Ghost crabs on a treadmill: Oxygen uptake and haemocyanin oxygen affinity

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Ghost crabs *Ocypode ceratophthalmus* were exercised on a specially constructed treadmill. At a running speed of 13,3 cm s^{-1}, most crabs ran for 2 h before getting fatigued. At this speed the oxygen consumption rate ($\dot{M}O_2$) was measured in time intervals for a total of 52 min. For exercised crabs the $\dot{M}O_2$ values are about eight times higher (28,5 ± 5,0 mmol $l^{-1}kg^{-1}h^{-1}$ compared with the values (3,5 ± 0,4 mmol $l^{-1}kg^{-1}h^{-1}$) for resting crabs. One hour after the exercising bout, the $\dot{M}O_2$ values were not back to normal resting values. The l-lactate levels in the haemolymph increase to 20,7 (± 4,8) mmol l^{-1} after 20 min exercise, whilst the pH decreases from 7,91 (± 0,05) to 7,71 (± 0,08). For resting crabs pre-branchial PO_2 (venous) is 35,9 (± 5,6) mmHg, total carbon dioxide concentration (C_{CO_2} tot.) 22,6 (± 2,6) mmol l^{-1}, l-lactate 0,68 (±0,5) mmol l^{-1}; haemocyanin oxygen capacity ($C_{max/HcyO_2}$) 1,50 (± 0,11) mmol l^{-1}; haemocyanin concentration 99,3 (± 21) mg ml^{-1} and haemolymph oxygen content 4,11 ml O_2 100 ml^{-1} haemolymph. Haemolymph from resting crabs has P_{50} values of 4,5 (± 1,0) mmHg, while for exercised crabs it is 10,0 (± 0,8) mmHg. Gel-chromatographed haemolymph increased the oxygen affinity of the haemocyanin to 1,30 (± 0,1) mmHg, an indication of the presence of oxygen affinity modulators in the haemolymph. A decrease in the pH of the haemolymph has a greater effect on oxygen affinity compared with the effects from either CO_2 or l-lactate. Hill-plot analysis of both native and gel-chromatographed haemolymph reveal that co-operativity (*n*) of the oxygen-binding sites in the haemocyanin sub-units increases to a value of 4 with a corresponding decrease in oxygen affinity. This effect has the result that large amounts of O_2 can be suddenly released by the haemocyanin to tissues such as muscle and ganglia for immediate mitochondrial use.

Ruiterkrappe *Ocypode ceratophthalmus* is op 'n spesiaal gemaakte trapmeul geoefen. By 'n hardloopspoed van 13,3 cm s^{-1} het die meeste krappe vir ten minste 2 h gehardloop voor moegheid ingetree het. By hierdie spoed is die suurstofverbruikkoers ($\dot{M}O_2$) gemeet in tydintervalle vir 'n totale tyd van 52 min. Die $\dot{M}O_2$-waardes vir geoefende krappe is ongeveer agt keer hoër (28,5 ± 5,0 mmol $l^{-1}kg^{-1}h^{-1}$) as dit vergelyk word met waardes (3,5 ± 0,4 mmol $l^{-1}kg^{-1}h^{-1}$) by rustende krappe. Een uur na oefening plaasgevind het, was die $\dot{M}O_2$ nog nie terug na die normale rustende vlakke nie. Die l-laktaatkonsentrasie in die hemolimf neem toe tot 20,7 (± 4,8) mmol l^{-1} na 20 min oefening. Die pH daarenteen, daal van 7,91 (± 0,05) tot 7,71 (± 0,08). Vir rustende krappe is verder ook die prebranchiale PO_2 (veneus) 35,9 (± 5,6) mmHg; totale koolstofdioksied-konsentrasie (C_{CO_2} tot.) 22,6 (± 2,6) mmol l^{-1}; hemosianiensuurstofkapasiteit ($C_{max/HcyO_2}$) 1,50 (± 0,11) mmol l^{-1}; l-laktaatkonsentrasie 0,68 (± 0,5) mmol l^{-1}; hemosianienkonsentrasie 99,3; (± 21,0) mg ml^{-1} en die hemolimfsuurstofinhoud 4,11 ml O_2 per 100 ml hemolimf, gemeet. Hemolimf van rustende krappe het 'n P_{50}-waarde van 4,5 (± 1,0) mmHg, terwyl dit vir geoefende krappe 10,0 (± 0,8) mmHg is. Gel-gechromatografeerde hemolimf laat die suurstofaffiniteit vir hemosianien tot 1,30 (± 0,1) mmHg toeneem, wat 'n aanduiding mag wees van die teenwoordigheid van suurstofaffiniteitsmoduleerders in die hemolimf. 'n Afname in die pH van die hemolimf het 'n groter uitwerking op die suurstofaffiniteit as dit vergelyk word met die effekte van beide CO_2 en l-laktaat. Hill-grafiekanalise van beide natuurlike en gel-gechromatografeerde hemolimf toon dat koöperatiwiteit (*n*) van die suurstofbindingsplekke van die hemosianien-subeenhede sterk toeneem tot 'n waarde van 4, met 'n ooreenstemmende afname in suurstofaffiniteit. Hierdie uitwerking het tot gevolg dat groot hoeveelhede O_2 skielik deur die hemosianien vrygestel kan word na weefsels soos spiere en ganglia vir onmiddellike mitochondrionale gebruik.

