Oxygen uptake and haemocyanin oxygen affinity of *Potamonautes warreni* Calman after exercise

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The $\dot{M}O_2$ values for exhaustively exercised crabs in water medium or air medium are nearly twice the values found for pre-exercised crabs. The l-lactate concentration in the haemolymph increases from 0,75 ± 0,37 mmol to 9,63 ± 2,1 mmol in *P. warreni* after air exercise, a value similar to those for other terrestrial and aquatic crabs. The same increase in the l-lactate concentration was found for water-exercised crabs. The pH in the haemolymph decreases from 7,51 (*SD* 0,07) to 7,11 (*SD* 0,08) after the 20 min air medium exercise and from 7,521 (*SD* 0,06) to 7,229 (*SD* 0,09) after 20 min exercise in water medium. In both respiratory media this causes a lowering in the haemocyanin oxygen affinity with a Bohr-factor of −1,05 whilst the haemocyanin-oxygen co-operativity increases from 3,0 to 3,7. Preliminary *in vitro* experiments indicate that l-lactate (as an acid metabolite) is mainly responsible for the Bohr-effect.

Die $\dot{M}O_2$-waardes vir krappe na strawwe oefeninge is ongeveer tweekeer hoër as derglike waardes vir niegeoefende krappe. Die l-laktaatkonsentrasie in die hemolimf neem van 0,75 ± 0,37 mmol tot 9,63 ± 2,1 mmol toe in *P. warreni* na strawwe oefening in lug. Dieselfde laktaatwaardes is verkry vir krappe wat in water geoefen is. Hierdie toename in laktaatwaardes is dieselfde wat gevind is vir ander terrestriële en akwatiese krapsoorte. Die pH van die hemolimf neem af van 7,51 (*SD* 0,07) tot 7,11 (*SD* 0,08) na die 20 min oefening in lug op die trapmeul en van 7,521 (*SD* 0,06) na 7,229 (*SD* 0,09) na 20 min oefening in 'n watermedium. 'n Direkte gevolg hiervan is 'n verlaging in die hemosianien- suurstofaffiniteit met 'n Bohr-faktor van −1,05 terwyl die hemosianien-suurstofkoöperatiwiteit van 3,0 na 3,7 toeneem. Voorlopige *in vitro*-eksperimente toon aan dat l-laktaat (as 'n suurmetaboliet) hoofsaaklik verantwoordelik is vir die Bohr-effek.

In recent experiments it was found that several members of the brachyuran decapods are capable of respiring equally well in air and in water (McMahon & Wilkens 1983). For the freshwater crab *Potamonautes warreni* the resting specific oxygen consumption rate ($\dot{M}O_2$) in water is 34,4 μmol O_2 kg^{-1} min^{-1} and in air it is 31,9 μmol O_2 kg^{-1} min^{-1} at 25°C (Van Aardt 1990). For the same experimental conditions $\dot{M}O_2$ values in air and in water for two crab species *Carcinus meanas* and *Cardisoma guanhumi* are not only the same but closely resemble the values found for *P. warreni* (Taylor & Wheatly 1979; McMahon & Wilkens 1983). An important condition to be met in obtaining these $\dot{M}O_2$ values is that the experimental crabs must be confined in the respirometer chamber with minimal body activity. In this way the resting metabolism (Herreid 1981) of the crab can be measured. Meaningful respiratory comparisons can then be made between the resting metabolism of crabs at physiologically different environments. It would, however, also be very interesting to compare the respiratory physiology of crabs subjected to exercise both in air and water as respiratory media with those of resting control crabs. Data on such experiments could be used to calculate the factorial aerobic metabolic scope [$\dot{M}O_2$ maximum/$\dot{M}O_2$ rest (= standard)], (Herreid 1981; Schmidt-Nielsen 1984). A high value of the factorial metabolic scope is a good indicator of the efficiency of gas exchange surfaces, blood capillary circulation, ventilation rates and cardial output (Piiper, Dejours, Haab & Rahn 1971). Furthermore any difference in the factorial aerobic metabolic scope between air-exercised and water-exercised crabs would be an indication of the efficiency of the gas transporting surfaces exposed to water or air as respiration media.

