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Oxygen-binding by haemocyanins from an ecological series of amphipod crustaceans

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Abstract The oxygen-binding properties of haemocyanins (Hc) from three species of gammaridean amphipods, *Gammarus locusta* (L.) (subtidal), *Echinogammarus pirloti* (Sexton and Spooner), (intertidal, marine) and *E. marinus* (Leach) (intertidal, estuarine), one species of hyalid amphipod *Hyale nilsonni* Rathke (high intertidal, marine) and the talitrid amphipod *Orchestia gammarellus* (Pallas) (semi-terrestrial) have been studied. All the species were collected from the Firth of Clyde, Scotland, during the spring of 1992. The oxygen-carrying capacity of haemolymph from each species was low, although variable, and was correlated with the low concentration of Hc present. The Hc oxygen-affinity of native gammarid haemolymph was relatively high [partial pressure of oxygen required for half-saturation, P_{50} = 4 to 5 torr (0.53 to 0.67 kPa)] at their respective in vivo pH values. At equivalent pH, however, Hc from *G. locusta* displayed a lower O_2-affinity than either *Echinogammarus* species. Gammarid Hcs had a large Bohr effect ($\Delta \log P_{50}/\Delta$ pH = −1.16 to −1.47). Resuspended Hc isolated from whole *H. nilsonni* showed similar O_2-binding properties to those of the gammaridean amphipods [P_{50} = 6.3 torr (1.44 kPa) at pH = 8.0; $\Delta \log P_{50}/\Delta$pH = −1.20]. Comparable data for haemolymph from *O. gammarellus* showed that the Hc had a lower affinity for O_2 [P_{50} = 14.1 torr (1.87 kPa) at in vivo pH] and exhibited a more moderate Bohr effect ($\Delta \log P_{50}/\Delta$ pH = −0.79). To eliminate the possibility that these differences were due to the different haemolymph constituents, each of the Hcs were pelleted and resuspended in physiological saline. The differences noted above persisted, demonstrating that they were due to inherent O_2-binding properties of the Hc molecules themselves. An increase in L-lactate resulted in an increase in Hc oxygen-affinity for both *Echinogammarus* species but not for *O. gammarellus*. This study has confirmed that there is a clear difference between Hcs from aquatic and semi-terrestrial amphipod genera. The results lend further support to the hypothesis that the move on to land by amphipod crustaceans is accompanied by a decrease in Hc oxygen-affinity, a decrease in the Bohr effect and a decrease in effector (in this case L-lactate) sensitivity.

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Introduction

Our understanding of oxygen transport by crustacean haemocyanins (Hc) has increased markedly over the past 10 yr (Mangum 1983a, 1990a, b; Morris 1990, 1991; Truchot 1992; Truchot and Lallier 1992). Understandably, most workers have examined the larger decapod species, to the general neglect of other smaller species. There is, however, a growing body of literature on the in vitro O_2-binding properties of Hcs of amphipod Crustacea (Taylor and Spicer 1986; Spicer and Taylor 1989; Spicer and McMahon 1990, 1991; Spicer et al. 1990), and an in vivo study has also been attempted (Spicer and McMahon 1992). One important feature to emerge from some of this recent work is that Hc from the aquatic *Apohyale* (*Hyale*) *pugettensis* (Dana) had a higher O_2-affinity and a more pronounced Bohr effect than the Hc of either the semi-terrestrial *Megalorchestia* (*Orchestoidea*) *californiana* (Brandt) or the semi-/euterrestrial *Traskorchestia* (*Orchestia*) *traskiana* (Stimpson). These differences persisted even when the respective Hcs were resuspended in the same physiological saline (Spicer and McMahon 1990, 1991).

In the present study, we examined O_2-binding by Hcs from an ecological series of amphipods to further determine any differences that may exist between aquatic and semi-terrestrial amphipod genera. All the amphipod species studied are native to British coasts and can co-occur within metres of each other on the shore (Lincoln 1979). *Gammarus locusta* (L.) is essentially a sublittoral species,

