

RESPIRATORY RATE, HAEMOLYMPH OXYGEN TENSION AND HAEMOCYANIN LEVEL IN THE SHRIMP *PALAEMON ADSPERSUS*

Rathke

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Abstract: The marine shrimp *Palaemon adspersus* Rathke, frequenting *Zostera* meadows, shows an oxygen consumption rate ($\dot{M}O_2$) that is independent of water oxygen tension (P_wO_2) as PO_2 decreases to a critical point (P_{cr}) near 70 mm Hg. This respiratory independence is associated with maintenance of a relatively constant "arterial" (post-branchial) haemolymph tension (P_aO_2) at 70-80 mm Hg. At lower P_wO_2 values, both $\dot{M}O_2$ and P_aO_2 fall, reflecting, in contrast to the above independence, a direct dependence of $\dot{M}O_2$ on internal PO_2 .

Haemolymph copper measurements demonstrate relatively high haemocyanin concentrations and oxygen-carrying capacities, but $\dot{M}O_2$ levels reflect an insignificant rôle for the pigment as an oxygen store.

The data are discussed as regards adaptations for aerobic metabolism.

INTRODUCTION

The published studies on crustacean respiration and on the respiratory qualities of the haemolymph almost invariably focus on large species, which generally live in relatively stable, polyhaline environments. Exceptions, however, include reports on the shrimp *Crangon vulgaris*, a burrowing callinassid and some oniscoid amphipods (see Djangmah & Grove, 1971; Miller *et al.*, 1976; Sevilla & Lagarrigue, 1979).

This study concerns the shrimp *Palaemon adspersus*, which during winter lives in fairly deep (2-20 m) and cold, oxygen-rich water, migrating in spring to warmer and shallower, vegetation-rich areas for reproduction (Fischer, 1978). The latter biotope may show strong diel variations in oxygen availability, varying from supersaturation during the day to pronounced hypoxia at night (Jørgensen, 1980). Also longer periods of water stagnancy may occur as a result of the formation of halo- and thermoclines. There is, however, evidence (Muus, 1967) that *P. adspersus* may leave the hypoxic *Zostera* meadows for open waters at night returning again in daytime.

The present study reports oxygen uptake rates and haemolymph oxygen tensions under conditions of declining oxygen tensions, and the haemolymph concentrations of the oxygen-transporting haemocyanin in *P. adspersus* Rathke, aiming to access

its capacity for aerobic metabolism in oxygen-unstable environments. An earlier study (Hagerman & Uglow, 1979) reported the heart and scaphognathite rates as indicative of ventilatory and circulatory responses. Another (Weber & Hagerman, in prep.) focuses on the respiratory gas-binding properties of the haemocyanin.

MATERIAL AND METHODS

P. adspersus was collected in shallow water among *Zostera* and *Fucus* in Aunø Fjord, South Sealand, where the mean yearly salinity is 10‰ (ranging from 6 to 22‰). Prior to experiments, the animals were kept at the Marine Biological Laboratory, Helsingør, for at least 3 wk in large aquaria with running sea water at 10°C and 15 to 20‰ S, and were fed with *Mytilus* flesh or mysids. The shrimps used weighed 1.8 to 2.2 g and were in the C-D₁ intermoult stages (classified according to Drach, in Passano, 1960).

Oxygen consumption rates ($\dot{M}O_2$) of the shrimps were measured individually with closed respirometry at 10°C using a Radiometer oxygen electrode and PHM 71 millivoltmeter and a Servogor recorder. The respiration chamber contained 125 ml water. A slowly rotating magnetic flea at the bottom ensured continuous water mixing, and was separated by a sieve from the upper animal compartment. The animal was allowed to decrease ambient (inhalant) oxygen tension (P_wO_2) to \approx 5 mm Hg. Each experiment lasted 2–3 h.

Post-branchial oxygen tensions were measured in haemolymph samples of 75 μ l or more, drawn with a Hamilton syringe from the pericardial cavity avoiding contact with air. Samples were taken from animals subjected to declining oxygen tensions (as above), and from animals maintained in water of constant oxygen tensions for at least 2 h. The haemolymph samples were injected into a thermostatted Radiometer blood gas micro-cuvette fitted with an oxygen electrode requiring 50 μ l for reliable readings.