Investigators studied locomotion on a diversity of crab species from different angles (Herreid & Full 1988). However, when it was discovered that crabs walk and run well on treadmills (Herreid 1981; Wheatly, McMahon, Burggren & Pinder 1985) it immediately opened the way to study the metabolism of crabs in motion. A discipline that benefitted handsomely from treadmill studies, was respiration. Respiratory responses, particularly from amphibious or land crabs, to moderate or severe exercise, will allow the experimenter to identify the strong as well as the weak points of the crab's respiratory make up. These points will be much more evident when the metabolic processes are substantially increased through exercise in comparison with the resting state. This approach may be applicable to crabs exercising both in water or air as respiratory medium. Thus Van Aardt (1990) measured the specific oxygen consumption rate ($\dot{M}O_2$) in an amphibious crab, *Potamonautes warreni*, in air and water. He found that the $\dot{M}O_2$ increased twofold after exercise both in air and in water. These results on *P. warreni* showed that the two different gas exchange processes operating in water and in air have the same capacity to exchange gases. Full (1987) studied the aerobic and anaerobic energetics of the ghost crab *Ocypode quadrata* during and after locomotion. He found considerable amounts of l-lactate production in both large (16 g) and small (2 g) crabs. Morris & Bridges (1985) studied the oxygen affinity of non-exercised *O. saratan* haemocyanin where l-lactate was experimentally added to the samples. They found that l-lactate increased oxygen affinity in both dialysed and undialysed haemolymph.

In this investigation *O. ceratophthalmus* was exercised on a treadmill. The $\dot{M}O_2$ was measured immediately after the exercise, followed by measurement of the haemocyanin oxygen affinity of native and gel-chromatographed haemolymph samples. These data, together with haemolymph measurements of total carbon dioxide, pH, pre-brancial PO_2, haemocyanin oxygen content and l-lactate were used to evaluate the ghost crab's reputation as a good runner (Hafemann & Hubbard 1969).

Materials and Methods

Adult (21 g–29 g) *Ocypode ceratophthalmus* were collected during late evening from a sandy beach (Indian Ocean) 6 km north of Port Edward (Natal), South Africa. Adhering sand particles were washed from individuals and each animal was subsequently restricted in a respirometer chamber kept open to the atmosphere but submerged at 25°C in a water bath. The volume of the respirometer chamber was not more than two or three times the volume of the crab. This arrangement, in part, allows for quick temperature equilibrium, restricted movement of the animal and a fast manometer response during the oxygen consumption rate measurements ($\dot{M}O_2$). Six to eight hours after the confinement of the crabs in the respirometer chambers, each of four chambers was connected to its individual respirometer (Scholander 1950). The respiration and compensation chambers were again left open to the atmosphere for at least 1 h. Subsequent $\dot{M}O_2$ measurements were made of the resting state of the crabs for the next hour at intervals of 15 min. A treadmill (Van Aardt 1990) was provided with a length-wise partition of its running surface. In this manner a pair of crabs could run simultaneously on its own running surface, 10,5 cm × 43 cm in area, surrounded by 20 cm high blackened walls made out of perspex. The crabs were run for 20 min at a speed of 13,3 cm s^{-1}. This value is about half the speed rate of a fast-running crab (Figure 1). Immediately after a treadmill run, the crabs were placed in the respirometer chambers. Measurements were made at 7-min intervals after an initial 10 min temperature equilibrium period between the respiration chambers and the water bath. Forty four minutes after the exercise, the crabs were weighed and a 1 ml haemolymph sample was taken from each animal (n = 20) and frozen at –17°C (Morris 1989). Control crabs (n = 12) were collected on the same beach and kept confined in the opened respirometers for 6–8 h before 1 ml haemolymph samples were taken from each control animal and immediately frozen. All haemolymph samples were collected by means of a 1-ml syringe through the arthrodial membrane at the base of the biggest chelipede and designated as pre-branchial or venous (v) haemolymph. Together with the samples twelve live adult crabs were transported to the laboratory. Each animal was kept separately in a 5-l container half filled with dampened sand and provided with a shallow cup of water. In the laboratory the crabs were kept in a climatic room (25°C, 82–95% relative humidity, 12hL : 12hD photoperiod) for 6–14 days before the start of the experiments. The containers were cleaned bi-weekly. Dry cat food (Catmor, S.A. Oil Mills) was given at a ration of five pellets (1 g) for each individual twice a week. The crabs survived for at least four months (Jan 1989–April 1989) and seemed in a healthy condition. However, no crabs molted during this period. All experiments were done at 25°C.

Haemolymph analysis

The pH of the haemolymph was immediately measured from samples taken from control crabs and crabs after the exercise with the aid of a micro-electrode unit (Type E5021, Radiometer, Copenhagen). With this unit the pH of haemolymph samples with added l-lactate (see later) was also measured.

L-lactate in the haemolymph for exercised and control crabs was determined according to Noll (1974) without deproteinization and neutralization with a fully enzymatic kit (no. 256773) from Boehringer-Mannheim. No interference by heavy metal ions (Cu^{++}) was observed using this method.

The partial oxygen tension (PO_2) of the prebranchial haemolymph and total carbon dioxide concentration, C_{CO_2} were determined as described (Van Aardt & Wolmarans 1987). Magnesium was determined with a Mercko-test kit no. 3338 (Germany) and calcium according to a Roche kit no. 1028 (Switzerland). The haemolymph oxygen content or total concentration of oxygen C_{O_2} tot., i.e. the sum of the dissolved O_2 (C_{O_2} diss.) and $HcyO_2$ concentrations (C_{HcyO_2}) (Dejours 1975) was measured at a barometric pressure of 656 mmHg in a 260 µl Tucker-cell according to Bridges, Bicudo & Lykkeboe (1979). The haemocyanin concentration in the haemolymph was determined spectrophotometrically with a 20 µl sample dissolved in 2 ml *Ocypode* Ringer with the following composition (mmol l^{-1}) NaCl 396, KCl 15, $MgCl_2$ 7,0, $CaCl_2$ 8,2 and $NaHCO_2$ 22,1.

