J. exp. Biol. 103, 225–236 (1983)
Printed in Great Britain © Company of Biologists Limited 1983

AQUATIC GAS EXCHANGE IN THE FRESHWATER/LAND CRAB, *HOLTHUISANA TRANSVERSA*

BY PETER GREENAWAY

School of Zoology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia

J. BONAVENTURA

Marine Biomedical Center, Duke University Marine Laboratory, Beaufort, North Carolina 28516, U.S.A.

AND H. H. TAYLOR

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

(*Received 16 August 1982—Accepted 20 September 1982*)

SUMMARY

Holthuisana transversa (von Martens), a freshwater/land crab from arid areas of Australia, is an efficient bimodal breather. In water, resting $\dot{M}O_2$ ($1{\cdot}65\,\mu mol\,g^{-1}\,h^{-1}$) and $\dot{V}_w$ ($15{\cdot}6\,ml\,g^{-1}\,h^{-1}$) at 25 °C were lower than in other aquatic crabs whilst percentage extraction of oxygen was quite high (47 %). $\dot{M}O_2$ was not regulated at low ambient PO_2 but could be increased at least three times during exercise. Normoxic P_aO_2 was low (18 Torr) compared with other water-breathing crabs. The haemolymph contained haemocyanin which had a moderate affinity for oxygen ($P_{50} = 8{\cdot}0$ Torr) and carried over 90 % of the oxygen transported. Oxygen content of post-branchial haemolymph was low ($346\,\mu mol\,l^{-1}$). There was a small positive Bohr shift (log P_{50}/pH-0·33). The strategy of gas exchange in water is discussed and compared with that of aquatic crabs.

INTRODUCTION

Recent studies have revealed the basic patterns involved in gas exchange in aquatic crabs although the details vary considerably between species due to morphological differences between taxa and adaptation to habitat. Circulation of blood and water through the gills is counter-current in *Carcinus* (Hughes, Knights & Scammell, 1969) and, given the common functional design of crab gills, this is possibly true of most aquatic crabs. Oxygen extraction in resting crabs is quite high e.g. *Callinectes* and *Libinia* (approx. 50 %) (Batterton & Cameron, 1978; Burnett, 1979), *Cancer* (>60 %) (McMahon & Wilkens, 1977), although values are much lower during hyperventilation. Resting oxygen consumption is regulated over a wide range of external oxygen tension (Arudpragasam & Naylor, 1964; Johansen, Lenfant & Mecklenburg, 1970;

Key words: Gas exchange, *Holthuisana*, land crab.

Taylor, 1976; Burnett, 1979). In response to hypoxia, ventilation of the gills is increased and, in *Libinia*, there is increased cardiac output. However, *Callinectes* is an oxygen conformer at rest, although ventilation and oxygen consumption are both elevated during exercise (Batterton & Cameron, 1978). Haemocyanin is normally saturated with oxygen in postbranchial haemolymph (Mangum, 1980), but the total oxygen capacity is variable because of interspecific variation in the quantity of pigment and the PO_2 levels of haemolymph. The oxygen affinity of the pigment is relatively high (P_{50} at normoxic pH = 10–16 Torr) (Mangum, 1980), and much oxygen is transported in simple solution by crabs in aerated water (Burnett, 1979; McMahon, McDonald & Wood, 1979). The oxygen gradient across the gill is high in the species examined, typically 60–100 Torr (Johansen *et al.* 1970; Taylor, 1976; McMahon *et al.* 1979). The PCO_2 levels of postbranchial haemolymph are considered to be uniformly low (< 5 Torr) in aquatic crabs (Rahn, 1966; Cameron, 1979).

The physiological and morphological requirements for gas exchange in water and air are very different (Rahn, 1966; Rahn & Howell, 1976) and adaptations which facilitate gas exchange in air may reduce efficiency in water. Thus it is of great interest to examine gas exchange in a species, such as *Holthuisana transversa*, which breathes effectively in both media. *Holthuisana* is a freshwater/land crab from the arid-zone of Australia. For long periods it breathes air under severe terrestrial conditions; to this end it has developed tidally ventilated lungs (Greenaway & MacMillen, 1978; Greenaway & Taylor, 1976; Taylor & Greenaway, 1979). In the brief wet periods, the crab forages actively in water (Greenaway, 1981). In this study the features of gas exchange in water by *Holthuisana* are examined and compared with the pattern established for aquatic crabs, whilst the accompanying paper is concerned with gas exchange in air.

