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MODULATION OF HAEMOCYANIN OXYGEN-AFFINITY BY L-LACTATE AND URATE IN THE PRAWN *PENAEUS JAPONICUS*

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Summary

The addition of either L-lactate or urate to dialysed haemolymph from the prawn *Penaeus japonicus* (Bate) increased the *in vitro* haemocyanin oxygen-affinity. The quantitative values of these two effects, expressed as $\Delta\log P_{50}/\Delta\log[\text{effector}]$, were found to be −0.077 for L-lactate and −0.032 for urate, at pH 7.6 and 25 °C. The normal, significant Bohr effect ($\Delta\log P_{50}/\Delta pH$ approx. −1.5 at pH 7.6, 25 °C) was not modified by the two effectors tested, nor was the cooperativity of haemocyanin oxygen-binding (n_{50} approx. 4).

Hypoxic exposure of the prawns to Pw_{O_2}=6.3 or 4.4 kPa (1 kPa=7.5 mmHg) for up to 48 h at 25 °C induced only a small, less than 2.5-fold, elevation of L-lactate concentration in the haemolymph, all values remaining below 0.5 mmol l^{-1}, but urate concentration increased to a greater extent (12-fold maximum increase from 0.01 to 0.12 mmol l^{-1}). Haemocyanin oxygen-affinity, measured *in vitro* on haemolymph samples drawn from hypoxic prawns, increased slightly during the first 3 h of hypoxia acclimation (ΔP_{50}=0.8–0.9 kPa at pH 7.6), returning to near normoxic control values after a 48 h hypoxic exposure.

The respective roles of L-lactate and urate in enhancing oxygen transport during hypoxia are discussed on the basis of their *in vitro* effects on haemocyanin oxygen-affinity and their *in vivo* concentration variations in haemolymph.

Introduction

During the last 15 years, many studies have dealt with the functions of crustacean haemocyanins and with the regulation of oxygen transport in the haemolymph under different kinds of stresses (e.g. osmotic, hypoxic, exercise) (reviewed in Mangum, 1983*a*; McMahon, 1985). Most of this work has concentrated on crabs, crayfishes and lobsters, with little attention paid to natantian decapods (e.g. Weber & Hagerman, 1981; Taylor *et al.* 1985; Morris *et al.* 1985,

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1988*c*). Moreover, there are no physiologically relevant data available for penaeid prawns, a group considered as the most primitive among decapods (Bowman & Abele, 1982).

Besides the well-known effects of inorganic ions, two organic cofactors reportedly increase haemocyanin oxygen-affinity in decapod crustaceans: L-lactate (Truchot, 1980) and urate (Morris *et al.* 1985). Numerous studies have shown that the L-lactate effect is present in many decapod crustaceans (Mangum, 1983*b*; Bridges & Morris, 1986). Since the recent finding that urate enhances the oxygen affinity of *Austropotamobius pallipes* haemocyanin (Morris *et al.* 1985), only a few other species have been investigated (Morris & Bridges, 1986; Lallier, 1988). To be considered as true modulators, these two molecules must be shown to appear in haemolymph under stress conditions and to modify *in vivo* haemocyanin oxygen-affinity. L-Lactate, the main end-product of anaerobiosis in Crustacea (Gäde, 1983), fulfils these requirements during muscular exercise (Booth *et al.* 1982) but its role during environmental hypoxia is not clear (Bouchet & Truchot, 1985; Lallier *et al.* 1987). Urate is a byproduct of purine catabolism, which is poorly documented in Crustacea (Claybrook, 1983).

Penaeus japonicus, a euryhaline species originating from southeast Asia, may encounter hypoxic water in the brackish, shallow lagoons where the adults live. This species is now cultured in brackish water ponds on the Atlantic coast of France. In this new environment, it may also face periodic hypoxic conditions of moderate amplitude, down to 9 kPa at 20°C at night (F. Lallier & J. P. Truchot, personal observations), or even more severe hypoxia, and possibly anoxic waters, in cases of eutrophication. Respiratory adaptations of the prawns to such decreases in environmental oxygen availability may involve an enhanced oxygen transport in the blood which could be mediated through an increased haemocyanin oxygen-affinity.

To examine how *P. japonicus* deals with such conditions, we (i) investigated the *in vitro* effects of L-lactate and urate on the oxygen-binding properties of *P. japonicus* haemocyanin and (ii) measured the variations of L-lactate and urate concentrations, together with the changes of haemocyanin oxygen-affinity *in vivo*, after exposure of the prawns to environmental hypoxia.

Materials and methods

Animals

Animals were obtained from a culture farm located near the Gironde estuary (southwest France) at the end of summer (mean mass 12–15 g) and kept in the laboratory in a large tank with continuously renewed natural sea water maintained at 20–22°C, salinity 27‰, for several weeks. They were fed on artificial pellets (Aqualim), specifically made for prawns and identical to those used during their growth in culture.

In vitro *experiments*

The effects of L-lactate and urate on haemocyanin oxygen-affinity were studied

on dialysed blood samples. A pool of haemolymph was sampled from the pericardial sinus of several individuals, using plastic syringes and G23 needles inserted between the thorax and the first abdominal segment. Since *Penaeus japonicus* haemolymph clots immediately, samples were treated by sonication and the liquid phase was collected after centrifugation before further analysis. The pooled sample (approx. 20 ml) was then dialysed at 4 °C, using an ultrafiltration method with immersible units (Millipore CX-10, cutoff point 10 000 Da). One unit was immersed in the haemolymph and connected to a peristaltic pump allowing withdrawal of water and low molecular weight solutes, which were automatically replaced with an equivalent volume of Ringer's solution (in mmol l^{-1}: NaCl, 430; $CaCl_2$, 15; KCl, 15; $MgCl_2$, 5; $MgSO_4$, 5; $NaHCO_3$, 5; pH 7.6 at 25 °C). After 12 h, when about 50 ml of Ringer had been filtered, the process was repeated with a new filter unit. Haemolymph was continuously stirred and its equilibration with Ringer was periodically checked by comparison of the ultrafiltrate with Ringer for Na^+, K^+ and Ca^{2+} concentrations and osmotic pressure. The dialysed pool (approx. 20 ml) was separated into identical 1 ml samples and frozen at −20 °C. After gentle thawing at 4 °C, each sample was prepared by adding various volumes of either a neutral 0.5 mol l^{-1} sodium L-lactate solution in Ringer or a saturated uric acid solution (approx. 2–3 mmol l^{-1}) in Ringer, diluted with Ringer to obtain the same final volume (1.2 ml). Using this procedure, the composition of all samples was the same, except for sodium L-lactate or urate concentration. L-Lactate and urate concentrations were measured for each sample after completion of the preparation procedure. The possible effects of freezing on oxygen-binding properties of haemocyanin have been assessed and no significant changes in affinity, cooperativity or the Bohr effect were found (Lallier, 1988).

