J. exp. Biol. 110, 319–322 (1984)
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SHORT COMMUNICATIONS

A NEW METHOD MEASURING LEG POSITION OF WALKING CRUSTACEANS SHOWS THAT MOTOR OUTPUT DURING RETURN STROKE DEPENDS UPON LOAD

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Accepted 6 January 1984

The basic movement of a walking leg consists of two parts, the power stroke (stance phase) and the return stroke (swing phase). The movement of a leg during the power stroke is determined not only by the sensory-neural system of the leg itself but also by the movement of the other supporting legs because of their mechanical coupling. In contrast the movement during the return stroke is under the sole control of the sensory-neural system of the leg itself. Therefore observation of the return stroke movement allows a more direct view of the motor output from the centre controlling the movement of the leg. Motor output can be recorded by electrophysiological methods. However, quantitative interpretation of these results is often difficult as the transformation of the recorded spikes (often from several superimposed motor units) to force is usually unknown. Whereas motor output during the power stroke clearly depends on walking speed and on load, some studies have found return stroke duration to be dependent upon walking speed, others found no such dependence (for reviews see Clarac, 1981; Evoy & Ayers, 1982). A solution to this discrepancy has been proposed on the basis of a model which assumes that load is an essential parameter affecting the movement of the individual walking leg (Cruse, 1983). A central assumption in this model is that motor output during both power stroke and return stroke is increased when the animal walks under load. It follows from this assumption that an increase in the load should lead to faster return stroke movements and, for constant leg amplitudes, to shorter return stroke durations. As mentioned above this prediction can be easily tested for the return stroke. Therefore, we developed a simple method to measure return and power stroke durations and step amplitude for a decapod walking under water.

The crayfish (*Astacus leptodactylus*) walked on a rubber belt driven by a motor (Ayers & Davis, 1977; Chasserat & Clarac, 1980) and was fixed on a holder at the carapace. The holder was counterbalanced and allowed the animal to choose its own distance to the ground. Different weights were fixed to the holder in order to change the vertical load under which the animal walked. Preliminary experiments showed that coordinated walking and clearly defined return stroke movements could only be

Key words: Crayfish, walking, motor output, load.

obtained for applied weights in the range from 5 g to $-3{\cdot}5$ g (an applied load of zero means that the holder without the animal was in balance, i.e. the animal carried its own weight; positive values mean increased load) and treadmill speeds between 9 cm s^{-1} and 14 cm s^{-1}. (When free in the aquarium animals walk at a speed of about 5–10 cm s^{-1}.)

The position of the walking legs was recorded in the following way. A thin copper wire (diameter, 0·1 mm), isolated but for the tip ('recording electrode'), was fixed by small pieces of adhesive tape on each leg to be measured. The tip of the wire was placed at the middle of the dactylopodite. The animal walked in an electrical field arranged parallel to the long axis of the body. This field was formed by two large grids (40 × 10 cm) positioned under water at the front and back end of the treadmill. These two field electrodes were connected to a sine wave generator producing alternating current (to avoid electrolysis) of 1 V and 10 kHz. The recording electrodes on the legs of the animal served as the third electrode of a potentiometer which records a voltage proportional to the position of the recording electrode relative to the grid electrodes. In order to obtain a d.c. signal corresponding to leg position, each recording electrode was connected to a rectifier and a low pass filter (corner frequency 1 kHz) to remove the high carrier frequency. This method produces completely linear position signals parallel to the long axis of the body and allows simultaneous recording with as many electrodes as desired. Marrelli & Hsiao (1976) proposed a similar method to measure the movement of a single leg joint. Our method is more appropriate for measuring movements of several legs in studies of coordination. Position signals from the legs were recorded on a multi-channel pen recorder (Fig. 1) and then analysed with a graphic-tablet connected to an Apple microcomputer.