The extinction coefficient of 2,69 $E^{1\% / 1 cm}$ at 335 nm (Nickerson & Van Holde 1971) was used. The remaining sample volume was diluted with an equal volume of 12% trichloroacetic acid, thoroughly mixed, and centrifuged for 3 min at 2000 G. The supernatant was used for determining sodium and potassium by flame photometry (Evans Electroselium, Model 227 integrating, UK) and chlorides by coulometric titration (CMT10 Chloride titrator, Radiometer, Copenhagen).

The oxygen-binding properties of haemocyanin were measured by means of a thin-layer optical cell described by Dolman & Gill (1978), at 335 nm (Van Aardt 1990). From the computerized data oxygen-haemocyanin dissociation curves (ODC) were constructed and Hill-plots made (Van Aardt & Naude 1990) in order to establish haemocyanin affinity (P_{50}) and oxygen-binding site co-operativity (n) between 25% and 75% oxygen saturation. ODC measurements were made on haemolymph samples treated in the following manner. In some experiments l-lactate, obtained as a free acid crystalline compound (98% purity; Sigma; USA) was neutralized and added to the haemolymph as described by Morris & Bridges (1985). In other experiments live *O. ceratophthalmus* were incubated for 5 h at 15°C in order to keep the l-lactate concentration in the haemolymph at a low level. After incubation, haemolymph samples were prepared for ODC measurements. Before use all haemolymph samples were declotted (Van Aardt 1990). Usually 1 ml haemolymph was gel-chromatographed in Sephadex G25

(columm 0,9c cm × 15,0 cm; Tris-HCl 100 mmol l^{-1}; $CaCl_2$ 20 mmol l^{-1}; $MgCl_2$ 10 mmol l^{-1}; flow rate 7 ml h^{-1}) at different pH values. Some declotted samples were dialysed for 24 h with *Ocypode* Ringer (see above) at pH 8,68. Oxygen dissociation curve measurements were also made of samples exposed to different temperatures and P_{CO_2} values. A total of 36 ODCs were made. From declotted haemolymph samples the pH was measured when exposed to 5,1% CO_2 in air (or no CO_2 in air) for 10 min in a tonometer (BMS 2, Radiometer, Copenhagen).

Results

Ocypode ceratophthalmus showed considerable endurance at a running speed of 13,3 cm s^{-1}. They could maintain treadmill exercise at this speed for more than 2 h before becoming fatigued (Figure 1). When the running speed on the treadmill was increased to 24,0 cm s^{-1}, mature crabs got fatigued after 15–25 min and let their abdomen parts drag on the moving canvas. In their behaviour on the treadmill, they normally run sideways, with one side leading, but can abruptly reverse their body position with the other side leading. During rapid running (more than 24,0 cm s^{-1}) the fourth pair of running legs did not participate in the running effort, a feature typical of ocypodids (Hafemann & Hubbard 1969). The oxygen consumption rate ($\dot{M}O_2$) increases nearly sixfold from a resting value of 3,43 mmol $l^{-1}kg^{-1}hr^{-1}$ to 20,0 mmol $l^{-1}kg^{-1}hr^{-1}$ measured 10 min after the 20-min exercise. $\dot{M}O_2$ rates could not be measured directly after exercise because time was needed for temperature equilibration of the respirometers. However, the $\dot{M}O_2$ of the crabs immediately after exercise could be indirectly inferred by interpolation of the data on the curve to zero time (Figure 2). This indirect inference by interpolation is not a reliable estimate of $\dot{M}O_2$ immediately after the excercise period. However, $\dot{M}O_2$ will not be less than 28 mmol $l^{-1}kg^{-1}hr^{-1}$ (Figure 1). Considerable variation of individual $\dot{M}O_2$ was encountered. One hour after the exercise the $\dot{M}O_2$ values were nearly back to normal resting values (Figure 2). During the 20-min exercise l-lactate levels in the haemolymph increased nearly fivefold to 20,7 mmol l^{-1}, compared with crabs kept for 8–10 h in the respiration chambers without exercise. (Table 1). This steep increase of the l-lactate levels in the haemolymph is all the more surprising because the running speed applied to the crabs by the treadmill, is at least 10 times slower than the maximum running speeds of which these animals are capable (Hafemann & Hubbard 1969).

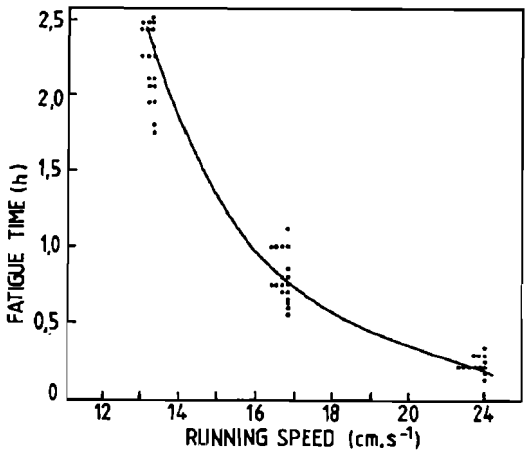

Figure 1 The relationship between running speed on a treadmill and fatigue time in *Ocypode ceratophthalmus* at 25°C. The size of the crabs was between 26 mm and 34 mm carapace length and a wet body mass between 19–27 g.