For each sample, dissociation curves were constructed using a diffusion chamber (Sick & Gersonde, 1969) and a step-by-step procedure (Lykkeboe *et al.* 1975; Bridges *et al.* 1979). Gas mixtures were obtained using Wösthoff pumps, and pH changes were induced by varying the CO_2 tension in the gas mixtures. pH was measured with a capillary pH electrode (G299, Radiometer) on a separate subsample equilibrated with the same gas mixture in the tonometers of a blood gas analyser (BMS2, Radiometer). Parabolic curves were fitted to data pairs (pH, $\log P_{50}$) using a quadratic polynomial least-squares regression, providing estimations of P_{50} and Bohr factor at any pH value in the range studied (7.1–7.7).

In vivo *experiments*

Six groups of 7–10 prawns were acclimated for 24 h in normoxic sea water in a 20-l experimental tank with a sandy bottom and supplied with sea water (3 l min^{-1}) from a 50-l thermostatted tank in which sea water was renewed continuously (0.3 l min^{-1}). This acclimation period was followed by either normoxic (control) or hypoxic exposure (Pw_{O_2}=6.3 and 4.4 kPa) for 3 or 48 h. Water oxygen partial pressure, Pw_{O_2}, was controlled by bubbling mixed air and nitrogen from mass flowmeters (FC260, Tylan), adjusted to obtain the desired value. Pw_{O_2} was checked periodically by measurements with a thermostatted P_{O_2} electrode.

Postbranchial blood samples (0.3–0.7 ml) were withdrawn from each individual as rapidly as possible after removing the animal from the water, and treated as described above to remove the clot. Since it was impossible to get enough haemolymph for individual measurements, a representative pool of whole haemolymph was constituted with equal volumes from each sample, and used for further determinations of L-lactate and urate concentrations and production of equilibration curves.

Measurements

L-Lactate concentrations were assessed with an enzymatic method (Boehringer, no. 139084) on samples deproteinized in 8% perchloric acid. This method reportedly provides stable readings for copper-containing samples (Gäde, 1984). Urate concentrations were determined using a spectrophotometric method (Sigma Diagnostic no. 685) at 520 nm. At this wavelength, haemocyanin absorbance interfered and test absorption measurements (At) had to be corrected correspondingly. For this purpose, a blank measurement (Ab) was made on a sample containing the same volume of haemolymph as the test sample diluted in a phosphate buffer (pH 7.8, 250 mmol l^{-1}) identical to the Sigma kit buffer. A standard sample measurement (As) served as the reference [Sigma Standard no. 685-1 diluted 1/1 (v/v) in distilled water, [Ustd]=0.149 mmol l^{-1}]. Urate concentration was calculated using the following formula: $[U]=[(At-Ab)/As]\times[Ustd]$. This method gave linear results over a wide range of urate concentrations (0–0.3 mmol l^{-1}), with an average coefficient of variation of 2–3%, amounting to a maximum 12.5% at the lowest concentrations.

Results

L-Lactate effect

L-Lactate increased the oxygen affinity of *Penaeus japonicus* haemocyanin (covariance analysis, $P<0.001$) (Fig. 1). At pH 7.6, which may be near the physiological value at the experimental temperature used, the drop in P_{50} amounts to 0.9 kPa when L-lactate concentration was increased from 0.05 to 4.72 mmol l^{-1}. *Penaeus japonicus* haemocyanin showed a large Bohr shift, reaching values of $\Phi=\Delta\log P_{50}/\Delta pH=-1.5$ at pH 7.6. The lactate effect seemed to become quickly saturated as there was no further increase of affinity at L-lactate concentrations above about 5 mmol l^{-1}. The cooperativity of oxygen binding to haemocyanin was not significantly affected by either L-lactate or pH (Fig. 1), with n_{50} values ranging from 3.5 to 4.5 and averaging 4.1±0.2 (S.E., $N=16$). The lactate effect, measured as the ratio $\Delta\log P_{50}/\Delta\log[\text{lactate}]$, was −0.067 at pH 7.2 compared with −0.077 at pH 7.6. However, this difference was not statistically significant ($P>0.5$), supporting the idea that the lactate effect may be independent of pH for *P. japonicus* haemocyanin.

Urate effect

Urate also enhanced haemocyanin oxygen-affinity (covariance analysis, $P<0.001$) (Fig. 2). From a qualitative point of view, the effect of urate was similar to that of L-lactate, although more moderate. For example, at pH 7.6, P_{50} decreased from 2.9 to 2.3 kPa when urate concentration was increased from 0.001 to 0.96 mmol l^{-1}. Raising urate concentration did not induce any change in cooperativity (Fig. 2). Saturation of the urate effect was not clearly evident. Whether it occurred at higher urate concentrations could not be tested owing to the low solubility of urate in haemolymph (McNabb & McNabb, 1980). The ratio $\Delta\log P_{50}/\Delta\log[\text{urate}]$, a numerical estimation of the urate effect, was −0.026 at

