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RESPIRATORY AND CIRCULATORY ADJUSTMENTS DURING AQUATIC TREADMILL EXERCISE IN THE EUROPEAN SHORE CRAB *CARCINUS MAENAS*

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Summary

Carcinus maenas (L.) were exercised using a novel design of aquatic treadmill respirometer. Tethered exercise was performed in sea water at 5.8 m min^{-1} for 5 min. The rate of oxygen consumption and the heart and scaphognathite beat rates increased at the onset of exercise, reaching a steady state within 180 s. The estimated haemolymph flow rate rose 2.6-fold during exercise, achieved by a 1.8-fold increase in heart rate and a 1.5-fold increase in the estimated cardiac stroke volume. The haemolymph total oxygen content difference increased significantly during exercise. Haemolymph total carbon dioxide content did not change significantly during exerise, but haemolymph pH deceased as a result of an L-lactate-induced metabolic acidosis. The acidosis may also have led to a reduced Bohr shift. It is concluded that O_2 and CO_2 exchange were not impaired during exercise and that *C. maenas* relied primarily upon O_2 to fuel underwater running at 5.8 m min^{-1}.

Introduction

Underwater locomotion, often involving slow-speed walking, is one of the characteristic features of marine Brachyura. Despite this, physiological changes during such activities have been studied in only a few brachyuran species (Houlihan and Innes, 1984), including *Carcinus maenas* (Houlihan *et al.* 1984; Houlihan and Mathers, 1985). These studies were primarily concerned with locomotory energetics, so many of the physiological changes occurring during exercise remain unknown. In these studies, a constant walking speed could not be induced as a probe was used to stimulate walking.

The present study was therefore designed to provide new information about changes in the physiology of *Carcinus maenas* during quantified submaximal

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exercise. Accurate control of the intensity and duration of exercise was achieved using a novel design of aquatic treadmill respirometer.

In addition, the data in the present study allow a better quantitative comparison to be made with the much larger volume of literature available on land crabs exercised in air. The evolution of crab air-breathing has resulted in a transition from diffusion-limited gills to perfusion-limited respiratory structures. This change, combined with the problems of desiccation and CO_2 excretion that land crabs potentially face (Innes and Taylor, 1986*c*), could conceivably lead to major differences in the response to exercise between aquatic and terrestrial crab species.

Materials and methods

Animals

Carcinus maenas (L.) of both sexes were collected from around the coast of Aberdeen, Scotland. The animals were held in recirculating seawater aquaria at the experimental temperature of 15 °C (salinity 31‰) for a maximum of 1 week. A short captivity time was desirable as the exercise performance of *C. maenas* declines with an increase in captivity time (Houlihan and Mathers, 1985). All animals had a complete complement of legs and chelae and were at the intermoult stage (Drach and Tchernigovtzeff, 1967). The mean dry mass of the animals was 12.01±0.76 g (34.38±3.09 g live mass; N=137).

The respirometer

Animals were exercised in a closed-system treadmill respirometer filled with sea water (total volume 2.97 l) (Fig. 1). During the observation periods, water was continuously circulated within the respirometer by the pump and the stirrer. The effect, if any, that turbulence may have had upon the exercise performance of the animal was not assessed. Following observation periods, a rubber bung in the roof of the respirometer could be opened, allowing the apparatus to be flushed with fresh, filtered sea water.

The animals were tethered to a balance arm and the counter-balance was adjusted so that the animals ran with a gait stereotypical of freely moving *C. maenas* (Bethe, 1930; Clarac and Coulmance, 1971). Free walking proved repeatedly unsuccessful as the animals allowed themselves to be dragged along by the treadmill belt.

Mixing time was assessed by injecting dye into the respirometer at a position close to where the animal's exhalant opening would normally be. After injection, 1 ml water samples were withdrawn from the sensor housing (Fig. 1) at 15 s intervals. During the experiment, water was continuously circulated within the respirometer by the pump and stirrer. The absorbance of the samples was measured at 580 nm (peak absorbance) and plotted against time. These data suggested that complete mixing was achieved within 50 s.