MATERIALS AND METHODS

Holthuisana (*Austrothelphusa*) *transversa* (von Martens) were collected from Bourke, Gulargambone and Lightning Ridge in western N.S.W. and maintained in the laboratory as described previously (Greenaway & MacMillen, 1978). Only large crabs (20–30 g) were used in experiments, which were carried out at 25 °C.

The rate of oxygen consumption ($\dot{M}O_2$), rate of ventilation ($\dot{V}_w$) and extraction of oxygen (% Ext) were measured in an overflow apparatus similar to those described by earlier workers (e.g. Larimer, 1961). This was a Perspex box of about 1·4 l capacity (14 × 10 × 10 cm). Masks were made from balloons attached to the carapace with Eastman 910 contact cement. The overflow method may underestimate $\dot{V}_w$ slightly due to increased resistance to water flow (Johansen *et al.* 1970). It also suffers from the disadvantage that the crabs are tethered and cannot move freely about the chamber. The use of electromagnetic flow probes, which reduces these problems, was investigated but proved to be unsuitable due to the small size of *Holthuisana* and the low conductivity of fresh water.

The overflow technique will also underestimate $\dot{V}_w$ if reversals of the direction of ventilatory flow occur during the measurement period. In several preliminary experiments the occurrence of reversals of scaphognathite beat were tested for with a Statham P23 AA pressure transducer connected to the mask by plastic cannula tubing. Scaphognathite activity was recorded on a Beckman Dynograph for crabs exposed

range of PO_2, over a period of several hours. No pressure changes due to reversals were observed and it was concluded that reversals were infrequent in *Holthuisana* and unlikely to cause significant error in measurement of $\dot{V}_w$.

Preliminary experiments showed that $\dot{V}_w$, $\dot{M}O_2$ and % Ext settled to stable, reproducible levels within a few hours of placing crabs in the overflow apparatus. An example is shown in Fig. 2. Subsequent measurements were made on crabs which had been in the apparatus for 24 h.

Blood-gas analysis

Samples of postbranchial haemolymph were taken anaerobically from the pericardial cavity in 1 ml plastic syringes in which the dead-space was filled with mineral oil. To avoid delay and disturbance at the time of sampling, holes were drilled in the carapace over the pericardium about 5 h before haemolymph was taken. Care was taken to avoid puncturing the hypodermis during drilling. Samples of prebranchial haemolymph were taken from the ventral thoracic sinus at the base of the chelae or pereiopods. All samples were taken whilst the crabs were submerged.

The PO_2 and PCO_2 of the haemolymph were measured separately on 0·2 ml samples using a Radiometer blood-gas analyser (pH27 plus PHM927B) equipped with separate water-jacketed electrodes maintained at 25 °C. For determinations of PCO_2, the electrode was calibrated with 2·9 % and 0·44 % CO_2, and output was measured on a mV recorder. Oxygen content was measured with a Lex O_2 Con TL oxygen analyser (Lexington Instrument Corporation). Both pH and [HCO_3] of haemolymph were measured on the same 0·3 ml sample. Immediately after sampling, the needle of the syringe was detached and haemolymph from the centre of the syringe was aspirated into a Radiometer G297/G2 capillary electrode and its pH determined. The remainder of each sample was used for the determination of the bicarbonate + carbonate concentration with a Radiometer autotitration system (PHM 64, TTT 80, TTA 80, ABU 80). A measured volume (0·2 ml) of haemolymph was added to a titration vessel containing 0·1 ml of 0·1 mmol l^{-1} HCl and 1 ml of saline. The samples were evacuated in a vacuum desiccator to remove the liberated CO_2 from solution and then titrated with 0·01 mmol l^{-1} NaOH until the original pH of the haemolymph was reached. Values obtained were corrected with blank samples containing only HCl and saline.