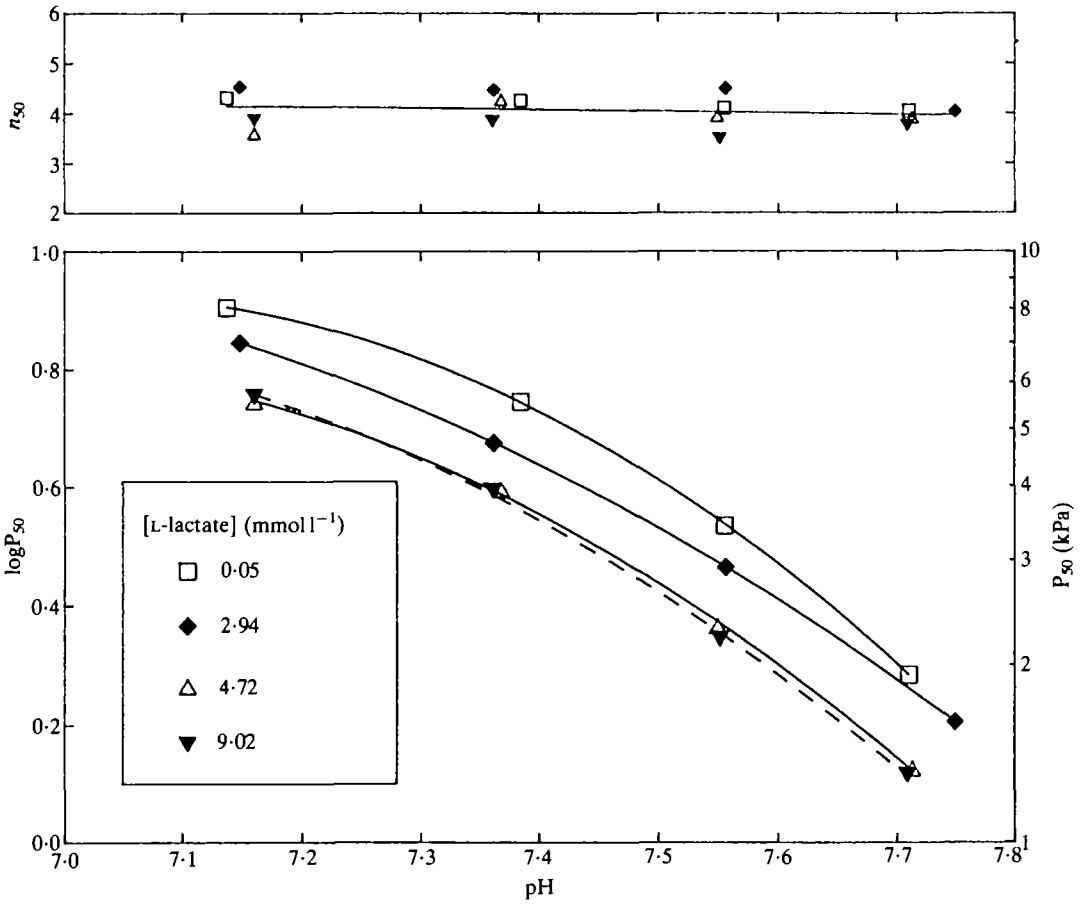

Fig. 1. Plot of $\log P_{50}$ *vs* pH showing the effects of various concentrations of L-lactate on the oxygen affinity of haemocyanin in the dialysed haemolymph of the prawn *Penaeus japonicus* at 25°C. Curves are fitted to experimental points by quadratic polynomial least-squares regression, describing the variations of the Bohr effect as a function of pH. The effects of L-lactate on haemocyanin cooperativity (n_{50}) within the pH range studied are shown above, with a linear regression line fitted to the data points.

pH 7.2 and −0.029 at pH 7.6, values which were not statistically different ($P>0.5$), again suggesting that the urate effect was independent of pH.

Hypoxia experiments

For each of the six experimental series, we determined L-lactate and urate concentrations. Data in Table 1 came from triplicate measurements on pooled haemolymph and did not allow evaluation of individual variations. Nevertheless, major trends could be observed. A 3-h hypoxic exposure elicited only a small rise in L-lactate level, which remained below 0.5 mmol l^{-1} (Table 1), whereas urate concentration increased to a greater extent, from about 0.01 mmol l^{-1} in normoxia to over 0.10 mmol l^{-1} in hypoxia, whatever the Pw_{O_2} (Table 1). The difference between normoxic and hypoxic levels tended to be reduced after 48 h of exposure