In this investigation the measurements outlined above were made on *P. warreni* exercised either in water or air as respiratory medium. The significance of l-lactate formed during exercise as an allosteric effector on the intrinsic O_2 affinity of *P. warreni* haemocyanin was also investigated.

Materials and Methods

Individuals of the freshwater crab *P. warreni* (56,9–92,8 g), which includes both female and male specimens, were collected from the northern part of the Mooi River situated in the municipal area of Potchefstroom (26°40'S / 27°05'E). In the laboratory the crabs were maintained in a climatic room with 12L : 12D photoperiod as described by Van Aardt & Wolmarans (1987). The crabs survived in a healthy condition for at least six months and regularly underwent molting. Crabs were used for the experiments two or three weeks after being collected in the field. Experimental crabs were starved overnight, had hardened carapaces and were judged to be in intermolt.

Experimental protocol. Groups of at least 15 crabs each were selected for the experiments. First a 0,3 ml prebranchial haemolymph sample was collected (Van Aardt & Wolmarans 1987) from crab one. Then the resting $\dot{M}O_2$ of the crab was determined in water with a respirometer. Three days later another prebranchial haemolymph sample was taken from the same crab after 20 min of exercise on a treadmill in air as respiratory medium and stored on ice. The $\dot{M}O_2$ of the crab was measured immediately afterwards in water with a

respirometer. Exactly the same procedure was followed for crabs exercised in water as respiratory medium. In another experiment a 1 ml haemolymph sample was taken from a crab at resting $\dot{M}O_2$. The l-lactic acid concentration was determined. The rest of the sample was divided into two equal parts. To one part l-lactic acid was added to give a total l-lactic acid concentration of 10 mmol l^{-1}. Each part was used to measure the oxygen-binding properties of haemocyanin in the haemolymph.

Analysis and determinations. The pH of the haemolymph samples was measured (before and after exercise and before and after the lactic acid was added) with the aid of a micro-electrode unit (Type E5021, Radiometer, Copenhagen). L-lactate in the haemolymph was determined according to Noll (1974) without deproteinization and neutralization with a fully enzymatic kit (no. 256773) from Boehringer-Mannheim. A stable reading at 365 nm was observed 30–40 min after the enzyme was added. No interference by heavy metal ions (Cu^{++}) was observed using this method (Graham, Mangum, Terwilliger & Terwilliger 1983).

The L-(+)-lactate added to the haemolymph was obtained from Sigma Chemical Co. as a free acid crystalline compound (98% purity). The oxygen-binding properties of haemocyanin were measured by means of a thin-layer optical cell. In essence the measurement was done by placing a thin layer (100 μm thick) of haemolymph (containing 50–80 mg/ml haemocyanin) in the optical path of a standard spectrophotometer and the absorbance of haemocyanin measured at 335 nm. The device that held the layer also functioned to provide decreasing amounts of oxygen partial pressure (PO_2) to the layer in dilution (air diluted with N_2) steps. In this manner the PO_2 after each dilution step could be controlled with an accuracy better than 0,1 mmHg (Dolman & Gill 1978). In another experiment the oxygen-binding properties of declotted haemolymph (haemolymph serum, Durliat 1985) were measured. Freshly drawn haemolymph (0,9 ml) was taken into a syringe and allowed to clot in a cuvette on ice. The jelly-like sample was put into a glass homogenizer and manually homogenized in ice and then centrifuged at 7000G. The supernatant was the haemolymph serum. The haemocyanin concentration in the haemolymph was determined spectrophotometrically with a 20 μl sample dissolved in 2 ml crab Ringer (NaCl 250 mmol; $CaCl_2$ 16 mmol; $MgCl_2$ 6,9 mmol; KCl 6,0 mmol; $NaHCO_3$ 10,0 mmol) using the extinction coefficient ($2{,}69^{1\%/1cm}$) of Nickerson & Van Holde (1971) on *Homarus americanus*.

Crabs were exercised in air on a treadmill (40 cm long × 22 cm wide) enclosed by four 20 cm high walls forming a black Perspex chamber. The moving floor of the treadmill consisted of a specially made silicone rubber impregnated canvas. Four fifths of the open roof part during a run was closed with black plastic sheeting. A light source (1236 lx at 45 cm) was positioned 45 cm above the canvas floor above the small opening. The crab usually ran so as to maintain its position in the darkened part of the chamber, coming back into the light part of the treadmill only when close to fatigue.