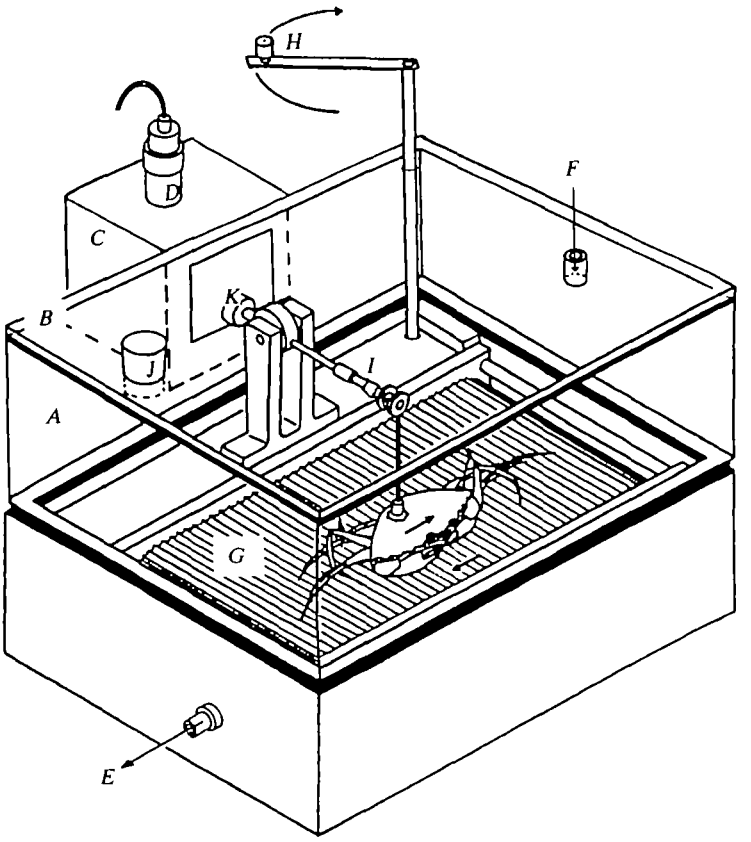

Fig. 1. Diagram of the treadmill respirometer. The respirometer was made of clear Perspex. The entire unit was submerged in a bath of sea water at 15°C. *A*, outer respirometer wall; *B*, removable roof; *C*, oxygen sensor housing containing oxygen sensor (*D*) and magnetic stirring bar (not shown). A submerged stirrer rotated the bar underneath the sensor, ensuring good mixing of water. Mixing was enhanced by circulating water from the output (*E*) to the input (*F*) using an external pump (not shown). The circulating circuit could be altered to flush fresh sea water through the respirometer as required. The animal was exercised on a ridged rubber belt (*G*) driven by the hand-turned handle (*H*) *via* a system of gears. The animal was tethered to a balance arm (*I*), which allowed limited vertical and horizontal rotation. The arm position could be altered vertically using the counterbalance (*K*). The rubber bung (*J*) was removed when flushing the respirometer with fresh sea water.

Oxygen consumption measurement

The output from the oxygen sensor was fed into the analogue-to-digital converter of a BBC microcomputer (Acorn Computers Ltd; see Hamilton, 1987, for computer program). The computer was used to calculate and store the value of $\dot{M}_{O_2}$ for each 1 min period of observation. Sensor drift was checked at the end of the experiment by comparing the computer-calculated P_{O_2} value to the P_{O_2} reading of the respirometer water measured using an independently calibrated

oxygen measurement cell. No problems with oxygen sensor drift were noted. At the end of each experiment, $\dot{M}_{O_2}$ was determined for 15 min without the animal in the respirometer (blank run). The blank value of $\dot{M}_{O_2}$ was then subtracted from the measured $\dot{M}_{O_2}$ of the animal. As filtered, fresh sea water was flushed through the respirometer prior to $\dot{M}_{O_2}$ determination during each experimental period, the value of the blank was found to be negligible and was not considered to be a source of measurement error.

Experimental regimes

The animals were allowed to rest, tethered, in the respirometer for a minimum of 12 h before determining the $\dot{M}_{O_2}$ of the resting animal over a 60 min period (pre-exercise value). The animal was then exercised at 5.8 m min^{-1} for 5 min (sub-burst speed walking for *C. maenas*; Houlihan *et al.* 1984). Following exercise, $\dot{M}_{O_2}$ was monitored until the pre-exercise value was reached. Fresh filtered sea water was flushed through the respirometer between observation periods to maintain the respirometer P_{O_2} above 80 % saturation. Experiments in which the animals were active for more than 2 min in any 5 min time block during the pre-exercise or post-exercise periods were aborted. Any animal that hesitated during exercise for more than 60 s was discarded.

Each animal was only used once. In some cases $\dot{M}_{O_2}$, heart rate or scaphognathite beat rate alone was monitored. In others, combinations of heart rate measurement, scaphognathite beat rate measurement, ventilatory water flow rate estimation and haemolymph variables were measured. The data from these different combinations have been pooled as no statistically significant differences were found when the same variables were compared between groups ($P>0.01$ in each case).

Oxygen consumption and mass relationships

In another series of experiments the relationship between mass and resting or peak $\dot{M}_{O_2}$ of *C. maenas* was investigated. Prior to $\dot{M}_{O_2}$ determination, the animals were allowed to rest as described above. Resting $\dot{M}_{O_2}$ was then determined with the animals untethered. Peak oxygen consumption rates were considered to represent maximal oxygen consumption rates ($\dot{M}_{O_2max}$) and were induced by tethering the animals to the balance arm and repeatedly stimulating the animals with a probe. $\dot{M}_{O_2max}$ was considered to have been achieved when the three highest $\dot{M}_{O_2}$ readings were observed over three consecutive minutes.

Ventilation and circulation

Heart and ventilation rates

Impedance techniques were used to measure heart (f_H) and scaphognathite (f_{SCAPH}) beat rates (Ansell, 1973; Depledge, 1978). Using measurements of $\dot{M}_{O_2}$, and inspired and expired water P_{O_2} values, ventilatory water flow rate ($\dot{V}_W$) was estimated using the Fick principle. Expired water was sampled using a respiratory

mask made from a commercially available rubber balloon (Butler *et al.* 1978; Hamilton, 1987).

Haemolymph gas analysis

Haemolymph was sampled after 12 h of tethered rest or at the end of the fifth minute of the exercise period. Postbranchial haemolymph samples (Pa_{O_2}) were taken *via* a hole previously drilled above the pericardial sinus on the dorsal side of the carapace. Prebranchial samples were taken *via* the arthrodial membrane at the base of the pereiopods. Each sample (approximately 0.7 ml) was withdrawn into a chilled plastic syringe, sub-sampled and then the various haemolymph gas variables were measured immediately. Sampling time was less than 60 s.