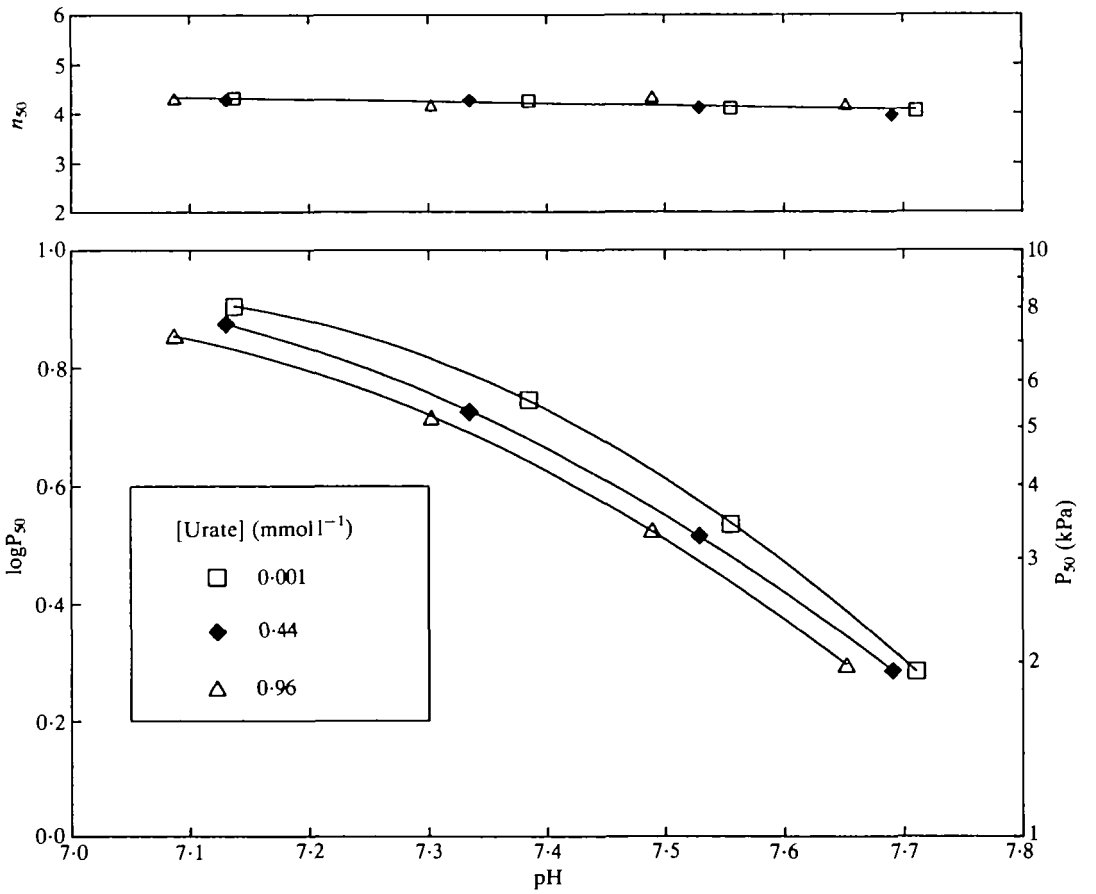

Fig. 2. Plot of $\log P_{50}$ *vs* pH showing the effects of various concentrations of urate on the oxygen affinity of haemocyanin in the dialysed haemolymph of the prawn *Penaeus japonicus* at 25°C. The effects of urate on haemocyanin cooperativity (n_{50}) within the pH range studied are shown above. Curves were fitted as described in Fig. 1.

Table 1. *Variations of* L-*lactate and urate concentrations as a function of water oxygenation and exposure time*

Pw_{O_2} (kPa)	Time (h)	[L-Lactate] ($mmol\,l^{-1}$)	[Urate] ($mmol\,l^{-1}$)
20.7	3	0.19	0.01
6.3	3	0.41	0.11
4.4	3	0.46	0.12
20.7	48	0.29	0.06
6.3	48	0.28	0.11
4.4	48	0.18	0.09

Values are means of triplicate measurements on pooled haemolymph from *Penaeus japonicus* at 25°C and salinity 27‰.

for both L-lactate and urate (Table 1). This seemed mainly to be due to a marked increase in the normoxic groups, especially for urate.

A 3-h exposure to environmental hypoxia increased the haemocyanin oxygen-affinity compared with that of normoxic blood, regardless of pH (covariance analysis, $P<0.001$) (Fig. 3A). At pH 7.6, P_{50} was about 3.7 kPa in the pooled blood of *Penaeus* exposed at $Pw_{O_2}=20.7$ kPa and about 2.9 kPa at a Pw_{O_2} of 6.3 kPa (Fig. 3A). A similar value prevails after 3 h at a Pw_{O_2} of 4.4 kPa. After a 48-h hypoxic exposure, only a slight, insignificant increase (covariance analysis, $P>0.25$) of haemocyanin oxygen-affinity was seen, whatever the Pw_{O_2} (Fig. 3B). Neither the Bohr effect nor the cooperativity were altered between normoxia and hypoxia.

Using *in vitro* values obtained for L-lactate and urate effects and *in vivo* variations of the concentrations of the two effectors, the respective roles of L-lactate and urate in the pH-independent increase of haemocyanin oxygen-affinity in the haemolymph during hypoxic exposure were estimated. Since the effects of L-lactate and urate were found to be independent of pH, the pH-independent coefficient, a_0, in the quadratic equation fitted to the normoxic data points (equation 1) was used to calculate the corresponding coefficient, a_0', for hypoxic conditions (equation 2), using the *in vitro* coefficient, $\Delta\log P_{50}/\Delta\log[\text{effector}]$, and the *in vivo* variation of concentrations, $\Delta\log[\text{effector}]$:

$$\log P_{50} = a_0 + (a_1 \times \text{pH}) + (a_2 \times \text{pH}^2)\,, \tag{1}$$

$$a_0' = a_0 + \left(\frac{\Delta\log P_{50}}{\Delta\log[\text{effector}]} \times \Delta\log[\text{effector}]\right)\,. \tag{2}$$

Taking data measured after 3 h of exposure at a Pw_{O_2} of 6.3 kPa as an example, we successively added the effects of L-lactate and urate (Fig. 4), obtaining a curve nearly identical to that obtained for the pooled blood of hypoxic prawns, with only small discrepancy at high pH values. Data from prawns exposed to a Pw_{O_2} of 4.4 kPa for 3 h produced similar results. However, it must be pointed out that such