although it can be found in some low shore pools (Lincoln 1979). *Echinogammarus* (*Chaetogammarus*) *pirloti* (Sexton and Spooner) and *E.* (*Marinogammarus* or *Chaetogammarus*) *marinus* (Leach) are found intertidally at different heights on the shore, occurring beneath rocks and in pools that are often subject to severe environmental hypoxia or even anoxia (Morris and Taylor 1983; Agnew and Taylor 1986). On the Isle of Cumbrae (Firth of Clyde, Scotland), *E. pirloti* is usually distributed between mean tide level and high-water neaps (Sexton and Spooner 1940; Agnew 1985), whereas *E. marinus* occurs over a much wider tidal range from high-water neaps down to low-water neaps (Agnew 1985). The hyalid *Hyale nilsonni* Rathke is found associated with the seaweed *Pelvetia canaliculata* in the high intertidal zone (Lincoln 1979). Hyalid amphipods have long been considered "intermediate" between gammarid and talitrid amphipods (Lincoln 1979), hence the importance of their inclusion in this study despite the technical difficulties involved in examining such small (even by amphipod standards) crustaceans. *Orchestia gammarellus* (Pallas) is a semi-terrestrial species that occurs beneath wrack, stones and debris near or above the high-water mark (Lincoln 1979). *O. gammarellus* has frequently been collected from more inland locations (e.g. Spicer et al. 1987).

The O_2-binding properties of haemolymph from *Orchestia gammarellus* have been studied before by the present authors (Taylor and Spicer 1986). They are re-examined here to compare the data obtained using Hc extracted by homogenising whole-animal preparations. In addition it was thought essential to make comparisons between the diffusion-chamber method for constructing O_2-binding curves with the Tucker method that we used in a previous study (Taylor and Spicer 1986). The final reason for the inclusion of *O. gammarellus* in this study was because the effect of L-lactate on Hc oxygen-binding has not been determined for this species.

The O_2-binding properties of both haemolymph and resuspended Hc from each of these species were determined in order to test the hypothesis that, with the adoption of increasingly terrestrial habits during the evolution of some members of this group, there is a decrease in Hc oxygen-affinity and a decrease in the magnitude of the Bohr effect (Spicer and McMahon 1990). In addition, the effect of L-lactate on O_2-binding by Hcs from the semi-terrestrial *Orchestia gammarellus* and the intertidal *Echinogammarus* species was examined to test the hypothesis that with the move onto land there is a decrease in effector sensitivity of Hc oxygen-binding (Spicer and McMahon 1990, 1991).

Materials and methods

Animal material and haemolymph collection

Gammarus locusta was collected from dead and decaying weed dredged from >10 m depth off the Isle of Cumbrae (Ordnance Survey Grid Ref. 165 535) and transferred within a week of capture to Glasgow. *Echinogammarus pirloti*, *Hyale nilsonni* and *Orchestia gammarellus* were collected from various heights on a boulder beach at Farland Bight on the Isle of Cumbrae, Firth of Clyde (Ordnance Survey Grid Ref. NS 172 542). *E. marinus* was collected from beneath stones in the middle of the intertidal zone on a boulder beach at Largs, Firth of Clyde (Ordnance Survey Grid Ref. NS 206 580). All amphipods were transported back to the Zoology Department, University of Glasgow, in plastic bags containing some of the substratum in which they were found. Haemolymph from individuals of each species was sampled within 5 h of arrival at Glasgow.

"Post-branchial" haemolymph was sampled from individuals of each species (with the exception of *Hyale nilsonni*) using a microsyringe (Hamilton, 25 µl capacity), the needle of which was inserted dorsally between the arthrodial membrane of Pereonite Segments 2 and 3. Haemolymph (0.2 to 15 µl per amphipod) was drawn up slowly into the syringe and then transferred to a 1.5 ml plastic centrifuge tube kept on ice. Pooled haemolymph samples for each species were thoroughly mixed before being centrifuged at 10 000 ×*g* for 15 min to remove any cells and coagulated protein. All samples were either examined immediately or stored at 3 °C for no more than 8 d.

Preparation of Hc from whole-animal material

Whole *Hyale nilsonni* and *Orchestia gammarellus* were homogenised with a Teflon homogeniser in a centrifuge tube containing a small volume of physiological saline at 4 °C (see following subsection). Since the presence of naturally occurring protease enzymes could potentially lead to disassociation of Hc aggregates, all samples were treated with both a serine protease inhibitor [1 mmol l^{-1} phenylmethansulphonyl fluoride (PMSF)] and a trypsin-like serine and cysteine protease inhibitor (0.1 mmol l^{-1} leupeptin). The resultant whole-animal homogenate was the centrifuged at 10 000 ×*g* for 15 min. The supernatant was then removed and treated as described in the following paragraph.

