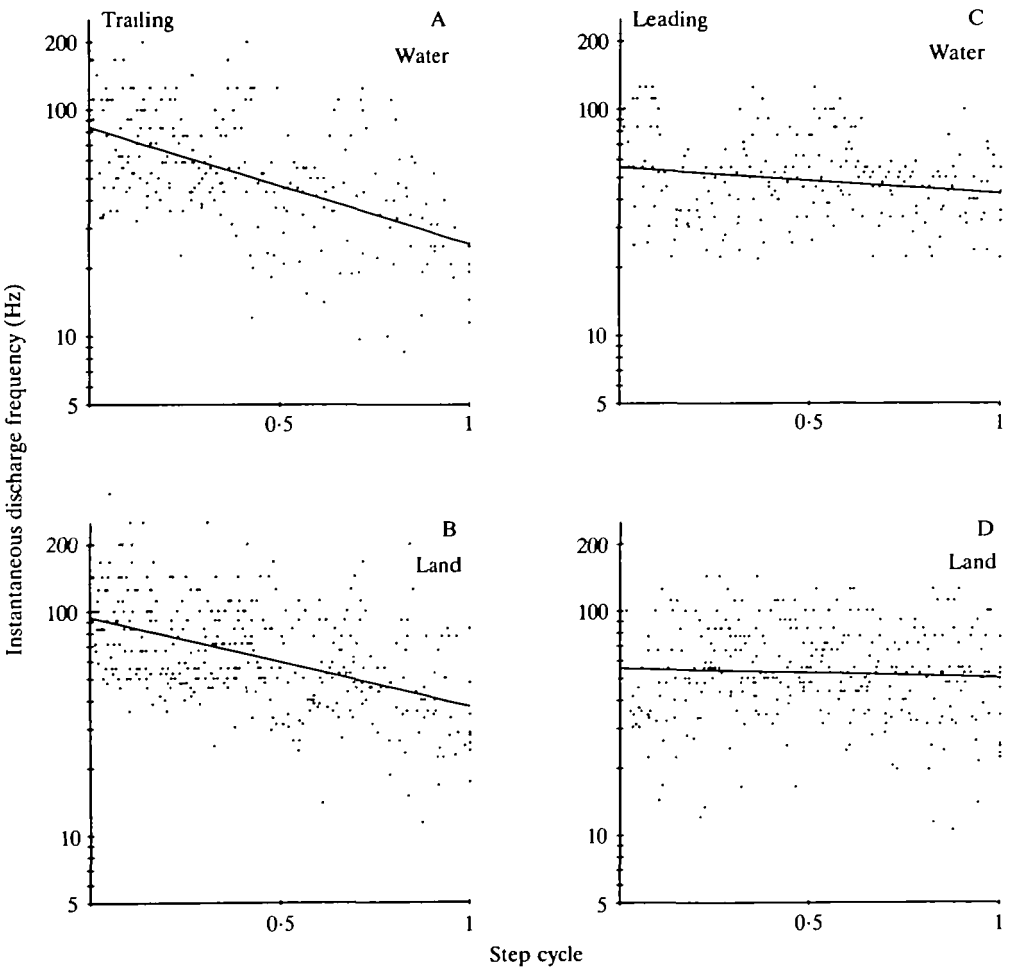

Fig. 8. Instantaneous frequency distribution of the discharge of the excitatory motor neurone innervating the stretcher muscle during normalized trailing steps under water (A) and on land (B) and normalized leading steps under water (C) and on land (D). The regression lines for each frequency distribution are given. Data are derived from consecutive steps of one animal. For details see text.

same animal during trailing steps on land. The activity of the closer muscle (dashed line, Fig. 9A) is recorded simultaneously. The recordings demonstrate that the two muscles in the three walking legs operate alternately and that there is no obvious difference in the activity pattern, although differences in burst duration occur (Fig. 9A). Usually the maximal frequency of the opener muscle occurred at the onset of the burst in all three legs (Fig. 9B–D, compare also with Fig. 8A,B). This was particularly obvious when the crab walked at slow speed, supporting the idea that the weight of the crab is equally distributed over all walking legs.