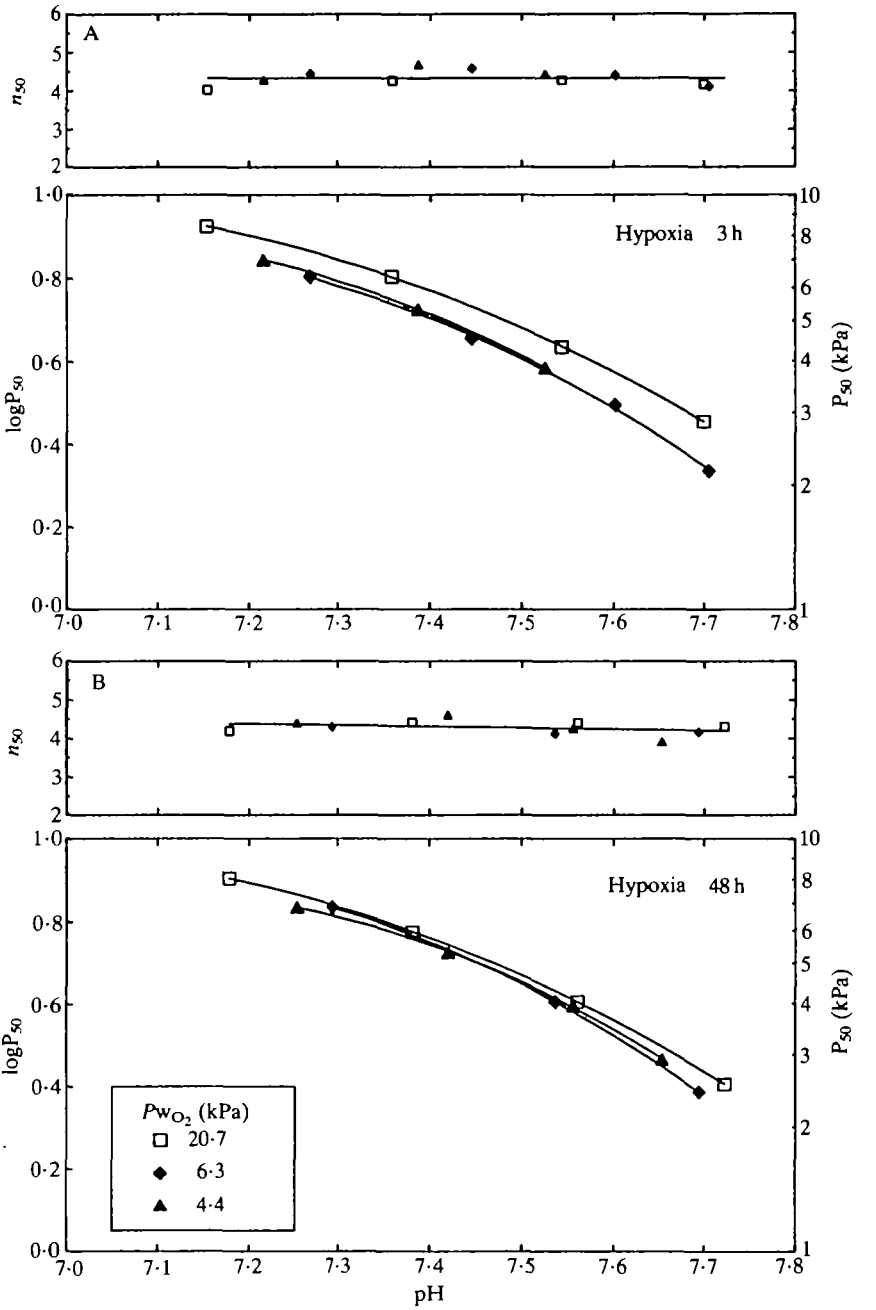

Fig. 3. Variations of P_{50} and n_{50} with pH in the haemolymph of *Penaeus japonicus* exposed to various water oxygen partial pressures (Pw_{O_2}) at 25°C. Curves were fitted as described in Fig. 1. (A) 3 h exposure; (B) 48 h exposure.

calculations assume that the effects of L-lactate and urate are independent. As noted by Morris *et al.* (1986) for the freshwater crayfish *Austropotamobius pallipes*, this may not be the case for our euryhaline prawn *Penaeus japonicus*.

Discussion

Although the prawns we had at our disposal were relatively large (12–15 g, about 10–12 cm long), only small volumes (approx. 0.3–0.7 ml) of haemolymph could be withdrawn from the pericardial cavity of any individual. Sampling in the abdominal sinus yielded even poorer results, presumably because of the considerable abdominal muscular mass. This prohibited paired design and individual measurement of all parameters. Moreover, *Penaeus japonicus* haemolymph forms a dense clot within a few seconds after withdrawal, requiring homogenization before liquid blood can be recovered, and precluding measurements such as *in vivo* pH and P_{O_2}, parameters which would have allowed further understanding of oxygen transport and hypoxia adaptation. Despite these limitations, our study produced data which will be discussed in the context of present knowledge concerning haemolymph oxygen-binding properties in decapod crustaceans and the *in vivo* responses of the oxygen transport system to environmental hypoxia.

Oxygen-binding properties of Penaeus *haemocyanin*

Oxygen binding to *Penaeus japonicus* haemocyanin is characterized by a high level of cooperativity ($n_{50} \approx 4$) and a large Bohr shift. The Bohr effect was similar

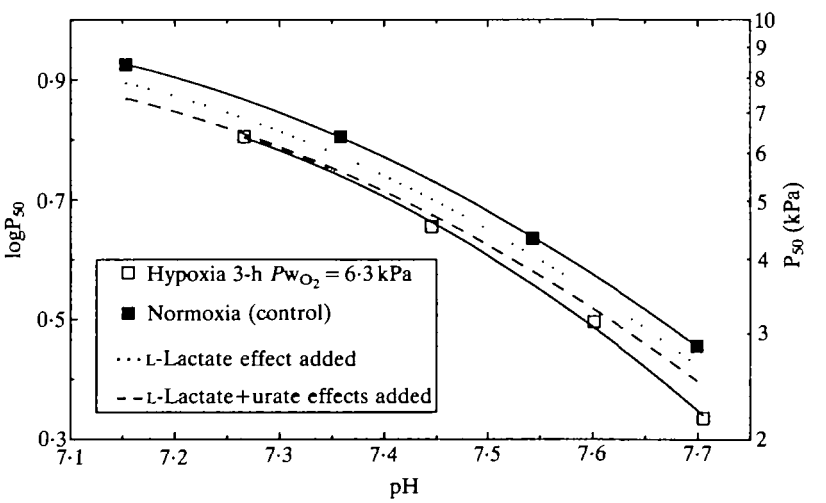

Fig. 4. Plots of $\log P_{50}$ *vs* pH showing that the difference in haemocyanin oxygen-affinity between hypoxic prawns and normoxic controls (redrawn from Fig. 3A for normoxia and Pw_{O_2}=6.3 kPa) is almost fully accounted for by the effects of lactate and urate. Dotted and dashed curves have been calculated as explained in the text, using *in vitro* values for L-lactate and urate effects (from Figs 1 and 2) and measured *in vivo* L-lactate and urate concentrations.