Haemolymph O_2 tension (P_{O_2}) and pH were measured using a BMS3 blood gas analysis system (Radiometer, Copenhagen). Total O_2 content (C_{O_2}) was measured using a Lex-O_2-Con TL O_2 content analyzer (Lexington Instruments). 40 μl haemolymph samples were used to avoid underestimation of O_2 content (Wood *et al.* 1979). Total CO_2 content (C_{CO_2}) was determined by injecting duplicate haemolymph samples (80 μl each) into a reaction chamber containing 0.01 mol l^{-1} HCl saturated with *n*-octanol and the increase in CO_2 units noted on a PHM 73 P_{CO_2} meter (Radiometer, Cophenhagen), pre-calibrated with 10 mmol l^{-1} $NaHCO_3$. C_{CO_2} was then calculated as described by Cameron (1971) and Houlihan *et al.* (1982).

P_{CO_2} was calculated using the equation:

$$P_{CO_2} = C_{CO_2}/[(X \times \alpha CO_2) + \alpha CO_2],$$

where X is the antilogarithm of (pH−apparent first dissociation constant of carbonic acid) and αCO_2 is the solubility coefficient of CO_2 (Truchot, 1976*b*). The values of the constants were for *C. maenas* at 15 °C at a salinity of 35 ‰.

$\dot{V}b$ was estimated using the values of $\dot{M}_{O_2}$, Ca_{O_2} and Cv_{O_2} substituted into the Fick equation. Estimates of $\dot{V}b$ obtained this way must be treated with caution because of the absence of a sampling site that yields a truly mixed venous haemolymph sample (Taylor, 1982; Taylor and Greenaway, 1984).

Oxygen equilibrium curves

Haemolymph oxygen equilibrium curves were determined using pooled (10 animals), declotted, mixed prebranchial and postbranchial haemolymph. Curves were determined for resting, untethered *C. maenas* or for tethered *C. maenas* exercised for 5 min at 5.8 m min^{-1} by mixing various ratios of oxygenated and deoxygenated haemolymph (BMS2 blood tonometry system, Radiometer; Copenhagen and Wöstoff gas-mixing pumps, Wöstoff, Germany). P_{O_2}, C_{O_2}, C_{CO_2} and the pH of each mixed sample were determined as described above. The oxygen-carrying capacity of the haemolymph (C_{O_2max}) was determined for each pooled sample after equilibration with air at 15 °C. The lactate concentration was also measured (see below). P_{50} values were derived from Hill plot regression equations.

Lactate concentration measurements

Whole-body L-lactate concentration was measured in untethered animals, animals that had been tethered for 20 min and animals that had been exercised at $5.8\,m\,min^{-1}$ for 5 min. Haemolymph L-lactate concentrations of resting and exercised animals was also measured in some cases. L-Lactate concentrations were determined using a specific spectrophotometric assay (Sigma diagnostic kit no. 826-UV; Sigma Chemical Co. Ltd), modified as suggested by Full and Herreid (1984).

Statistical analysis

Parametric statistical tests were performed according to formulae outlined by Snedecor and Cochran (1972) and Sokal and Rohlf (1969). The 5% level of statistical significance has been used throughout.

Results

Response to exercise

Steady state

At the onset of exercise, $\dot{M}_{O_2}$, f_H and f_{SCAPH} increased until a steady state was achieved (Table 1, Fig. 2). The half-times for these responses were 91±8 s, 73±4 s and 72±6 s, respectively. The mean half-time of the $\dot{M}_{O_2}$ response was significantly slower (one-tailed *t*-test; $P<0.05$) than the mean half-times of the heart and scaphognathite response for the same animal. This discrepancy was probably due to inherent delay in the measuring system.

At steady state, the $\dot{M}_{O_2}$ had increased 3.4-fold ($N=10$ animals). $\dot{V}w$ was estimated to have increased by 3.5-fold (Table 2, $N=4$ animals), resulting from the 3.7-fold increase in f_{SCAPH} ($N=8$ animals). The $\dot{M}_{O_2}$ of animals wearing the mask was not significantly different from that of unmasked animals ($P>0.01$). As the scaphognathite of *C. maenas* is known to be a variable-volume pump (Wilkens *et al.* 1984; Mercier and Wilkens, 1984), scaphognathite stroke volume (V_S) changes may also have contributed to the $\dot{V}w$ increase.

Ventilation

The $\dot{V}w$ increase ensured a large P_{O_2} gradient between the water and haemolymph. This diffusion gradient into the haemolymph would have been further enhanced by the 0.59 kPa decrease in the prebranchial haemolymph O_2 tension ($N=7$ animals). The convection requirement for water ($\dot{V}w/\dot{V}_{O_2}$) was maintained during exercise ($N=4$ animals), and no significant change in the percentage of O_2 extracted from the ventilatory current ($\%Extr_w$) was noted (Table 2). This suggests that any increase in the physiological, anatomical or diffusional dead space resulting from the increase in $\dot{V}w$ did not create an O_2 transport problem during exercise.