DISCUSSION

The present study deals with three new aspects of walking in the shore crab. First, EMGs were recorded from crabs walking as freely as possible in an environment which resembled the natural habitat. Second, the EMG patterns of walking behaviour under water were compared with those obtained during locomotion on land. Third, motor patterns during trailing and leading sequences were compared.

The EMGs of the muscles responsible for the movements of the trailing side consist of sharply defined high-frequency bursts with a clear alternation between antagonistic muscles operating at the same joint. This is consistent with previous cinematographic studies (Clarac & Coulmance, 1971). During the PS (stance phase) the depressor, the extensor and the opener muscles are simultaneously active, even though the onset of their bursts is not synchronized. During the RS (swing phase), the levator, flexor and closer muscles are active together, and again the activity onsets are not synchronous. The very short flexor burst is characteristic for a trailing leg and occurs at the end of the RS. However, if cinematographic recordings are analysed, the flexion of the M–C joint starts well before the activity of the flexor muscle. This suggests that, concomitant with the elevation of the leg caused by the levator muscle operating at the C–B joint and initiating the RS during the end of the PS, a passive flexion at the M–C joint occurs (cinematographic study: Clarac & Coulmance, 1971). Only the final part of the flexion movement is generated by muscular activity.

The motor patterns during leading steps are different from those during trailing steps. Passive movements due to gravitational forces could occur during leading steps at the C–B and the P–D joints. The contact of the tip of the dactyl with the substrate is short and not clearly defined during leading steps. As the crab moves, the dorsal edge of the dactyl makes contact with the ground and provides an increased surface for bearing weight in leading (Libersat *et al.* 1987). The P–D joint is closed maximally, and opens during the PS.

It has been shown previously that the frequency of discharge of motor neurones is well correlated with the force developed by the muscle (Clarac & Cruse, 1982). From the data presented here it is possible that the propulsive forces produced by the muscles of the trailing side are greater than the forces exerted by the muscles of the leading side; during walking, a crab seems rather to push itself (trailing legs) than to pull (leading legs). This type of force separation is most extreme in the ghost crab *Ocypode*, where the legs of the leading side are used only as a support and lateral

