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AN INVESTIGATION OF HAEMOCYANIN OXYGEN AFFINITY IN THE SEMI-TERRESTRIAL CRAB *OCYPODE SARATAN* FORSK

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SUMMARY

The oxygen affinity of the haemocyanin in the supralittoral crab *Ocypode saratan* was investigated at temperatures between 20 and 35 °C. The effect of L-lactate on dialysed and undialysed haemolymph oxygen affinity was also examined.

In general, the temperature sensitivity of the haemocyanin was low: ΔH was $-3{\cdot}1\,kJ\,mol^{-1}$, between 25 and 30 °C. Temperature sensitivity was temperature-dependent, being larger at the extreme temperatures ($\Delta H = -26\,kJ\,mol^{-1}$). The Bohr effect ($\Delta \log P_{50}/\Delta pH$) was temperature-independent and averaged $-0{\cdot}67$.

No specific effect of CO_2 on oxygen affinity was observed but L-lactate increased oxygen affinity in both dialysed and undialysed haemolymph. The maximal effect of lactate on oxygen affinity was similar in dialysed and undialysed haemolymph, but was evident at a lower lactate concentration ($4\,mmol\,l^{-1}$) in dialysed, compared with undialysed, haemolymph ($7\,mmol\,l^{-1}$). Dialysed haemolymph showed a higher oxygen affinity than undialysed haemolymph at low lactate concentration ($<4\,mmol\,l^{-1}$). The Bohr effect and buffer value both decreased with increasing lactate concentration in both dialysed and undialysed haemolymph. The physiological implications of these findings are discussed.

INTRODUCTION

Although the ghost crabs, family Ocypodidae, have been the subject of numerous ecological studies (Bliss, 1968; Wolcott, 1978; see Powers & Bliss, 1983 for review) few studies have been made of the respiratory physiology of this group (Storch & Welsch, 1975; Burnett, 1979). Some characteristics of haemolymph gas transport in land crabs have been described by Redmond (1968), Young (1972) and Burnett & Infantino (1984). A significant contribution to the study of air breathing in crabs was made by a series of investigations during the *Alpha Helix* cruise of 1979 (cf. Cameron, 1981*a*). These investigations were, however, restricted to the genera present on the Palau islands and did not include any member of the Ocypodidae nor did they investigate extensively the oxygen-binding properties of the haemocyanin.

Key words: Land crabs, oxygen affinity, haemocyanin, temperature effect, lactate effect.

Since the work of Truchot (1980), the existence of specific, organic modulators of haemocyanin oxygen affinity has become an area of increasing interest. Some comparative information is available about the distribution of these modulating effects throughout the Crustacea (Mangum, 1983*a*; Bridges, Morris & Grieshaber, 1984), but to date no terrestrial decapod has been considered.

The subtropical land crab, *Ocypode saratan*, although not fully terrestrial, is confined to a supralittoral habitat (von Linsenmair, 1967) and can be found up to 1 km inland. The object of the present study was to characterize some of the oxygen-transporting properties of the haemolymph of this species and to investigate the specific modulation of haemocyanin O_2 affinity in a terrestrial decapod. The results of the investigation are discussed in terms of the possible physiological advantages to *Ocypode saratan*.

MATERIAL AND METHODS

Adult *Ocypode saratan* were collected from local beaches by personnel of the University of Jeddah, Saudia Arabia and individually packed for transport in cooled, moist styrofoam cases. The animals were then air freighted to the Department of Zoology in Glasgow and installed in a warm aquarium room at 25 ± 2 °C. The crabs were maintained for 2 weeks in tanks containing autoclaved sand dampened with sea water. Fresh water and sea water were both provided and the animals were fed with fresh salad/fruit twice each week and occasionally with chopped meat.

Blood sampling

Prior to sampling, intermoult male crabs of carapace width 35–49 mm (weight range 21–36 g) were maintained in separate polystyrene ice buckets, with moist sand, for a period of 1 day to ensure that L-lactate in the haemolymph was at resting levels. Venous haemolymph samples were then withdrawn, in less than 10 s, from the quiescent crabs *via* the arthrodial membrane at the base of the third walking leg. The samples were then pooled and frozen at − 70 °C prior to transport to Düsseldorf.

Initial measurements

The concentrations of the key inorganic ions were determined in the undialysed haemolymph samples. The concentrations of Mg^{2+} and Ca^{2+} were determined spectrophotometrically (Merkotest 3338, Darmstadt, F.R.G. and Roche, No. 1028, Basel Switzerland, respectively) and that of Cl^- using a chloride titrator (CMT 10, Radiometer, Copenhagen, Denmark). The concentration of L-lactate in the haemolymph samples and Ringer solutions used was estimated using the method of Gutmann & Wahlefeld (1974) modified according to Graham, Mangum, Terwilliger & Terwilliger (1983).

Haemolymph protein and haemocyanin concentrations were calculated from the absorbance peaks of spectrophotometric scans between 250–400 nm using 1 : 100 dilutions of haemolymph in Ringer solution. The absorbance maxima at 280 nm and 335 nm were used to determine concentrations of total protein and haemocyanin respectively using the extinction coefficients of 14·3 $E^{1\%}_{1\,cm}$ and 2·69 $E^{1\%}_{1\,cm}$ (Nickerson & van Holde, 1971).

Preparation

Native *Ocypode* haemolymph, in aliquots of 1 ml, was dialysed at 4 °C for 24 h against two changes (2 l) of *Ocypode* Ringer (pH = 8·2) with the following composition (in mmol l^{-1}): NaCl, 386; KCl, 7; $MgSO_4$, 10; $MgCl_2$, 5; and $NaHCO_3$, 22. Dialysis was carried out twice on two separate haemolymph aliquots (A and B). The different haemolymph lactate concentrations were achieved in the following manner. Samples (100 μl) from the appropriate test haemolymph were centrifuged at 136 000 ***g*** (Airfuge ultracentrifuge, Beckman, California, U.S.A.) for 30 min to pellet the haemocyanin. A portion of the plasma (5 μl) was then removed and replaced with 5 μl of neutralized (pH = 8·2) solutions of L-(+)-lactate (Boehringer, Mannheim, F.R.G.) of varying concentrations. The haemocyanin was then remixed with the plasma. The precise concentration of lactate in the haemolymph was subsequently determined by enzymatic assay. Control haemolymph solutions were made by using similar (5 μl) amounts of Ringer solution without lactate.