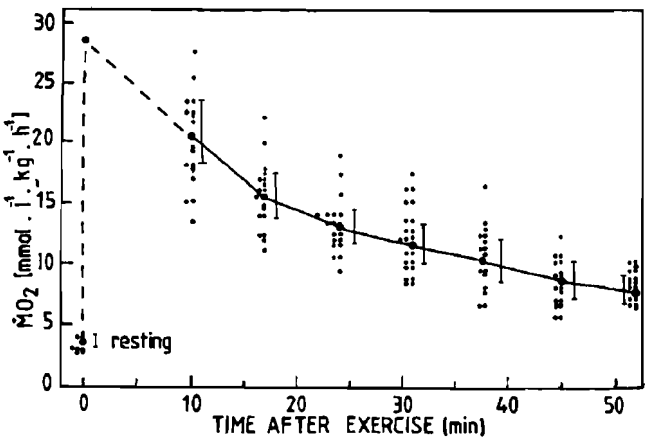

Figure 2 The oxygen consumption rate ($\dot{M}O_2$) from resting (n = 9) and 20 min exercising (n = 19) ghost crabs (*Ocypode ceratophthalmus*). The vertical bars denote the standard deviation from the mean. Broken lines are extrapolations to find the $\dot{M}O_2$ value at zero time after exercise.

Table 1 Haemolymph physiological properties of exercising and non-exercising ghost crabs; v = venous; + = calculated (Morris & Bridges 1985), n = number of animals

Physiological property			n
Haemocyanin concentration (mg ml^{-1})			
— exercised	=	95,6 (± 16)	20
— non-exercised	=	99,0 (± 22)	12
Haemolymph oxygen content			
(C_{O_2} tot.), mmol l^{-1}	=	1,83 (± 0,11)	8
Haemocyanin oxygen capacity*			
($C_{max/HcyO_2}$) mmol l^{-1}	=	1,50 (± 0,11)	
Pre-branchial PvO_2 (mm Hg)	=	35,9 (± 5,6)	7
Pre-branchial (v) total CO_2 content			
(C_{CO_2} tot.) mmol l^{-1}	=	22,6 (± 2,6)	8
L-lactate (mmol l^{-1})			
—5 h chilled crabs	=	0,68 (± 0,5)	3
—8–10 h resting crabs	=	4,5 (± 1,5)	12
—20 min exercising crabs	=	20,7 (± 4,8)	20
Pre-branchial haemolymph pH:			
—not exercised	=	7,91 (± 0,05)	11
—20 min exercised	=	7,71 (± 0,08)	9
—tonometered (5,1% CO_2 in air)	=	7,52 (± 0,08)	4
Sodium (mmol l^{-1})	=	542,6 (± 20,1)	10
Potassium (mmol l^{-1})	=	7,1 (± 0,5)	10
Magnesium (mmol l^{-1})	=	6,9 (± 0,2)	10
Calcium (mmol l^{-1})	=	8,2 (± 0,5)	10
Chlorides (mmol l^{-1})	=	369 (± 10,0)	10

The 8–10 h of resting after the crabs were caught, resulted in haemolymph l-lactate levels of 4,5 mmol l^{-1}, a value that clearly does not represent normal resting values of about 1 mmol l^{-1} or less for *O. saratan* (Morris & Bridges 1985) or 0,68 mmol l^{-1} found for three chilled specimens of *O. ceratophthalmus* (Table 1). L-lactate levels 44 min after exercise showed large variation from the mean of 20,7 mmol l^{-1}. However, this l-lactate concentration compares favourably with the l-lactate concentration of 15 mmol g^{-1} wet body mass found for *O. quadrata* after 20 min of exercise (Full 1987).

The oxygen dissociation curves obtained for native haemolymph show that the haemocyanin of *O. ceratophthalmus* is very sensitive to pH changes, caused by increasing the $[H^+]$ by adding CO_2 or HCl (Figure 3). At pH 7,92 with 0,68 mmol l^{-1} l-lactate, a haemocyanin with high oxygen affinity (P_{50} is 4,0 mmHg) was found. When 16 mmol neutralized l-lactate was added, the P_{50} value was found to be nearly the same (Figure 3). Morris & Bridges (1985) showed that neutralized l-lactate increases the oxygen affinity of *O. saratan* haemocyanin. This effect was demonstrated for haemolymph dialysed at 4°C for 24 h (Morris & Bridges 1985). For *O. ceratophthalmus* oxygen dissociation curves made with haemolymph containing 20,7 mmol l^{-1} l-lactate (and no CO_2) from crabs exercised for 20 min, show haemocyanin with high oxygen affinity at pH of 7,50. When the pH of the haemolymph with an intrinsical l-lactate level of 2 mmol l^{-1} is lowered to pH 7,39 by adding HCl, the P_{50} value increases to 21,5 mmHg. With 5,1% CO_2 in the gas mixtures for ODC measurements, the pH of the haemolymph decreased to 6,90 and a P_{50} value of 31,0 mmHg was obtained (Figure 3E). The oxygen dissociation curve represented in Figure 3A is typical of native haemolymph of *O. ceratophthalmus* during resting conditions and low l-lactate levels. Pre-branchial haemolymph PO_2 determined from seven laboratory-kept crabs under normoxic conditions (Table 1) has a mean value of 35,9 mmHg with large variation. For *O. quadrata* pre-branchial PO_2 was about half the value found in this study (Burnett 1979). The oxygen content of *O. ceratophthalmus* haemolymph is 4,11 ml O_2 per 100 ml haemolymph (1,83 mmol l^{-1}) and is twice as much compared with the same values in *O. quadrata* (Burnett 1979) and *O. saratan* (Morris & Bridges 1985).