Samples of native haemolymph from *Orchestia gammarellus* and supernatant derived from whole-animal homogenate of both *O. gammarellus* and *Hyale nilsonni* were applied to a Superose 6 gel filtration column connected to a fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) in order to partially purify the Hcs. To obtain maximum resolution, a flow rate of either 0.1 or 0.2 ml min^{-1} was employed and 50×0.5 ml fractions were collected. Native haemolymph, both treated and untreated with protease inhibitors, was included to control for any possible effect of such inhibitors on Hc oxygen-binding.

The column was calibrated using the following protein markers: blue dextran (M_r = 2000 kdaltons), thyroglobulin (669 kdaltons), apoferritin (443 kdaltons), albumin (68 kdaltons) and cytochrome C (6.3 kdaltons) (Sigma Chemical Company). The column was eluted, and the haemolymph sample was diluted, with a modified saline solution (see following subsection for details) at pH = 7.80.

Eluted fractions were examined using a scanning spectrophotometer, and the absorbencies at λ = 280 nm and 335 nm were recorded to enable the relative concentrations of Cu^{2+} and protein to be determined. Fractions containing Hc were then pooled, pelleted (see following subsection), and immediately placed in a refrigerator (3 °C) where they were stored until the O_2-binding properties of the Hc could be examined (see following subsection for details).

Oxygen-binding characteristics of haemolymph and resuspended Hc

In vitro O_2-binding curves were constructed at 10 °C for both untreated haemolymph and for resuspended Hc of each species using a diffusion chamber technique (Spicer et al. 1990). The relationships between log P_{50} and pH were compared using ANCOVA. The level of significance chosen was P = 0.05 in each case.

Experiments were also carried out to examine both the effects of resuspending Hc in physiological saline and of the allosteric effects of L-lactate on O_2-binding (cf. Spicer and McMahon 1990) of Hcs from *Echinogammarus pirloti*, *E. marinus* and *Orchestia gammarellus*. Unfortunately, insufficient material was available to allow similar analyses on haemolymph from *Gammarus locusta*. Two aliquots (140 µl) of untreated haemolymph from each species were centrifuged at 144 000 ×*g* (Airfuge, Beckman, California, USA) for 90 min to pellet the Hc. The supernatant was carefully removed and replaced with modified saline. The ionic composition of this saline was identical to that used by Spicer and McMahon (1990, 1991), which was

based on the ionic composition of the haemolymph of the beach hopper *Traskorchestia traskiana*. This saline was used rather than salines based on the ionic composition of the haemolymph of the respective species, since it enabled direct comparisons to be made between these species and with the data for resuspended Hcs of other species examined previously. The stock *T. traskiana* saline (STS) had the following composition (mmol l^{-1}): NaCl, 449; KCl, 13; $CaCl_2$, 12; $MgCl_2$, 13; $NaHCO_3$, 3; the pH was adjusted (using NaOH) to pH = 7.90 ± 0.01 at 10 °C. The pellet was then resuspended and this procedure repeated. The effect of L-lactate on Hc oxygen-affinity was assessed by adding differing quantities of L-lactate (as the lithium salt) to the saline.

Haemolymph pH and O_2-content measurements

The pH of post-branchial haemolymph was determined for each species (with the exception of *Hyale nilsonni*) using a collection procedure similar to that described above (see subsection "Animal material and hemolymph collection"). However, instead of drawing the haemolymph sample into a syringe, the haemolymph was drawn directly into the capillary pH electrode of a Radiometer BMS II connected to a PHM 73 pH meter (Radiometer, Copenhagen, Denmark). This technique was used successfully in a previous study on other amphipod species (Taylor and Spicer 1986). Only large amphipods could be used, since at least 20 µl of haemolymph was required for each analysis. The remaining volume needed to fill the electrode was made up by introducing electrolyte. Individuals were quiescent prior to sampling and the haemolymph sample was extracted within a few seconds in attempt to minimise disturbance.

The BMS II, thermostatted at 10 °C, was also for tonometry of haemolymph samples with air, and the total oxygen content of the haemolymph (C_{O_2}) was determined in duplicate 15 µl samples using the modified "Tucker" method of Bridges et al. (1979).

