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HAEMOLYMPH FLOW DISTRIBUTION, CARDIAC PERFORMANCE AND VENTILATION DURING MODERATE WALKING ACTIVITY IN *CANCER MAGISTER* (DANA) (DECAPODA, CRUSTACEA)

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Summary

Adult male *Cancer magister* (Dana) were equipped with pulsed-Doppler flowmeters and pressure transducers for simultaneous measurement of heart and ventilation frequencies, haemolymph flow through each of the major arterial systems and cardiac output and for calculation of stroke volume. Each variable was measured at rest and during two consecutive periods of moderate treadmill walking activity and recovery. During activity, haemolymph flow through the sternal and anterolateral arteries increased, while flow through the hepatic arterial system decreased. This resulted in a redistribution of haemolymph flow in which a proportion of cardiac output was shifted from the anterior, posterior and hepatic arterial systems to the sternal arterial system. The relative proportion of the cardiac output flowing through the anterolateral artery remained constant. This indicated that oxygen supply was shifted away from the digestive system to the muscles of the walking legs and the respiratory system. Cardiac output, heart rate and stroke volume all increased in response to activity. The increase in cardiac output is the result of a large increase in stroke volume and a small increase in heart rate. A doubling of ventilation rate also occurred during activity. Both the circulatory and ventilatory systems were restored to pre-activity values by 60 min of recovery.

Key words: *Cancer magister*, crustacean, activity, circulation, cardiac output, heart rate, blood flow, ventilation, pulsed-Doppler flowmeter

Introduction

Satisfying the increase in oxygen demand associated with increased activity involves responses in both ventilatory and circulatory systems (for reviews on crustaceans, see McMahon, 1981; McMahon and Wilkens, 1983; Vernberg, 1983). Three main strategies are known to be involved. First, increases in scaphognathite performance increase ventilation volume and hence oxygen supply to the gills. Second, increases in gill perfusion increase oxygen transport away from the gills. Third, modification of the oxygen-binding properties of the haemocyanin increases the P_{O_2} gradient across the gills (McMahon *et al.* 1979; McMahon and Wilkens, 1983). Very few studies have focused on the effects of activity on the circulatory performance of crustaceans.

The circulatory system of decapod crustaceans consists of a single ventricle which pumps haemolymph through five arterial systems: anterior, anterolateral, hepatic, posterior and sternal (Maynard, 1960). Each artery originates at a muscular, independently innervated cardioarterial valve (Alexandrowicz, 1932; Kuramoto and Ebara, 1989). These valves respond to both neural and neurohormonal stimulation (Kuramoto and Ebara, 1984; Kuramoto *et al.* 1995). The arteries, which lack smooth muscle control (Shadwick *et al.* 1990), perfuse the tissues through capillary-sized vessels which empty into tissue lacunae. The return pathway is through venous sinuses into the pericardial cavity.

Insight into the control of the crustacean open circulatory system has increased rapidly during the last decade, with the demonstration of precise regulatory mechanisms at several levels (for reviews, see McMahon and Wilkens, 1983; McMahon and Burnett, 1990). For example, the availability of micro pulsed-Doppler flowprobes now allows the study of simultaneous changes in haemolymph flow through each of the different major arterial systems in crustaceans exposed to environmental or metabolic changes. An advantage of the pulsed-Doppler technique is that it results in a much lower level of disturbance and stress to the animal (Airriess *et al.* 1994). The resulting direct measurements of cardiovascular performance allow us to study the fine regulation of the

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crustacean circulatory system in resting undisturbed animals and at various levels of activity.

The hypotheses advanced at the beginning of this study were as follows: that crabs exposed to moderate walking activity would increase cardiac output; that this increase would be attained mainly by an increase in stroke volume; and that haemolymph supply to the locomotor muscles would increase preferentially over that to other body regions by adjustment of flow into specific arterial systems.