Oxygen binding

Oxygen-binding curves were determined at several different pH values at 25 °C. Haemolymph samples (1–2 ml) were taken from the ventral sinuses of five crabs and allowed to clot. The clots were removed by centrifugation (35 000 r.p.m.) and the supernatant containing the haemocyanin was retained. A few µl of each sample were used for electrophoresis (acrylamide gel) and, as no evidence of polymorphism between subunits of haemocyanin was apparent, we pooled the samples for the oxygen binding experiments, keeping the pooled haemolymph of male and female crabs separate. The pooled samples were diluted with saline containing the same major ions as the haemolymph (270 mmol l^{-1} NaCl, 15 mmol l^{-1} $CaCl_2$, 5 mmol l^{-1} $MgCl_2$) (Greenaway & MacMillen, 1978) and were buffered with 50 mmol l^{-1} Tris (pH 7·68 and above) or 50 mmol l^{-1} Bis-Tris buffer. A vacuum-spectrophotometric method, similar to that of Riggs & Wolbach (1956), was used to determine the binding curves.

Values of the percentage saturation of the haemocyanin with oxygen were calculated from absorption spectra collected over the wavelength range 400–300 nm using a scanning spectrophotometer (Cary 14).

Further samples of haemolymph from each crab were diluted with saline as described above and examined with a Beckman model E analytical ultracentrifuge at 60 000 r.p.m. to provide information on the molecular species of haemocyanin present.

The symbols used in the text follow Dejours (1981). Values are given as means $\pm 1 \times$ S.E.M..

RESULTS

Oxygen consumption

The $\dot{M}O_2$ in air-saturated water was $1{\cdot}65\ \mu mol\ g^{-1}\ h^{-1}$. In response to declining oxygen tension in the water, $\dot{M}O_2$ fell in a more or less linear fashion for each crab tested (Fig. 1). Regression analysis was performed on the pooled data for $\dot{M}O_2$ of all crabs and a significant linear relationship was found between $\dot{M}O_2$ and PO_2 of the water ($\dot{M}O_2 = 0{\cdot}25 + 0{\cdot}089 \times PO_2$, $\mu mol\ g^{-1}\ h^{-1}$, $P < 0{\cdot}001$). *Holthuisana* is an oxygen conformer, at least whilst at rest.

Rate of ventilation

The mean value of $\dot{V}_w$ for resting crabs in air-saturated water was $15{\cdot}6 \pm 2{\cdot}0\ ml\ g^{-1}\ h^{-1}$. Individual crabs showed a small increase in $\dot{V}_w$ when the PO_2 of the water fell below 100 Torr (Fig. 1) but no general response was apparent when the data were pooled for linear regression. The mean value for $\dot{V}_w$ at the lowest PO_2 tested ($22{\cdot}2\ ml\ g^{-1}\ h^{-1}$ at 26 Torr) was, however, significantly higher than that obtained in air – saturated water ($0{\cdot}02 > P > 0{\cdot}05$ using a paired 't' test), an increase of 42 %.

Oxygen extraction

Extraction of oxygen from the respired water varied considerably between individuals (21·9–73·4 %) but in all cases was relatively high with a mean in air-saturated water of $46{\cdot}4\ \% \pm 5{\cdot}1$ S.E.M.. The individual response to reduced PO_2 was rather variable but the % Ext was generally maintained until low PO_2 was reached. The level of individual variability prevented any statistical demonstration of responses to low PO_2, and, indeed some crabs showed elevation of % Ext whilst others showed decreased % Ext at the lowest PO_2 levels tested (Fig. 1).

Blood-gas tensions

The P_aO_2 values for crabs maintained in a resting state in aerated water (155 Torr) were very low with a mean of only 17·8 Torr (Table 1). Crabs which retained air bubbles in their branchial chambers had much higher values of P_aO_2, similar in fact to the values found for crabs breathing air (Greenaway, Taylor & Bonaventura, 1983). Care was taken to ensure that all air was expelled from the gill chambers well before samples of haemolymph were taken.