Construction of oxygen equilibrium curves

Oxygen equilibrium curves were constructed spectrophotometrically using 10 μl samples in a diffusion chamber (Sick & Gersonde, 1969) with the modifications of Lykkeboe, Johansen & Maloiy (1975) used by Bridges, Bicudo & Lykkeboe (1979). Gas mixtures were supplied to the chamber by serially connected Wösthoff gas mixing pumps (303 a/f Wösthoff, Bochum, F.R.G.). The pH of the haemolymph was varied by changing the partial pressure of CO_2 in the gas mixture (P_{CO_2} = 2–36 Torr). The pH of the haemolymph was measured in separate sub-samples (60 μl) tonometered in a BMS 2 (Radiometer) which was supplied with the same gas mixtures as the diffusion chamber. The pH of the equilibrated haemolymph was measured near the P_{50} using a microcapillary electrode (Type G299, Radiometer) thermostatted at the experimental temperature in BMS 2. These data were then used to calculate the magnitude of the Bohr shift.

Oxygen equilibrium curves were constructed for native, untreated *Ocypode saratan* haemolymph at temperatures of 20, 25, 30 and 35 °C to assess the effect of environmental temperature change (R. J. A. Atkinson, personal communication) on the O_2 binding of the haemocyanin. The changes in the heat of oxygenation (ΔH) of the pigment accompanying an increase in temperature, at a constant pH, were calculated according to the equation:

$$\Delta H = -2{\cdot}303R \frac{\Delta \log P_{50}}{\Delta(T^{-1})} \text{ (kJ mol}^{-1}\text{)},$$

where R is the gas constant and T is the absolute temperature.

Measurements on the effect of lactate were made at 30 °C. The P_{50} values were estimated from regression analyses of saturation values between 25 and 75 % according to the Hill equation. The cooperativity (n_{50}) was also determined from these points.

The possible existence of a specific effect of CO_2 on the affinity of *Ocypode saratan* haemocyanin was investigated by comparing the fixed acid and CO_2 Bohr effect for

native haemolymph at 30 °C. A similar method to that of Morris, Taylor, Bridges & Grieshaber (1985) was used to make these determinations. This technique involved first centrifuging the haemolymph and then replacing a small amount of the supernatant plasma (5 μl) with 0·1 mol l^{-1} HCl or 0·1 mol l^{-1} $NaHCO_3$ made up in Ringer solution. Control determinations were made using replacements of Ringer alone. Oxygen equilibrium curves were then constructed using two CO_2 concentrations, 0·4 % and 3 %, in order to compare the effects of changing pH with and without changes in CO_2.

Buffer values

Estimates of the buffer value of undialysed haemolymph and of dialysed haemolymph were made from CO_2 titration measurements. The P_{CO_2} was varied using gas mixing pumps (see above) and the pH measured. In each case the haemolymph bicarbonate concentration was calculated using the Henderson-Hasselbalch equation together with pK_1' and αCO_2 estimated from the nomograms of Truchot (1976) for 30 °C ($pK_1' = 5{\cdot}946$ and $\alpha CO_2 = 0{\cdot}334$ mol l^{-1} $Torr^{-1}$).

RESULTS

Calculations made using data from the spectrophotometric scans of the haemolymph of *Ocypode saratan* revealed a protein concentration of 53·3 mg ml^{-1} and a haemocyanin concentration that was virtually the same at 53·4 mg ml^{-1}. Using this information and assuming a molecular weight for haemocyanin of between 72 000 and 75 000 Da (Rochu & Fine, 1984) the oxygen-carrying capacity of the haemocyanin ($C^{max}_{HcyO_2}$) was calculated to be 0·72–0·74 mmol l^{-1} (1·59–1·62 vol%).

The concentration of Cl^- in the native haemolymph was 425 ± 15 mmol l^{-1} and the concentration of Mg^{2+} was 14·6 ± 0·8 mmol l^{-1} compared to 9·4 ± 2·3 mmol l^{-1} for Ca^{2+} ion concentration. The calculated concentration of HCO_3^- in the haemolymph after equilibration with 7·24 Torr CO_2 was, at 30 °C, 23·1 ± 1·8 mmol l^{-1}.

The effect of temperature on oxygen affinity

A large number of equilibrium curves resulted from this investigation and two typical sets of data for haemolymph at 20 and 35 °C are shown in Fig. 1. From these data it is apparent that the oxygen affinity of the haemolymph was temperature dependent; for example, the P_{50} at 35 °C was 12·2 Torr (pH = 7·91) but decreased to a value of 8·3 Torr at 20 °C (pH = 7·90). The same curves also clearly demonstrate the presence of a significant Bohr effect.

The CO_2 Bohr factor ($\varphi = \Delta \log P_{50}/\Delta pH$) was calculated for native *Ocypode* haemolymph at all experimental temperatures using the data presented in Fig. 2. The value of φ was found to be essentially temperature-independent (Fig. 2) and a mean CO_2 Bohr factor of $-0{\cdot}670 \pm 0{\cdot}02$ could be calculated for the temperature range 20–35 °C.

The temperature sensitivity of the haemocyanin was analysed further by comparing the ΔH values (Table 1) at two different pH values using data calculated from the regression lines presented in Fig. 2. The similarity of ΔH at the two pH values (7·4 and 7·8) confirms the low temperature sensitivity of φ. At a constant pH 7·8,