Haemolymph absorbances, as reflective of haemocyanin concentrations were measured at 335 nm using a Unicam SP-1800 UV-spectrophotometer. Copper contents were measured with a Perkin-Elmer Atomic absorption spectrophotometer at 324.7 nm after 100-fold dilution of the haemolymph in 0.1 N HNO₃.

RESULTS

OXYGEN CONSUMPTION IN DECLINING OXYGEN TENSIONS

The relation of O_2 consumption rates ($\dot{M}O_2$) to external O_2 tension (P_wO_2) for 16 intermoult specimens of *P. adspersus* are shown in Fig. 1. Although the pooled data indicate that the rates of aerobic metabolism increase with O_2 tension over the entire range studied (regression equation, $\dot{M}O_2$ (ml · g⁻¹ · h⁻¹) = 0.00127 · PO_2 (mm)

+ 0.0927; correlation coefficient, 0.737), analysis of the rates for individual shrimps show clear evidence for independence of O_2 uptake between 70 and 130 mm Hg O_2 tension (see Fig. 1). In most cases a critical point (P_{cr}) occurs near 70 mm Hg below

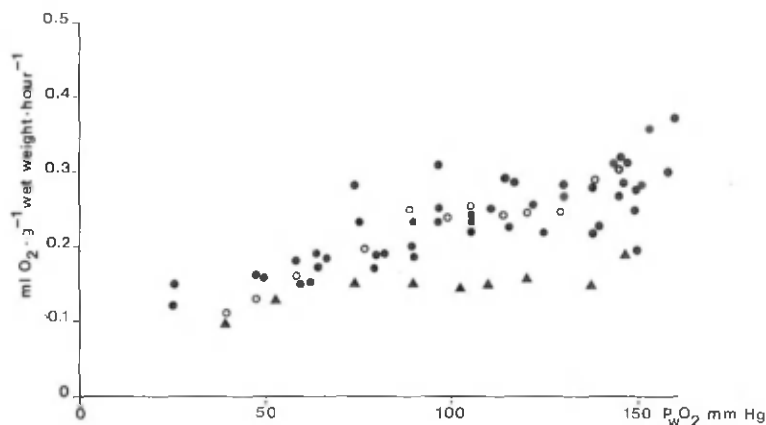


Fig. 1. Oxygen consumption rates at 10 °C of 16 specimens (●) of *Palaemon adspersus* in relation to the oxygen tension in the medium (salinity, 15‰): ○ and ▲ rates of individual animals (the latter representing a large 2.5-g individual that showed no visible activity during the experiment).

which metabolic conformity sets in. It is of interest to note that aerobic metabolism also increased with O_2 tension above ≈ 140 mm Hg.

POST-BRANCHIAL HAEMOLYMPH OXYGEN TENSION

Oxygen tensions of post-branchial haemolymph samples (P_aO_2) taken from the heart under conditions of declining O_2 tensions are shown in Fig. 2. In aerated water (P_wO_2 , 150–160 mm Hg) the haemolymph tension approximates 80 mm Hg. This shows a pronounced gradient ($\Delta PO_2 = 70$ –80 mm) indicating the existence of significant barriers to O_2 -uptake across the exchange membranes, or mixing of "arterialized" haemolymph in the heart region with O_2 -poor fluid from the tissues. P_aO_2 stays relatively constant as P_wO_2 declines to about 70 mm, then both tensions fall in parallel. We note the paradoxical situation (Fig. 2) that some haemolymph values in this region exceed those in the surrounding water. If this were the result of air contamination during sampling and PO_2 measurement (despite stringent precautions taken), it would be justified to assume that the lowest measured values are closest to the real in vivo values. Similar anomalies were, however, evident from previous investigations. Under conditions of declining P_wO_2 , the coelomic fluid PO_2 ($P_{cl}O_2$) in the sea urchin, *Strongylocentrotus droebachiensis*, first increases to a level significantly above P_wO_2 then falls in parallel while remaining higher than P_wO_2 (Johansen & Vadas, 1967). Newell & Courtney (1965) describe a similar phenomenon in *Holothuria forskali* attributing it to a delayed transfer of oxygen

from the respiratory trees to the coelomic fluid. We cannot offer a similar explanation for *Palaemon adspersus*. Irrespective of the exact explanation for the high P_aO_2