Materials and methods

All experiments were carried out at the Bamfield Marine Station, British Columbia Canada. Adult male *Cancer magister* (Dana) (N=10, wet mass 720±143 g, mean ± S.E.M.) were purchased from local suppliers and held at low densities in tanks with aerated, running, natural sea water. Water temperature in the holding tanks over the experimental period (February to May) was 12±1 °C and salinity was 32±2 ‰. The crabs were held for 2 weeks before experimentation to ensure good health. Crabs were normally fed twice a week on chopped mussels but were starved for 2 days before and during experimental recordings.

Arterial haemolymph flows were assessed using a pulsed-Doppler flowmeter (University of Iowa, Bioengineering) by the method described by Airriess *et al.* (1994) and by Airriess and McMahon (1994). Airriess *et al.* (1994) verified these techniques for accurate measurement of blood flow in equivalent-sized *C. magister* and showed a very high correlation between actual flow and perfusion rate (r^2=0.996). In short, this method is as follows. Flow within two arterial systems (anterolateral and anterior) was assessed using a non-invasive technique, whereby piezoelectric crystal transducers (1 mm^2, Tritonics Medical Instruments) were fixed in position directly above the desired artery after a small part of the carapace had been removed without penetrating any dermal tissue. The sternal, hepatic and posterior arteries are located more deeply. In these cases, haemolymph flow was measured using implanted transducers. The transducers were guided into position adjacent to the arteries by polyethylene tubing (o.d. 0.9 mm, i.d. 0.6 mm). Transducer and leads were passed through the catheters before insertion. In the case of the sternal artery, the catheter passed through the body wall of the first abdominal somite and was guided anteriorly along the median groove of the sella turcica and the median plate of the endophragmal system. In the case of the hepatic artery, the catheter passed through the dorsal body wall 1 cm anterior of the pericardial cavity and between the anterior and anterolateral arteries and was directed vertically downwards. In the case of the posterior artery, the catheter passed through the body wall of the first abdominal somite. Haemolymph loss was prevented by inserting the catheter through latex dental dam glued over the whole site. The probes were held in place with cyanoacrylate glue and dental periphery wax. Measurement of centre-stream velocity was accomplished by lateral positioning of the probe in combination with vertical focusing of the Doppler signal.

Ventilation frequency was measured using pressure transducers (Statham Gould PB23Db) connected with the epibranchial chambers through seawater-filled 2 mm diameter tubing (after McDonald, 1977). Ventilation frequency was determined by counting the peaks in the ventilation pressure waveform, which correspond to scaphognathite movement. Animals survived the experiments for at least 4 weeks, after which they were released in the wild.

Flowmeter output for each artery and left and right ventilation pressures were recorded continuously using a computerized data-acquisition system. Raw data were sampled at 100 Hz per channel by a data-acquisition board (National Instruments ATMI016X), displayed in real time and simultaneously stored to hard disk. The computer program was developed in-house using the Labview data-acquisition package (National Instruments). Data were filtered using a median filter (rank=8) to improve the signal-to-noise ratio. These filtered data were then used to calculate instantaneous haemolymph flow. Since the arterial radii of crabs showed no statistically significant correlation with the mass of the animals over the limited size range used, the following mean arterial radius values were used (I. J. McGaw, unpublished results): anterior 0.58 mm; posterior 0.61 mm; hepatic 0.81 mm; anterolateral 0.74 mm; sternal 1.56 mm (N=30). The mean flow of each arterial system was calculated for each 5 s interval and formed the basis for subsequent analysis. Cardiac output was calculated as the sum of all arterial flows. Heart rate was determined by counting the peaks in the flow records and stroke volume was calculated by dividing cardiac output by heart rate.

Statistical analyses consisted of repeated-measures analysis of variance (ANOVA) and contrast analysis. Differences were considered significant at P<0.05.