Most probably the high oxygen content values in *O. ceratophthalmus* could be attributed to the much higher haemocyanin concentrations of 95,6 mg ml^{-1} and 99,3 mg ml^{-1} found respectively in the haemolymph of exercised and resting *O. ceratophthalmus* compared with the value of 53,4 mg ml^{-1} for *O. saratan* (Morris & Bridges 1985). However, large haemocyanin concentration fluctuations among haemocyanin-carrying invertebrates seems to be a common feature (Senozan & Briggs 1989).

The *in vivo* pH of the haemolymph of resting crabs is 7,91. This is in accordance with similar pH values found for other ocypodids (Morris & Bridges 1985; Burnett 1979). A contributing factor for the high pH values of the haemolymph in these crabs is the high C_{CO_2} tot. value of 22,6 mmol l^{-1} determined for *O. ceratophthalmus* and of 23 mmol l^{-1} found for *O. saratan* (Morris & Bridges 1985). Haemolymph that is gel-chromatographed with Sephadex G25 (pH 7,88, no CO_2, 10 mmol l^{-1} Mg^{2+}, 20 mmol l^{-1} Ca^{2+}, 0,1 mmol l^{-1} Tris-HCl, no l-lactate) has haemocyanin with a high oxygen affinity (Figure 4C) with values between 1,45 mmHg and 1,49 mmHg. When 12,6 mmol l^{-1} neutralized l-lactate is added to the gel-chromatographed haemocyanin, the P_{50} did not change. Sephadex-cleaned

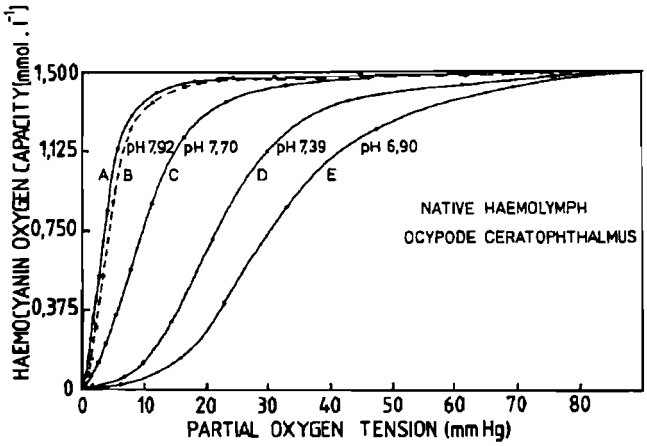

Figure 3 Oxygen dissociation curves of native haemolymph of *Ocypode ceratophthalmus* before and after exercise. A: 5 h chilled crabs, pH 7,92 with 0,68 mmol l^{-1} l-lactate, P_{50}, 4,0 mmHg; B: pH 7,92 with 16,0 mmol l^{-1} l-lactate experimentally added, P_{50} 4,5 mmHg; C: pH 7,70 after 20 min exercise with 20,7 mmol l^{-1} l-lactate accumulation. Measurements made without CO_2 in samples, P_{50} 10 mmHg; D: pH 7,39 with 2 mmol l^{-1} l-lactate, (no CO_2) and HCl experimentally added according to Morris & Bridges (1985), P_{50} 21,5 mmHg; E: pH 6,90 with 2 mmol l^{-1} l-lactate and 5,1% CO_2 in sample during measurement, P_{50} 31,0 mmHg.

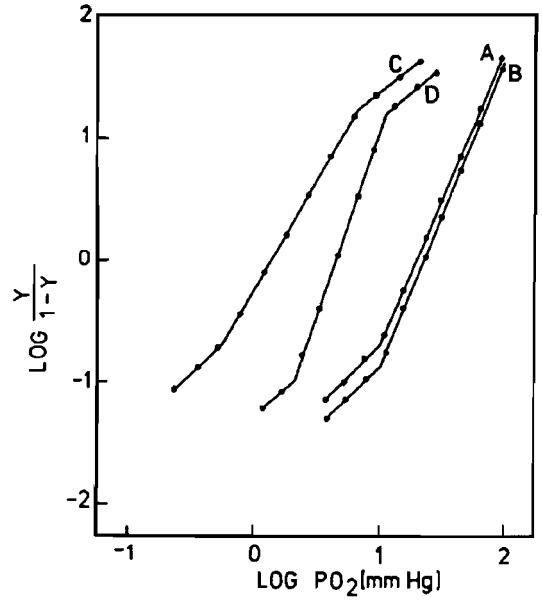

Figure 4 Hill-plots of gel-chromatographed haemolymph. Eluant: (in mmol l^{-1}) Tris-HCl 100, $CaCl_2$ 20, $MgCl_2$ 10 with a flow rate of 5–7 ml h^{-1}; A: pH 7,03; CO_2 5,1%; Temp. 25°C; P_{50} 20 mmHg; *n* 2,5; B: pH 7,03; CO_2 nil; Temp. 25°C; P_{50} 23,7 mmHg; *n* 2,5; C: pH 7,88; CO_2 nil; Temp. 25°C; P_{50} 1,45 mmHg; *n* 1,8; D: pH 7,88; CO_2 5,1%; Temp. 25°C; P_{50} 4,5 mmHg; *n* 3,2.