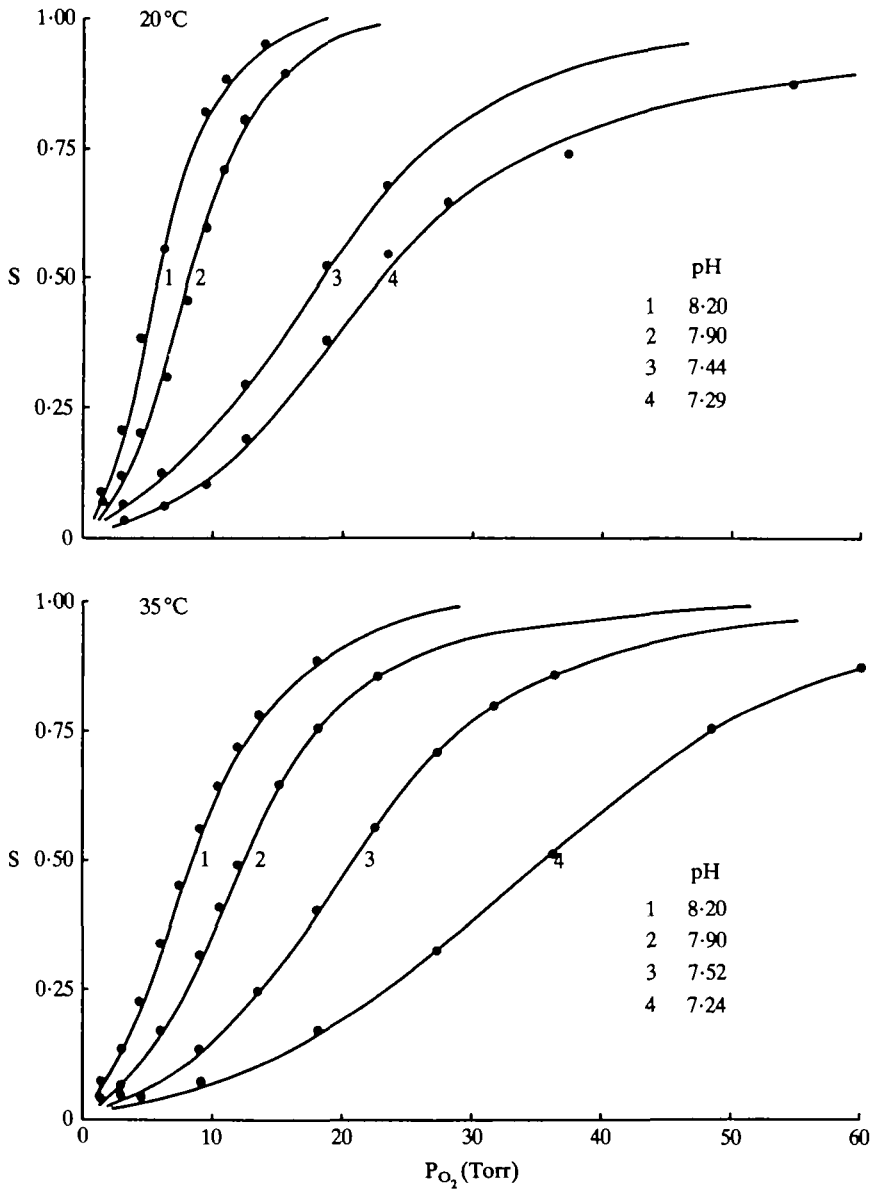

Fig. 1. Oxygen dissociation curves for *Ocypode saratan* haemocyanin constructed at 20 and 35 °C, using P_{CO_2} (2–36 Torr) to vary pH.

the change in the heat of oxygenation of the respiratory pigment accompanying a rise in temperature from 20 to 35 °C was found to be $-18{\cdot}4\,kJ\,mol^{-1}$. An examination of the change in ΔH accompanying increases of 5 °C within this temperature range showed, however, a distinct decrease in temperature sensitivity near to the normal environmental temperature. Increasing the temperature of the haemolymph from 25 to 30 °C produced a ΔH value of less than $-4\,kJ\,mol^{-1}$.

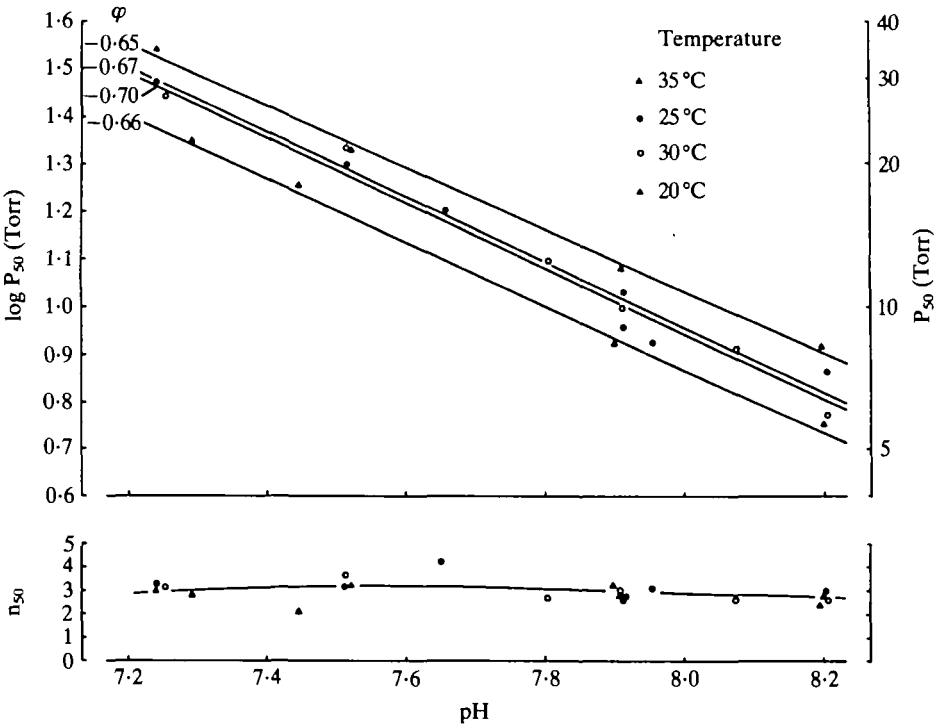

Fig. 2. The pH dependence of $\log P_{50}$ for *Ocypode saratan* haemolymph at 20, 25, 30 and 35 °C. The CO_2 Bohr values (φ) are the calculated slopes of the regression lines. The pH dependence of cooperativity measured at n_{50} is shown in the lower part of the figure.

Using P_{50} values from the O_2 equilibrium curves made at various temperatures an estimate was made for the temperature dependence of pH at a constant P_{CO_2}. This calculated *in vitro* value obtained at a P_{CO_2} of 7·4 ± 0·1 Torr (a CO_2 tension selected from the literature as being close to the *in vivo* tension) was extremely low (< 0·001 pH units °C^{-1}). Interestingly, the calculated n_{50} values exhibited no obvious dependence on temperature (Fig. 2) and changed minimally, if at all, with a change in haemolymph pH.