After placement of the probes, which involved approximately 60 min of handling in a shallow seawater-filled pan, animals were allowed to recover for approximately 18 h from handling and surgery in an aquarium with a thick sandy bottom. Animals were placed in the treadmill 4 h before experimental recording. Experimental recording periods lasted 120 min. Baseline measurements were recorded for the first 10 min. The animals were then forced to walk on a rotating treadmill at constant speed (3.5 m min^{-1}) for 10 min. After 30 min of recovery, animals were forced to walk for another 10 min. Recovery was monitored during the following 60 min.

The treadmill consisted of a round plastic children's swimming pool (1.7 m diameter and 0.25 m high) mounted on a rotating disk, which was driven by a slow motor (27.6 revs min^{-1}). Inside the pool, a runway (0.25 m wide) was created by mounting an inner wall parallel to the outer wall of the pool. This prevented the animal from escaping sideways. Linear speed in the middle of the runway was 3.5 m min^{-1}. The bottom of the runway was roughened to increase grip for the crabs' legs, using silicone rubber and sand. To minimise the need for manual stimulation, a cage, 0.8 m long and without a

bottom, was hung in the runway. To encourage continual movement, one end of the cage was darkened using black plastic. The animals showed a tendency to remain under the darkened area and away from the other end of the cage. Continuous inflow of vigorously aerated fresh sea water ensured constant water temperature (12 °C) and aeration.

Results

Arterial haemolymph flow

Moderate treadmill activity (10 min) affects haemolymph flow rates through several arterial systems (Fig. 1). Flow through the sternal and anterolateral arteries increased significantly (250–300 % and 100 % respectively, $P<0.05$) during both activity periods. The increase in flow during the second period of activity appeared to be slightly greater, but the differences were not significant ($P>0.05$). Haemolymph flow in the sternal artery decreased the moment walking ceased and returned slowly towards initial values, but did not return completely to control levels during the first 30 min recovery period. After the second (60 min) recovery period, haemolymph flow had returned to levels not significantly different from control values ($P>0.05$). The decrease in haemolymph flow in the anterolateral artery was delayed until 10 min after walking stopped. As for the sternal artery, complete recovery required 60 min.

Haemolymph flow through the hepatic artery showed the opposite response, decreasing drastically during activity ($P<0.05$). A decrease in variability was also observed at this time. Hepatic artery flow was re-established during recovery. A transient overshoot, compared with the control values, occurred in both recovery periods but was most pronounced during the second period. As previously, control values were not reached until the end of the second recovery period (60 min).

Haemolymph flow in the sternal, anterolateral and hepatic arterial systems responded rapidly at the onset of enforced activity, reaching the respective maximum or minimum values within 2 min after the onset of walking. Flow in the hepatic and sternal arteries, but not in the anterolateral artery, also responded quickly on cessation of activity. Flow through the anterior artery decreased slightly over the whole experimental period, while haemolymph flow through the posterior artery was highly variable, both over time and between different animals. Flow in the posterior and anterior arteries showed no clear relationship with walking activity.

The relative importance of each arterial system during control and activity periods can best be compared in terms of the percentage of cardiac output passing through that system (Fig. 2). The sternal artery delivers the majority of the haemolymph during rest and activity, but the proportion