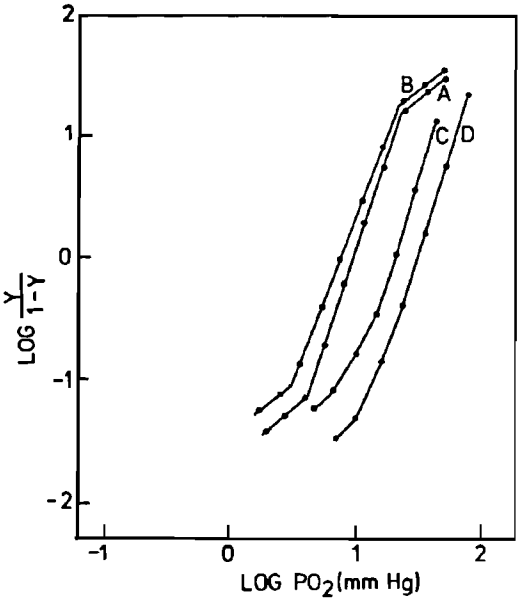

Figure 5 Hill-plots made of native (A) and dialysed haemolymph (B, C, D,). Dialysis medium (in mmol l^{-1}) NaCl 396; KCl 7,1; $MgCl_2$ 7,0; $CaCl_2$ 8,2 and $NaHCO_3$ 22,1. A: pH 7,91; CO_2 nil; Temp. 25°C; P_{50} 8,7 mmHg; *n* 3,7; B: pH 8,68; CO_2 nil; Temp. 25°C; P_{50} 6,5 mmHg; *n* 2,6; C: pH 8,68; CO_2 2,5%; Temp. 25°C; P_{50} 17,7 mmHg; *n* 3,6; D: pH 8,68; CO_2 5,1%; Temp. 25°C; P_{50} 27,3; *n* 3,5.

haemolymph with 5,1% CO_2 resulted in a P_{59} value of 4,59 mmHg at pH 7,88 (Figure 4D). The effect of low pH on oxygen-haemocyanin binding is to decrease affinity. This is more pronounced than the 5,1% CO_2 has on the haemocyanin-oxygen affinity (Figures 4A, B). At high pH values the effect of 5,1% CO_2 is much more pronounced compared with 5,1% CO_2 at low pH values of the haemolymph (Figure 5). At this pH (7,8–7,9) most *in vivo* pH values of the haemolymph for resting *O. ceratophthalmus* were found.

The effect of temperature at a low pH value of 7,035 on haemocyanin-oxygen binding for gel-chromatographed haemolymph was to decrease affinity by 1 mmHg partial pressure per degree Celcius increase, while co-operativity increases from 2,4 to 3,3. An increase in co-operativity was also found when CO_2 was increased or pH lowered for all samples tested.

Discussion

The eightfold increase in the oxygen consumption of moderately exercised *O. ceratophthalmus* above resting values points to a well operating gas exchange system for an animal such as *O. ceratophthalmus* with an open blood circulatory system and a chitin layer (although a thin one) covering the gas exchange surface. Compared with the $\dot{M}O_2$ values of active ectothermic vertebrates such as lizards (Garland 1982) the $\dot{M}O_2$ maximum values for *O. ceratophthalmus* is nearly on the same level. However, compared to mammals of a similar size, the $\dot{M}O_2$ values are about 10 times higher (Koteja 1987). This much lowered $\dot{M}O_2$ in crabs may be explained by the large differences found in the crabs' O_2 conductance compared with insects or mammals. Oxygen conductance (expressed in units of microlitres of oxygen delivered per gram animal per hour per mmHg (Piiper, Dejours, Haab & Rahn 1971) from the outside of the body to the mitochondria is 1,43 µl for a 45 g exercised crab which increased its $\dot{M}O_2$ threefold from a resting value of 3,12 $mlO_2\ kg^{-1}hr^{-1}$. For a 21 g exercised mouse and a 5 g exercised cockroach, this value is, respectively, 35,3 µl and 6,93 µl (Herreid 1981). Other factors, apart from lower oxygen conductance, may also influence $\dot{M}O_2$ values in *O. ceratophthalmus*.

Despite the large $\dot{M}O_2$ increase during exercise (usually an indication of aerobic metabolism) *O. ceratophthalmus* accumulates a considerable amount of l-lactate (20,7 mmol l^{-1}) in the haemolymph. For *O. quadrata* the l-lactate accumulation was about 15 mmol g^{-1} (Full 1987) for the same exercise period. The use of anaerobic energy during the 20-min exercise of ghost crabs is a clear indication that haemolymph gas transport is functionally insufficient. Specific tissues, particularly the muscles, may operate under hypoxic conditions while the whole crab itself remains at normal oxygen concentrations. On the other hand, the particular gas exchange surface could limit gas diffusion into the animal. Piiper & Scheid (1975) made an analysis of gas exchange systems. If applied to *O. ceratophthalmus*, it seems that the branchiostegal 'lung' (the gills do not partake in gas exchange) and the open circulatory system calls for a diffusion limited (i.e. rate of gas exchange is limited by diffusion at the exchange surface) system (Piiper & Scheid 1975). Furthermore, the relatively low speed rate (0,25 m s^{-1}) that *O. ceratophthalmus* and *O. quadrata* (Full 1987) can sustain during exercise, is further evidence of the poor design of the respiratory system compared to mammals and insects. For comparative purposes, the upper sustainable speed in similarly sized mammals is 1,60 m s^{-1} (Herreid & Full 1988).