Table 1. *The effect of temperature on the binding of oxygen by the haemocyanin of* Ocypode saratan, *determined by the change in the heat of oxygenation (ΔH) accompanying temperature changes calculated for two constant pH values*

	ΔH (kJ mol^{-1})	
°C	pH 7·8	pH 7·4
20–25	− 25·75	− 32·10
25–30	− 3·11	− 1·04
30–35	− 26·45	− 22·87
20–35	− 18·43	− 18·09

$\Delta pH/\Delta T < 0{\cdot}001$ (*in vitro*, $P_{CO_2} = 7{\cdot}4 \pm 0{\cdot}1$).

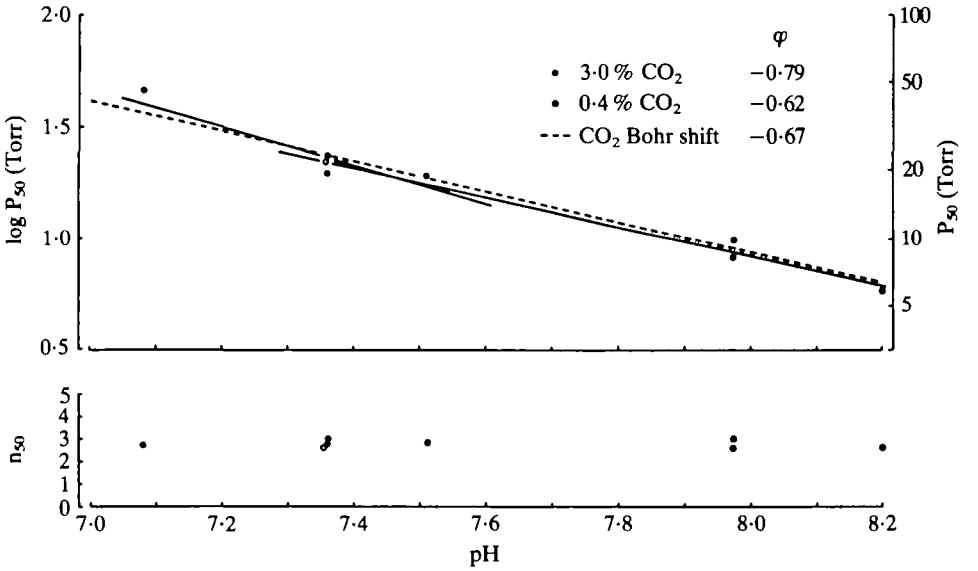

Fig. 3. Demonstration of the absence of a specific effect of CO_2 on the binding of oxygen by haemocyanin in *Ocypode saratan* at 30 °C. The slopes of the solid lines show fixed acid Bohr shifts at 0·4 % CO_2 (open symbols) and 3·0 % CO_2 (closed symbols). The CO_2 Bohr shift (P_{CO_2} = 2–36 Torr) is indicated by the broken line. The response of cooperativity (n_{50}) is shown in the lower part of the figure.

Specific effects on the binding of oxygen

Carbon dioxide

No detectable specific effect of carbon dioxide on O_2 binding by the haemocyanin of *Ocypode saratan* was found within the CO_2 concentration range 0·4–3·0 % (Fig. 3). The P_{50} values calculated from equilibrium curves made at these two concentrations and at different fixed acid concentrations were judged to describe one line for the relationship between pH and log P_{50} (the fixed acid Bohr shift). The calculated regression line for the combined data from the fixed acid determinations was; $\log P_{50} = 6{\cdot}31 - 0{\cdot}67\,\mathrm{pH}$ ($r = 0{\cdot}97$). A similar Bohr value was found for untreated haemolymph in which the Bohr shift was induced by CO_2 (see Figs 2, 3). The identity of the CO_2 and fixed acid Bohr shifts is clearly evident in Fig. 3, as is the absence of any effect of the small dilutions of the native plasma by the addition of acid or base.

L-(+)-*lactate*

The measured L-lactate concentration of native *Ocypode saratan* haemolymph was $1{\cdot}12 \pm 0{\cdot}01\ \mathrm{mmol\,l^{-1}}$, a value sufficiently low to permit the addition of neutralized L-lactate to give concentrations in the physiological range (Wood & Randall, 1981; Smatresk & Cameron, 1981; Taylor & Davies, 1982). The error of the lactate estimates was at all times less than 1 %. Increasing the lactate concentration of the undialysed haemolymph was found to increase the haemocyanin oxygen affinity (Fig. 4). Increasing the haemolymph lactate concentration to $9{\cdot}51\ \mathrm{mmol\,l^{-1}}$ resulted in a