The pH of the haemolymph of *Holthuisana* (Table 1) was low compared with th

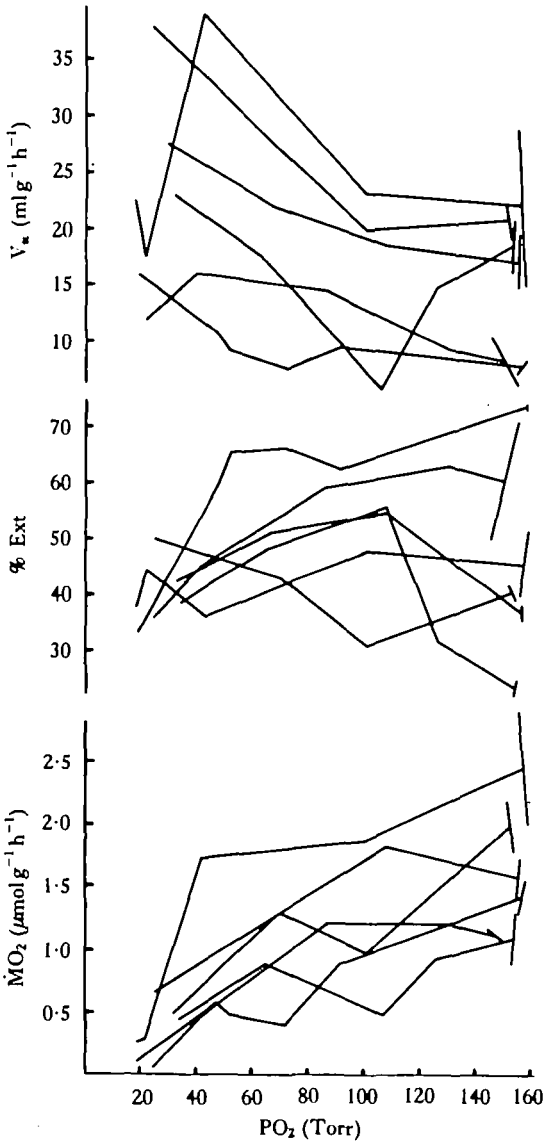

Fig. 1. The effect of oxygen tension on $\dot{V}_w$, $\dot{M}O_2$, and % Ext of individual crabs. Initial and final values in normoxic water are linked by bars.

of other crabs (Mangum & Schick, 1972; McMahon *et al.* 1978; Aldridge & Cameron, 1979; Taylor & Wheatly, 1979). At these pH levels the CO_3^{2-} concentration was negligible (Truchot, 1976) and CO_2 in the haemolymph was present as HCO_3^- and dissolved CO_2. Dissolved CO_2 in the haemolymph was calculated from PCO_2, using a value of 0·041 mmol l^{-1} Torr^{-1} for the solubility coefficient of CO_2 taken from the data for *Carcinus* at 12‰ and 25 °C (Truchot, 1976). At this salinity the osmotic concentration of the haemolymphs of *Carcinus* and *Holthuisana* was similar and their respective solubility coefficients for CO_2 would have been very close. Both $\bar{P}_aCO_2$ and

ured gas tensions (Torr), bicarbonate and CO_2 concentrations ($mmol\,l^{-1}$) and pH in the blood of water crabs

						Bicarbonate Conc.		Dissolved CO_2		
P_aO_2	P_vO_2	P_aCO_2	P_vCO_2	pH_a	pH_v	a	v	a	v	
17·8	13·0	6·0	6·8	7·33	7·36	9·54	9·93	0·247	0·279	9·
2·03	0·85	0·51	0·68	0·029	0·024	0·73	0·72	0·021	0·028	–
10–28	9–16	4–7·8	4·3–9·1	7·22–7·44	7·26–7·47	5·1–13·2	7·1–14·3	0·164–0·32	0·176–0·373	–
10	9	6	6	10	10	10	10	6	6	–

P_vCO_2 of haemolymph samples were higher than recorded in other aquatic crabs (McMahon *et al.* 1978; Taylor & Wheatly, 1979). However, P_aCO_2 values were almost certainly too high. Several minutes necessarily elapsed between removing a sample of haemolymph and obtaining its PCO_2. In this time, even in the absence of carbonic anhydrase, a new equilibrium would have been established between CO_2 and HCO_3^- in the sample with a resultant increase in PCO_2 from the expected low level of post-branchial haemolymph and a slight decrease in the concentration of HCO_3^-. This is discussed in more detail below. Values for P_vCO_2 are likely to be more accurate, erring on the low side if at all, as equilibrium between CO_2 and HCO_3^- would have been more complete. These considerations have no effect on the values for CCO_2 and it was apparent that the total loss of CO_2 across the gills was about 4 % of that carried in prebranchial haemolymph.