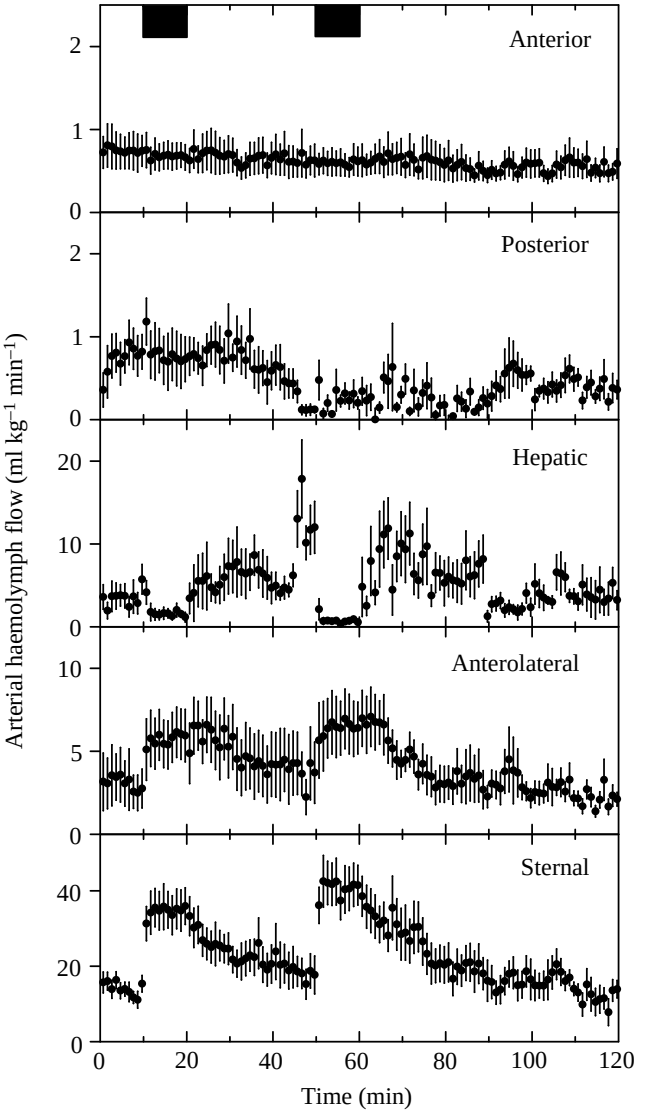

Fig. 1. Variation in mean arterial flow, measured using pulsed-Doppler probes, in each arterial system for *Cancer magister* over the whole experimental period. The black boxes at the top indicate periods of treadmill walking. Data are given as means ±1 S.E.M. (N=10) over 1 min periods.

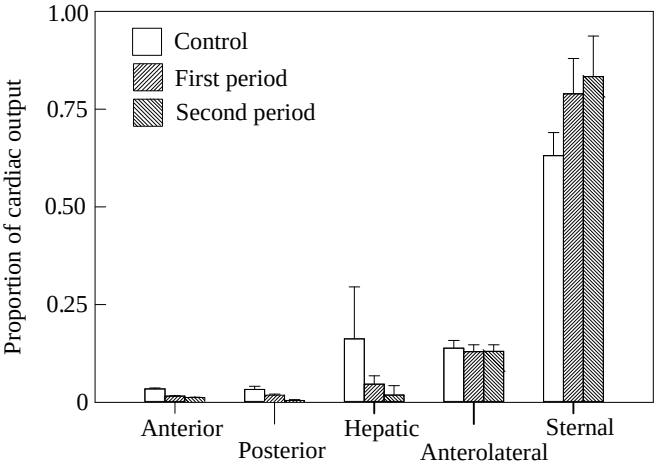

Fig. 2. Changes in the proportion of the cardiac outflow of *Cancer magister* into each arterial system during the control period (first 10 min, open bars) *versus* the proportion observed during the first and second 10 min periods of activity (hatched bars). Data are given as means +1 S.E.M. (N=10).