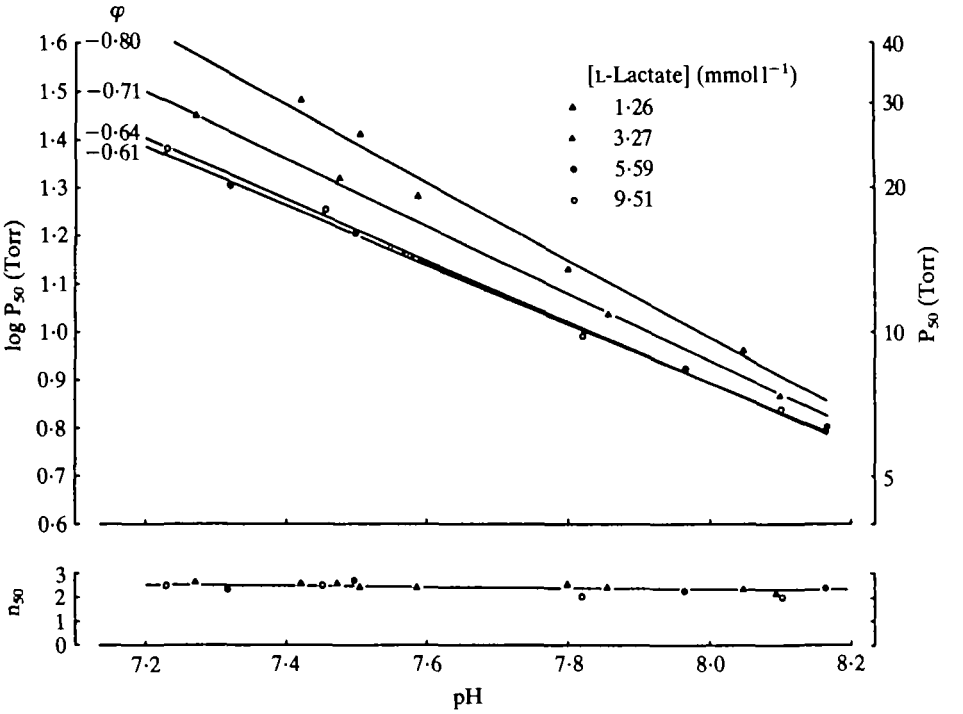

Fig. 4. The pH dependence of $\log P_{50}$ in *Ocypode saratan* whole haemolymph at varying lactate concentrations measured at 30 °C. The CO_2 Bohr values (φ) are the calculated slopes of the regression lines. The pH dependence of cooperativity measured at n_{50} and varying lactate concentrations is shown in the lower part of the figure.

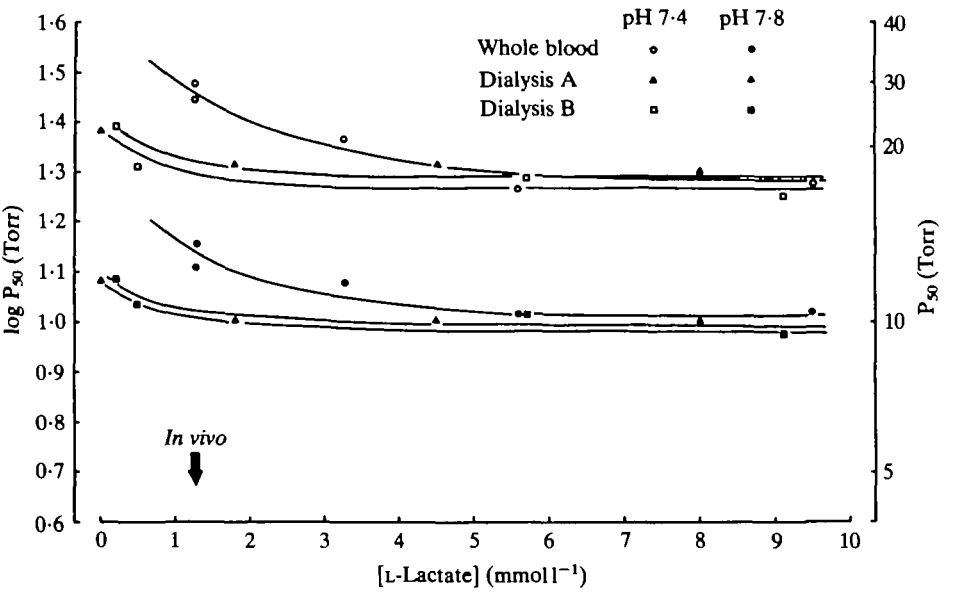

Fig. 5. The dependence of $\log P_{50}$ on L-lactate concentration for undialysed whole haemolymph and in the dialysed haemolymph (A and B) of *Ocypode saratan*, calculated for pH 7·4 (open symbols) and pH 7·8 (closed symbols) using the data shown in Fig. 3 together with similar data for dialysed haemolymph obtained at 30 °C. The dark arrow (⬇) indicates the measured *in vivo* lactate concentration of the undialysed whole haemolymph.

pH-dependent decrease in the log P_{50} value. For example $\Delta \log P_{50}$ is 0·123 at pH 7·8 ($\Delta P_{50} = 3{\cdot}7$ Torr) but increases to 0·195 at pH 7·4 ($\Delta P_{50} = 10{\cdot}5$ Torr) Increasing the concentration of haemolymph lactate not only increases affinity but also significantly (covariance analysis gives $0{\cdot}01 < P < 0{\cdot}05$) reduces the size of the Bohr factor (Fig. 4). The n_{50} values calculated from the same data showed no evidence of lactate-dependent cooperativity.

For the purposes of determining if other effectors of O_2 affinity were present in the haemolymph of *Ocypode saratan*, similar experiments were carried out on haemolymph dialysed against *Ocypode* Ringer. Lactate exponentially increased O_2 affinity in both dialysed and undialysed haemolymph. For comparison, plots of log P_{50} against log L-lactate concentration for dialysed and undialysed haemolymph have been presented in Fig. 5. The data were in all cases calculated using values from the regression lines for log P_{50} *vs* pH and include data shown in Fig. 4. The response of haemocyanin affinity to increasing lactate was dissimilar in dialysed and undialysed haemolymph. Firstly, the O_2 affinity of the undialysed haemolymph never exceeded that of haemolymph that had been dialysed and secondly, at lactate concentrations lower than approximately 7 mmol l^{-1}, the affinity of undialysed haemolymph became progressively lower. This effect resulted in a large difference in O_2 affinity between dialysed and undialysed haemolymph at low lactate concentrations. Near to the measured lactate concentration for the original native haemolymph, this difference was calculated to be 8·8 Torr and 3·5 Torr at pH 7·4 and pH 7·8, respectively. Similar results were obtained with the two separately dialysed aliquots (Fig. 5: A, B). The relationship between log P_{50} and log L-lactate concentration was calculated for native haemolymph and dialysed haemolymph by regression analysis (Table 2). The calculated coefficients in Table 2 indicate that dialysed haemolymph exhibited a reduced sensitivity to L-lactate when compared to that of undialysed haemolymph. A maximum effect of lactate was achieved at much lower concentrations (approx. 4 mmol l^{-1}) in dialysed blood than in undialysed blood (approx. 7 mmol l^{-1}). At concentrations greater than 7 mmol l^{-1} lactate, the effect was maximal in all preparations and the oxygen affinity was essentially the same at any given pH value.