Analysis of haemolymph ionic composition

The concentrations of both Ca and Mg in untreated haemolymph from each of the species (with the exception of *Hyale nilsonni*) were estimated after appropriate dilution with double-distilled water and addition of lanthanum chloride, using a Philips PU 9200 atomic absorption spectrophotometer. The concentrations of Na and K in the haemolymph were estimated using a flame photometer (Corning 410). Calibration curves were constructed using an appropriate range of standards. The concentration of Cl^- was estimated by electrochemical titration (Jenway PCLM3 Chloride meter). The concentration of L-lactate in untreated haemolymph and in the saline containing resuspended Hc was determined in duplicate 10 µl sub-samples using the method of Engel and Jones (1978).

The absorption spectra (λ = 250 to 700 nm) of untreated haemolymph from each species were determined using a Philips PU 8700 series UV/visible scanning spectrophotometer following dilution (1 : 50) with deionized water. The total protein was then estimated using a previously calculated extinction coefficient of $E^{1\%}_{1\,cm} = 14.42$ at λ = 278 nm (J. I. Spicer unpublished data).

Results

Oxygen-binding by untreated haemolymph and resuspended Hcs

Fig. 1 presents the relationships between P_{50} and the pH, at 10 °C, of native haemolymph from the three gammarid species examined. The O_2-binding properties of haemolymph from *Gammarus locusta* and from both *Echinogammarus* species were similar but not identical; the Hc of *G. locusta* displayed a significantly lower O_2-affinity compared with those of both *Echinogammarus* species at equivalent pH (Table 1). The Bohr values for Hcs from both *E. pirloti* and *E. marinus* were the same but smaller than that recorded for *G. locusta* (Table 1).

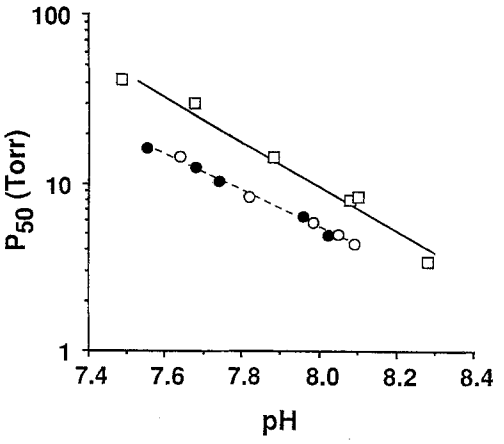

Fig. 1 *Gammarus locusta* (□), *Echinogammarus pirloti* (●) and *E. marinus* (○). Relationship between oxygen-affinity (P_{50}) and pH in untreated haemolymph at 10 °C

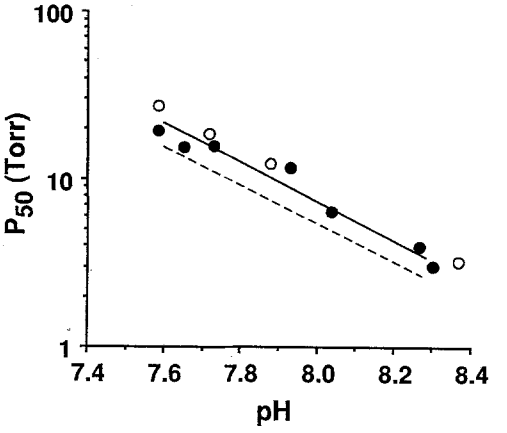

Fig. 2 *Echinogammarus pirloti* (●), and *E. marinus* (○). Relationship between oxygen-affinity (P_{50}) of Hc (haemocyanin) resuspended in STS (= stock *Traskorchestia traskiana* saline) and pH at 10 °C (*dashed line* untreated haemolymph)

The relationships between P_{50} and the pH for pelleted Hc from both *Echinogammarus pirloti* and *E. marinus*, resuspended in saline, are presented in Fig. 2. The magnitude of the Bohr effect remained the same although the elevations of the regression lines fitted to the data were significantly different from those of untreated haemolymph.

The relationship between P_{50} and pH for resuspended Hc from *Hyale nilsonni* is presented in Fig. 3. The resuspended Hc exhibited a high affinity for O_2 and a large Bohr effect (see Table 1).

