

HAEMOLYMPH OXYGENATION AND OXYGEN CONSUMPTION IN A HIGH SHORE CRAB, *LEPTOGRAPSUS VARIEGATUS*, BREATHING IN AIR AND WATER

M.E. FORSTER

Department of Zoology, University of Canterbury, Private Bag, Christchurch, New Zealand.

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ABSTRACT

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Post-branchial haemolymph was sampled in specimens of the high shore crab, *Leptograpsus variegatus*, held under two sets of conditions. The partial pressure of oxygen (P_{aO_2}) in crabs immersed for 24 h at 20-22°C in running sea water measured 15.9 ± 1.2 mmHg (2.1 kPa). After 24 h in air at the same temperature the P_{aO_2} measured 24.9 ± 1.6 mmHg. P_{vO_2} (pre-branchial) haemolymph measured 6.6 ± 1.5 mmHg in sea water.

The higher P_{aO_2} in air is associated with the animal's observed preference for emersion. The low apparent diffusing capacity of the gas exchange system may minimise passive water losses in air. Resting oxygen consumptions ($\dot{V}O_2$) were also measured in the two media and rates were similar. A 100 g crab would have a $\dot{V}O_2$ of 5 ml $O_2 \cdot h^{-1}$.

KEYWORDS: crustacean - haemolymph - oxygen - $\dot{V}O_2$.

INTRODUCTION

Leptograpsus variegatus, the purple or large shore crab is one of the largest invertebrate animals of the New Zealand sea shore, being found well above high-water marks. An active predator it shelters in rock crevices by day, emerging at night to forage on the shore (Trenery 1982).

One indicator of its adaptation to a terrestrial existence is the acid-base regulating ability of *L. variegatus*, which is not dependent upon seawater for restoration of its haemolymph pH following exhausting exercise (Forster *et al.* 1989). This contrasts with the physiology of the smaller, sympatric species *Cyclograpsus lavauxi* (Waldron *et al.* 1986). When forced to exercise to exhaustion on a treadmill in air both species showed a pronounced acidosis, but only *L. variegatus* was able to restore pH to pre-exercise values when held in both air and seawater.

L. variegatus is generally found emersed when placed in a container in which it has access to either sea water or air (Forster *et al.* 1989). As a

gill breathing animal it is of interest to know whether gas exchange is as efficient in air as it is in water. Gas exchange might be affected by several factors, including differences in the ventilation of the gills, their perfusion with haemolymph and their diffusive capacity in the two media. The partial pressure of oxygen in the post-branchial haemolymph (P_{aO_2}) is a good indicator of the efficiency of gas exchange. The more closely this approaches the P_{O_2} of the branchial water or air, the more efficient an exchanger the gill is. Generally, in crabs that use their gills for air-breathing, haemolymph P_{aO_2} is lower on emersion than it is when immersed (Dejours & Truchot 1988). Gill lamellae may collapse or coalesce when unsupported by water, thus reducing the effective gill area available for gas exchange. Then, with time in air there can be a separation of the gill filaments allowing improved gas exchange (Innes *et al.* 1986).

In these experiments gas exchange in the two media was investigated by measuring both the P_{aO_2} of the haemolymph and oxygen consumption

($\dot{V}O_2$) of crabs held for 24 h either in air or submerged in seawater. Comparisons of these two parameters should indicate whether oxygen uptake in resting crabs is limited in either medium.

MATERIALS AND METHODS

Adult *L. variegatus* of both sexes were collected at night from rocky shores in the vicinity of Leigh (36°6'S, 174°48'E) and were taken to the Leigh laboratory and placed into containers surrounded by running seawater of relatively constant temperature (range 20 - 22°C). The crabs were either totally immersed in running seawater without access to air, or were placed in 15 l containers in which there was no access to seawater. In both cases the animals were held in these conditions for 22 to 24 h before blood sampling.