Buffer values (β) calculated using pH and P_{CO_2} data from the oxygen equilibrium curves for undialysed blood are given in Table 3. It would appear that an increase in haemolymph lactate concentration results in a progressively decreasing buffer value. Furthermore, this relationship was not linear but exponential, like the

Table 2. *The relationship between haemocyanin oxygen affinity (log P_{50}) and haemolymph lactate concentration* (log [lactate$^-$]) *at 30 °C in* Ocypode saratan

	$\Delta \log P_{50}/\Delta \log$ [lactate$^-$]	
Blood treatment	pH 7.4	pH 7.8
Whole blood	−0·240	−0·162
Dialysis A	−0·035	−0·040
Dialysis B	−0·062	−0·048

Table 3. *Buffer values for the undialysed haemolymph of* Ocypode saratan *at 30 °C containing varying concentrations of* L-*lactate*

L-lactate (mmol l^{-1})	Buffer value (mmol pH $unit^{-1}$)
1·26	19·0
3·27	14·0
5·59	11·7
9·51	11·2

relationship between log P_{50} and lactate concentration. A regression equation calculated for these data by the method of least squares is given below:

$$\log[\text{lactate}^-] = 1{\cdot}296 - 0{\cdot}274\,\beta \; (r = -0{\cdot}979).$$

Dialysed haemolymph showed a similar trend with β decreasing with increasing lactate concentration.

DISCUSSION

Blood pH and oxygen affinity

Although no *in vivo* measurements were made during this study it would appear, from the literature, that the Ocypodidae might differ from other terrestrial decapods in at least one respect, the pH of the haemolymph. Data for *Ocypode quadrata,* which exhibits a similar mode of life to *Ocypode saratan* (von Linsenmair, 1967; Wolcott, 1978), indicate an *in vivo* pH value in excess of pH 7·9 (Burnett, 1979). Investigation of other semi-terrestrial crustaceans (Burggren & McMahon, 1981; McMahon & Burggren, 1981; Smatresk & Cameron, 1981; Cameron, 1981*b*; Wood & Randall, 1981; Taylor & Davies, 1982) all report pH values lower than 7·8. These workers agree, however, that haemolymph P_{CO_2} levels normally lie within the range 6–12 Torr. The *in vitro* pH 7·89 measured at a P_{CO_2} of 7·4 Torr (30 °C) in *Ocypode saratan* haemolymph is in close agreement with the data of Burnett (1979) and may be important in confirming that the Ocypodidae maintain a high haemolymph pH. This may be facilitated in *Ocypode saratan* through a high haemolymph bicarbonate level which can be up to 50 % higher than those reported in other land crabs (McMahon & Burggren, 1981; Cameron, 1981*b*; Wood & Randall, 1981; Taylor & Davies, 1982).

The maintenance of a high blood pH is of significance when estimating oxygen affinity *in vivo*. At pH 7·8 and near environmental temperatures (30 °C) the P_{50} is approximately 12 Torr. This is not an exceptionally high affinity in comparison with the P_{50} values from other terrestrial crabs, e.g. 3·9 Torr (pH 8·18) in *Cardisoma guanhumi* (Young, 1972) and 4·5 Torr (pH 7·53) in the same species (Redmond, 1962), 20 Torr (pH 7·3) in *Gecarcinus lateralis* (Taylor & Davies, 1981), 13 Torr (pH 7·46) and 25 Torr (pH 7·46) in *Cardisoma carnifex* and *Birgus latro*, respectively (Burggren & McMahon, 1981). The mean CO_2 Bohr factor of $-0{\cdot}67$ in *Ocypode saratan* is lower than the $-0{\cdot}76$ reported for *Ocypode quadrata* (Burnett, 1979) and the $-0{\cdot}95$ found for *Ocypode occidentalis* (Burnett & Infantino, 1984).

The effect of temperature

It is evident from Fig. 1 and Table 1 that oxygen affinity is temperature dependent in *Ocypode saratan*. The ΔH values in Table 1 are, however, low in comparison with values from other crustaceans ($\Delta H = -12$ to $-143\,kJ\,mol^{-1}$; Mangum, 1980). Young (1972) reports values, calculated at constant pH, of $-45\,kJ\,mol^{-1}$ for *Cardisoma*, $-43\,kJ\,mol^{-1}$ for *Gecarcinus* and $-46\,kJ\,mol^{-1}$ for *Goniopsis* within the temperature range 25–30 °C. Maximum values for ΔH (approx. -26 $kJ\,mol^{-1}$) are observed in the present study at extreme temperatures. A similar temperature-dependent effect has been shown in *Palaemon elegans* (Morris *et al.* 1985), and low temperature sensitivity of oxygen binding has been reported in a number of crustaceans, including *Callianasa californiensis* (Miller & van Holde, 1974), *Pagurus bernhardus* (Jokumsen & Weber, 1982), *Euphasia superba* (Bridges *et al.* 1983) and *Palaemon elegans* (Morris *et al.* 1985). Values given for ΔH in the present study are calculated for a constant pH. *In vivo* pH values will change with temperature: McMahon & Burggren (1981) found a $\Delta pH/\Delta T$ of $-0{\cdot}023$ and $-0{\cdot}017$ in *Birgus latro* and *Cardisoma carnifex*, respectively. *In vitro* measurements in *Ocypode saratan*, however, indicate a far lower temperature sensitivity for pH. The Bohr effect may remain temperature-independent (Fig. 2), but if affinity is decreased at higher temperature then the same pH change will produce a more pronounced change in P_{50} (cf. $\log P_{50}$) than at lower temperatures.

Specific effects of CO_2 and L-lactate

Investigation of a specific CO_2 effect on oxygen binding by haemocyanin in *Ocypode saratan* did not reveal any changes in oxygen affinity, which is in agreement with other recent work (Burnett & Infantino, 1984; A. C. Taylor, S. Morris & C. R. Bridges, in preparation). Differences between fixed acid and CO_2 Bohr effects have seldom been reported (Truchot, 1973; Weber & Hagerman, 1981; Morris *et al.* 1985). Young (1972) has suggested, however, that his findings and those of Redmond (1955) possibly indicate the presence of a specific CO_2 effect in land crabs. No effect was detected in *Ocypode* (see also Burnett & Infantino, 1984), but this group (Ocypodidae) was not considered by Young and may well be phylogenetically different.