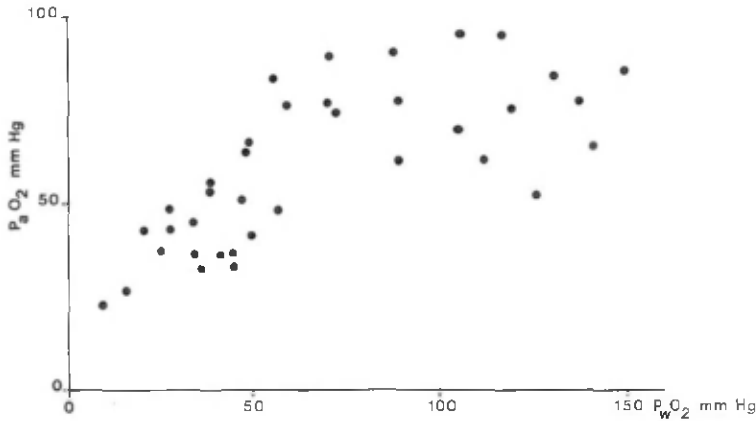


Fig. 2. Oxygen tension in post-branchial haemolymph of *Palaemon adspersus* in relation to declining oxygen tension in the medium (10°C , $15\%_{\text{O}_2}$).

values, the data clearly show that the PO_2 gradient at the gills is drastically reduced as P_wO_2 falls to 70 mm and below (Fig. 2).

HAEMOLYMPH HAEMOCYANIN LEVELS

The haemolymph shows distinct absorption maxima at 280, 335, and 580 nm, as characterize haemocyanin. Upon de-oxygenation by nitrogen equilibration the latter two bands disappear.

The haemolymph of individual shrimps shows large variations both in absorbances at 335 nm and in copper content (Fig. 3). This presumably relates to nutritional state and moulting stage (Djangmah, 1970; Djangmah & Grove, 1970). The calculated regression relating the spectroscopic data and the copper values does not pass through the origin indicating the presence of some non-haemocyanin copper.

The mean haemolymph copper content is 170 mg/l (range, 97 to 236 mg/l). Considering that crustacean haemocyanin contains 75 000 daltons of protein and 2 copper atoms per O_2 -binding site (Van Holde & Van Bruggen, 1971) these values indicate a mean haemocyanin concentration of 10 g/100 ml (10.0 g%), and a mean O_2 -carrying capacity as 3.0 ml/100 ml (3.0 vol%) (range, 1.7 to 4.2 ml/100 ml). The real O_2 capacities may, however, be lower due to the presence of non-haemocyanin copper. The stoichiometry means that the average haemolymph (Cu, 170 mg/l; A_{335} , 17.8 — see Fig. 3) contains 1.34 mmol subunit, and thus that, when neglecting effects of residual absorbance and light scattering, a millimolar extinction coefficient of 13.3 is applicable for estimations from copper of the maximum O_2 -carrying capacity values of the haemocyanin in the native haemolymph. This

value is lower than those (≈ 16 – 20) calculable from values reported for the other arthropods (cf. Antonini & Brunori, 1974); the difference is in accordance with the occurrence of non-haemocyanin copper. Applying a millimolar extinction coefficient of 17.3 as in *Carcinus maenas* haemocyanin, then the mean A_{335} value of 17.3 (Fig. 3) gives 1.03 mmol subunits compared with 1.34 mmol as calculated from copper, indicating 30% non-haemocyanin copper.