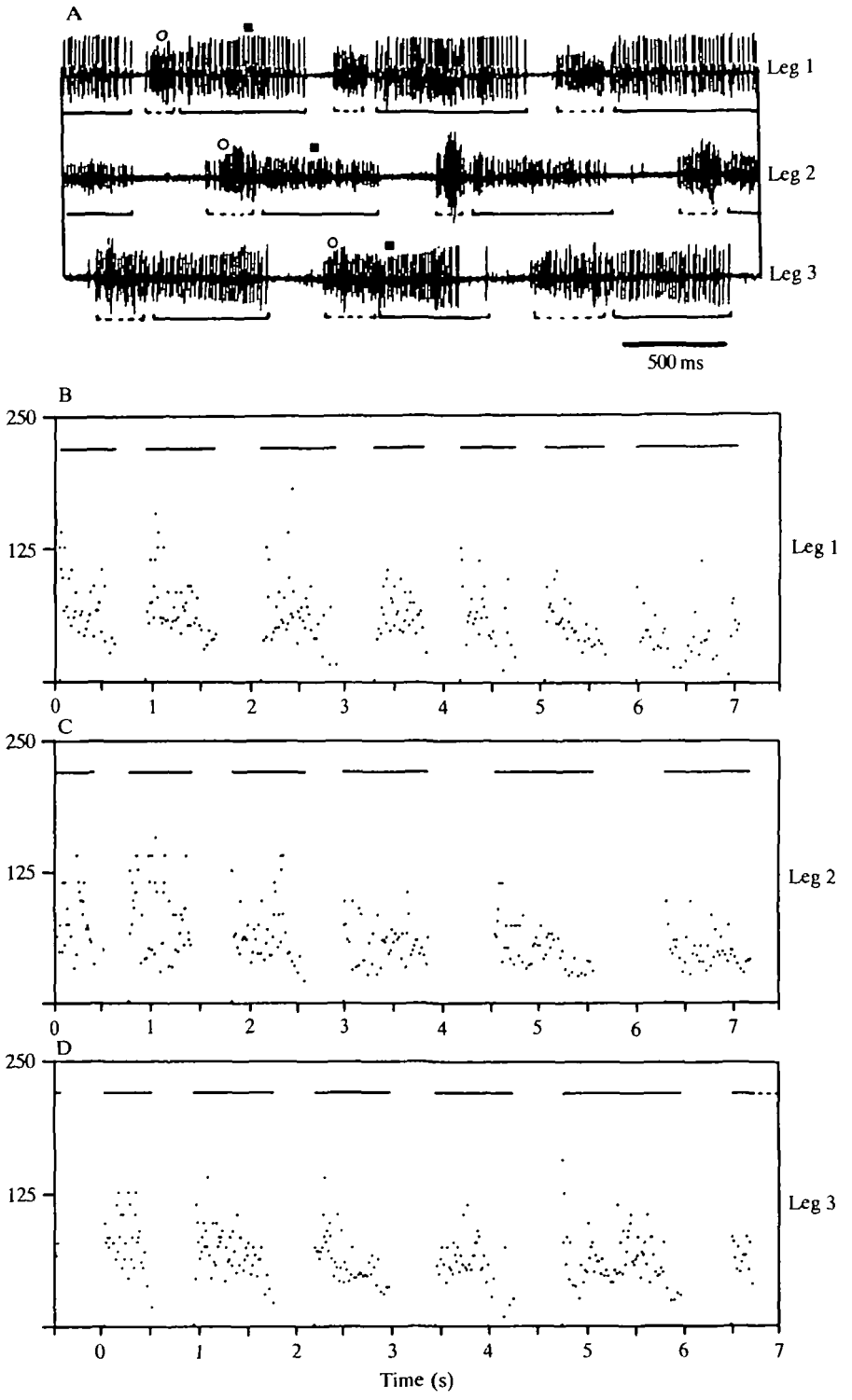
A
Leg 1
Leg 2
Leg 3
500 ms
B
250
125
Leg 1
0
1
2
3
4
5
6
7
C
250
125
Leg 2
0
1
2
3
4
5
6
7
D
250
125
Leg 3
0
1
2
3
4
5
6
7
Time (s)

Fig. 9

displacement during locomotion is achieved by the alternating activity of two legs of the trailing side (Burrows & Hoyle, 1973).

Lateral walking is a characteristic form of locomotion of brachyuran crabs. However, it also occurs in astacuran Crustacea. This aspect has been investigated in the rock lobster *Jasus lalandii* (Ayers & Clarac, 1978; Clarac, 1982) and in the lobster *Homarus americanus* (Ayers & Davis, 1977) walking on treadbands. The EMG patterns are similar to those obtained from crabs. The morphology of the legs in brachyuran decapods, however, favours lateral walking. In *Carcinus*, the P–D joint is particularly important for the development of propulsive forces during trailing. In other decapod Crustacea, such as *Astacus*, *Homarus* and *Jasus*, the dactyl is usually reduced in size compared with other leg segments and during lateral walking performs only small movements. During forward and backward walking, which are the preferential directions of locomotion in these species, the dactyl also moves only slightly and serves as a strut.