Recovery of $\dot{M}O_2$ to pre-exercised values are completed within 1 h in *O. ceratophthalmus* with similar findings by Full (1987) for *O. quadrata* and *O. guadichaudii* by Full & Herreid (1983). For the other land crabs *Cardisoma carnifex* (Wood & Randall 1981a) and *C. guanhumi* (Herreid, Lee & Shah 1979), recovery of $\dot{M}O_2$ to resting values was completed within 3 h. Recovery of l-lactate levels to normal values in the haemolymph of land or aquatic crabs after exercise takes at least 12 h to complete (Phillips, McKinney, Hird & Macmillan 1977; Van Aardt 1988). Elimination rates for l-lactate in ocypodids are not known but most probably it is a slow process and could explain the relatively high lactate levels found in *O. ceratophthalmus* 8–10 h after they had been captured (Table 1).

Notwithstanding the above, several investigators (Wood & Randall 1981b; McMahon, McDonald & Wood 1979) found that the time recovery curves for oxygen consumption rate and l-lactate break down rate show only a general correspondence. These authors (see McMahon 1987) strongly suggest that oxygen consumption and lactic acid removal are not closely coupled. These facts may explain the high l-lactate retention in the haemolymph and a relatively fast $\dot{M}O_2$ decrease of the post-exercising animal.

Despite the high l-lactate levels found in *O. ceratophthalmus* after exercise, the pH of pre-branchial haemolymph decreases only by 0,2 units to 7,714. However, it must be

remembered that haemolymph samples were taken for pH measurements 35 min after the exercises on the treadmill had stopped. During this period, the pH could have again increased. A high pH of the haemolymph was also found for the land crab *Cardisoma carnifex* after severe exercise (Wood & Randall 1981b), but not for the river crab *Potamonautes warreni* where the pH dropped from 7,51 to 7,11 (Van Aardt 1990). It seems that land crab haemolymph is well buffered and this may explain the high total CO_2 concentration (C_{CO_2} tot. values generally found in land crabs. In this connection the contribution of haemocyanin as a buffer substance in itself should not be overlooked. It is known that haemocyanin concentration and bicarbonate buffer capacities tend to increase with terrestriality (McMahon & Burggren 1988). When native haemolymph was tonometered with 5,1% CO_2 in air, a further decrease of 0,2 pH units was observed in *O. ceratophthalmus*. This may indicate that a 5,1% CO_2 in air (P_{CO_2} = 32,1 mmHg) is physiologically too high and cannot be buffered. No experimental data on what the P_{CO_2} in the haemolymph of ghost crabs is, exists. For other land crabs, P_{CO_2} in post-branchial haemolymph is between 4,0 mmHg and 10,5 mmHg (McMahon & Burggren 1988). The empirically determined oxygen content of the haemolymph ($HcyO_2$ + free O_2 in solution) averages 1,83 mmol l^{-1} (4,11 ml O_2 per 100 ml haemolymph). The haemocyanin oxygen capacity, when calculated (Morris & Bridges 1985), is 1,50 mmol l^{-1} (Table 1). These figures for *O. ceratophthalmus* compare favourably with the highest values found for any land crab investigated (McMahon & Burggren 1988). A possible advantage of increased O_2 content for terrestrial crabs is that the higher O_2 content allows more O_2 to be taken up and transported per unit haemolymph flow. According to McMahon & Burggren (1988), this in turn could allow reduced haemolymph flow through gas-exchange sites with consequently reduced respiratory water loss. The oxygen affinity at 50% saturation (P_{50}) for native haemolymph (pH 7,92) in the absence of CO_2 was below 5 mmHg. For crabs exercised for 20 min the P_{50} was 10 mmHg and is an indication of a relatively high oxygen affinity haemocyanin, a characteristic also shared by *O. saratan* (Morris & Bridges 1985) and *O. quadrata* (Burnett 1979) at haemolymph pH of 7,9. There seems to be no concensus among investigators about the significance of a high oxygen affinity haemocyanin for terrestrial crabs. Well designed and functional aerial gas exchange surfaces should result in low oxygen affinity haemocyanins, whereas the amphibian type of terrestrial crabs need a high oxygen affinity haemocyanin to cope with less advanced gas exchange surfaces. To complicate matters further, modulators such as l-lactate may increase or decrease haemocyanin oxygen affinity, depending on their concentration (Truchot 1980). From the data (Figure 3) it is clear that l-lactate does not affect the oxygen affinity of *O. ceratophthalmus* haemocyanin where 0,68 mmol l^{-1} and 16 mmol l^{-1} l-lactate in native haemolymph gave nearly the same P_{50} values. For *O. saratan*, native haemolymph, (at high pH) the oxygen affinity increases from 12,5 mmHg to 10 mmHg when l-lactate was increased from 1,26 mmol l^{-1} to 9,51 mmol l^{-1} (Morris & Bridges 1985). In native haemolymph of anomuran land crabs investigated, l-lactate had no effect on haemocyanin oxygen affinity. A dialysable component in the haemolymph of these crabs may be operating, diminishing the l-lactate effect on oxygen binding (Morris, Greenaway & McMahon 1988). The Hill plots (Figures 4–7) made of *O. ceratophthalmus* haemocyanin under various conditions of pH, CO_2, temperature and state of purity all indicate an increase in co-operativity (n = 1,9 to 4,5) when affinity decreases. Low pH on haemocyanin solutions decreases the oxygen affinity to a much greater extent than CO_2 (Figure 4). The insensitivity of CO_2 may be attributed to a terrestrial life where CO_2 levels are regulated by ventilation and / or buffered by the high total CO_2 content in the haemolymph.