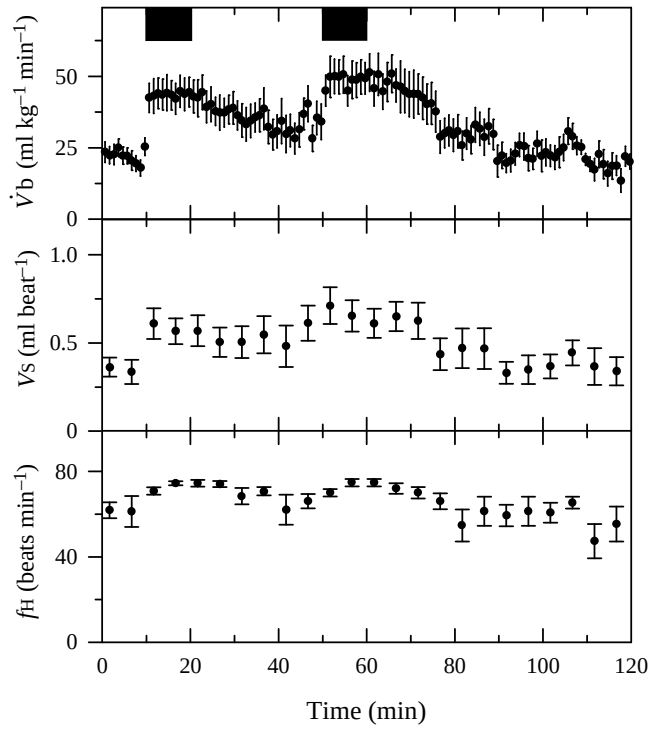

Fig. 3. Changes in cardiac output ($\dot{V}$b), stroke volume (VS) and heart rate (fH) as a function of time for *Cancer magister*. The black boxes at the top indicate periods of walking. Data are given as means ±1 S.E.M. (N=10) over 1 min periods.

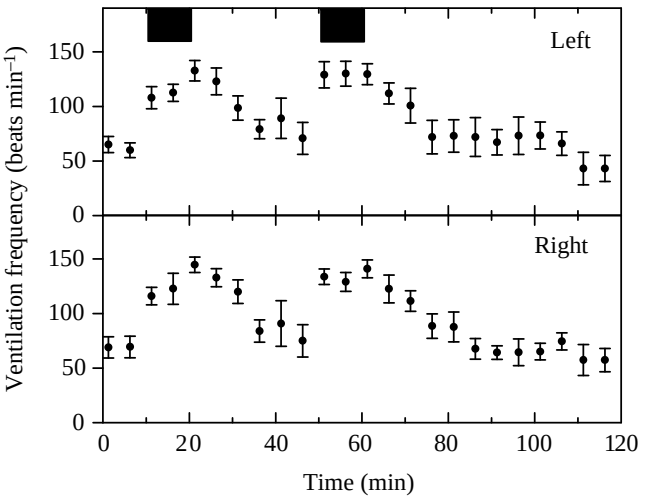

Fig. 4. Changes in ventilation frequency in the left and right branchial chambers of *Cancer magister* as a function of experimental time. The black boxes indicate periods of walking. Data are given as means ±1 S.E.M. (N=10) over 10 s.

delivered during activity is also significantly elevated (P<0.05). The relative importance of haemolymph flow through the anterolateral artery did not change significantly with activity (P>0.05), while the proportion of cardiac output through the hepatic artery decreased (P<0.05). The decrease in the proportion of the cardiac output flowing through the hepatic arterial system accounts for the majority of the increase through the sternal system. The proportion of the cardiac output passing through the anterior arterial system was small, but also appeared to decrease during activity. Although no clear relationship was found between posterior haemolymph flow and activity, the proportion of the cardiac output delivered by the posterior artery showed a significant decrease during the second period of activity (P<0.05).

Heart performance

Cardiac output increased significantly during activity (Fig. 3) (P<0.05). In general, the time course for the cardiac output response was similar to that for the sternal artery (Fig. 1) and complete recovery was reached only at the end of the second recovery period (60 min of recovery). Heart rate and stroke volume also increased significantly with activity (Fig. 3) (P<0.05). The increase in the heart rate was relatively small (17 %), but stroke volume almost doubled. Both heart rate and stroke volume returned to control values after 60 min of recovery but each followed a different time course. Heart rate showed a tendency to react more slowly and the increased rate persisted into the recovery period (Fig. 3). The time course for change in stroke volume was much more similar to the pattern for cardiac output, with sharp changes occurring at the onset of activity.