The relationships between P_{50} and pH at 10 °C for the Hc of *Orchestia gammarellus* isolated from whole-animal homogenate and resuspended in saline, and for untreated haemolymph are presented in Fig. 4. There were no significant differences in these relationships between the different experimental treatments employed, i.e. resuspended Hc, resuspended Hc with added protease inhibitors and re-

Table 1 *Gammarus locusta*, *Echinogammarus pirloti*, *E. marinus*, *Hyale nilsonni* and *Orchestia gammarellus*. Oxygen-binding data for whole haemolymph and for resuspended haemocyanins (Hc). Calculated Bohr coefficients are for untreated haemolymph, with exception of *H. nilsonni*. Values for half saturation, P_{50} (at pH=8), and in vivo P_{50} were interpolated from Bohr plots for whole haemolymph. Half-saturation value (at pH=8.0) for resuspended Hc (*Res* P_{50}) was interpolated from Bohr plots for resuspended Hcs *na* (not available)

Species	C_{O_2} (mmol l^{-1})	P_{50} (at pH=8) [torr (kPa)]	Res. P_{50} (at pH=8) [torr (kPa)]	Bohr effect ($\Delta \log P_{50}/\Delta$ pH)	Haemolymph pH	In vivo P_{50} [torr (kPa)]
G. locusta	0.40 ± 0.12	8.7 (1.16)	n/a	−1.47	8.16 ± 0.12	5.1 (0.68)
E. pirloti	0.59 ± 0.14	4.9 (0.65)	8.7 (1.16)	−1.16	8.07 ± 0.11	4.0 (0.53)
E. marinus	0.53 ± 0.16	5.2 (0.69)	8.7 (1.16)	−1.16	8.04 ± 0.09	4.7 (0.63)
H. nilsonni	na	na	6.3 (0.84)	−1.20	na	na
O. gammarellus	0.54 ± 0.10	11.0 (1.47)	11.9 (1.59)	−0.77	7.86 ± 0.06	14.9 (1.99)

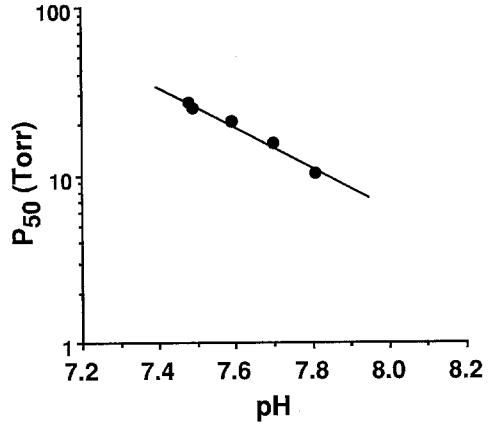

Fig. 3 *Hyale nilsonni*. Relationship between oxygen-affinity (P_{50}) of resuspended Hc and pH at 10 °C

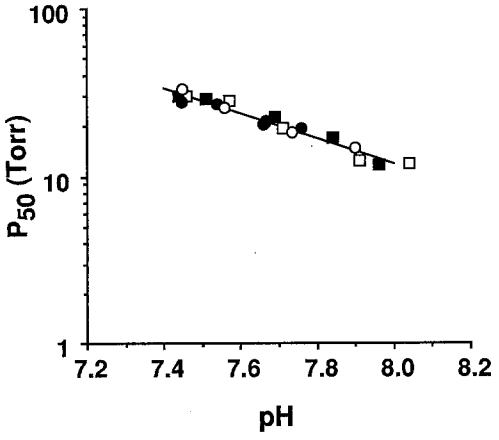

Fig. 4 *Orchestia gammarellus*. Relationship between oxygen-affinity (P_{50}) and pH of haemolymph and resuspended Hc at 10 °C (□ untreated haemolymph; ■ resuspended Hc; ○ resuspended Hc with protease inhibitors; ● resuspended Hc from whole-animal homogenate with protease inhibitors)

suspended Hc with added protease inhibitors separated from whole-animal homogenate. These data confirmed that the O_2-binding properties of Hc obtained by homogenising whole individuals did not differ significantly from those of Hc obtained by direct sampling. These results strengthen the case for the inclusion of the data obtained using homogenised *Hyale nilsonni* presented in the preceding paragraph. The untreated haemolymph of *O. gammarellus* possessed a lower O_2-affinity (at fixed pH) and more moderate Bohr effect than the haemolymph of the gammarid amphipods examined (Table 1).

Effect of L-lactate

The relationships between P_{50} and pH for resuspended pellets of Hc from *Echinogammarus pirloti*, *E. marinus*, and *Orchestia gammarellus* in the presence of different amounts of L-lactate, are presented in Fig. 5. L-lactate was responsible for a marked increase in Hc oxygen-affinity for both *Echinogammarus* species but not for *O. gammarellus*. Increasing the concentration of L-lactate resulted in a significant decrease in the Bohr effect (from −1.16 to −0.89) for Hc from *E. marinus*, but not for *E. pirloti*.