Table 1. *Measured variables (±S.E.M.) for* Carcinus maenas *prior to exercise and during steady-state exercise at 5.8 m min^{-1}*

Variable	Pre-exercise	Exercise	Significance
$\dot{M}_{O_2}$ (μmol O_2 g^{-1} dry mass h^{-1})	7.14±0.45 (10)	24.10±0.45 (10)	$P<0.002$
f_H (beats min^{-1})	84.4±2.2 (14)	150.9±1.1 (14)	$P<0.002$
f_{SCAPH} (total) (beats min^{-1})	206.3±5.9 (8)	769.6±4.3 (8)	$P<0.002$
$P_{I_{O_2}}$ (kPa)	18.50±0.36 (4)	18.14±0.17 (4)	NS
$P_{E_{O_2}}$ (kPa)	14.80±0.71 (4)	15.00±0.28 (4)	NS
Pa_{O_2} (kPa)	10.30±0.70 (10)	8.97±0.59 (8)	NS
Pv_{O_2} (kPa)	1.39±0.08 (8)	0.80±0.07 (7)	$P<0.002$
Ca_{O_2} (mmol O_2 l^{-1} haem)	0.63±0.02 (9)	0.60±0.02 (7)	NS
Cv_{O_2} (mmol O_2 l^{-1} haem)	0.18±0.03 (6)	0.02±0.01 (6)	$P<0.002$
$Ca-v_{O_2}$ (mmol O_2 l^{-1} haem)	0.44±0.01 (6)	0.57±0.02 (6)	$P<0.002$
Ca_{CO_2} (mmol CO_2 l^{-1} haem)	5.21±1.13 (4)	4.30±0.26 (4)	NS
Cv_{CO_2} (mmol CO_2 l^{-1} haem)	5.32±0.24 (4)	4.51±0.32 (4)	NS
pHa	7.828±0.002 (4)	7.762±0.018 (4)	$P<0.01$
pHv	7.800±0.005 (4)	7.729±0.006 (4)	$P<0.05$
L-Lactate (μmol g^{-1})	2.96±0.28 (15)	4.29±0.38 (15)	$P<0.01$
L-Lactate (mmol l^{-1} haem)	3.89±0.70 (6)	9.77±1.16 (4)	$P<0.05$

haem, haemolymph; (N), number of animals used in the experiment.

NS, not statistically significant.

$\dot{M}_{O_2}$, rate of oxygen consumption; f_H, heart rate; f_{SCAPH}, scaphognathite beat rate; $P_{I_{O_2}}$, P_{O_2} of inhaled water; $P_{E_{O_2}}$, P_{O_2} of exhaled water; Pa_{O_2}, postbranchial P_{O_2}; Pv_{O_2}, prebranchial P_{O_2}; Ca_{O_2}, postbranchial oxygen content of the haemolymph; Cv_{O_2}, prebranchial oxygen content; $Ca-v_{O_2}$, difference between Ca_{O_2} and Cv_{O_2}; Ca_{CO_2}, postbranchial CO_2 content; Cv_{CO_2}, prebranchial CO_2 content; pHa, postbranchial haemolymph pH; pHv, prebranchial haemolymph pH.

Circulation

The observed 1.8-fold increase in f_H, coupled with a calculated 2.6-fold increase in $\dot{V}b$, suggests that cardiac stroke volume increased 1.5-fold during steady-state exercise. The ventilation perfusion ratio ($\dot{V}w/\dot{V}b$) increased slightly during exercise, indicating that perfusion of the gills was increased to facilitate the increase in available O_2 at the gills. The efficiency of O_2 uptake by the haemolymph ($\%Eb_{O_2}$) increased and the convection requirement for haemolymph ($\dot{V}b/V_{O_2}$) decreased as a result of changes in the amount of O_2 transported and released by the haemolymph (Table 2). The increase in $\dot{V}b$ coupled with the maintenance of a high Ca_{O_2}, resulted in an increase in the amount of O_2 transported by the haemolymph to the tissues during exercise, as indicated by the capacity rate increase ($\dot{V}b \times Ca_{O_2}$).

Haemolymph gas

Postbranchial O_2 content and tension did not change significantly during exercise ($P>0.05$ in each case; $N=7$ and 8 animals, respectively; see Table 1). In contrast, prebranchial O_2 content and tension values after exercise were signifi-

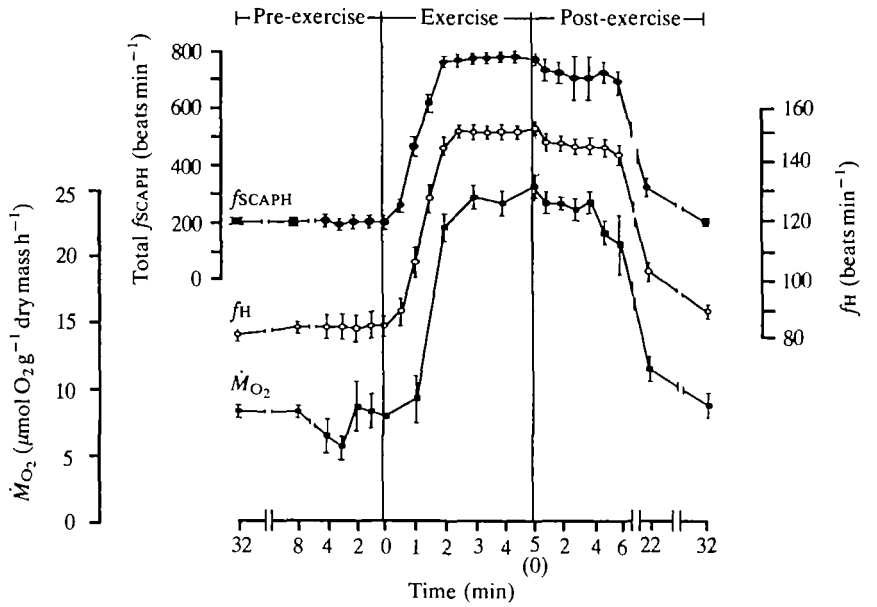

Fig. 2. Mean (±S.E.M.) $\dot{M}_{O_2}$, f_H and f_{SCAPH} of *Carcinus maenas* exercised in the treadmill respirometer at 5.8 m min^{-1} for 5 min, plotted as a function of time. The pre-exercise and post-exercise periods were divided into 5-min time blocks. Each block consisted of five 1-min observations made for each animal. The observations in a time block were pooled and the mean was plotted against the third minute in the time block. In the case of the exercise period, the time blocks span 1 min ($\dot{M}_{O_2}$) or 30 s (f_H and f_{SCAPH}), with each block consisting of one observation per animal.

cantly different from the pre-exercise values ($P<0.002$ in each case; $N=6$ and 7 animals, respectively). The $Ca-v_{O_2}$ difference therefore increased during exercise ($P<0.002$).

