Effects of short-term hypoxia on metabolism and haemocyanin oxygen transport in the prawns *Palaemon adspersus* and *Palaemonetes varians*

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ABSTRACT: In summer, high temperatures are combined with severe nocturnal hypoxia in the shallow water of Danish fjords. Under these conditions, the prawns *Palaemonetes varians* (Leach) and *Palaemon adspersus* (Rathke) are spatially separated, with the former occupying the areas of highest temperature and lowest oxygen tension. If kept together, *P. adspersus* shows aggressive behaviour towards *P. varians*. Aerobic/anaerobic respiration and haemocyanin characteristics were measured in laboratory experiments (24°C, 14 ppt). Although the 2 species have similar metabolic rates, *P. varians* survives severe hypoxia better and has a lower P_c (critical oxygen tension, below which respiration is partly or fully anaerobic) than *P. adspersus*. Both species accumulate high levels of lactate during anaerobiosis and remove it at similar rates. The haemocyanin concentration and total oxygen carrying capacity (0.78 ± 0.25 mmol l^{-1} and 2.25 ± 0.56 vol%) of *P. varians* was higher than *P. adspersus* (0.28 ± 0.07 mmol l^{-1} and 1.14 ± 0.15 vol%). Haemocyanin oxygen affinity was always higher in *P. varians* regardless of pH, but the Bohr factor (−1.44 and −1.46) and cooperativity (n_{50}: 2.61 ± 0.20 and 2.82 ± 0.49) were similar for *P. varians* and *P. adspersus*, respectively. *P. varians* is thus better adapted to severe hypoxia than *P. adspersus*.

KEY WORDS: Aerobic/anaerobic metabolism · Lactate Oxygen affinity

INTRODUCTION

Large fluctuations in water oxygen tension occur in shallow brackish waters on a diel basis, especially in areas with dense vegetation and a low degree of water mixing. Large variations in other physico-chemical parameters [temperature, salinity, pH, pCO_2 (CO_2 partial pressure)] also take place in these waters (Ganning 1971, Morris & Taylor 1983, Grieshaber et al. 1994). Such more or less regular changes in the physico-chemical parameters challenge the physiological capacities of animals living in these habitats (Taylor 1990, Grieshaber et al. 1994, Hagerman 1998), and it is of vital importance for the animals to possess the necessary behavioural and/or physiological adaptations. Some of these might be enhanced blood-oxygen-transporting qualities, tolerance of high levels of anaerobic metabolites, an enhanced ability for osmoregulation or tolerance of changes in internal ion concentrations, and a high degree of temperature tolerance.

The palaemonid prawn *Palaemonetes varians* is found typically in very shallow waters near freshwater outlets in Danish fjords (e.g. the Roskilde Fjord) (Rasmussen 1973). The related species *Palaemon adspersus* dominates in seagrass (*Zostera marina*) beds at ca 1 to 5 m depth (Rasmussen 1973); this species leaves the seagrass beds during the night and moves into more open or shallower areas to feed (Hagerman & Østrup 1980). The 2 species are rarely observed together, even though their behaviour and ecology are very similar (Rasmussen 1973, Hagerman & Uglow 1984). The somewhat smaller *P. varians* is probably excluded from the 'protected' *Z. marina*-areas by *P. adspersus*, which is aggressive towards *P. varians* when they are kept together in the laboratory (authors' unpubl. obs.).

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Palaemonetes varians migrates to shallow waters with low salinity and high summer temperatures, where they survive better than *Palaemon adspersus* due to a greater tolerance of low salinities at high temperatures (Hagerman & Uglow 1983). There are also indications that *P. varians* is more able to tolerate periods of severe hypoxia (Hagerman & Uglow 1984) and is possibly able to utilise low oxygen tensions better, by a more efficient uptake of oxygen across the gills and, perhaps, by having a higher haemolymph oxygen carrying capacity and a respiratory pigment with a higher affinity for oxygen.

The aim of the present work is to evaluate the respiratory physiology of *Palaemonetes varians* and *Palaemon adspersus* under conditions prevailing in shallow water during summertime in an attempt to explain their distributions.