In vivo pH, P_{50} and haemolymph O_2 capacity

Values for the in vivo pH, the P_{50} at the in vivo pH interpolated from the regression lines depicted in Figs. 1 and 4, and the total O_2 content of the haemolymph are also presented in Table 1. One-way analysis of variance showed that there were small, but significant, differences in haemolymph pH between species ($df=3$, 40; $F=20.42$; $P<0.001$). There was, however, no significant difference in haemolymph pH between the *Echinogammarus* species. The pH of the haemolymph of *Gammarus locusta* was significantly greater, and that of *Orchestia gammarellus* significantly lower, than that of *Echinogammarus* spp. This resulted in differences in the calculated in vivo P_{50}s between species. Haemocyanins from all three gammarid species exhibited a quite high O_2-affinity and these values were greater than the comparable value for *O. gammarellus* (Table 1).

The total O_2 content of untreated haemolymph varied between species, although there was considerable variation between individuals of the same species (Table 1). One-way analysis of variance of the data for *Gammarus locusta*, both *Echinogammarus* species and *Orchestia gammarellus* indicated that there were significant differences in O_2-carrying capacity between the species ($df=3$, 70; $F=4.39$; $P=0.007$). When, however, the data for *G.*

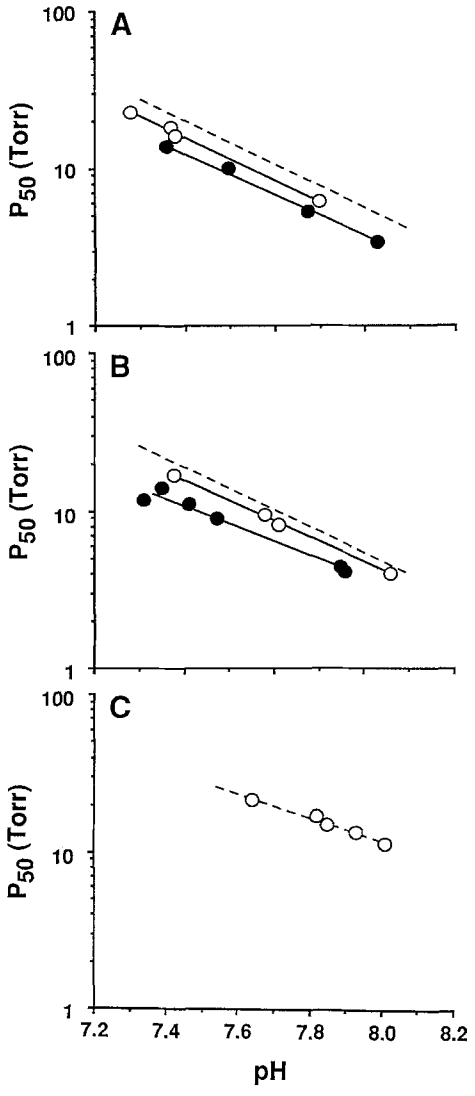

Fig. 5 *Echinogammarus pirloti* (**A**; ● 4.36 mmol l^{-1}, ○ 1.04 $mmol^{-1}$); *E. marinus* (**B**; ● 4.88 mmol l^{-1}, ○ 1.17 $mmol^{-1}$); *Orchestia gammarellus* (**C**; ○ 4.17 mmol l^{-1}). Effect of L-lactate on oxygen-affinity of Hc resuspended in STS (*dashed line* resuspended Hc)

locusta were removed from the analysis, no significant differences were detected ($df=2, 63$; $F=1.88$; $P>0.05$).

Ionic composition of the haemolymph

The ionic composition of untreated haemolymph for each of the amphipod species (with the exception of *Hyale nilsonni*), together with the concentrations of protein and L-lactate in the haemolymph are presented in Table 2. Values for the concentrations of the major ions in the physiological saline (STS) used in this study are also given for comparison. There were very few differences between the concentrations of the major ions in the haemolymph of the four species examined. The exceptions were Ca, which was present at slightly higher concentrations in the haemolymph of *Gammarus locusta* and *Orchestia gammarellus* than in either of the *Echinogammarus* species, and Mg, which was considerably lower in the haemolymph of *O. gammarellus* than in any of the other amphipods examined. It is of particular interest that the protein concentrations in the haemolymph of each of the species were comparatively low. *E. pirloti* had a significantly greater total haemolymph protein concentration than any of the other species (see Table 2).