Under pre-exercise conditions, the haemocyanin transported 78 % of the O_2 to the tissues. During exercise, the haemocyanin transported 84 % of the O_2 to the tissues; however, this is not a statistically significant difference ($P>0.05$).

Of the O_2 made available to the tissues, 71.8 % diffused from the haemolymph to the tissues (%$Extr_b$) during the pre-exercise period, rising to 96.6 % during exercise. An increase in %$Extr_b$, despite an increase in $\dot{V}b$ as noted in *C. maenas*, is a typical crustacean response to exercise (McMahon, 1981; McMahon *et al.* 1979).

Haemolymph pH

Both post- and prebranchial pH values ($N=4$ animals in each case) decreased significantly as a result of exercise ($P<0.01$ and $P<0.05$, respectively). CO_2 content did not change significantly as a result of exercise ($N=4$ animals in each case). The estimated pre-exercise values for postbranchial and prebranchial CO_2

Table 2. *Calculated variables and indices for* Carcinus maenas *prior to exercise and during steady-state exercise at 5.8 m min^{-1}*

Variable	Pre-exercise	Exercise
$\dot{V}w$ (ml H_2O g^{-1} h^{-1})	154.7±5.0 (4)	546.4±8.8 (4)***
$\dot{V}b$ (ml haem min^{-1})†	3.2	8.3
Vs (ml haem beat^{-1})†	0.04	0.06
%$Extr_w$ (%)	20.3±3.8 (4)	18.4±0.8 (4)^{NS}
%$Extr_b$ (%)	71.8±3.2 (6)	96.6±1.5 (6)***
($\dot{V}w/\dot{V}_{O_2}$) (l H_2O ml^{-1} O_2)	1.06±0.18 (4)	1.07±0.04 (4)^{NS}
($\dot{V}w/\dot{V}b$)† (ml H_2O ml^{-1} haem)	9.7	13.2
($\dot{V}b/\dot{V}_{O_2}$)† (ml haem ml^{-1} O_2)	99.3	77.0
($\dot{V}b \times Ca_{O_2}$)† (μmol O_2 min^{-1})	2.00	4.99
%Ew_{O_2} (%)†	22.0	19.1
%Eb_{O_2} (%)†	64	81

haem, haemolymph.

(N), number of animals used in the experiment; NS, not statistically significant.

† Calculated for a 12.01 g dry mass animal (mean dry mass in this study).

*** Statistically significant difference, $P<0.002$.

$\dot{V}w$, rate of water flow through the gills; $\dot{V}b$, haemolymph flow rate; Vs, stroke volume; %Extr, percentage extraction of oxygen from the water (w) or haemolymph (b); $\dot{V}_{O_2}$, rate of oxygen uptake; Ca_{O_2}, postbranchial oxygen content of haemolymph; %Eb_{O_2}, effectiveness of oxygen uptake by the tissues; %Ew_{O_2}, effectiveness of oxygen uptake from the water.

tension were 0.21 kPa and 0.24 kPa, respectively. There was no significant change in these values following exercise (Pa_{CO_2}=0.25 kPa, Pv_{CO_2}=0.28 kPa).

L-Lactate concentrations

Five minutes of exercise resulted in a significant 1.4-fold increase in whole-body L-lactate concentration ($P<0.01$; N=15 animals) and a significant 2.5-fold increase in haemolymph L-lactate concentration ($P<0.05$; N=4 animals; see Table 1). Anaerobiosis accounted for 29 % of the total ATP production during exercise, calculated using ATP equivalents (Bennett and Licht, 1972).

Oxygen equilibrium curves

Prior to exercise, haemolymph P_{50} was 1.68 kPa (12.6 Torr) at a pH of 7.798. After 5 min of exercise, P_{50} had increased to 2.08 kPa (15.6 Torr) at a pH of 7.733, resulting in a Bohr shift of −1.43 (Fig. 3). The estimated P_{CO_2} values at P_{50} of the haemolymph were 0.23 kPa (1.7 Torr) for the pre-exercise samples and 0.24 kPa (1.8 Torr) for the samples from exercised animals. Cooperativity decreased from 4.8 to 3.9. No Root effect was exhibited by either of the pooled haemolymph samples.