The effect of temperature (Figure 6) on the oxygen binding of haemocyanin at low and high pH values, indicates a pH dependence, because oxygen affinity decreases measurably at low pH (Figure 6A, B, C) compared with the high pH haemocyanin (Figure 6D, E, F). The right shift of the curves is caused by a decrease in O_2 affinity of haemocyanin owing to change in the specific heat of oxygenation (McMahon & Burggren 1988). High temperature presumably promotes the unloading of oxygen to the tissues and can be verified by the right shift of the oxygen dissociation curve. A decrease in O_2 affinity is due to an increase of proton (H^+) concentration. Temperature and pH in tandem can have dramatic effects on the ability of the ghost crab's oxygen transport system to deliver sufficient O_2 at extremes of the crab's temperature range Figure 7D).

McMahon & Burggren (1988) strongly emphasize the need in crab research for *in vivo* measurements of respiratory parameters, especially haemocyanin oxygen-binding

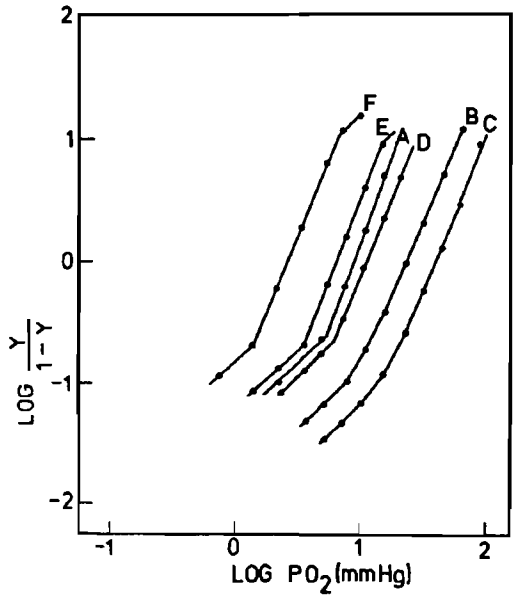

Figure 6 Hill-plots made of gel-chromatographed (A, B, C,) and dialysed (D, E, F) haemolymph. Eluant: as in Figure 4. Dialysis medium: as in Figure 5. A: pH 7,03; CO_2 nil; Temp. 15°C; P_{50} 10,9 mmHg; n 2,4; B: pH 7,03; CO_2 nil; Temp. 25°C; P_{50} 23,7 mmHg; n 2,6; C: pH 7,03; CO_2 nil; Temp. 35°C; P_{50} 39,8 mmHg; n 3,3; D: pH 8,68; CO_2 nil; Temp. 35°C; P_{50} 9,17 mmHg; n 3,6; E: pH 8,68; CO_2 nil; Temp. 25°C; P_{50} 6,5 mmHg; n 2,6; F: pH 8,68; CO_2 nil; Temp. 15°C; P_{50} 2,66 mmHg; n 1,9;

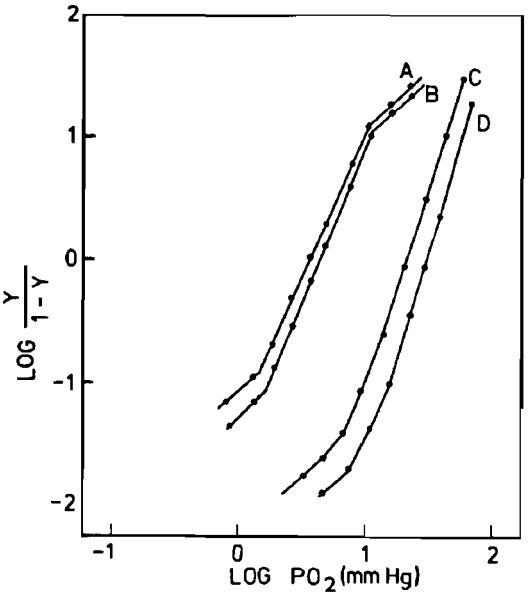

Figure 7 Hill-plots of native haemolymph from *O. ceratophthalmus*. A: l-lactate 0,68 mmol l^{-1}; pH 7,92; CO_2 nil; Temp. 25°C; P_{50} 4,65 mmHg; *n* 2,6; B: 16,0 mmol l^{-1} l-lactate; pH 8,10; CO_2 nil; Temp. 25°C; P_{50} 3,65 mmHg; *n* 1,8–2,1; C: 2,0 mmol l^{-1} l-lactate; pH 7,36 (HCl); CO_2 nil; Temp. 25°C; P_{50} 21,3; D: 2,0 mmol l^{-1} l-lactate; pH 7,36 (HCl); CO_2 5,1%; Temp. 25°C; P_{50} 30,3 mmHg.

data whenever practically possible. In this manner physiological comparisons can be made on a direct organismal basis, thus allowing a better ecophysiological judgement on such animals. For instance, the relatively small terrestrial *O. ceratophthalmus* has an *in vivo* PaO_2 of 35,6 mmHg (this study) and *O. quadrata* a PaO_2 of 20,0 mmHg (Burnett 1979) compared with the *in vivo* PaO_2 of 130–140 mmHg of the highly terrestrial crab *Pseudotelphusa garmani* (Innes, Taylor & Haj 1987).

It can be concluded that the respiratory properties in *O. ceratophthalmus* do not differ much from what is known of other similar ocypodids. Despite the large lactate accumulation in the haemolymph during exercise, ghost crabs have aquired a well-adjusted set of respiratory processes to cope efficiently with running behaviour.

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