Ventilation

Scaphognathite (ventilatory) rate also increased significantly (100 %) (P<0.05) during activity (Fig. 4) and returned to control values only at the end of the second recovery period. Although the increase in scaphognathite frequency was of similar magnitude to that seen for cardiac output, the time course was more similar to that observed for heart rate (Fig. 3). Ventilatory rate increased progressively during activity and reached its peak at the beginning of the recovery period.

Discussion

Moderate treadmill walking increased cardiac output and changed the distribution of haemolymph flow in *C. magister*, confirming the hypothesis that active regulation of haemolymph flow and distribution occurs during activity in brachyuran decapods. During each 10 min period, haemolymph was diverted away from the regions supplied by the hepatic, anterior and posterior arteries and into the sternal artery. The proportion of the cardiac output perfusing regions supplied by the latter artery thus increased dramatically. Perfusion of the regions supplied by the anterolateral arteries increased only in proportion to the increase in cardiac output. Flow through the anterior and posterior arteries showed no correlation with activity.

The sternal arterial system perfuses both the ventral nerve cord and the muscles of most appendages, including both the walking legs and the scaphognathites (Pearson, 1908; B. De Wachter, C. N. Airriess and I. J. McGaw, unpublished data). The additional

Table 1. *Comparison of cardiac performance parameters, measured using pulsed-Doppler flow probes, under three different experimental treatments in the crab* Cancer magister

	Activity		P_{O_2} (kPa)		Temperature (°C)		
	Rest	Walking	18.17	2.96	4	12	20
Heart rate (beats min^{-1})	60.7±3.4	72.0±2.7	74.5±2.6	38.2±4	37.8±1.2	73.2±3.6	110.4±4.8
Cardiac output (ml kg^{-1} min^{-1})	22.9±3.4	43.5±3.4	9.8±1.6	11.9±1.2	8.56±3.93	9.95±1.79	13.21±2.31
Stroke volume (ml $beat^{-1}$)	0.36±0.02	0.62±0.03	0.15±0.03	0.41±0.06	0.22±0.10	0.13±0.02	0.12±0.02
References	This study		Airriess and McMahon (1994)		De Wachter and McMahon (1995)		

haemolymph diverted through this network could serve to supply the increased oxygen demand expected in these structures during walking. This may be particularly important in the case of the scaphognathites, which beat twice as fast during walking activity and have highly vascularised muscles. Much of the additional sternal arterial flow was diverted from the hepatic arteries, which perfuse the hepatopancreas and other alimentary structures. In fact, flow through this system virtually ceased during walking, falling to below 2% of cardiac output. Flow through the anterolateral arteries increased during walking but only in proportion to the increase in cardiac output. These arteries perfuse many visceral structures in the anterior cephalothorax, including the antennary glands, the suboesophageal ganglion, the antenullae, the antennae and the base of the eyestalks (B. De Wachter, C. N. Airriess and I. J. McGaw, unpublished results).

Similar redirection of flow away from the hepatic arteries and into the sternal system has been reported in hypoxic *C. magister* (Airriess and McMahon, 1994). In contrast, no redirection of haemolymph flow was induced during temperature change (De Wachter and McMahon, 1995). A comparison of these three studies on cardiovascular performance in *C. magister* reveals interesting differences in cardiovascular responses to variation in temperature, activity and hypoxia (Airriess and McMahon, 1994) (Table 1). In each case, readjustment of cardiac performance and/or haemolymph flow is needed to deliver sufficient oxygen. Cardiac output increased in response to all treatments, but the relative contributions to this increase of heart rate and stroke volume differed markedly. Heart rate increased principally in response to increased temperature, less in response to activity and decreased in response to hypoxia. Stroke volume, in contrast, increased greatly during hypoxia and moderately during activity but decreased in response to increased temperature. Temperature variation may act primarily on the central nervous system (CNS) or cardiac ganglion to alter heart performance. No alteration in the distribution of haemolymph flow is needed since the basal metabolic rate of all tissues increases similarly. The data for other decapod crustacean species are fragmentary (McMahon, 1992; McMahon and Burnett, 1990; McMahon and Wilkens, 1983; Reiber *et al.* 1992) but generally support these conclusions. Taken together, these data strongly suggest that heart rate and stroke volume are controlled separately, with heart rate changing largely in response to primary stimuli such as temperature and stroke volume responding more closely to variation in metabolic demand.