The speed of the treadmill can be varied between 0 to 35 cm s^{-1} with the aid of a Slo-syn steppingmotor (S525) responding 1,8 degrees per electric pulse through a voltage to frequency converter.

The relationship between running speed on a treadmill and time to get fatigued in *P. warreni* was investigated in preliminary runs. Individuals between 60–80 g in mass and carapace length between 6,2–6,6 cm showed sustained exercise for at least 10 min at a speed of 3,2 cm s^{-1} (0,5 carapace length per second) (Figure 1). For crabs smaller or larger than 6,4 cm carapace length the treadmill speed was adjusted with a potentiometer to 0,5 of the carapace length per second.

Crabs were exercised in a water medium according to the method described by Houlihan & Innes (1984). The same speed of 0,5 carapace length per second, used for the treadmill, was applied to crabs exercised in water.

The specific oxygen consumption rate ($\dot{M}O_2$ during rest and immediately after exercise) was determined in

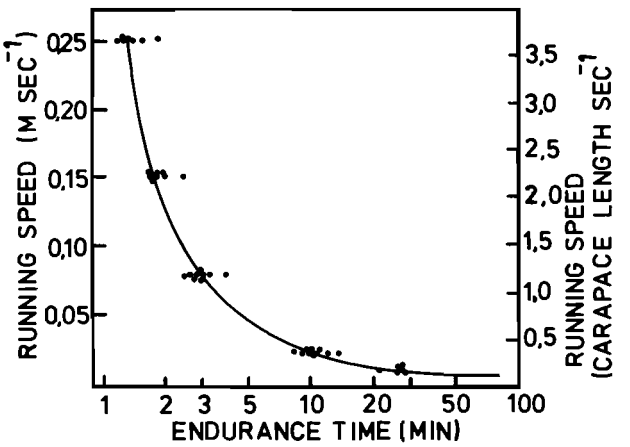

Figure 1 The relationship between running speed on a treadmill and endurance time in *Potamonautes warreni* at 25°C.

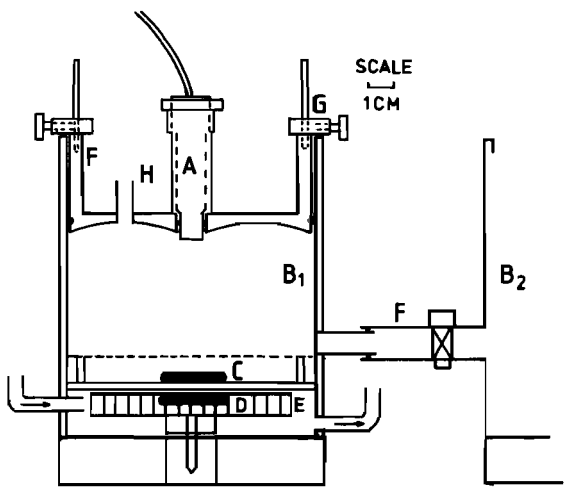

Figure 2 A diagrammatic drawing of part of the closed system respirometer used to determine the oxygen consumption rate of *P. warreni* in water after exercise on a treadmill and during resting conditions. See text for details.