MATERIAL AND METHODS

Palaemonetes varians and *Palaemon adspersus* were collected in the Roskilde Fjord area, west Zealand, Denmark. *P. adspersus* (mean wet weight 1.622 ± 0.376 g) were caught in prawn traps in the deeper part of the fjord. *P. varians* (mean wet weight 0.658 ± 0.116 g) were collected with a hand-held push-net in the innermost part of the fjord, close to the sluice leading to the Selsø lake. The prawns were transported to the Helsingør laboratory within a few hours and kept in 200 l tanks with fully aerated running seawater (S: 32 ppt; T: 12°C). The prawns were acclimated to laboratory conditions for at least 1 wk before use. Prawns were fed regularly with fish pellets and minced meat of *Mytilus edulis*. All experimental prawns were acclimated to temperature (24°C) and salinity (14 ppt) in well-aerated water for at least 4 d before experiments. The prawns were not fed during this acclimation period. Only healthy intermoult prawns were used.

Field measurements. Diel variations in pH, water pO_2, temperature and salinity were measured every hour in shallow water (0.5 m) in May, June and August 1996 at 3 different places close to the sluice leading to the Selsø lake. pH was measured with a combination pH electrode (PHC2005, Radiometer, Copenhagen, Denmark) connected to a portable pH meter (PHM80, Radiometer). Water temperature and pO_2 were measured with an oxygen meter (Oxi96-B, WTW, Germany; or YSI58, Yellow Springs Instrument Co., Ohio, USA). Air temperature was measured in areas sheltered by trees close to the water's edge. Water samples, for salinity measurements, were brought back to the laboratory within 4 to 5 h and analysed with a refractometer (Atago Co., Tokyo, Japan).

Laboratory experiments. Aerobic metabolism was measured as oxygen consumption in a closed system. The respiration chamber was placed in an aquarium (24°C, 14 ppt) acting as water supply during the flushing periods and as a temperature bath. An oxygen electrode (E5047, Radiometer) was placed between the respiration chamber and a pump (Gilson minipuls3, USA) and connected to a computer via an oxygen meter (Radiometer PHM71 acid-base analyser with an O_2 module PHA930). The oxygen tension in the chamber was recorded continuously during the experiments. Continuous water flow was provided by the Gilson pump to ensure a homogeneous oxygen distribution in the chamber and a continuous flow over the electrode. Respiration (MO_2: weight specific respiratory rate) was measured on single individuals. Due to the smaller size of *Palaemonetes varians* it was impossible to obtain individuals of both species within the same narrow weight range.

Prawns were allowed to acclimatise to the conditions within the chamber for 1 to 2 h until the respiratory rate was stable. During this period the chamber was flushed with well-aerated water. The experiments ran until the oxygen tension in the chamber had decreased below 5.3 kPa or when the prawn appeared to be near death. All data (oxygen tension vs time) were corrected for electrode drift and the MO_2 calculated after subtraction of the values of oxygen consumption recorded in controls run without prawns.

Anaerobic metabolism was studied by measurements of haemolymph lactate accumulation. After acclimation for 4 d to 24°C and 14 ppt, the prawns were transferred to a test aquarium with clean, well-aerated water 1 d before the experiment was carried out. Hypoxia was achieved by bubbling N_2 through the water; oxygen tension was regulated by a microprocessor-controlled regulator (constructed by Ejner Larsen, Århus University, Denmark) to 0.66 kPa for *Palaemonetes varians* and 1.6 kPa for *Palaemon adspersus* and monitored during the experiment with the same equipment as used for the respiration experiments. The difference in the oxygen tension chosen for the 2 species was based on the difference in P_c (critical oxygen tension) found in the respiration experiments (see below). The tension chosen was well below the P_c values recorded for each species. During the lactate accumulation experiments, individuals were removed for blood sampling at fixed time intervals. Each prawn was sampled only once. Blood samples were taken by penetrating the pericardium with a 0.1 ml Hamilton hypodermic syringe and the volume of blood sampled was measured (40 to 70 µl). The blood was diluted immediately in 120 µl 0.6 N perchloric acid and frozen at −18°C prior to analysis. Lactate was measured on a spectrophotometer at 340 nm (Pharmacia Ultrospec II,

Cambridge, England) with a L-lactic acid test-kit (no. 139 084, Boehringer-Mannheim).