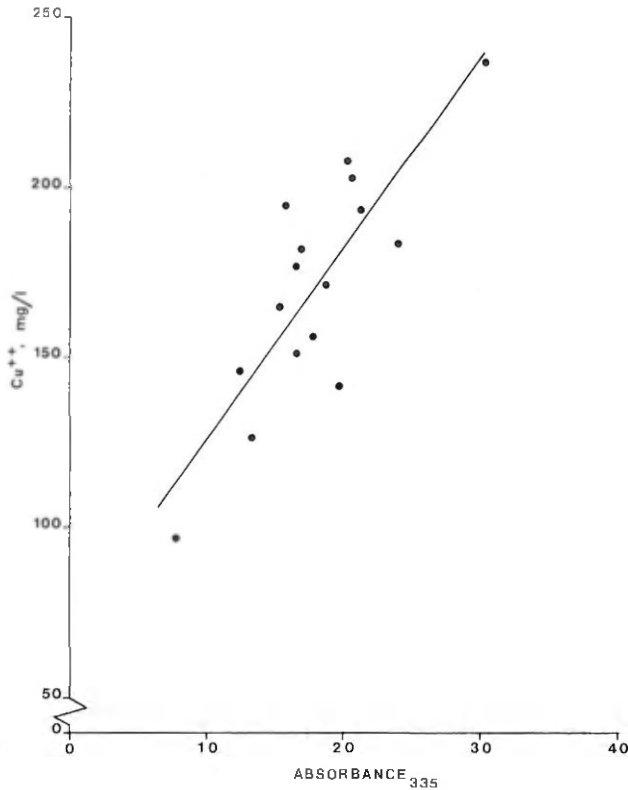


Fig. 3. Copper content and absorbance at 335 nm of oxygenated haemolymph of *Palaemon adspersus*; calculated linear regression: $[Cu] \text{ (mg/l)} = 5.54 \cdot A_{335} + 71.43$ (corr. coeff. = 0.824).

That the relationship between haemocyanin concentration and the O_2 -carrying capacity in body fluids is not yet clear, is also evident from Ainslie's (1980) data showing non-linearity between these values in the abalone *Haliotis*.

DISCUSSION

Our data show that the oxygen consumption rate of individual specimens of *Palaemon adspersus* is independent of oxygen saturation of the water over the range

of inhalant oxygen tensions (70–140 mm Hg) which spans those most frequently encountered in nature. Below a critical point near 70 mm Hg aerobic metabolism becomes directly dependent on ambient oxygen tension. This pattern of metabolic dependence is similar to those previously reported in *Carcinus maenas*, *Cancer magister*, and *Homarus vulgaris* and the burrowing shrimp *Callinassa californiensis* (Taylor, 1976; Johansen *et al.*, 1970; McMahon & Wilkens, 1975; Miller *et al.*, 1976). Curiously we also find evidence for respiratory dependence above 140 mm. It is, however, probable that animals at rest or those executing spontaneous activity normally show an independent type of respiration in oxygen-rich water and that the dependency observed here at high P_wO_2 is attributable to stress. This is suggested by the greater degree of respiratory independence observed in large specimens that remained inactive throughout the measurement (see Fig. 1). It is also suggested by increases in heart and scaphognathite frequencies observed after handling (even in the absence of visible increases in locomotor activity, Hagerman & Uglow, 1979), and by the fact that the metabolic rates at high oxygen tensions were measured at the start of the respirometry. *Carcinus maenas* similarly shows metabolic conformity after 1 h in the respirometer but independence after 5 h (Taylor, 1976). Again, *Callinassa* displays conformity in postmoult stages (and in intermoult stages with haemolymph removed) but independence in unstressed intermoult animals (Miller *et al.*, 1976).