The recent finding (Truchot, 1980) that L-lactate increases the oxygen affinity of some haemocyanins has prompted more detailed investigations of this effect (Mangum, 1983*a*; Bridges *et al.* 1984). The calculated coefficients for the lactate effect ($\Delta \log P_{50}/\Delta \log [lactate^-]$) of $-0{\cdot}240$ and $-0{\cdot}162$ in undialysed haemolymph at pH 7·4 and pH 7·8, respectively (Table 2), represent a marked effect of lactate when compared to the known range of values of 0·00 to $-0{\cdot}630$ (Bridges *et al.* 1984). Some care should be taken when interpreting these values as the coefficient can be influenced by several parameters (Bridges *et al.* 1984). The coefficient does, however, demonstrate the pH sensitivity of the effect seen in some (see Bridges *et al.* 1984) but not all species (Truchot, 1980; Graham *et al.* 1983). The apparent reduction in the magnitude of the lactate effect in dialysed blood at low lactate concentration was unexpected. Although both sets of data (Fig. 5) show typical saturation kinetics the effect is near maximum at a much lower lactate concentration in dialysed haemolymph

(4 mmol l^{-1}) than in undialysed haemolymph (7 mmol l^{-1}). The cause of this difference is unclear.

The presence of an unidentified modulator of oxygen affinity has been shown in a number of crustaceans (Mangum, 1983*a,b*; Bridges *et al.* 1984), but these all increased the oxygen affinity. Lactate binding to haemocyanin does not show a 1 : 1 stoichiometry and lactate is thought to bind either between subunits or to a particular subunit (Johnson, Bonaventura & Bonaventura, 1984). It is possible that competitive inhibition could occur at either of these sites.

The exponential decrease in the buffer value with increasing lactate concentration (Table 3) also suggests an allosteric change in the structure of the haemocyanin such that the number of available proton buffering sites are decreased. This is corroborated by the decrease in the CO_2 Bohr effect with increasing lactate concentration. Much speculation surrounds the identity and mechanism of haemocyanin oxygen affinity modulators (Bridges *et al.* 1984; Johnson *et al.* 1984): the results for *Ocypode saratan* raise new questions which await further investigation at the molecular level.

Physiological implications

Exercise or environmental hypoxia may exert substantial demands on the oxygen transport system of crustaceans and in particular air-breathing species like *Ocypode saratan*. Cardiac output together with ventilation may control the efficiency of gas exchange and therefore modulate Pa_{O_2} (Wood & Randall, 1981), but venous oxygen tensions will depend upon the position of the equilibrium curve and on oxygen demand and supply.

A right shift of the curve, due to respiratory or metabolic acidosis, will aid unloading at the tissues to the detriment of arterial saturation, if Pa_{O_2} values are initially low due to gas exchange limitations or if the haemocyanin has an intrinsically low oxygen affinity. A left shift of the curve, due to respiratory alkalosis or to the presence of lactate, will enhance loading at the gill, if arterial saturation was previously impaired, and also defend the venous reserve, depending upon oxygen demand (Booth, McMahon & Pinder, 1982; Graham *et al.* 1983).

Inequalities in the efflux rates of protons and lactate from the tissues and their removal from the haemolymph after exercise have been reported in two species of land crab, *Cardisoma carnifex* (Wood & Randall, 1981) and *Gecarcinus lateralis* (Smatresk, Preslar & Cameron, 1979). Lactate was found to have a longer residence time in the haemolymph after exercise and may therefore play a significant role during recovery. This may represent an ideal adaptation for *Ocypode saratan*, which exhibits short periods of rapid locomotion and more sustained burrowing activity (von Linsenmair, 1967) the lactate effect would prevent the immediate depletion of haemolymph oxygen stores and could help maintain oxygen delivery under conditions of high oxygen demand.

The magnitude of both the Bohr and lactate effects together with the buffer value of the haemolymph, which is lactate-dependent in *Ocypode saratan* (Table 3), will all play a decisive role in determining the response of the oxygen transport system to both internal and external demands.

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REFERENCES

Bliss, D. E. (1968). Transition from water to land in decapod crustaceans. *Am. Zool.* **8**, 355–392.

Booth, C. E., McMahon, B. R. & Pinder, A. W. (1982). Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab *Callinectes sapidus*. *J. comp. Physiol.* **148**, 111–121.

Bridges, C. R., Bicudo, J. E. P. W. & Lykkeboe, G. (1979). Oxygen content measurement in blood containing haemocyanin. *Comp. Biochem. Physiol.* **62**A, 457–462.

Bridges, C. R., Morris, S. & Grieshaber, M. K. (1984). Modulation of haemocyanin oxygen affinity in the intertidal prawn *Palaemon elegans* (Rathke). *Respir. Physiol.* **57**, 189–200.

Bridges, C. R., Savel, A., Stöcker, W., Markl, J. & Linzen, B. (1983). Structure and function of Krill (*Euphausia superba*). Haemocyanin–adaptation to life at low temperature. In *Structure and Function of Invertebrate Respiratory Proteins*, (ed. E. J. Wood), pp. 353–356. Life Chemistry Reports, Suppl. 1, Chur, London, New York: Harwood Academic Publishers.

Burggren, W. W. & McMahon, B. R. (1981). Hemolymph oxygen transport, acid-base status and hydromineral regulation during dehydration in three terrestrial crabs, *Cardisoma, Birgus* and *Coenobita*. *J. exp. Zool.* **218**, 53–64.

Burnett, L. E. (1979). The effects of environmental oxygen levels on the respiratory function of haemocyanin in the crabs, *Libinia emarginata* and *Ocypode quadrata*. *J. exp. Zool.* **210**, 284–300.

Burnett, L. E. & Infantino, R. L. (1984). The CO_2-specific sensitivity of haemocyanin oxygen affinity in the decapod crustaceans. *J. exp. Zool.* **232**, 59–65.

Cameron, J. N. (1981*a*). Brief introduction to the land crabs of the Palau islands: stages in the transition to air breathing. *J. exp. Zool.* **218**, 1–5.

Cameron, J. N. (1981*b*). Acid-base responses to changes in CO_2 in two Pacific crabs: The Coconut crab, *Birgus latro*, and a Mangrove crab, *Cardisoma carnifex*. *J. exp. Zool.* **218**, 65–73.

Graham, R. A., Mangum, C. P., Terwilliger, R. C. & Terwilliger, N. (1983). The effect of organic acids on oxygen binding of haemocyanin from the crab *Cancer magister*. *Comp. Biochem. Physiol.* **74**A, 45–50.