The same procedure was used to measure the time-dependent loss of lactate from the haemolymph due to oxidation or the formation of glycogen via gluconeogenesis after exposure to severe hypoxia for 5 h for *Palaemonetes varians* and 2 h for *Palaemon adspersus*. Blood sampling for these measurements began immediately after the oxygen tension was raised from hypoxic levels to normoxic tensions. The differences in the time that the prawns were exposed to hypoxia was necessary to ensure that sufficient lactate had accumulated in the haemolymph of *P. varians*.

In vivo haemolymph pH was measured in individual prawns using a pH G299A electrode (BMSII, mk2, Radiometer) connected to a PHM73 Blood Gas Monitor (Radiometer). The polycarbonate capillary of the electrode was inserted directly into the pericardium of the prawn and the blood drawn directly into the electrode, thus avoiding any contact with air. The electrode was calibrated using Radiometer precision pH buffers.

Oxygen carrying capacity was calculated from haemocyanin (hc) concentrations measured on pooled haemolymph samples. The absorbance of 20 µl haemolymph diluted in 400 µl Tris-HCl buffer (pH: 7.9) was measured at 335 nm using an UV-spectrophotometer (Pharmacia Ultrospec II). Hc concentration was calculated using an extinction coefficient ε_{mmol}^{cm} of 17.26 as applies to *Carcinus maenas* hc (Hagerman & Weber 1981). Assuming haemolymph osmolarities equivalent to salinities of 23 and 25 ppt for *Palaemonetes varians* and *Palaemon adspersus* respectively (Potts & Parry 1964, Hagerman & Uglow 1979) and a barometric pressure of 101 kPa, the total oxygen-carrying capacity of the haemolymph was calculated by adding the physically dissolved oxygen to the calculated hc oxygen-carrying capacity.

Haemocyanin oxygen affinity: Pooled haemolymph samples were centrifuged in 1.5 ml Eppendorf tubes for 5 min at 14 000 × *g* and the supernatant used immediately for the construction of oxygen equilibrium curves (Weber & Hagerman 1981). Oxygen equilibrium curves were constructed by stepwise oxygenation using a thin-layer technique (Sick & Gersonde 1969). A small volume of sample (5 µl for *Palaemonetes varians* and 10 µl for *Palaemon adspersus*, the difference was due to differences in the hc concentrations) was withdrawn from the supernatant and the oxygen equilibrium curves measured at 335 nm in a gas diffusion chamber (Eschweiler, Kiel, Germany) connected to a photometer (Eppendorf 1101M, Hamburg, Germany). The signal from the photometer was recorded on a potentiometric recorder via an antilogarithmic converter (Eschweiler). The appropriate gas mixtures were delivered by serially connected gas-mixing pumps (Wösthoff Digamix

SA27/3A and 2M201/a-F, Bochum, Germany) keeping constant pCO_2 and increasing pO_2 to obtain the different equilibration steps. Full oxygenation and de-oxygenation were measured after equilibration with O_2 and N_2 respectively at the beginning and end of each curve. At each step the haemolymph in the diffusion chamber was allowed to equilibrate with the gas mixture for at least 20 min. Haemolymph pH was measured at several steps near half-saturation. A small volume of the pooled haemolymph sample was equilibrated to the appropriate gas mixture for 20 min and pH measured with a MI410 micro-combination electrode (Microelectrodes Inc., USA) connected to a PHM73 blood gas monitor (Radiometer).

Statistics: Differences between regression lines in the hc oxygen-affinity experiments were tested with comparisons of regression coefficients and analysis of covariance (ANCOVA). All other differences were tested with single classification of analysis of variance (ANOVA) (Sokal & Rohlf 1981). Differences were considered significant if p < 0.05.

RESULTS

Field measurements

Temperature, water pO_2 and pH showed large diel variations with high diurnal and low nocturnal values for all 3 sampling locations (Table 1). The largest fluctuations in all these parameters occurred in August when water temperatures were between 16.2 and 24.0°C, oxygen tension of water (P_wO_2) between 14.3 and 176.7 % saturation and pH between 7.76 and 8.62 at location 3. Salinity showed no diel variation.