for dialysed and whole blood (see Figs 1 and 3, for example), amounting to $\Phi=-0.99$ at pH 7.4 and $\Phi=-2.01$ at pH 7.8. These data agree with previous studies of penaeid haemocyanin (Brouwer *et al.* 1978; Mangum, 1982; Mangum & Burnett, 1986) which also found the combination of a high Bohr effect and a high cooperativity in *P. setiferus*, *P. monodon* and *P. duorarum*. Such a high pH-sensitivity of oxygen affinity seems to be typical of natantian decapods, since similarly high values have been reported for *Palaemon adspersus* ($\Phi=-2.0$ and -0.9 at pH 7.85 and 7.40, respectively, Weber & Hagerman, 1981) and *Palaemon elegans* ($\Phi=-1.17$ for pH between 7.4 and 8.0, Taylor *et al.* 1985).

Penaeus japonicus haemocyanin exhibits a moderate lactate effect compared with other species. In a recent review, Bridges & Morris (1986) reported values of $\Delta\log P_{50}/\Delta\log[\text{lactate}]$ ranging from -0.04 in *Ocypode saratan* to -0.66 in *Palaemon serratus*. Our value of -0.077 for *Penaeus japonicus* is in the lower range among crustaceans. Furthermore, as is the case in most decapods investigated, this coefficient appears to be relatively independent of pH in the physiological range. It is also interesting to note that, in *Penaeus japonicus*, a moderate lactate effect occurs in combination with a strikingly large Bohr effect. The roughly proportional relationship between these two effects suggested by Bridges & Morris (1986) could not therefore be confirmed in *Penaeus*. This, however, makes sense physiologically, since only small amounts of lactate appear in the haemolymph during hypoxia in this species.

Oxygen affinity was increased when urate was added to dialysed *Penaeus* haemolymph. The presence of a urate effect enhancing oxygen binding by crustacean haemocyanins, first discovered in the crayfish *Austropotamobius pallipes* (Morris *et al.* 1985), has been reported in some decapod species – *Callinectes sapidus*, *Carcinus maenas*, *Palaemon serratus*, *Homarus vulgaris* – but seems to be absent in others, for example *Coenobita clypeatus* (Morris & Bridges, 1986). Because of the recent finding of the effect of urate, there are few complete studies reporting values of $\Delta\log P_{50}/\Delta\log[\text{urate}]$. Morris *et al.* (1985) found a value of -0.39 for the crayfish *Austropotamobius pallipes*, and Lallier (1988) measured a value of -0.22 in *Carcinus maenas* at pH 7.8 and 15 °C. Compared with these data, the urate effect found in *Penaeus japonicus* seems to be quite small (-0.029 at pH 7.6 and 25 °C). Nevertheless, it is interesting to note that, despite the relative primitiveness of penaeids among decapod crustaceans (Bowman & Abele, 1982), both lactate and urate effects are present in *Penaeus japonicus*. This suggests that lactate and urate sensitivities may be early characteristics of decapod crustacean haemocyanins: lack of one or both of these effects in some species (e.g. *Procambarus clarkii*, Mangum, 1983*b*; *Glyphocrangon vicaria*, Arp & Childress, 1985; *Holthuisiana transversa*, Morris *et al.* 1988*a*; and more generally air-breathing species, Morris *et al.* 1988*b*) may therefore indicate that they have subsequently disappeared.

Responses to environmental hypoxia

The physiological significance of the modulation of the haemocyanin oxygen

affinity by organic cofactors remains unclear. That L-lactate is the main anaerobic end-product in decapod crustaceans suggests that the lactate effect might participate in the adjustment of haemolymph oxygen transport in situations such as environmental hypoxia or muscular exercise. However, for lactate and urate to be considered as physiological modulators, one must demonstrate first that they accumulate in haemolymph, and then that this results in an increase of the oxygen affinity *in vivo*.

Accumulation of L-lactate in decapod crustaceans during environmental hypoxia has been documented largely by biochemical (Zebe, 1982; Gäde, 1983) or physiological studies (Bridges & Brand, 1980; Spotts, 1983; Mauro & Malecha, 1984). None of these studies, however, describes a steady-state relationship between haemolymph lactate concentration and ambient oxygen levels, which would ensue, at least to a certain extent, if the lactate effect was stabilizing oxygen transport to the tissues. In a recent work on resting crabs, *Carcinus maenas*, under precisely controlled Pw_{O_2}, L-lactate has been shown to accumulate in haemolymph only under severely hypoxic conditions (Pw_{O_2} below 2.3 kPa at 15 °C), suggesting that lactate is produced in substantial amounts or, alternatively, is released from the tissues only below a Pw_{O_2} threshold (Lallier *et al.* 1987). At least during environmental hypoxia, the lactate effect may provide only an emergency response. Present data show that there is no large increase in L-lactate concentration in the haemolymph of quiescent *Penaeus japonicus* exposed to a Pw_{O_2} as low as 4.4 kPa at 25 °C. These conditions can be considered as a relatively severe hypoxia, since the critical partial pressure (Pc) is about 4–5 kPa for *Penaeus japonicus* at this temperature (Truchot & Jouve-Duhamel, 1985). In the shrimp *Crangon crangon*, haemolymph L-lactate concentration increases only below a Pw_{O_2} of 2 kPa at 20 °C (Hagerman & Szaniawska, 1986), also supporting the notion of a Pw_{O_2} threshold. To test whether more severe hypoxia could induce a marked accumulation of L-lactate in *Penaeus japonicus* haemolymph, an experiment was conducted at a Pw_{O_2} of 2.7 kPa (25 °C). This resulted in considerable mortality among prawns within a few hours, suggesting that resting *Penaeus japonicus* do not rely significantly upon anaerobic metabolism under sublethal hypoxic conditions.