water with the aid of a Clark-polarographic oxygen sensor (POS) (Figure 2A) sealed in a closed chamber respirometer (Figure $2B_1$). The water as respiratory medium, was continuously agitated with a magnetic stirring bar (Figure 2C). Stirring of the bar was achieved by magnetically induced rotation with a magnetic head (Figure 2D) fixed onto a water-driven turbine (Figure 2E). The respirometer lid (Figure 2F) that holds the POS could quickly be fitted on top of the respirometer at a desired depth with the aid of the three adjusting rods with screws (Figure 2G). The water overflow (Figure 2H) also serves as an air bubble escape route for the respiratory water medium kept at a volume of 955 ml. With the aid of the interconnecting hose and tap (Figure 2F) a new quantity of air-saturated water could be transferred from one respirometer (B_2) to another (B_1). This procedure was followed when duplicate runs were made of the $\dot{M}O_2$ measurements. The respirometers were placed in a waterbath at 25°C during measurements. The decrease in the PO_2 values as measured by the POS was registered with the aid of a REC 61 Servograph recorder fitted with a REA 112 Sensitivity Unit both from Radiometer, Copenhagen. For the oxygen consumption rate measurements the PO_2 value of the water was not allowed to decrease to below 60% of the starting value of 15,9–17,3 kPa. The displacement volume of each crab was measured in water and subtracted from the 955 ml water volume of the respirometer. The values found by Benson & Krause (1980) for the concentration of oxygen dissolved in a quantity of fresh water in equilibrium with the atmosphere at a particular barometric pressure (87,3–87,9 kPa, Potchefstroom) were used for $\dot{M}O_2$ calculations at BTPS. Oxygen consumption rates were also measured in air for crabs (n = 5) at rest and after 20 min of treadmill exercise in air with the aid of a manometric method (van Aardt 1988a).

Oxygen-haemocyanin dissociation curves were constructed (Figure 3) and Hill-plots made to quantify haemocyanin-oxygen affinity (P_{50}) and oxygen-binding site co-operativity (n_{max}) between 25% and 75% saturation. The results were subjected to Student's t test. Means (SD) and the 5% level of significance were used throughout.

Results

The 20 min exercise period chosen causes maximal energy expenditure for the crab, because after 10–15 min exercise the crabs responded very slowly or not at all to tactile stimuli, became refractory and often tried to rest with their carapaces on the floor of the exercising device. The results (Table 1) show that $\dot{M}O_2$ values for exercised

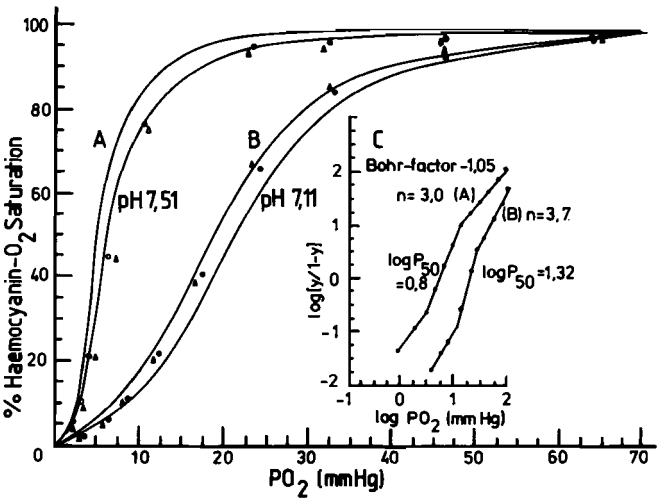

Figure 3 The effect of exercise and l-lactate on oxygen binding of hemocyanin from the crab *P. warreni*. A: Oxygen equilibrium curves of pre-exercising crabs (n = 6) with 0,10–0,73 mmol l-lactate in the haemolymph (pH 7,51 ± 0,09). B: Oxygen equilibrium curves of exercising crabs (n = 5) with 7,19–11,01 mmol lactate in native heamolymph (pH 7,11 ± 0,13). △: Oxygen equilibrium curve of haemocyanin (n = 3) pH 7,56 with 0,5 mmol l-lactate added. ▲: Oxygen equilibrium curve of haemocyanin (n = 2) pH 7,13 with 10 mmol l-lactate acid added. ○: Oxygen equilibrium curve of declotted haemolymph from pre-exercising crabs. ●: Oxygen equilibrium curve of declotted haemolymph from exercising crabs. C: Hill-plots made from data obtained from curves A and B.