Laboratory measurements

Aerobic and anaerobic metabolism. The critical oxygen tension (P_c, below which declining P_wO_2 results in a decreased MO_2) at 24°C and 14 ppt was found to be 6.56 ± 0.6 kPa (n = 6) and 7.77 ± 1.1 kPa (n = 9) for *Palaemonetes varians* and *Palaemon adspersus*, respectively. The differences in the P_c values were found to be significantly different (p < 0.005). Routine metabolic rate at $P_wO_2 > P_c$ was found to be 0.166 ± 0.034 mg O_2 h^{-1} g^{-1} wet wt (n = 6) and 0.163 ± 0.035 mg O_2 h^{-1} g^{-1} wet wt (n = 9) for *P. varians* and *P. adspersus*, respectively (Table 2).

Both species resorted to anaerobic metabolism when exposed to a P_wO_2 well below the P_c. In *Palaemonetes varians* there was a tendency towards a lower lactate accumulation rate during the first 120 to 150 min. In *P. varians* the maximum concentrations of haemolymph

Table 1. Minimum and maximum values of some physico-chemical parameters during three 24 h periods at 3 locations (1 to 3) close to the sluice leading to the Selsø lake, Roskilde Fjord, Denmark. P_wO_2: oxygen tension of water

	Water temperature (°C)	P_wO_2 (% sat.)	pH	Salinity (ppt)
May				
Location 1	10.3 17.6	70 100	7.96 8.36	17 17
Location 2	10.9 18.6	61 100	7.90 8.30	– –
Location 3	11.0 17.5	64 100	7.88 8.26	16 16
June				
Location 1	17.9 24.1	39 144	7.91 9.06	12 15
Location 2	18.8 24.6	21 160	7.96 8.70	14 15
Location 3	18.8 25.0	42 167	7.95 8.48	14 15
August				
Location 1	16.5 25.0	45 155	7.99 8.63	15 17
Location 2	16.1 24.9	26 180	7.82 8.76	16 17
Location 3	16.2 24.0	15 177	7.76 8.62	16 17

an appropriate number of blood samples. A lactate accumulation rate of 0.162 μmol ml⁻¹ min⁻¹ was, however, calculated for *P. adspersus* for the 120 min of hypoxia that they survived. This rate was not significantly different from the value of 0.141 μmol ml⁻¹ min⁻¹ found for *P. varians* during the first hypoxic period, which increased to 0.272 μmol ml⁻¹ min⁻¹ after 150 min of hypoxia (Table 2).

The time-dependent loss of haemolymph lactate in *Palaemonetes varians* and *Palaemon adspersus* (Fig. 1B) during normoxia following severe hypoxia showed a decrease of 0.162 μmol ml⁻¹ min⁻¹ for *P. adspersus* (although very few data could be obtained due to the high mortalities)

lactate (45.4 μmol ml⁻¹) were reached after ca 260 min of hypoxia (Fig. 1A). A high mortality of *P. adspersus* during the hypoxia exposure made it difficult to obtain and 0.145 μmol ml⁻¹ min⁻¹ for *P. varians*. For both species the rates of lactate loss were thus very similar to the lactate accumulation rates.

Table 2. *Palaemonetes varians* and *Palaemon adspersus*. Summary of experimental results on respiratory physiology at 24°C, and 14 ppt. P_c: critical oxygen tension; MO_2: respiration; P_{50}: haemocyanin oxygen affinity; and n_{50}: cooperativity coefficient. Statistics are shown as differences between the 2 species (a: $p < 0.001$; b: $p < 0.005$; ns: no significant difference)