Post-branchial haemolymph was sampled by piercing the carapace above the heart with a 21 gauge needle and withdrawing approximately 150 μ l of haemolymph into a 1 ml syringe. The sample was then immediately introduced into a Strathkelvin MC100 microcell, with IL 1302 oxygen electrode and Strathkelvin oxygen meter (model 781). Samples were taken without removing the animal from the medium in which it had been held. After sampling the puncture holes were sealed with vaseline. The crab was then placed into the alternative medium (sea water if previously emerged and *vice versa*) and was left for a further 22 to 24 h before a second set of haemolymph samples were taken. As a check that the cardiac puncture was indeed sampling post-branchial blood, a pre-branchial haemolymph sample was taken from seven immersed crabs, through a needle that punctured the arthroal membranes at the bases of the legs.

Crabs were weighed after cardiac puncture, but to minimise handling disturbance only superficial water was removed using paper towels. The weight changes in the two media are given as indicators of desiccatory stresses and to augment points in the discussion, but do not represent a definitive statement on water balance in *L. variegatus*.

Where $\dot{V}O_2$ was to be measured the animals were held from between 30 and 36 h in the appropriate medium. $\dot{V}O_2$ was measured using the IL1302 electrodes and Strathkelvin meters and

sealed, water-jacketed, perspex respirometers containing a crab in either seawater or air. For $\dot{V}O_2$ in air the PO_2 was measured directly in the chamber and the output from the oxygen meter was displayed on a Servoscribe recorder. For $\dot{V}O_2$ in sea water, 250 μ l samples of the medium were taken at intervals and injected into the microcell to determine the PO_2 drop over time. The biological oxygen demand of the seawater was determined in respirometers without crabs and this was subtracted from the values obtained with the animals *in situ*. There was no attempt to assess spontaneous activity in these measurements of $\dot{V}O_2$.

A total of 49 animals (28 males) were used, ranging in body weight (wt.) from 26.6 to 202.4 g. The mean wt. was 86.1 ± 5.6 g (\pm SE). Most of the female crabs carried eggs. After use, the animals were returned to the shore.

RESULTS

The PO_2 of post-branchial haemolymph was higher in air than in sea water (Table 1). Sampling some individuals twice, once in each medium, confirms this difference. In immersed crabs the PO_2 of pre-branchial haemolymph was lower than that of post-branchial haemolymph (Table 1), indicating that the cardiac puncture technique did indeed collect arterialised blood. Care was taken

Table 1. Oxygen partial pressures in haemolymph samples taken from *Leptograpsus variegatus* maintained for 24 h in either seawater or air. "n" values in brackets. PO_2 values in mmHg.

		PaO_2	PvO_2
Emerged	(23)	24.9 ± 1.6	
Immersed	(16)	$15.9 \pm 1.2^*$	$6.6 \pm 1.5^{\S}$ (7)
Emerged ¹	(12)	24.5 ± 2.1	
Immersed ¹	(12)	$15.4 \pm 1.3^*$	

¹ A subset of the larger group, where haemolymph was sampled in the same individual after 24 h in both media.

* Significantly different from emerged crabs, $P < 0.001$

^{\S} Significantly different from arterial values in sea water, $P < 0.001$.

to collect haemolymph without applying strong negative pressures and indeed once the pericardium was punctured the haemolymph generally flowed freely, particularly in water-breathing crabs. It is not likely that the samples were deoxygenated as they were collected.

Crabs transferred to air lost weight and those immersed in sea water after aerial exposure gained weight. The mean change in wt. (positive or negative) was $4.8 \pm 0.4\%$ ($n = 24$). Though much of this weight change might be attributed to water loss from the branchial chambers it could also include urinary losses.

Oxygen consumption in air and seawater is graphed in Fig. 1. The size range of animals used in measurements of $\dot{V}O_2$ in the emersed group was too restricted to fit a regression line. The regression line for the immersed group indicates that a

100 g crab would have had an oxygen consumption of c. 5 ml h^{-1} . The mass exponent was 0.63 for the water breathers, whose wt.s ranged from 28.3 to 170.8 g.