Table 1 The specific oxygen consumption rate ($\dot{M}O_2$), haemolymph pH and haemolymph l-lactate concentration of crabs exercising for 20 min in air or water as respiratory medium. * manometric method for measuring specific oxygen consumption rate in air, n = number of animals used per measurement

	pH		l-lactate (mmol l^{-1})		$\dot{M}O_2$ (μmol kg^{-1} min^{-1})	
	before exerc.	after exerc.	before exerc.	after exerc.	before exerc.	after exerc.
Air-exercised crabs	n = 15	n = 15	n = 15	n = 15	n = 15	n = 15
	7,510 ± 0,07	7,110 ± 0,08	0,75 ± 0,37	9,6 ± 2,1	40,5 ± 5,9	75,8 ± 10,9
Water-exercised crabs	n = 20	n = 20	n = 20	n = 20	n = 20	n = 20
	7,521 ± 0,06	7,229 ± 0,09	0,63 ± 0,26	10,9 ± 1,2	45,1 ± 7,7	71,3 ± 11,3
Air-exercised crabs*	–	–	–	–	n = 5	n = 5
	–	–	–	–	40,2 ± 4,9	74,8 ± 12,2

crabs in air and water are nearly twice the values for pre-exercised crabs. The $\dot{M}O_2$ values for exercised crabs in water or air are nearly the same and when compared, do not differ significantly ($p < 0,05$).

The $\dot{M}O_2$ values for air-exercised crabs measured in air by the manometric method are the same compared with the $\dot{M}O_2$ values for air-exercised crabs measured in water by the polarographic method (Table 1).

Decapods are very susceptible to stress during handling with the result that unusually high $\dot{M}O_2$ values are encountered directly after handling (Greenaway, Taylor & Bonaventura 1983b; McMahon 1981. Thus Greenaway *et al.* (1983b) found that $\dot{M}O_2$ decreased at least five times, 150 h after the crabs were disturbed. In the light of this information pre-exercised $\dot{M}O_2$ values found for *P. warreni* may be too high to be described as standard or routine metabolism. This is so because specimens were taken from their containers and, after haemolymph sampling, were immediately subjected to the $\dot{M}O_2$ measurements.

It may be argued that the $\dot{M}O_2$ values obtained after the exercise do not represent the actual oxygen consumption rate of crabs during a sustained exercise period. However, it is known from the work of Herreid (1981) on crabs that oxygen consumption does not reach a steady state during exercise. In fact, peak $\dot{M}O_2$ values were obtained several minutes after the run was terminated. Furthermore our data during the $\dot{M}O_2$ measurements of exercised *P. warreni* showed a linear decrease of the PO_2 of the water as traced on the recording Servograph. This lasted for at least 20 min after the crabs started to consume the oxygen in the water.

The $\dot{M}O_2$ values for *Holthuisana transversa,* the only other freshwater/land crab investigated, are about three times lower at resting conditions and twice as low for active crabs when compared to the $\dot{M}O_2$ values for *P. warreni*. No difference was found between $\dot{M}O_2$ values for *H. transversa* respiring in air and in water during rest and activity (Greenaway, Bonaventura & Taylor 1983a, 1983b). Compared with other air-breathing decapods such as *Coenobita clypeatus* (McMahon & Burggren 1979), *Birgus* (Cameron & Mecklenburg 1973), *Gecarcinus* and *Cardisoma* (Cameron 1975) and *Ocypode quadrata* (Bennet 1978), $\dot{M}O_2$ values from resting *P. warreni* (40,51 μmol kg^{-1} min^{-1}) are very much the same. The factorial aerobic metabolic scope ($\dot{M}O_2$ maximum/$\dot{M}O_2$ standard) (Bennet 1978) is nearly two in *P. warreni*. This figure is about two times lower compared to the factorial metabolic scope of several terrestrial and aquatic decapods after exercise (McMahon 1981; Herreid 1981; McMahon & Wilkens 1983).

The mean l-lactate levels (0,75 ± 0,37 mmol) of prebranchial haemolymph in pre-exercised *P. warreni* is higher compared with l-lactate levels (0,05–0,06 mmol found by Graham, Mangum, Terwilliger & Terwilliger (1983) and Albert & Ellington (1985) (0,19 ± 0,17 mmol). Both these authors modified their method for l-lactate analysis to avoid unstable reaction end points. After 40 min, stable end points were always found for our samples when the analytical method of Noll (1974), without deproteinization with 12% trichloric acetic acid was used. The relatively high l-lactate values obtained for *P. warreni* at rest may be attributed to a stress factor or body activity during handling and during the haemolymph sampling procedure. It has been proved by Truchot (1980) that l-lactate levels less than 2 mmol in the haemolymph of *Cancer pagarus* have a pronounced effect on the haemocyanin oxygen affinity.