The effect of disturbance

Immediately after crabs had been placed in the overflow apparatus $\dot{M}O_2$ and $\dot{V}_w$ were very high (Fig. 2). These parameters declined steadily from their initial values and became stable well within a 24 h period. The initial $\dot{V}_w$ for crab 748 (Fig. 2) was $40{\cdot}6\ ml\ g^{-1}\ h^{-1}$, five times the level seen in resting metabolism (24 h). Clearly the capacity existed to increase ventilation substantially, although this ability was largely unused by resting crabs during hypoxia. Initial $\dot{M}O_2$ for crab 748 ($3{\cdot}08\ \mu mol\ g^{-1}\ h^{-1}$), was about 3·5 times the resting level.

In a separate experiment, crabs were kept moving for a 5 min period and their P_aO_2 was measured immediately afterwards. The mean value obtained ($22{\cdot}2 \pm 2{\cdot}6$ Torr) was not significantly different from that found in resting crabs ($0{\cdot}4 > P > 0{\cdot}2$).

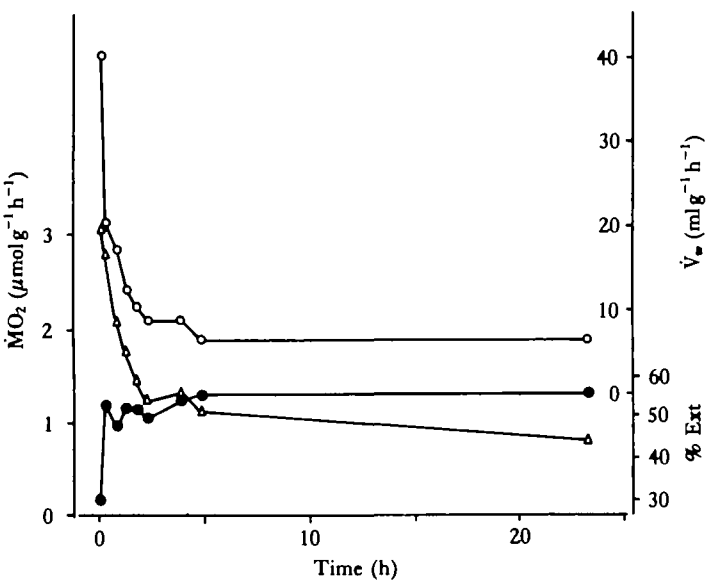

Fig. 2. Post-exercise records of $\dot{V}_w$ (open triangles), % Ext (closed circles) and $\dot{M}O_2$ (open circles) for a single crab.

Oxygen binding

The haemocyanin had a moderate affinity for oxygen at 25 °C with a P_{50} of 8·0 Torr at normoxic pH, in the absence of CO_2. A small positive Bohr effect was apparent with a log P_{50}/ pH value of −0·33 (Fig. 3).

The data for oxygen binding (Fig. 3) revealed that the pigment was nearly saturated with oxygen at approximately 20 Torr PO_2, at normoxic pH and in the absence of CO_2. The exact P_aCO_2 was not known but was likely to have been about 2·5 Torr and thus had little effect on oxygen binding. Thus, haemocyanin was about 90% saturated with oxygen at $\bar{P}_aO_2$ (17·8 Torr) and about 80% saturated at $\bar{P}_vO_2$ (13·0 Torr), which indicated a large venous oxygen reserve.