Discussion

Hc oxygen-affinity

The Hcs of all three gammarid species were characterised as having comparatively high O_2-affinities in vivo [P_{50} = 4 to 5 torr (0.53 to 0.67 kPa)]. This is also likely to be true for the hyalid amphipod *Hyale nilsonni*. These P_{50} values were considerably lower than those calculated for *Orchestia gammarellus* [P_{50} = 14.1 torr (1.87)] and those recorded previously for semi/euterrestrial talitrid amphipods [P_{50} = 9.5 to 43.4 torr (1.27 to 5.79 kPa)] (Taylor and Spicer 1986; Spicer and Taylor 1989; Spicer and McMahon 1992; J. I. Spicer unpublished observation). Such differences persist even when O_2-affinities are compared at identical pH values. Such findings are consistent with the suggestion that among Hcs of amphipod crustaceans there appears to be a correlation between the possession of Hc with a low O_2-affinity and the adoption of terrestrial habits (Spicer and Taylor 1989; Spicer and McMahon 1990). This is notwithstanding Mangum's (1982) valid criticism of decapod and isopod data, to the effect that earlier generalisations linking low O_2-affinity with aerial gas exchange in crustaceans were based on comparisons of geographically and taxonomically separate species. Indeed, within the amphipods examined here, the differences in Hc oxygen-affinity persisted even when the Hc from each gammarid species was resuspended in the same physiological saline (cf. Spicer and McMahon 1990).

Relationship between gas-exchange surfaces and Hc oxygen-binding

The possession of a high Hc oxygen-affinity by gammarids (which have a characteristically large gill area: Moore and Taylor 1984) relative to talitrid amphipods (in which there is marked reduction in gill area and number: Spicer and Taylor 1986), could appear anomalous; particularly if we assume, as would seem reasonable, that there are no major differences in gill-diffusion distances between the groups. Consequently, our data may lend further support to the suggestion made on a number of occasions (Spicer et al. 1987; Spicer and McMahon 1992, 1994; Spicer and Taylor 1994) that the gills are not the only sites of gas exchange in amphipod crustaceans, particularly in semi- and euterrestrial talitrids. The role of extrabranchial gas-ex-

Table 2 *Gammarus locusta*, *Echinogammarus pirloti*, *E. marinus*, *Hyale nilsonni* and *Orchestia gammarellus*. Ionic composition (mmol l^{-1}) and total protein (mg ml^{-1}) of haemolymph. Ionic composition of physiological saline (STS = stock *Traskorchestia traskiana* saline) used to resuspend Hcs is also given. Values are mean ± standard deviation of 2 to 6 pooled damples (– not applicable)

Species	Na	K	Ca	Mg	Cl^-	Protein	L-lactate
G. locusta	441 ± 18	9.3 ± 0.7	14.9 ± 1.0	8.6 ± 0.3	483 ± 31	19.2 ± 3.7	0.193 ± 0.021
E. pirloti	462 ± 22	9.5 ± 1.2	10.3 ± 2.0	9.8 ± 1.0	493 ± 35	30.4 ± 3.1	0.233 ± 0.030
E. marinus	450 ± 19	10.1 ± 2.1	11.4 ± 1.1	9.7 ± 1.4	466 ± 29	23.2 ± 4.2	0.201 ± 0.035
O. gammarellus	438 ± 16	12.4 ± 1.3	15.4 ± 0.3	6.6 ± 1.3	469 ± 26	21.1 ± 4.0	0.110 ± 0.035
Physiological saline	449	13.0	12.0	12.0	510	–	–

change surfaces in amphipods is the subject of continuing studies.

The O_2 content of haemolymph from each of the four species examined was low and was comparable to equivalent data for other gammarid and talitrid amphipods (Taylor and Spicer 1986; Spicer and Taylor 1989; Spicer et al. 1990; Spicer and McMahon 1992).

Bohr effect

The Hcs of *Gammarus locusta*, *Echinogammarus pirloti*, *E. marinus* and *Hyale nilsonni* all exhibited comparatively large Bohr effects ($\Delta \log P_{50}/\Delta pH = -1.16$ to -1.47) similar to those in the upper range of values found for other crustacean species (see Mangum 1983 a and Truchot 1992 for examples). Two other fully aquatic amphipods, *Apohyale pugettensis* (a hyalid allied to *Hyale nilsonni*) and *Gammarus lacustris lacustris* Sars (the only other non-talitrid amphipods for which we have data), were also characterised by Hcs exhibiting large Bohr effects (Spicer and McMahon 1990). This contrasts with the data for *Orchestia gammarellus* and all the talitrid amphipods examined to date, which possess Hcs with a characteristically moderate (and surprisingly conservative) Bohr effect (average $\Delta \log P_{50}/\Delta pH = -0.82$).