The mechanisms involved are not fully understood, but neural stimulation of cardioregulatory nerves increases heart rate but not stroke volume in crayfish (Wilkens and Walker, 1992), and heart rate increases during walking activity in lobsters largely under the control of the cardioaccelerator nerves (Guirguis and Wilkens, 1995), while peptide hormones such as proctolin are more effective in boosting stroke volume rather than heart rate in crabs (McMahon, 1992; McGaw *et al.* 1994). Clearly, adjustment of the balance between neural and neurosecretory stimulation could duplicate the responses noted here.

Regional distribution of cardiac output *via* specific arterial systems also differs between experimental treatments. No change is seen in response to temperature, but in both activity and hypoxia, flow through the sternal artery increases at the expense of flow to anterior and visceral structures. Flow through the anterolateral artery responds differently, increasing in proportion to cardiac output in response to activity and temperature change but decreasing during hypoxia (Airriess and McMahon, 1994).

Bourne and McMahon (1989) provided preliminary evidence for shunting of haemolymph between arterial systems during activity in *C. magister*. These authors showed increased flow through the anterior (ophthalmic) aorta, while the flow through the anterolateral (antennary) artery decreased. Flows through the sternal artery and hepatic arteries were not assessed. The differences seen between this and the present study result from the different type (struggling) and very short duration (30 s) of their activity stress, and thus could be more related to behavioural than to metabolic factors.

The redirection of haemolymph flow observed probably results from neuronal and/or neurohormonal control of the cardioarterial valves at the origin of each artery (Alexandrowicz, 1932). Experiments with isolated decapod crustacean hearts show differential responses of the anterior and posterior cardioarterial valves to neural stimulation and to amine and peptide neurohormones (Kuramoto and Ebara, 1984; Kuramoto *et al.* 1995; Wilkens, 1995; Fyjiwara-Tsukamoto *et al.* 1992). More directly, infusion of cardioactive peptides *in vivo* results in redirection of flow between arterial systems in both the crab *C. magister* (McGaw *et al.* 1992, 1994) and the lobster *Homarus americanus* (McMahon and Reiber, 1991; McMahon, 1992). Infusion of proctolin, for instance, mimics many of the responses seen during both activity and hypoxia (McGaw *et al.* 1992, 1994; McMahon, 1992) and may be involved in the natural response.

Limited data for the effects of activity on this crab and other larger decapods are available in the literature. These studies focused on respiratory responses to activity and were limited to indirect estimates of most circulatory variables. McMahon *et al.* (1979) studied responses to enforced exhaustive activity stress in *C. magister*. Despite the much more strenuous activity regime in this earlier study, overall responses were very similar to those reported here. Both studies showed a doubling of ventilation rate after activity. The increases in heart rate were almost identical. Cardiac outputs and stroke volumes, estimated indirectly *via* the Fick principle, also increased but the magnitudes of the changes were greater in the earlier study.

Comparison of measured cardiac performance values from the two studies reveals a major difference. The crabs in the present study had slightly higher heart rates, as would be expected from the higher ambient temperature (12 *versus* 8 °C) but the resting values for stroke volume (0.36 *versus* 1.6 ml $beat^{-1}$) and cardiac output (22.9±3.4 *versus* 72±25 ml kg^{-1} min^{-1}) were markedly lower. Similarly low direct measurements for cardiac output in *C. magister* are presented in Airriess and McMahon (1994) and McGaw *et al.* (1994). The difference probably stems from the different methodologies used. The accuracy of the pulsed-Doppler technique for crab arteries has been verified (Airriess *et al.* 1994). Utilisation of the Fick principle involves considerable disturbance to the animals, with intermittent handling and haemolymph sampling and constant disturbance from the ventilatory mask needed to assess oxygen consumption. These conditions would certainly have increased cardiac output (McMahon and Wilkens, 1983) and, following the reasoning above, this increase would occur principally through an increase in stroke volume. It thus seems likely that the use of the Fick principle or other indirect methods for calculating stroke volume and cardiac output in the crab may lead to substantial overestimates.