DISCUSSION

The higher P_{aO_2} values in emersed *L. variegatus* might be expected from its observed preference for this medium, rather than sea water. The difference in P_{aO_2} values between *L. variegatus* breathing in air and sea water could reflect a difference in the relative importance of alternative sites of oxygen uptake. Methods of aerial ventilation in *L. variegatus* have not been described, but the appearance of the branchiostegal membranes suggests that these might function as a lung (H.H. Taylor, Department of Zoology, University of

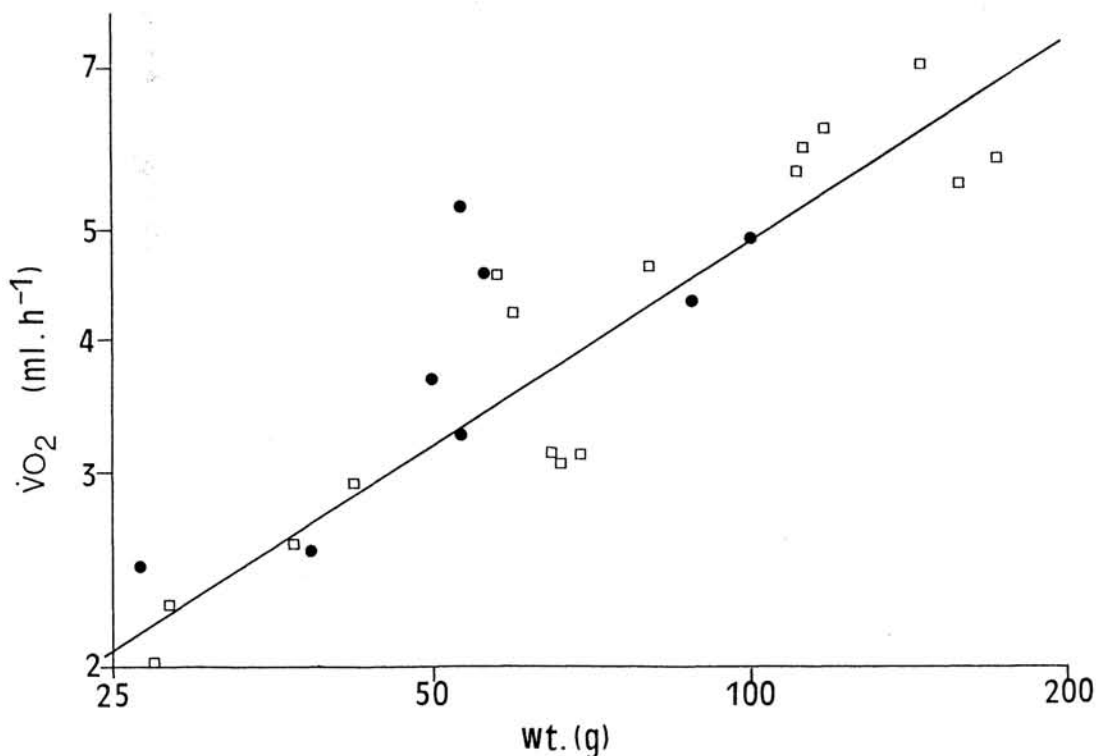


Figure 1. Oxygen consumption in either sea water (□) or air (●) in different sized individuals of the high shore crab *Leptograpsus variegatus*. The regression line for the group immersed in sea water is given by the formula:

$$\log \dot{V}O_2 = -0.56 + 0.625 \log \text{wt.} \quad (r^2 = 0.85)$$

where $\dot{V}O_2$ is expressed as $\text{ml O}_2 \text{ (STPD)} \cdot \text{h}^{-1}$ and wt. is the wet weight of the animal in grams.