The pattern of urate release in haemolymph of crustaceans during hypoxia seems to be quite different from that of lactate, though little studied. In a previous work on *Carcinus maenas* (Lallier *et al.* 1987), urate levels in haemolymph were found to be closely related to water oxygenation, increasing proportionately with Pw_{O_2} decrease after hypoxic exposure, and decreasing after hyperoxia. An increase of urate concentration has also been reported in hypoxic crayfish (Czietrich *et al.* 1987). These studies also emphasized the large variations of urate concentrations among individuals, though they were apparently in the same physiological conditions. Such individual variability could not be assessed in our study because of the small volume of haemolymph sampled from each prawn. Nevertheless, measurements on pooled samples clearly show an important and rapid increase in haemolymph urate concentration upon hypoxic exposure, suggesting that the urate response in *P. japonicus* may be similar to that observed

in *Carcinus*. There are, however, few data concerning purine catabolic pathways in crustaceans, especially for urate production in the cells and subsequent release into haemolymph under hypoxic conditions.

Increased haemocyanin oxygen-affinity has been described previously in the haemolymph of hypoxic crustaceans (McMahon *et al.* 1978; Burnett, 1979), but this was attributed solely to the concomitant elevation of blood pH. Whether oxygen affinity increases *in vivo* independently of pH, as would be expected if there is an action of lactate and urate, and possibly of other unidentified organic cofactors, remains poorly documented. In the crayfish *Orconectes rusticus* exposed for 3.5 weeks to moderate ambient hypoxia, Wilkes & McMahon (1982) found an increased oxygen affinity, of which only 50 % was accounted for by pH variations, the remaining 50 % being unrelated to changes in ionic composition of haemolymph (i.e. no effect of calcium). In *Homarus vulgaris*, Bouchet & Truchot (1985) also found a pH-independent increase of oxygen affinity in haemolymph sampled from hypoxic lobsters, which was only partly due to elevated L-lactate concentrations. In the present study, there was also a moderate, pH-independent increase of oxygen affinity after a 3-h hypoxic exposure in *Penaeus*, which had disappeared after 48 h. This increase may be almost fully explained by the concomitant *in vivo* variations of L-lactate and urate concentrations in the haemolymph. This would exclude the possibility of another unidentified cofactor involved during hypoxia in *Penaeus*. However, it must be recalled that this interpretation relies upon the assumption that the effects of urate and lactate are additive, a factor not assessed in this study. It should be noted that, in the crayfish *Austropotamobius pallipes*, the effect of L-lactate was found to be markedly reduced in the presence of high urate concentrations (Morris *et al.* 1986), suggesting that these effects are agonistic.

Most water breathers, such as fishes and crustaceans, increase their ventilatory flow rate when faced with declining ambient oxygen tensions (Dejours, 1981). Hyperventilation generally results in a hypocapnic alkalosis, as has been observed in many crustacean species (see Truchot, 1987). Thus, although blood pH could not be measured in this study, it may increase during environmental hypoxia in *Penaeus japonicus*. Because of the large Bohr shift of *Penaeus japonicus* haemocyanin, this elevation of pH should lead to a large increase in haemocyanin oxygen-affinity. Therefore, the *in vivo* increase of affinity during hypoxia should be the result of the synergistic action of pH, L-lactate and urate increases.

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References

ARP, A. J. & CHILDRESS, J. J. (1985). Oxygen-binding properties of the blood of the deep-sea shrimp *Glyphocrangon vicaria*. *Physiol. Zool.* **58**, 38–45.

BOOTH, C. E., MCMAHON, B. R. & PINDER, A. W. (1982). Oxygen uptake and the potentiating effects of increased haemolymph lactate on oxygen transport during exercise in the blue cr[illegible] *Callinectes sapidus*. *J. comp. Physiol.* B **148**, 111–121.

BOUCHET, J. Y. & TRUCHOT, J. P. (1985). Effects of hypoxia and L-lactate on the haemocyanin oxygen affinity of the lobster, *Homarus vulgaris*. *Comp. Biochem. Physiol.* **80**A, 69–73.

BOWMAN, T. E. & ABELE, L. G. (1982). Classification of the recent Crustacea. In *The Biology of Crustacea* (ed. D. E. Bliss), vol. 1, *Systemics, the Fossil Record, and Biogeography* (ed. L. G. Abele), pp. 1–28. New York: Academic Press.

BRIDGES, C. R., BICUDO, J. E. P. W. & LYKKEBOE, G. (1979). Oxygen content measurements in blood containing haemocyanin. *Comp. Biochem. Physiol.* **62**A, 457–462.

BRIDGES, C. R. & BRAND, A. T. (1980). The effect of hypoxia on oxygen consumption and blood lactate levels of some marine Crustacea. *Comp. Biochem. Physiol.* **65**A, 399–409.

BRIDGES, C. R. & MORRIS, S. (1986). Modulation of haemocyanin oxygen affinity by L-lactate; a role for other cofactors. In *Invertebrate Oxygen Carriers* (ed. B. Linzen), pp. 341–352. Heidelberg: Springer-Verlag.

BROUWER, M., BONAVENTURA, C. & BONAVENTURA, J. (1978). Analysis of the effect of three different allosteric ligands on O_2 binding by hemocyanin of the shrimp *Penaeus setiferus*. *Biochemistry, N.Y.* **17**, 2148–2154.

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**, 289–300.

CLAYBROOK, D. L. (1983). Nitrogen metabolism. In *The Biology of Crustacea* (ed. D. E. Bliss), vol. 5, *Internal Anatomy and Physiological Regulation* (ed. L. H. Mantel), pp. 163–213. New York: Academic Press.

CZIETRICH, H. M., BRIDGES, C. R. & GRIESHABER, M. K. (1987). Purine metabolism of the crayfish *Astacus leptodactylus*. *Verh. dt. Zool. Ges.* **80**, 207.

DEJOURS, P. (1981). *Principles of Comparative Respiratory Physiology*, 2nd edn (first edn 1975). Amsterdam: Elsevier/North-Holland. 265pp.