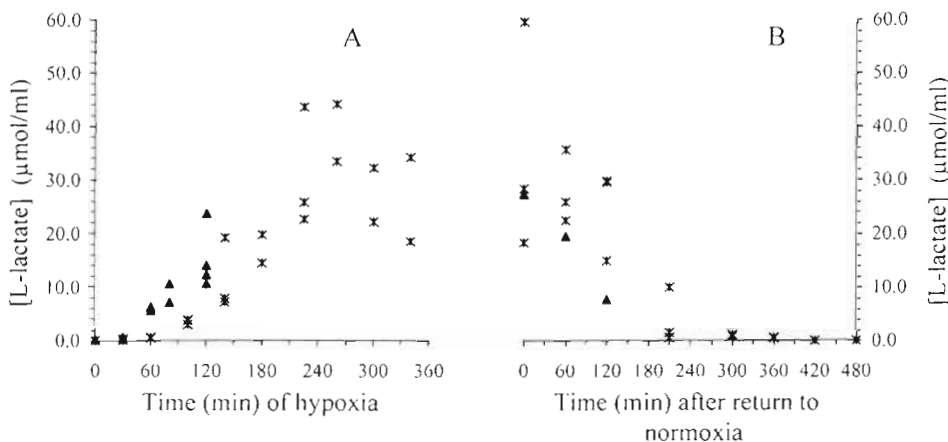| Variable	P. varians		P. adspersus
P_c (kPa)	6.55 ± 0.6	b	7.77 ± 1.1
MO_2 (mg O_2 h⁻¹ g⁻¹ wet wt)	0.166 ± 0.034	ns	0.163 ± 0.035
Lactate accumulation rate (μmol ml⁻¹ min⁻¹)	0.141	ns	0.162
Lactate reoxidation rate (μmol ml⁻¹ min⁻¹)	0.145	ns	0.162
Haemocyanin concentration (mmol l⁻¹)	0.776 ± 0.252	a	0.282 ± 0.068
Haemocyanin O_2 carrying capacity (vol%)	1.74 ± 0.56	a	0.63 ± 0.15
Total O_2 carrying capacity (vol%)	2.25 ± 0.56	a	1.14 ± 0.15
In vivo pH	7.438 ± 0.074	ns	7.427 ± 0.111
Log P_{50} (dependence of pH)	$-1.44 \times pH + 12.5$	b	$-1.46 \times pH + 12.81$
Bohr factor ($\Delta log P_{50}/\Delta pH$)	-1.44	ns	-1.46
n_{50}	2.61 ± 0.20	ns	2.82 ± 0.49

Fig. 1 *Palaemonetes varians* and *Palaemon adspersus*. (A) Haemolymph lactate accumulation during exposure to severe hypoxia of 0.66 kPa (x, *P. varians*) and 1.6 kPa (▲, *P. adspersus*) at 24°C, and 14 ppt. (B) Haemolymph lactate reoxidation after return to normoxia; symbols as in (A)

Fig. 2. *Palaemonetes varians* and *Palaemon adspersus*. (A) Haemocyanin oxygen affinity (P_{50}) and cooperativity (n_{50}) vs pH at 24°C and 14 ppt in *P. varians* (×) and *P. adspersus* (▲). (B) Oxygen dissociation curves of haemocyanin at 24°C and 14 ppt for *P. varians* (thick line) and *P. adspersus* (thin line). Curves are calculated from the P_{50} values at the *in vivo* pH and the mean values of n_{50}
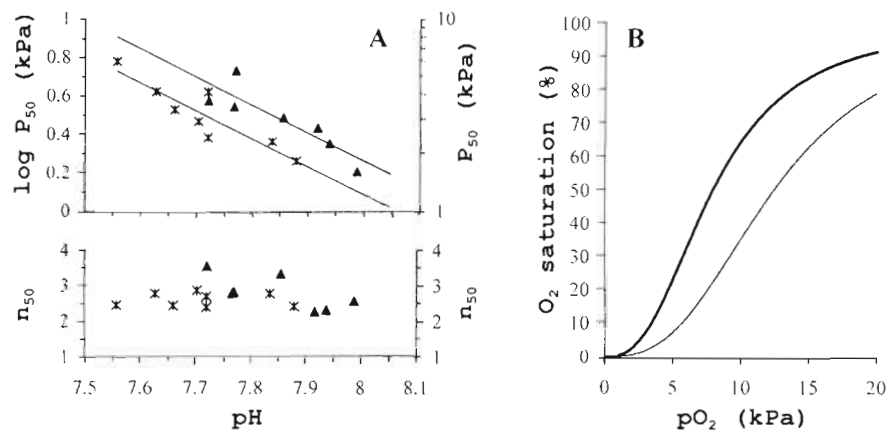
In vivo haemolymph pH was 7.44 ± 0.07 (n = 6) for *Palaemonetes varians* and 7.43 ± 0.11 (n = 7) for *Palaemon adspersus* (Table 2).