When the motor patterns during walking on land and under water are compared, the differences in the general patterns for the leading and trailing sides are maintained. A slight increase in the discharge frequency of motor neurones innervating the PS muscles, the recruitment of additional units (e.g. extensor muscle) as well as an increase in the duration of the bursts are the most prominent features during walking on land. This could compensate for the increase in load acting on the legs (Grote, 1981). Loading an animal changes the relationship between the RS and the PS within a stepping cycle (Evoy & Ayers, 1982). During walking on land the increased load supported by the legs changes the afferent activity of the proprioreceptors and thus the efferent motor control to the leg muscles. Strains and forces acting on the leg cuticle are known to affect the activity of the cuticular stress detectors (Klärner & Barnes, 1986) and of the funnel canal organ (FCO) in the dactyl of the crab (Libersat *et al.* 1987). It has been shown for the FCOs that they can contribute to equal load distribution on the different walking legs as well as to the motor control of the timing between PS and RS.

The EMGs recorded from the stretcher and opener muscles mirror the discharge of the single excitatory motor neurone innervating these two muscles. The maximal discharge frequency observed during walking reached 350 Hz, mainly during doublet discharges. High frequencies (150–250 Hz) have also been reported for the motor neurone innervating the opener and stretcher muscles of the crab *Cardisoma* walking on a treadmill (Barnes *et al.* 1972) and for the slow motor neurone innervating the closer muscle in the crab *Eriphia* (Ballantyne & Rathmayer, 1981). In the fast-running ghost crab *Ocypode*, the motor neurone to the extensor muscle fires at 300–350 Hz (Burrows & Hoyle, 1973). The frequency does not increase linearly with

Fig. 9. (A) Simultaneous EMG recordings from the opener (■) and closer (○) muscles of the walking legs 1, 2 and 3 are shown for trailing sequences when the crab walks on land. In each trace the opener burst is indicated by a solid line and the closer burst by a dashed line. (B–D) Instantaneous frequency distribution in the EMGs of the opener muscles during trailing steps of walking legs 1 (B), 2 (C) and 3 (D) on land. Black bars in each graph indicate the duration of the burst of the opener muscle in each step.

the speed of locomotion. The burst duration is inversely proportional to the walking speed.

The high discharge frequency often occurs at the onset of the muscle burst and is particularly well-suited to enhance the efficiency of neuromuscular transmission at muscle fibres, where the excitatory junctional potentials are small in amplitude upon low-frequency discharge of the motor neurone, but facilitate to large amplitudes at high frequencies (Atwood & Bittner, 1971; Sherman & Atwood, 1972; Rathmayer & Hammelsbeck, 1985) or with patterned rhythmic discharge of the motor neurone (type II muscle fibres: Rathmayer & Erxleben, 1983; Rathmayer & Maier, 1987). The observed high discharge frequency of the slow motor neurone to the closer muscle during trailing steps results in a fast closing movement of the dactyl and demonstrates the difficulties often arising from the terms 'fast' or 'slow' for motor neurones in arthropod neuromuscular systems.

The innervation of the stretcher and opener muscles through a common excitatory motor neurone seems to be a strange evolutionary quirk. The lack of separate excitors is compensated by a specific inhibitory motor neurone innervating each muscle, which is used to decouple the two muscles from common excitatory input (Wiens & Rathmayer, 1985). In the present paper it has been shown that these two muscles indeed receive identical excitatory input. Thus it is unlikely that axonal conduction block at the M–C joint, as observed in isolated preparations of the crayfish upon repetitive stimulation (Hatt & Smith, 1975; Smith & Hatt, 1976), occurs in the crab and that this effect could be important for differential impulse channelling to the stretcher and the opener muscles *in vivo* (Hatt & Smith, 1975).

The rhythmicity found in the discharge of the motor neurone innervating the stretcher and opener muscles is understandable when the function of the opener muscle during lateral walking is regarded. The P–D joint undergoes excursions of up to 60°, while the C–P joint displays little displacement except in the first walking leg (less than 20°) during lateral walking. The activation of specific inhibitory neurones has been shown to be centrally linked to the activation of the excitors to the antagonistic muscles (Wiens, 1976; Wiens & Atwood, 1978). The role of the specific inhibitors and the importance of the reciprocal activation of the bender and the stretcher muscles, however, need further experimentation.

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