GÄDE, G. (1983). Energy metabolism of arthropods and mollusks during environmental and functional anaerobiosis. *J. exp. Zool.* **228**, 415–429.

GÄDE, G. (1984). Effects of oxygen deprivation during anoxia and muscular work on the energy metabolism of the crayfish, *Orconectes limosus*. *Comp. Biochem. Physiol.* **77**A, 495–502.

HAGERMAN, L. & SZANIAWSKA, A. (1986). Behaviour, tolerance and anaerobic metabolism under hypoxia in the brackish water shrimp *Crangon crangon*. *Mar. Ecol. Prog. Ser.* **34**, 125–132.

LALLIER, F. (1988). Adaptation de la fonction de transport de l'oxygène par l'hémocyanine en milieu hypoxique: étude chez la crevette *Penaeus japonicus* et chez le crabe *Carcinus maenas*. Thèse de l'Université de Bordeaux I, France, no. 258, 133pp.

LALLIER, F., BOITEL, F. & TRUCHOT, J. P. (1987). The effect of ambient oxygen and temperature on haemolymph L-lactate and urate concentrations in the shore crab *Carcinus maenas*. *Comp. Biochem. Physiol.* **86**A, 255–260.

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

MCMAHON, B. R. (1985). Functions and functioning of crustacean haemocyanin. In *Respiratory Pigments in Animals. Relation Structure–Function* (ed. J. Lamy, J. P. Truchot & R. Gilles), pp. 35–58. Heidelberg: Springer-Verlag.

MCMAHON, B. R., BUTLER, P. J. & TAYLOR, E. W. (1978). Acid–base changes during recovery from disturbance and during long term hypoxic exposure in the lobster *Homarus vulgaris*. *J. exp. Zool.* **205**, 361–370.

MCNABB, R. A. & MCNABB, F. M. A. (1980). Physiological chemistry of uric acid solubility, colloid and ion-binding properties. *Comp. Biochem. Physiol.* **67**A, 27–34.

MANGUM, C. P. (1982). On the relationship between P_{50} and the mode of gas exchange in tropical crustaceans. *Pacif. Sci.* **36**, 403–410.

MANGUM, C. P. (1983*a*). Oxygen transport in the blood. In *The Biology of Crustacea* (ed. D. E. Bliss), vol. 5, *Internal Anatomy and Physiological Regulation* (ed. L. H. Mantel), pp. 373–429. New York: Academic Press.

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

MANGUM, C. P. & BURNETT, L. E. (1986). The CO_2 sensitivity of the hemocyanins and its relationship to Cl^- sensitivity. *Biol. Bull. mar. biol. Lab., Woods Hole* **171**, 248–263.

MAURO, N. A. & MALECHA, S. R. (1984). The effects of hypoxia on blood pH and lactate levels in *Macrobrachium rosenbergii* (De Man). *Comp. Biochem. Physiol.* **77**A, 627–630.

MORRIS, S. & BRIDGES, C. R. (1986). Novel non-lactate cofactors of haemocyanin oxygen affinity in crustaceans. In *Invertebrate Oxygen Carriers* (ed. B. Linzen), pp. 353–356. Heidelberg: Springer Verlag.

MORRIS, S., BRIDGES, C. R. & GRIESHABER, M. K. (1985). A new role for uric acid: modulator of haemocyanin oxygen affinity in crustaceans. *J. exp. Zool.* **235**, 135–139.

MORRIS, S., BRIDGES, C. R. & GRIESHABER, M. K. (1986). The potentiating effect of purine bases and some of their derivatives on the oxygen affinity of haemocyanin from the crayfish *Austropotamobius pallipes*. *J. comp. Physiol.* B **156**, 431–440.

MORRIS, S., GREENAWAY, P. & MCMAHON, B. R. (1988*a*). Oxygen and carbon dioxide transport of an amphibious crab, *Holthuisiana transversa*. *J. comp. Physiol.* B **157**, 873–882.

MORRIS, S., GREENAWAY, P. & MCMAHON, B. R. (1988*b*). Adaptations to a terrestrial existence by the robber crab, *Birgus latro*. I. An *in vitro* investigation of blood gas transport. *J. exp. Biol.* **140**, 477–491.

MORRIS, S., TAYLOR, A. C. & BRIDGES, C. R. (1988*c*). Response of haemocyanin oxygen affinity to simultaneous salinity and oxygen stress in the intertidal prawn *Palaemon elegans* (Rathke). *Comp. Biochem. Physiol.* **90**A, 31–39.

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

SPOTTS, D. G. (1983). Oxygen consumption and whole body lactate accumulation during progressive hypoxia in the tropical freshwater prawn, *Macrobrachium rosenbergii* (de Man). *J. exp. Zool.* **226**, 19–27.

TAYLOR, A. C., MORRIS, S. & BRIDGES, C. R. (1985). Modulation of haemocyanin oxygen affinity in the prawn *Palaemon elegans* (Rathke) under environmental salinity stress. *J. exp. mar. Biol. Ecol.* **94**, 167–180.

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

TRUCHOT, J. P. (1987). *Comparative Aspects of Extracellular Acid–Base Balance*. Berlin, Heidelberg: Springer-Verlag. 248pp.

TRUCHOT, J. P. & JOUVE-DUHAMEL, A. (1985). Consommation d'oxygène de la crevette japonaise, *Penaeus japonicus*, en fonction de l'oxygénation du milieu: effets de la température et de l'acclimatation à des conditions ambiantes hypoxiques. In *Bases Biologiques de l'Aquaculture*. Montpellier, 1983. IFREMER, Actes de Colloques, **1**, 245–254.

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

WILKES, P. R. H. & MCMAHON, B. R. (1982). Effect of maintained hypoxic exposure on the crayfish *Orconectes rusticus*. II. Modulation of haemocyanin oxygen affinity. *J. exp. Biol.* **98**, 139–149.

ZEBE, R. (1982). Anaerobic metabolism in *Upogebia pugettensis* and *Calianassa californensis* (Crustacea, Thalassanidea). *Comp. Biochem. Physiol.* **73**B, 613–617.