L-lactate levels in the haemolymph for exhaustively exercised *P. warreni* are 9,63 ± 0,37 mmol. These values are in agreement with l-lactate values found in similar experiments for several terrestrial and aquatic crab species investigated (McMahon 1981). In our experiments the effect of l-lactate on *P. warreni* haemocyanin was to decrease the oxygen affinity (Figure 3) when l-lactate as lactic acid was added to the native haemolymph sample. The result is that the pH of the sample is drastically lowered from 7,51 to 7,11 (Figure 3). It is known that l-lactate concentrations from 2–15 mmol (neutralized in the form of Na-l-lactate) increase the oxygen affinity of the haemocyanin of various decapods (Truchot 1980; Mangum 1983). The large Bohr-effect of –1,05 (Figure 3) found for *P. warreni* when lactic acid is added or when lactic acid is produced during exercise (proton influence on haemocyanin-oxygen binding) probably completely obscures the lactate influence on haemocyanin-oxygen binding, first described by Truchot (1980). However, it is also possible that the haemocyanin in *P. warreni*, like some other crustacean haemocyanins, (Mangum 1983; Bridges 1988), is not modulated by the neutralized lactate. The co-operativity in oxygen binding at P_{50} increases from 3,0 to 3,7 when lactic acid is added *in vitro* or when lactic acid is produced during exercise (Figure 3). This observation is in agreement with those in similar experiments on *Callinectes sapidus* (Booth, McMahon & Pinder 1982) and *Palaemon elegans* (Bridges, Morris & Grieshaber 1984). From Figure 3 it is clear that no difference in oxygen affinity was found between haemocyanin in haemolymph serum and haemocyanin with clotting proteins in the haemolymph sample.

Discussion

P. warreni fatigued much quicker compared with other terrestrial crabs. Wood & Randall (1981) reported that the land crab *Cardisoma carnifex* (100 g body mass) maintained 0,2 carapace lengths s^{-1} (0,045 m s^{-1}) for at least 2 h whilst *Ocypode cerato-opthalmus* (18,5 g body mass), an active terrestrial shore crab, is able to maintain running speed for at least 1 ½ h at 5,3 carapace lengths s^{-1} (0,17 m/s^{-1}) (personal observation).

The accumulation of l-lactate in the haemolymph during strenuous exercise in *P. warreni* clearly demonstrates the dependance of this crab on anaerobic processes for its energy supply, during severe activity. The anaerobic capacity i.e. the amount of lactate produced during strenuous exercise (Bennett & Licht 1972) is about the same as those found in other decapods (McMahon 1981). These values (11–17 mmol kg^{-1}) fall in the same concentration range reported for small reptiles

(Bennett 1978). The decrease of the haemolymph pH from 7,510 to 7,110 for *P. warreni* is probably not only caused by lactic acid (metabolic acidosis) but also by CO_2 production during aerobiosis to form carbonic acid. An increase in the PCO_2, both in the 'venous' and 'arterial' compartments has been found for other crabs after exercise (McMahon 1981; Wood & Randall 1981).

A further decrease in the haemolymph pH during exercise could be counteracted by a H^+ absorbing mechanism where bicarbonates and calcium are used as neutralizing agents thus changing their concentrations in the haemolymph (Van Aardt & Wolmarans 1987; Defur, Wilkes & McMahon 1980). Succinic acid formed during anaerobiosis in bivalves, was neutralized by their shell (Crenshaw & Neff 1969) while $CaCO_2$ deposits play a major role in neutralization of lactic acid formed during anoxia in turtles (Ultsch & Jackson 1982).

The value of the factorial aerobic scope of two found for *P. warreni* undoubtedly points to the fact that the maximal rate at which oxygen is used is low and the maximal capacity of the oxygen supply system is rather poorly developed. This is clear when the factorial aerobic metabolic scope of two for *P. warreni* is compared with that of the active terrestrial shore crab *Ocypode quadrata* of about eight. In this regard *O. quadrata* does not differ from an ectothermic vertebrate of the same size that uses lungs and a closed circulatory system (Full 1987). Apart from some agility, *P. warreni* does not endure long bouts of body activity (Figure 1). According to Taylor & Weibel (1981) each animal type adjusts its functional system to the level required for maximal performance and that no more structure is designed or maintained than is required to fulfill this maximum need.