With this method, the duration and amplitude of both power stroke and return stroke were measured. This was done for legs 3 and 4 for forward walking for three different treadmill speeds (9, 11 and 14 cm s^{-1}) and for three different load values (5, 0 and $-3{\cdot}5$ g). Initially, the animal was adapted to the experimental situation with a pre-test walk of about 30 min at a constant speed and zero load. The test procedure was as follows. First, about 100 steps were recorded. Next, the two non-zero load

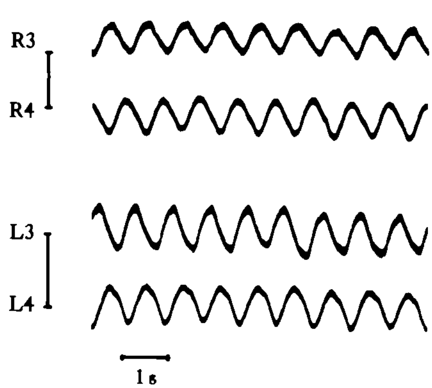

Fig. 1. An example of a record of four legs. Return stroke movement is upwards. L, left; R, right legs; legs are numbered from front to rear. Left and right legs were recorded with different gain; vertical bars indicate 5 cm. Treadmill speed, 11 cm s^{-1}; load, 0 g.

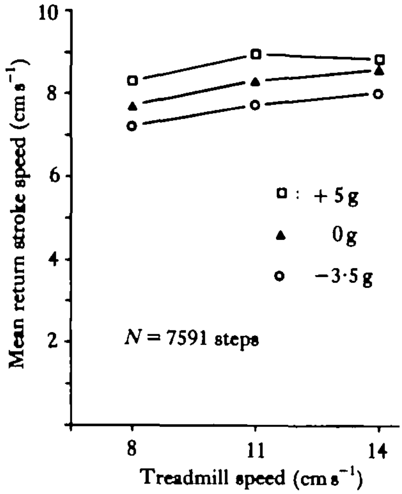

Fig. 2. Mean return stroke speed of leg 3 for different walking speeds and different vertical loads. All differences for a given walking speed are significant ($P < 0{\cdot}1\,\%$; Behrens-Fisher test). Standard deviations range from $\pm 1{\cdot}13$ to $\pm 1{\cdot}59\,\mathrm{cm\,s^{-1}}$.

values were applied consecutively and another 100 steps were recorded for each. Finally, the animal was again unloaded and a further 100 steps were recorded. This procedure was repeated at least three times for all four animals and all three walking speeds tested. Thus, nine experimental conditions (three load values × three walking speeds) were tested.

The intention was to use only return stroke duration as a measure for the strength of motor output. The results showed that step amplitude was not constant in all experimental situations. Therefore as a second measure for the strength of motor output the mean return stroke speed (= step amplitude/return stroke duration) was calculated. For leg 3, the step amplitude consistently increased with load but was nearly constant for the three walking speeds tested. Return stroke duration decreased with both increasing speed and increasing load. This is in full agreement with the predictions of the model calculation. The dependence of motor output upon these two parameters was even more obvious in the mean return stroke speed (Fig. 2). For both return stroke duration and mean return stroke speed no further change was observed for the highest walking speed and the highest load value.

For leg 4 the values of step amplitude showed considerable scatter in the nine experimental conditions. Therefore the two measurements – 'return stroke duration' and 'mean return stroke speed' – gave different results. For return stroke duration, the results agreed with the model predictions in eight out of nine cases. They did not agree in the case of the highest load value and the highest walking speed. For mean return stroke speed, two results contradicted the prediction (load 5 g, walking speed 11 cm s^{-1} and 14 cm s^{-1}). Altogether, in only one or two (depending on the measure used) of 18 experimental series (nine conditions for the two legs) did the results differ from the model predictions. These deviations occurred: (a) only in leg 4, which also showed much less regular stepping behaviour, and (b) only for high parameter values. Therefore, the results are considered to support the predictions of the model calculation.

From electrophysiological investigations, different results are described in the literature concerning the influence of load on the motor output during return stroke. No such influence was found by Grote (1981) when comparing crayfish walking under water and on land. Barnes (1977) found an increase in spike frequency and a shorter burst duration when the animal was loaded. Our behavioural results agree with the latter. However, both authors allowed their animals to choose the walking speed freely and made no direct comparison for identical walking speeds. In the crab *Cardisoma guanhumi* Evoy & Fourtner (1973) found that the return stroke duration of loaded animals was longer than that of unloaded animals. However, loaded animals walked at different speeds from unloaded ones. Because return stroke duration and walking speed may be correlated (see MacMillan, 1975) it is possible in these investigations that load did not influence the return stroke duration directly but only *via* changes in walking speed. This possibility is excluded for our experiments as we compared results obtained from walks with constant speed.

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