Oxygen carrying capacities were calculated from haemolymph hc concentrations, which were 0.78 ± 0.25 mmol l^{-1} (n = 8) and 0.28 ± 0.07 mmol l^{-1} for *Palaemonetes varians* and *Palaemon adspersus*, respectively. These values are significantly different (p < 0.001). The oxygen carrying capacities calculated from the hc concentrations correspond to 1.74 ± 0.56 vol% and 0.63 ± 0.15 vol%, and when the physically dissolved oxygen is added this gives a total oxygen carrying capacity of 2.25 ± 0.56 vol% for *P. varians* and 1.14 ± 0.15 vol% for *P. adspersus*.

Haemocyanin oxygen affinity (P_{50}: oxygen tension for half saturation of hc) and the cooperativity coefficient (n_{50}) in relation to pH are shown in Fig. 2A, and oxygen dissociation curves calculated at *in vivo* pH for both species are shown in Fig. 2B. *Palaemonetes varians* hc shows a significantly higher affinity for oxygen than *P. adspersus*. Bohr factors ($\Delta \log P_{50}/\Delta pH$) were large, −1.44 for *P. varians* and −1.46 for *P. adspersus*. $\log P_{50}$ was found to be significantly higher (p < 0.005) for *P. adspersus* regardless of pH, i.e. there is a higher haemocyanin oxygen affinity in *P. varians*. The n_{50} values were similar in the 2 species, 2.61 ± 0.20 (n = 8) in *P. varians* and 2.82 ± 0.49 (n = 7) in *P. adspersus*.

DISCUSSION

The diel fluctuations in physico-chemical parameters in the shallow waters at the Selsø sluice locality were large, especially with regard to P_wO_2 and pH, the latter reflecting variations in P_wCO_2 (CO_2 tension of water) with low diurnal and high nocturnal values. The changes are typical for shallow, sheltered areas and rock pools (Ganning 1971, Truchot & Duhamel-Jouve 1980, Morris & Taylor 1983). In all 3 field sampling periods, the weather was a little windy, so fluctuations

in these parameters may be expected to be even larger in calm weather. This highly variable environment, and especially the high water temperatures, requires special adaptations from organisms living there. *Palaemonetes varians* can thus be expected to have the physiological adaptations typical for species of highly variable environments, while *Palaemon adspersus*, living in a less variable environment, might not be expected to have such a high degree of physiological adaptability.

Palaemonetes varians was shown to have a significantly lower P_c than *Palaemon adspersus*, but this value is considerably higher than the values (1 3–2.6 kPa at 20°C) found in an earlier study of *P. varians* (Hagerman & Uglow 1984). The higher temperature decreases the concentration of oxygen and increases the metabolic rate, shifting P_c towards a higher value (Herreid 1980). The shore crab *Carcinus maenas*, for example, increased its P_c by 2.6 kPa when the temperature was increased from 10 to 18°C (Taylor et al. 1977). Such interspecific differences in P_c were also noted for the intertidal (rock-pool) *Palaemon elegans* compared with the subtidal *Palaemon longirostris* and *Palaemon serratus* (Taylor & Spicer 1989, Taylor 1990). The non-significant difference in metabolic rates is possibly due to a better metabolic regulation of the smaller *P. varians* at high temperatures, as also noted for *P. vulgaris* by McFarland & Pickens (1965).

During severe experimental hypoxia (1.6 and 0.66 kPa for *Palaemon adspersus* and *Palaemonetes varians*, respectively) at 24°C, *P. varians* survived considerably longer, although oxygen tension was lower. Both species change their metabolism to anaerobic glycolysis with lactate as the end product under severe hypoxia/anoxia, and haemolymph lactate accumulation rates can be used as a measure of anaerobic metabolism (Gäde 1983). Lactate accumulation rates for the 2 species were the same, probably due to the difference in experimental P_wO_2, i.e. their metabolism may have been only partly anaerobic. Taylor & Spicer

(1987) found a lower lactate accumulation rate at a P_wO_2 of 1.3 kPa than of 0.66 kPa in *Palaemon elegans*. The concentrations of haemolymph lactate after the hypoxic period were high in both species compared to the lactate concentrations in *P. elegans* and *P. serratus* at 0.66 and 1.3 kPa, respectively (Taylor & Spicer 1987). The rates found for both species in the present investigation are similar to the lactate accumulation rate found for *Crangon crangon* at 1.1 kPa (Hagerman & Szaniawska 1986, Hagerman & Vismann 1995).