The intrinsic oxygen affinity of haemocyanin in native haemolymph of *P. warreni* at pH 7,4 (25°C) was 6,36 mmHg. This value was obtained in the absence of CO_2 in the thin layer sample used to construct the oxygen equilibrium curves. It is known (Truchot 1973; Weber & Hagerman 1981; Morris, Taylor, Bridges & Grieshaber 1985; Mangum & Burnett 1986) that at constant pH, the O_2 affinity of the haemocyanin was increased by CO_2. Obviously the CO_2 must preferentially combine with the oxy-R form of the pigment. Mangum (1980), however, is of the opinion that the CO_2 effect on oxygen affinity is small in decapods within the physiological range of variation.

Recently, investigators could not find any haemocyanin-CO_2 sensitivity in a terrestrial brachyuran (*Ocypode saratan*), a land hermit crab (*Coenobita clypeatus*) and in tarantula haemocyanin (Morris & Bridges 1985; Morris & Bridges 1986; Bridges 1988). Nothing is known of the effect of CO_2 on haemocyanin-oxygen affinity of *P. warreni*.

The haemocyanin affinity for oxygen in *P. warreni* under similar physiological conditions is the same for haemolymph without clotting proteins and haemolymph with clotting proteins (Figure 3).

It is interesting to note that for several decapods (investigated) non-dialysed haemolymph was found to exhibit a higher oxygen affinity than dialysed haemolymph at the same pH and lactate concentrations. This effect has been defined as the effect of unidentified factor(s) (UF). No change in oxygen affinity was observed when known anaerobic metabolites were added to dialysed blood (Bridges & Morris 1986). However, urate has been implicated as one of the UF factors (Morris, Taylor, Bridges & Grieshaber 1985).

The Hill value, *n*, gives an indication of the minimum number of active sites involved in the co-operativity of the haemocyanin molecules. The n_{max} at P_{50} is 3,7 for *P. warreni*, an indication that the haemocyanin molecule has more than four active sites. Like other brachyurans it probably consists of dodecamers or hexamers (Mangum 1983b). From the Hill plot (Figure 3A) the following can be deducted: By intersecting the two asymptotical straight lines with the X-axis, the values (in mmHg) of KT and KR, the affinity constants of the pigment molecule, can be found in the T- and R-states respectively (Van Holde & Miller 1982). In the case of *P. warreni* KT is 1,67 mmHg and KR is 7,79 mmHg. Other allosteric effectors such as calcium and magnesium, at physiological concentrations, increase the oxygen affinity for haemocyanin in most crab species investigated (Mangum 1983a). Calcium increases from about 10 mmol to 16 mmol during metabolic acidosis in *P. warreni* (Van Aardt 1988b). This increased calcium concentration does not only act as a buffer system as proposed by Truchot (1973) but also stabilizes the haemocyanin molecule. Removal of calcium to less than 10 mmol causes considerable dissociation of the dodecamer of the freshwater brachyuran *Potamon edulis in vitro* (Chantler, Harris & Bannister 1973).

An important result from this investigation is that the metabolic scope values for water- or air-exercised crabs is the same. From this it can be concluded that the gas exchange surfaces and ventilation structures operating during air respiration function at the same exchange rate compared with the gas exchange surfaces and ventilation structures for crabs when exercised in water as respiration medium. Whether these gas exchange surfaces used for air respiration are topographically the same when used for water respiration remains to be investigated.

The results of the effect of exercise on the physiology and haemocyanin affinity of *P. warreni* presented here show that this freshwater crab cannot be compared to the highly active terrestrial crab species that posess a much higher aerobic metabolic scope. However, *P. warreni* uses a haemocyanin that is generally much more sensitive to internal changes such as l-lactate accumulation compared to the haemocyanin in terrestrial crabs. The large normal Bohr-shift found for exercised *P. warreni* is advantageously used to provide efficient unloading of oxygen at the tissue side at a stage when oxygen in transit is badly needed.

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