Post-exercise response

After exercise, $\dot{M}_{O_2}$, f_H and f_{SCAPH} decreased, eventually reaching the original

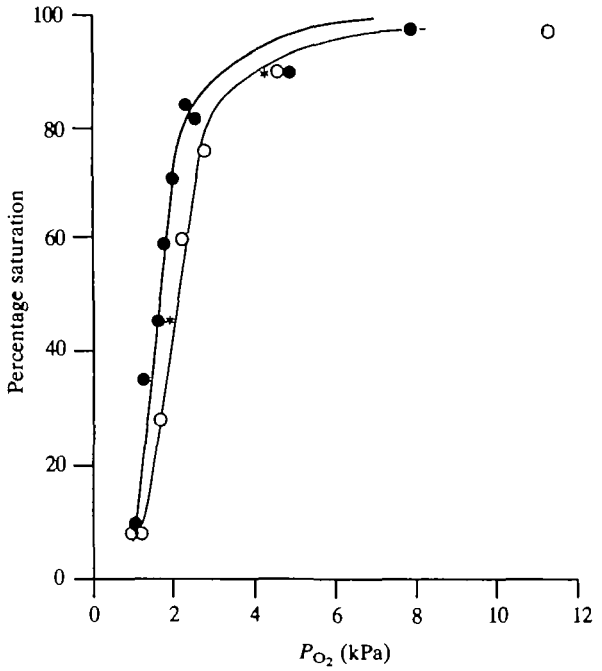

Fig. 3. *In vitro* haemolymph oxygen equilibrium curves for *Carcinum maenas* expressed as percentage saturation. (●) Pre-exercise conditions; (○) exercised for 5 min at 5.8 m min^{-1}. Two overlapping data points are indicated by an asterisk.

pre-exercise value. The half-times for these responses were 752±67 s, 771±63 s and 1030±245 s, respectively (each significantly longer than the corresponding half-time at the beginning of exercise, $P<0.002$; paired t-test).

Oxygen consumption and live mass

The relationship between the rate of oxygen consumption and live mass has been expressed in units of ml O_2 $animal^{-1}$ h^{-1} ($\dot{V}_{O_2}$) to allow easy comparison with the relationships noted in the established literature. The $\dot{V}_{O_2}$ values of resting and maximally exercised animals were linearly related to live mass (Fig. 4). The slopes of the two lines are not significantly different ($P>0.05$; F slope=0.033) whereas the elevations are significantly different ($P<0.05$; F elevation=337.47). The relationship between resting $\dot{V}_{O_2}$ and mass was: $\log\dot{V}_{O_2rest}$ (ml O_2 $animal^{-1}$ h^{-1})=0.655 × log(live mass in grams) − 0.706; (r=0.85; N=32; $P<0.01$).

The relationship between maximal $\dot{V}_{O_2}$ and mass was: $\log\dot{V}_{O_2max}$ (ml O_2 $animal^{-1}$ h^{-1}) = 0.704×log(live mass in grams) − 0.116; (r=0.88; N=12; $P<0.05$). The aerobic expansibility (ratio of $\dot{V}_{O_2max}$ to $\dot{V}_{O_2rest}$) was 4.6 for the mean live mass of animal used in the exercise experiments.

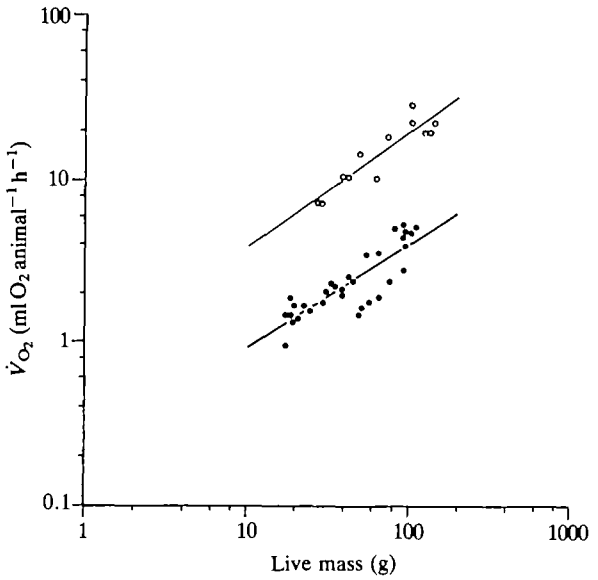

Fig. 4. $\dot{V}_{O_2}$ of resting and maximally respiring *Carcinus maenas* at 15°C plotted as a function of live mass. Lines were fitted by least-squares regression analysis. (●) Resting animals (N=32); (○) maximally respiring animals (N=12).

Discussion

Response to exercise

Oxygen consumption

$\dot{M}_{O_2}$ increased at the onset of exercise and rapidly reached a steady state (Fig. 2). This is characteristic of primarily aerobically dependent decapod crustaceans running in air (Full, 1987; Full and Herreid, 1983; Herreid, 1981; Herreid *et al.* 1979) and primarily aerobic vertebrates and insects (Brackenbury and Avery, 1980; Cerretelli *et al.* 1977; Chadwick and Gilmour, 1940; Krough and Weis-Fogh, 1951; Morton, 1985). The latter animals usually have high aerobic expansibility values ranging up to 20 times resting $\dot{M}_{O_2}$ value (Brett, 1964; Pasquis *et al.* 1970; Priede, 1985; Schmidt-Nielsen, 1984; Thomas, 1975). Like vertebrates, primarily aerobically dependent crustaceans have higher aerobic expansibility values than more anaerobically reliant species (see Hamilton, 1987, for a brief review), although comparison is difficult as truly basal metabolic rates have not been measured in the majority of crustacean studies. Aerially respiring *Ocypode* spp., renowned as the world's fastest crustaceans (Hafemann and Hubbard, 1969), have expansibilities between 6.4 to 11.9 times the resting rate (Full, 1987; Full and Herreid, 1983). In contrast, the land crab *Sesarma cinereum* has an expansibility of just 1.5 times the resting rate, a very limited exercise capability and a heavy reliance upon anaerobiosis during exercise (Full *et al.* 1985). *C. maenas* fits somewhere between these two species, with an expansibility of 4.7 for a 50 g live mass animal (Fig. 4).