Gutmann, I. & Wahlefeld, A. W. (1974). L-(+)-lactate determined with lactic dehydrogenase and NAD. In *Methods in Enzymatic Analysis* (2nd edition), (ed. H. U. Bergmeyer), pp. 1464. New York, London: Verlag Chemie Weinheim & Academic Press Inc.

Johnson, B. A., Bonaventura, C. & Bonaventura, J. (1984). Allosteric modulation of *Callinectes sapidus* hemocyanin by binding of L-lactate. *Biochemistry, N.Y.* **23**, 872–878.

Jokumsen, A. & Weber, R. E. (1982). Haemocyanin oxygen affinity in hermit crab blood is temperature independent. *J. exp. Zool.* **221**, 389–394.

Lykkeboe, G., Johansen, K. & Maloiy, G. M. O. (1975). Functional properties of haemoglobins in the teleost *Tilapia grahami*. *J. comp. Physiol.* **104**, 1–11.

McMahon, B. R. & Burggren, W. W. (1981). Acid-base balance following temperature acclimation in land crabs. *J. exp. Zool.* **218**, 45–52.

Magnum, C. P. (1980). Respiratory function of the haemocyanins. *Am. Zool.* **20**, 19–38.

Mangum, C. P. (1983*a*). On the distribution of lactate sensitivity among haemocyanins. *Mar. Biol. Lett.* **4**, 139–149.

Mangum, C. P. (1983*b*). Adaptability and inadaptability among HcO_2 transport systems: an apparent paradox. In *Structure and Function of Invertebrate Respiratory Proteins*, (ed. E. J. Wood), pp. 333–352. Life Chemistry Reports, Supp. 1, Chur, London, New York: Harwood Academic Publishers.

Miller, K. & van Holde, K. E. (1974). Oxygen binding by *Callianasa californiensis* haemocyanin. *Biochemistry, N.Y.* **13**, 1668–1674.

Morris, S., Taylor, A. C., Bridges, C. R. & Grieshaber, M. K. (1985). Respiratory properties of the haemolymph of the intertidal prawn *Palaemon elegans* (Rathke). *J. exp. Zool.* **233**, 175–186.

Nickerson, K. W. & van Holde, K. E. (1971). A comparison of molluscan and arthropod hemocyanin. I. Circular dichroism and absorption spectra. *Comp. Biochem. Physiol.* **39**B, 855–872.

Powers, L. W. & Bliss, D. E. (1983). Terrestrial adaptations. In *Environmental Adaptations, Biology of the Crustacea*, Vol. 8, (eds F. J. Vernberg & W. B. Vernberg), pp. 271–334. New York, London: Academic Press.

REDMOND, J. R. (1955). The respiratory function of haemocyanin in Crustacea. *J. cell. comp. Physiol.* **46**, 209–247.

REDMOND, J. R. (1962). The oxygen-haemocyanin relationships in the crab *Cardisoma guanhumi*. *Biol. Bull. mar. biol. Lab., Woods Hole* **122**, 252–262.

REDMOND, J. R. (1968). Transport of oxygen by the blood of the land crab *Gecarcinus lateralis*. *Am. Zool.* **8**, 471–479.

ROCHU, D. & FINE, J. M. (1984). The molecular weights of arthropod hemocyanin subunits: influence of Tris buffer in SDS-PAGE estimations. *Comp. Biochem. Physiol.* **79B**, 41–45.

SICK, H. & GERSONDE, K. (1969). Method for continuous registration of O_2-binding curves of hemoproteins by means of a diffusion chamber. *Analyt. Biochem.* **32**, 362–376.

SMATRESK, N. J. & CAMERON, J. N. (1981). Post-exercise acid-base balance and ventilatory control in *Birgus latro*, the Coconut crab. *J. exp. Zool.* **218**, 75–82.

SMATRESK, N. J., PRESLAR, A. J. & CAMERON, J. N. (1979). Post exercise acid-base disturbance in *Gecarcinus lateralis*, a terrestrial crab. *J. exp. Zool.* **210**, 205–210.

STORCH, V. & WELSCH, U. (1975). Über Bau und Funktion der Kiemen und Lungen von *Ocypode ceratophthalma* (Decapoda: Crustacea). *Mar. Biol.* **29**, 363–371.

TAYLOR, A. C. & DAVIES, P. S. (1981). Respiration in the land crab, *Gecarcinus lateralis*. *J. exp. Biol.* **93**, 197–208.

TAYLOR, A. C. & DAVIES, P. S. (1982). Aquatic respiration in the land crab, *Gecarcinus lateralis* (Freminville). *Comp. Biochem. Physiol.* **72A**, 683–688.

TRUCHOT, J.-P. (1973). Action specifique du dioxyde de carbone sur l'affinité pour l'oxygène de l'hémocyanine de *Carcinus maenas* (L.) (Crustacé Décapode Brachyoure). *C.R. hebd. Séanc. Acad. Sci., Paris.* **276**, 2965–2968.

TRUCHOT, J.-P. (1976). Carbon dioxide combining properties of the blood of the shore crab, *Carcinus maenas* (L.): carbon dioxide solubility coefficients and carbonic acid dissociation constants. *J. exp. Biol.* **64**, 45–57.

TRUCHOT, J.-P. (1980). Lactate increases the oxygen affinity of crab haemocyanin. *J. exp. Zool.* **214**, 205–208.

VON LINSENMAIR, K. E. (1967). Konstruktion und Signalfunktion der Reiterkrabbe *Ocypode saratan* Forsk (Decapoda, Brachyura, Ocypodidae). *Z. Tierpsychol.* **24**, 403–456.

WEBER, R. E. & HAGERMAN, L. (1981). Oxygen and carbon dioxide transporting qualities of haemocyanin in the hemolymph of a natant decapod *Palaemon adspersus*. *J. comp. Physiol.* **145**, 21–27.

WOLCOTT, T. G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fabricius) on an ocean beach: scavengers or predators. *J. exp. mar. Biol. Ecol.* **31**, 67–82.

WOOD, C. M. & RANDALL, D. J. (1981). Haemolymph gas transport, acid-base regulation and anaerobic metabolism during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 23–35.

YOUNG, R. E. (1972). The physiological ecology of haemocyanin in some selected cabs. II. The characteristics of haemocyanin in relation to terrestrialness. *J. exp. mar. Biol. Ecol.* **10**, 193–206.