When Hcs from both *Echinogammarus* species and *Hyale nilsonni* were resuspended in a physiological saline solution having the same ionic composition as the haemolymph of the talitrid *Traskorchestia traskiana* (Hc from this species: $\Delta \log P_{50}/\Delta pH = -0.70$), the large Bohr effect was retained (cf. Spicer and McMahon 1990 and present study). Similarly, when Hc from *Orchestia gammarellus* was resuspended in this saline, the Bohr effect remained unchanged.Taken together with the results of Spicer and McMahon (1990, 1991), this further strengthens the suggestion that differences observed in the Bohr effect were due to differences in the Hc molecules themselves and that gammarid and hyalid amphipods probably possess Hcs that are structurally dissimilar to that of talitrids.

It is interesting that all the gammarid and hyalid Hcs examined to date exhibited comparatively large Bohr effects. Both *Echinogammarus* species, and perhaps also *Gammarus locusta* and *Hyale nilsonni*, inhabit environments which are periodically exposed to hypoxia (see "Introduction" for literature references), and so the possession of Hc that displays a large Bohr effect may well be advantageous in some circumstances (Truchot 1992).

L-lactate as an effector of Hc oxygen-affinity

L-lactate is produced in crustaceans during exercise and environmental anaerobiosis and is known to affect Hc oxygen-affinity (Truchot 1980, 1992). Hc from both *Echinogammarus* species, together with Hcs from the other aquatic amphipods that have been examined (Spicer and McMahon 1990, 1991) display increasing O_2-affinity with increasing concentration of L-lactate in the haemolymph. The calculated coefficients for the effect of L-lactate [$\Delta \log P_{50}/\Delta \log$ (L-lactate)] on Hc from both the *Echinogammarus* species fall well within the known range of values (0 to –0.63) calculated for decapods and amphipods (Mangum 1983 b; Bridges and Morris 1986; Spicer and McMahon 1990). Bridges and Morris (1986), reviewing literature current at that time, concluded that, while it was not possible to establish a direct correlation between Bohr and L-lactate effects, a general trend could be discerned in which the occurrence of a large Bohr effect was generally associated with a large L-lactate effect. The present study, taken together with that of Spicer and McMahon (1990), confirms such a trend for amphipod Hcs.

Interestingly, an L-lactate effect was absent in Hc from *Orchestia gammarellus*, and from Hcs from most of the semi-/euterrestrial talitrids examined (Spicer and McMahon 1990; Spicer et al. 1990). Such a finding is paralleled in decapod crustaceans. Morris (1991), in a recent review of the ecological determinants of gas transport in crustaceans, noted that for more than 20 species of terrestrial decapod studied the Hc shows little or no sensitivity to L-lactate.

Conclusions

There appear to be few qualitative differences between the O_2-binding properties of Hcs of amphipod crustaceans and those of decapod and isopods (Mangum 1983 a; Morris 1991; Truchot 1992). The present study showed that, in general, the aquatic gammarids and hyalids possessed Hcs that were characterised by the presence of a high O_2 af-

finity, a large Bohr effect and, in the case of the gammarids, a large effect of L-lactate. The semi-/euterrestrial talitrids, as represented by *Orchestia gammarellus* in this study, possessed Hcs with a low O_2-affinity, a moderate Bohr shift and a low sensitivity to the allosteric effects of L-lactate. These differences would seem to be intrinsic to the Hc molecules themselves and not due to their ionic environment. A decrease in effector sensitivity with the transition from sea to land has also been noted for decapod crustaceans (Morris 1991). For both decapods and amphipods, this means that terrestrial species have recourse to a limited repertoire with regard to modulation of Hc oxygen-affinity. Such modulation, in their more aquatic relatives, may be used to optimise O_2 transport when the animal is under stress, e.g. exercise (McMahon 1986; Truchot 1992). It may be assumed, therefore, that the benefits associated with the large O_2 content of air outweighs the requirement for such modulation in terrestrial decapods and amphipods.

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