In *C. maenas* the expansibility was relatively constant (4.4–4.8) over a mass range of 10 g to 100 g live mass. In contrast, aerobic metabolic scope ($\dot{M}_{O_2max}-\dot{M}_{O_2rest}$) increases progressively with body mass (0.17 mmol O_2 g^{-1} live mass h^{-1} to 0.70 mmol O_2 g^{-1} live mass h^{-1}; 3.9 ml O_2 g^{-1} live mass h^{-1} to 15.5 ml O_2 g^{-1} live mass h^{-1}) over the same mass range (Fig. 4). Consistent with this relationship is the observation that aerobic metabolic enzymes in this species all scale with exponents that are not significantly different from those of the resting and $\dot{M}_{O_2max}$ regression lines (N. M. Hamilton and D. F. Houlihan, personal observation). The scaling of aerobic scope has been reported to be mass-dependent in many crabs (Houlihan *et al.* 1984, 1985; Innes *et al.* 1986). Such scaling effects must be investigated if meaningful comparisons of crab exercise performance are to be made using $\dot{M}_{O_2max}$ as an indication of aerobic capacity.

Ventilation

With an increase in $\dot{M}_{O_2}$, there was a concomitant increase in f_{SCAPH} and $\dot{V}_W$ of *C. maenas* (Fig. 2). This is a typical response to exercise in *Cancer magister* (McDonald, 1977; McMahon *et al.* 1979) and *Callinectes sapidus* (Booth *et al.* 1982; McMahon and Wilkens, 1983). In these three species, and in the aerially respiring crabs *Cardisoma guanhumi* (Herreid *et al.* 1979) and *Cardisoma carnifex* (Wood and Randall, 1981*a*), increases in ventilation volume contribute more to increased O_2 provision than do increases in the percentage of O_2 extracted from the respiratory medium. In contrast, the $\%Extr_a$ increases much more than the rate of air flow through the gills during exercise in *Gecarcinus lateralis* (Herreid *et al.* 1983).

Circulation

The cardiac response of submerged *C. maenas* to exercise, in which f_H increases more than V_S (Tables 1 and 2), is the opposite to its cardiac response at rest in progressively increasing external hypoxia (Taylor, 1976; Taylor and Butler, 1978). f_H also shows a greater proportional increase than V_S during exercise in *Callinectes sapidus* (Booth *et al.* 1982) and in the lobster *Homarus vulgaris* (Hamilton, 1987). In contrast, the proportional increase in V_S is greater than that of f_H during exercise in *Carcinus mediterraneus* and *Pachygrapsus marmoratus* (Houlihan and Innes, 1984), *Cancer magister* (McMahon *et al.* 1979) and in the land crab *Cardisoma carnifex* (Wood and Randall, 1981*a*). A bradycardia in response to exercise in *Cardisoma guanhumi* (Herreid *et al.* 1979) is yet another variation that has been reported. The reason for these differences is not clear.

Haemolymph gas

No significant decrease in postbranchial O_2 tension and content was noted in *C. maenas* following exercise (Table 1). *C. sapidus* has a similar response (Booth *et al.* 1982). *Cardisoma carnifex* (Wood and Randall, 1981*a*) and *Cancer magister* (McMahon *et al.* 1979; Johansen *et al.* 1970) have significantly decreased Pa_{O_2}

values during exercise; however, Ca_{O_2} shows little change as the haemocyanin remains virtually fully saturated in each case. In *Carcinus mediterraneus* and *Pachygrapsus marmoratus*, both haemolymph O_2 tension and O_2 content decrease significantly as a result of exercise (Houlihan and Innes, 1984).

The change in the Cv_{O_2} of *C. maenas* during exercise resulted in an increase in the $Ca-v_{O_2}$ difference (Table 1). Several other authors have noted an increase in the $Ca-v_{O_2}$ difference during exercise, primarily as a result of a Pv_{O_2} decrease (Johansen *et al.* 1970; McMahon *et al.* 1979; Wood and Randall, 1981*b*). *Callinectes sapidus* is a notable exception as its Pv_{O_2} shows no significant change after 25 min of swimming (Booth *et al.* 1982).

The release of O_2 to the tissues is enhanced in some species by a normal Bohr shift (see McMahon and Wilkens, 1983). In *C. maenas*, the Bohr shift probably plays a minimal role in the release of O_2. The Bohr shift noted in *C. maenas* exercised at 5.8 m min^{-1} was −1.43 (Fig. 3). This is lower than the value of −1.54 reported by Taylor and Butler (1978). The lower value in the former case may have been caused by the L-lactate accumulation opposing the rightward equilibrium curve shift induced by the haemolymph pH drop. L-Lactate is known to modulate the affinity of *C. maenas* haemocyanin *in vitro* (Truchot, 1980). Modulation of O_2-binding by other haemolymph cofactors during exercise cannot be ruled out (Lallier *et al.* 1987; Morris *et al.* 1985). Cooperativity decreased during exercise. A decrease was also reported in swimming *C. sapidus* (Booth *et al.* 1982); however, the relationships between cooperativity, haemolymph pH and lactate ion concentration are not understood.

The post- and prebranchial haemolymph CO_2 tensions did not change significantly as a result of exercise, suggesting that gas exchange at the respiratory surface was not seriously impaired. This was possibly facilitated by the reduced Bohr shift, which would assist the maintenance of a high Pa_{O_2}. This, in turn, would aid CO_2 unloading as O_2 uptake and CO_2 excretion are thought to be linked in *C. maenas* (Truchot, 1976*a*). As the P_{CO_2} difference between the prebranchial and postbranchial haemolymph was small (<0.03 kPa; 0.2 Torr), unloading of CO_2 would be aided further by the Haldane effect (Truchot, 1976*a*). In contrast, *Callinectes sapidus* and *Cancer magister* both show increases in haemolymph CO_2 tension during exercise (Booth *et al.* 1984; McDonald *et al.* 1979).