Several studies have examined respiratory and cardiac responses to activity in other decapod crustaceans engaged in both swimming (Booth *et al.* 1982) and walking (Hamilton and Houlihan, 1992; Guirguis and Wilkens, 1995; and see McMahon and Wilkens, 1983, for a review of earlier work). Because of major differences in the type and duration of activity, these results cannot be compared too rigorously. A general comparison (Table 2) reveals similar overall changes in cardio-respiratory performance, but also reveals differences in the regulatory mechanisms. Portunid crabs, e.g. *Callinectes sapidus* and *Carcinus maenas*, whether swimming or walking, showed a greater increase in heart rate and a smaller increase in stroke volume than the present results for a cancerid crab. Large increases in heart rate accompanied walking activity in the lobster *H. americanus* (Guirguis and Wilkens, 1995). More studies on a greater variety of genera and activity patterns are required, however, before these differences can be assigned to a particular group, pattern of activity or experimental protocol.

Table 2. *Measured and calculated mean circulatory, ventilatory and respiratory data for three different aquatic crabs during activity*

Species	Duration of activity (min)	Temperature (°C)	Cardiac output (ml kg^{-1} min^{-1})			Stroke volume (ml $beat^{-1}$)			References
			Rest	Active	Ratio	Rest	Active	Ratio	
Carcinus maenas	5	20	(F) 94	(F) 244	2.6	(F) 1.17	(F) 1.76	1.5	Hamilton and Houlihan (1992)
Callinectes sapidus	60	20	(F) 151	(F) 345	2.3	(F) 1.7	(F) 2.4	1.4	Booth *et al.* (1982)
Cancer magister	20	8	(F) 72	(F) 185	2.3	(F) 1.6	(F) 2.5	1.6	McMahon *et al.* (1979)
Cancer magister	10	12	(D) 23	(D) 43	1.9	(D) 0.36	(D) 0.62	1.7	This study, first activity period
	10	12		(D) 48	2.1		(D) 0.67	1.9	This study, second activity period
			Heart rate (beats min^{-1})			Ventilation rate (beats min^{-1})			
			Rest	Active	Ratio	Rest	Active	Ratio	
Carcinus maenas	5	20	(i) 84	(i) 151	1.8	(i) 206	(i) 770	3.7	Hamilton and Houlihan (1992)
Callinectes sapidus	60	20	(i) 89	(i) 143	1.6	(i) 94	(i) 312	3.3	Booth *et al.* (1982)
Cancer magister	20	8	(i) 59	(i) 69	1.2	(p) 74	(p) 151	2.0	McMahon *et al.* (1979)
Cancer magister	10	12	(D) 61	(D) 72	1.2	(p) 65	(p) 116	1.8	This study, first activity period
	10	12		(D) 72	1.2		(p) 129	2.0	This study, second activity period
			Ratio (active/resting) of ventilation volume			Ratio (active/resting) of oxygen consumption			
Carcinus maenas	5	20		(F) 3.5			(r) 3.4		Hamilton and Houlihan (1992)
Callinectes sapidus	60	20		(s) 2.9			(F) 2.6		Booth *et al.* (1982)
Cancer magister	20	8		(e) 2.3			(F) 2.3		McMahon *et al.* (1979)

(F), calculated based on the Fick principle; (D), measured with pulsed-Doppler flow probes; (i), measured with the impedance technique; (p), measured with pressure transducers; (r), measured by closed-system respirometry; (s), calculated from scaphognatite frequency; (e), measured with electromagnetic flowmeters.

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