Canterbury, pers. comm.). Another amphibious grapsid crab, *Hemigrapsus nudus*, which occurs lower on the shore than *L. variegatus*, has a substantial venous circulation in its branchiostegal lining (Greenaway & Farrelly 1990).

What is noteworthy is that P_{aO_2} values in both media are low. P_{aO_2} tends to fall in intertidal decapods when emersed (DeFur 1988). In the intertidal shore crab *Carcinus maenas* P_{aO_2} in emersed animals was only 40% of the value when immersed in seawater, but even the P_{aO_2} in emersed crabs measured 41 mmHg (Dejours & Truchot 1988). Higher P_{aO_2} values are reported for species that are primarily either aquatic or air breathing (McMahon 1981, Taylor & Innes 1988). The P_{aO_2} values in *L. variegatus* are slightly higher than those reported for *Coenobita clypeatus*, an anomuran crab also using phyllobranchiate gills for aerial gas exchange (McMahon & Burggren 1979, Innes & Taylor 1986). It is possible that the mechanisms that result in the low P_{aO_2} in *L. variegatus* also minimise passive water loss in air in the species, which is found well above the high-tide mark. Crustaceans generally are susceptible to desiccatory water losses (Innes *et al.* 1986). The c. 5% weight change after 24 h of emersion suggests that *L. variegatus* might also be susceptible to dehydration, although in its natural habitat it wedges itself into rock crevices, a behaviour which may limit water losses. As mean water content has not been determined for *L. variegatus*, it is not possible to convert the measured weight losses and gains to a change in total body water (see Innes *et al.* 1986).

For immersed *L. variegatus* it is possible to calculate the apparent diffusion limitation index for gas exchange (Piiper 1982). This is given by the formula:

$$L_{diff} = (P_{iO_2} - P_{aO_2}) / (P_{iO_2} - P_{vO_2}).$$

For immersed gill-breathing crustaceans, L_{diff} values range from 0.5 to 0.8 (Taylor & Innes 1988). The value for immersed *L. variegatus*, based on mean arterial and venous P_{O_2} s is 0.937, similar to the values reported for gill-breathing land crabs in air and indicating a marked diffusion limitation. We cannot calculate L_{diff} for emersed *L. variegatus*, as P_{vO_2} was not measured in air. However, as P_{aO_2} values are also low, L_{diff} must be high in *L. variegatus* in air. We should note that the L_{diff} values referred to above may overestimate the true diffu-

sion limitation if the branchial chambers are not well ventilated.

The low diffusing capacity in the gas exchange system of *L. variegatus* may not compromise oxygen uptake at rest. If the haemolymph contains significant amounts of the respiratory pigment, haemocyanin, and its affinity for oxygen is high, then it may well be that oxygen transport by the haemolymph is not disadvantaged by the low P_{aO_2} values. However the P_{O_2} gradient from haemolymph to myocyte mitochondria, for example, must be low, which implies a constraint upon aerobic metabolism. Measurements of P_{aO_2} in active individuals might indicate whether the apparent limitation to the diffusion of oxygen at the gas exchange surfaces can be lessened. Activity can change P_{aO_2} in other crustaceans, but some species show a fall, rather than a rise (Wood & Randall 1981).

$\dot{V}O_2$ at rest in *Leptograpsus* at 20-22°C was similar to that recorded for other brachyurans at these temperatures (McMahon 1981). The high shore crab *Cyclograpsus lavauxi* also consumes oxygen at rest at a similar rate when emersed and immersed (Innes *et al.* 1986). Resting $\dot{V}O_2$ decreased in two intertidal species on emersion (Burnet & McMahon 1987). $\dot{V}O_{2max}$ values would be informative and would indicate the animal's aerobic scope. Certainly *L. variegatus* is an active predator, foraging widely over the shore (Trenery 1982 and pers. obs.), which suggests that at such times it has a high rate of expenditure of metabolic energy.

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