Assuming the animals were in acid–base equilibrium at the end of the exercise period, the insignificant changes in haemolymph CO_2 content and calculated P_{CO_2} suggest that *C. meanas* did not experience a respiratory acidosis during exercise. The significant decrease in haemolymph pH would therefore be due largely to a metabolic acidosis induced by the significant increase in L-lactate concentration. The majority of crab species that have been examined show a mixed metabolic/respiratory acidosis during exercise (McDonald *et al.* 1979; Smatresk and Cameron, 1981; Smatresk *et al.* 1979; Waldron *et al.* 1986; Wheatly *et al.* 1986; Wood and Randall, 1981*a*,*b*).

The L-lactate concentration increase in *C. maenas* indicates the anaerobiosis contributed 29 % of the total ATP equivalent production, as noted in the Results

section. Accumulation of this amount of lactate suggests that the exercise intensity was approaching burst speed levels.

Recovery from exercise

The recovery from exercise in crustaceans follows a monoexponential curve (Full and Herreid, 1983). The reason why the half-times of the $\dot{M}_{O_2}$, f_H and f_{SCAPH} responses were each significantly longer at the end of exercise than at the beginning is not clear. The most likely explanation is that the responses during the recovery period were periodically elevated during the observed short bursts of spontaneous activity (duration <1 min). There is no significant difference between these half-times for $\dot{M}_{O_2}$ in *Ocypode gaudichaudii* (Full and Herreid, 1983).

Interspecific differences in response to exercise

The reason why some species of crustaceans show markedly different physiological responses to submaximal exercise remains unclear, largely due to the dearth of information in this field. One contributing factor may be whether the animal relies upon rapid neurophysiological or much slower-acting humoral mechanisms to initiate the ventilatory responses during exercise (Herreid, 1981).

The O_2 diffusion rate across the gills will also be a limiting factor during exercise. The amount of O_2 that can diffuse into the haemolymph will be a function of the magnitude of the O_2 diffusion gradient, the area available for diffusion, the diffusion pathlength and the barrier to diffusion created by the physical structure of the gas exchange apparatus. *Callinectes sapidus* has a relatively large gill area (Gray, 1957) and short diffusion pathlength (Aldridge and Cameron, 1979) compared to many other species of crab (Gray, 1957), including *C. maenas* (Butler, 1976; Taylor and Butler, 1978). These factors may explain why *C. sapidus* has a shorter half-time for $\dot{M}_{O_2}$ at the beginning of exercise than *C. maenas* (Booth *et al.* 1982).

Associated with adaptation to a terrestrial environment is a reduction in gill surface area (Gray, 1957; Cameron, 1981; Hawkins and Jones, 1982) in addition to the evolution of alternative gas exchange structures (Bliss, 1968, 1979; Diaz and Ridriguez, 1977; Innes and Taylor, 1986*c*, 1987; El Haj *et al.* 1986; Maitland, 1986). The effect of these morphological adaptations upon exercise performance and the accompanying physiological adjustments are not clear. *Ocypode gaudichaudii* shows exceptional exercise capabilities in air, has a fast half-time for $\dot{M}_{O_2}$ at the beginning of exercise and reaches a steady state (Full and Herreid, 1983). This may be due to the invaginations of its branchial chamber lining, which act as an accessory respiratory surface (Diaz and Rodriguez, 1977). However, *Pseudothelphusa garmani* has evolved an invaginated 'lung', which enables it to achieve the highest Pa_{O_2} values recorded for crustaceans (approximately 18.7 kPa; Innes and Taylor, 1986*a*, 1987), but this species does not have exceptional locomotory capabilities (Innes and Taylor, 1986*b*). Tolerance to desiccation may also determine the exercise responses of aerially respiring species (Innes *et al.* 1986).

The magnitude of phosphate and O_2 stores, in addition to the rate of phosphate

utilization, is also likely to determine the rate of the $\dot{M}_{O_2}$ response. Unfortunately, accurate information concerning these variables is limited and is of little help in clarifying the reasons for the interspecific differences in response time.

Circulatory response data are also sparse in the literature and there is no clear correlation between physiological responses, habitat and exercise performance. It is worth noting that aquatic crustaceans are diffusion-limited in their O_2 transport, whereas aerially respiring crustaceans are perfusion-limited (reviewed by Innes and Taylor, 1986*c*). Perfusion limitation may explain the extremely limited exercise capabilities of *Sesarma cinereum*, although the exact nature of the limitation is unknown (Full *et al.* 1985).

The present study has revealed the major ventilatory and circulatory adjustments that occur in *C. maenas* in response to controlled submaximal exercise. These results suggest that O_2 and CO_2 exchange were not impaired during exercise and that *C. maenas* relied primarily upon O_2 to fuel exercise at $5.8\,m\,min^{-1}$. *C. maenas* shows $\dot{M}_{O_2}$ responses that are similar to primarily aerobic land crabs. From the aquatic species that have been studied, it would appear that the aquatic medium does not confer any particular advantage to marine crustaceans in terms of exercise performance when compared to aerially respiring species exercised in air. This implies that the evolution of air-breathing in crabs *per se* is not necessarily limiting to exercise performance. Owing to a lack of published information on detailed physiological responses under a controlled exercise regime, the reason for interspecific differences in crustacean exercise response remains unknown.

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