The data show a drastic decrease in the water/post-branchial PO_2 difference as P_wO_2 falls. It should, however, be borne in mind that oxygen transfer primarily depends on the water/pre-branchial PO_2 difference (which will be greater than the water/post-branchial difference measured). The decreased ΔPO_2 with falling P_wO_2 , demonstrates that alternative compensatory responses, such as increases in gill ventilation or in haemolymph circulation, must be invoked to explain the constancy in oxygen uptake as PO_2 falls to the critical point. Earlier data on *Palaemon adspersus* (Hagerman & Uglow, 1979) show increased scaphognathite rates, but a decrease in heart rate, as P_wO_2 declines from 120 to 40 mm. It is possible that the effect of the bradycardia is compensated by increases in stroke volume. The small size of the shrimps, however, precluded measurement of "venous" PO_2 for evaluation of cardiac output via the Fick equation. Although stroke volume decreases in *Carcinus maenas* as P_wO_2 falls to ≈ 80 mm (Taylor, 1976), McMahon *et al.* (1979) observed a marked increase in stroke volume, which increases cardiac output rate in *Cancer magister* during enforced activity. Again, the effect of the increased scaphognathite rates may be compounded by parallel augmentation in stroke volume. Increases in ventilation volume as P_wO_2 falls also occur in *Carcinus maenas* (Arudpragasam & Naylor, 1964; Taylor, 1976) and *Homarus vulgaris* (Butler *et al.*, 1978).

The results permit speculation on the meaning of P_{cr} . A widely held hypothesis is that it reflects the PO_2 where the haemocyanin is no longer saturated when it leaves the gills (see Redmond, 1955). Pointing out that this interpretation is marred

by demonstrations that crustacean blood often remains poorly saturated when it leaves the gills, Young (1973) suggests instead that P_{cr} corresponds to the point where the low values of P_aO_2 and post-branchial pigment saturation starts to limit tissue uptake during decreasing P_wO_2 . Data of Weber & Hagerman (in prep.) show almost 90% O_2 saturation for *Palaemon adspersus* haemolymph at 15°C, pH 7.7 and an arterial PO_2 of 70 mm. Coincident decreases in O_2 saturation and $\dot{M}O_2$ at lower P_wO_2 values are suggestive of a causal relationship. The fact that both $\dot{M}O_2$ and P_aO_2 show inflections at the same P_wO_2 (cf. Figs. 2 and 3) moreover argues for a direct controlling influence of P_aO_2 on tissue $\dot{M}O_2$. This allows the animal to control its metabolic rate via changes in the ventilatory effort (see Johansen & Vadas, 1967).

The evidence for low ΔP values at water PO_2 values below 70 (Fig. 2) and the finding that the shrimps can reduce water PO_2 down to 5 mm, suggest high efficiency in oxygen transfer under hypoxia, contrary to the generally held view that crustacean respiratory surfaces greatly impede the diffusion of oxygen. This will obviously extend the range of oxygen tensions under which aerobic metabolism can occur.

Our data reflect relatively high haemocyanin concentration and thus high oxygen-carrying capacities in *P. adspersus* haemolymph. The haemocyanin levels, however, exhibit large individual variations. Studies of other crustaceans suggest that external and endogenous factors contribute to this variability. In the shrimp *Crangon vulgaris* the pigment concentration varies with season and locality, moulting stage, and nutritional state; during starvation the haemocyanin is catabolized to fuel the metabolism, while the copper level increases in the hepatopancreas indicating that this organ plays a rôle in storing the metal (Djangmah, 1970; Djangmah & Grove, 1970). In *Carcinus maenas* haemocyanin content increases in response to hypo-osmotic stress (Boone & Schoffeniels, 1979).

The copper concentrations here reported in *Palaemon adspersus* haemolymph (97–236 mg/l) are similar but more variable than those (124–217 $\mu\text{g/ml}$) found in *P. squilla* in corresponding moult stages (C–D₁), but significantly higher than the value for *P. serratus* (69.7 ± 9.2) in stage C (cf. Djangmah & Grove, 1970).

The haemocyanin concentrations permit estimation of the significance of the haemolymph as an oxygen store during periods of oxygen lack. With blood volumes in shallow-water decapods averaging 35% of body weight (Prosser *et al.*, 1952) a 2-g shrimp will contain 0.7 g blood which, containing a 1.34 millimolar concentration of functional unit will be able to bind 0.210 ml O_2 . At a haemolymph PO_2 of 75 mm and 15°C this haemocyanin-bound O_2 component will be augmented by 0.02 ml of physically dissolved oxygen. At a $\dot{M}O_2$ of 0.25 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ (Fig. 1), a 2-g shrimp will thus consume the total haemolymph oxygen within 28 min, showing that it will not contribute significantly during diel oxygen shortages.

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