Both *Palaemonetes varians* and *Palaemon adspersus* remove lactate at approximately the same rate as they accumulate it. This has been observed in a number of decapods, such as the related *Palaemon elegans* (Bridges & Brand 1980, Taylor & Spicer 1987). Some crustacean species, e.g. *Galathea strigosa* and *Homarus gammarus*, have, however, a considerably lower rate of lactate removal than accumulation (Bridges & Brand 1980). The lactate reoxidises to pyruvate and can enter the citric acid cycle, some might also be used in gluconeogenesis (Bridges & Brand 1980, Stryer 1988). The fraction used for gluconeogenesis varies between species and this might account for interspecific variations in lactate removal rates. It has been suggested that species most likely to encounter environmental hypoxia are better adapted physiologically to remove accumulated lactate on the return to normoxic conditions (Bridges & Brand 1980, Grieshaber et al. 1994, Astall et al. 1997, Hagerman 1998).

The lower P_c for *Palaemonetes varians* indicates an enhancement in the oxygen-transporting properties of the hc compared to that of *Palaemon adspersus*. The former species had a significantly higher hc oxygen-carrying capacity and thereby also a higher total haemolymph oxygen carrying capacity. In earlier works, *P. adspersus* was found to have a higher oxygen carrying capacity (Hagerman & Weber 1981, Weber & Hagerman 1981) than in the present study. Haemocyanin concentration can show large fluctuations between populations in relation to factors such as season, salinity and the nutritional state of the animal (Taylor 1990, Truchot 1992), and this might be the reason for the noted discrepancies in hc concentrations. Other palaemonid prawns have hc values intermediate to those found here (Taylor & Spicer 1989).

The *in vivo* haemolymph pH values were very low for both species, but such low values have been reported for a few reptant decapods at high salinities and high temperatures (Weiland & Mangum 1975, Truchot 1978, 1992). Even if handling stress was minimised it cannot be totally excluded and this might contribute to a low haemolymph pH.

The hc of *Palaemonetes varians* had a higher oxygen affinity than did the hc of *Palaemon adspersus*; P_{50} was lower for *P. varians* at all *in vitro* pH values measured. The P_{50} values and the Bohr shifts found are within the ranges typical for palaemonid prawns at this temperature, and the lower P_{50} for *P. varians* compared to *P. adspersus* is what might be expected for a species more tolerant of both the frequency and duration of severe hypoxia (Taylor 1990, Truchot 1992). A large normal Bohr shift will enhance oxygen affinity during hypoxia, due to initial hyperventilation resulting in an increase in haemolymph pH (Truchot 1975). During prolonged severe hypoxia with partly aerobic metabolism, however, lactate production and the associated acidification of the haemolymph will decrease hc oxygen affinity. Even if lactate *per se* will counteract this decrease slightly (Truchot 1980) a deoxygenation of hc would occur and thereby transfer the last hc-bound oxygen reserve to the tissues.

Temperature, salinity and low oxygen are important environmental factors that contribute to the spatial demarcation of the habitats of marine species. All factors are believed to restrict the distribution of *Palaemon adspersus* in shallow and sheltered waters during the warm summer months. The metabolic and respiratory adaptations found in the present study clearly show that *Palaemonetes varians* is physiologically better adapted than *P. adspersus* to survive periodic hypoxia during summertime. The high temperatures and low oxygen tensions recorded in shallow water in the Danish fjord are thus not a problem for *P. varians*. The higher carrying capacity and stronger oxygen affinity will enhance the uptake of oxygen during hypoxia, making it possible for this species to survive, while *P. adspersus* has to migrate to deeper, colder water. *P. varians* also shows more pronounced behavioural adaptations to hypoxia. Both in the laboratory and in the field *P. varians* could often be seen to adopt an inverted position at the water surface during periods of low water oxygen tension. In this way, the prawns are able to ventilate their gills with water that can be expected to have a higher oxygen tension than water deeper in the water column. Hagerman & Uglow (1984) also noted that during hypoxia *P. varians* jumped out of the water and for a short time adhered to plants etc. Taylor & Spicer (1988) noted also that during severe hypoxia, *Palaemon elegans* exhibited a partial emersion response, remaining quiescent at the air water interface.

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