Cooperativity of the haemocyanin was calculated from Hill plots and lay between 2 and 3 over the range of pH studied (Fig. 3). These values are characteristic of decapod crustaceans (Mangum, 1980). Ultracentrifugation of the haemolymph gave Schlieren peaks at 23–24 *s* and 15·5–16·5 *s* which corresponded to dodecameric and hexameric aggregation states of haemocyanin molecules respectively. This was again characteristic of decapod haemocyanins and similar to values for other freshwater crabs (Bonaventura *et al.* 1979). In female crabs, a third peak was evident at 10–11 *s* but did not represent haemocyanin.

Oxygen content

C_aO_2 was measured in five crabs kept in aerated water and gave a mean value of

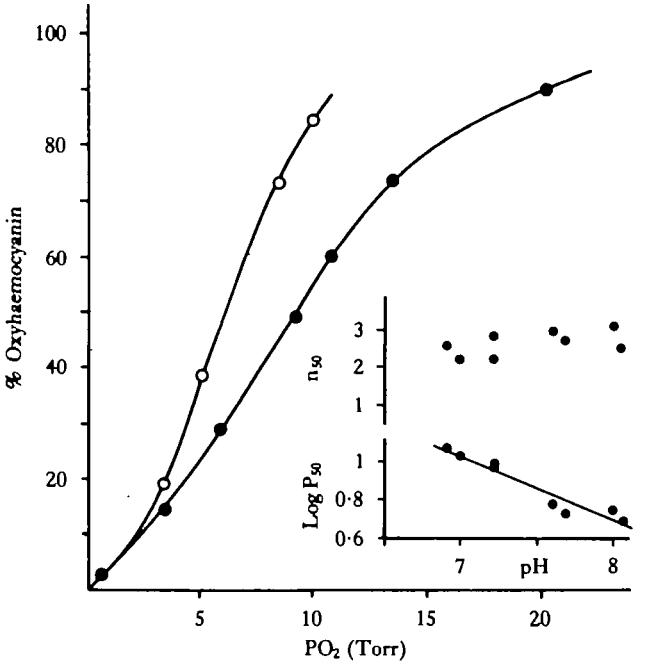

Fig. 3. The effect of pH on oxygen binding by the haemocyanin of *Holthuisana*. Closed circles (pH 7·22), open circles (pH 7·6). The effect of pH on the affinity (P_{50}) and cooperativity (n_{50}) of haemocyanin from *Holthuisana* are shown as an inset.

$\pm 3{\cdot}6\,\mu molO_2\,l^{-1}$ (0·774 vol %). Using the mean values for P_aO_2 and C_aO_2 and treating the haemolymph as half strength sea water, the oxygen carried in simple solution was calculated to be $26\,\mu mol\,l^{-1}$, 7·5 % of C_aO_2. The haemocyanin was approximately 90 % saturated at $\bar{P}_aO_2$ and carried the remaining 92·5 % (320 μmol l^{-1}) of the measured C_aO_2. Thus saturated haemocyanin had a capacity of 355 μmol l^{-1}.

DISCUSSION

Weight-specific log $\dot{M}O_2$ decreases linearly with increasing log body mass within crab species (Taylor & Wheatly, 1979; MacMillen & Greenaway, 1978; Kotaiah & Rajabai, 1975) and this might be expected to hold interspecifically for crabs generally. Thus a small species should have a relatively high $\dot{M}O_2$ but, in practice, *Holthuisana* has a lower $\dot{M}O_2$ than any of the larger species studied (Table 2). Indeed, $\dot{M}O_2$ was three times lower than that found in *Carcinus* of similar body weight (25 g) at the same temperature (25 °C) (Taylor & Wheatly, 1979). The $\dot{V}_w$ of *Holthuisana* was also the lowest of the species studied (Table 2), even allowing for an expected decrease in $\dot{V}_w$ with increasing body size. Comparison with slightly larger *Carcinus* at 18 °C yielded a value 2·2 times lower in *Holthuisana* (Table 2). The % Ext of oxygen by *Holthuisana* was quite high, similar to that of the aquatic crabs *Callinectes, Cancer* and *Libinia* and higher than in *Carcinus* (Taylor, Butler & Al-Wassia, 1977). The emergent pattern for *Holthuisana* was of an animal with a low $\dot{M}O_2$ and a very low $\dot{V}_w$ but with efficient extraction of oxygen from the water. Like *Callinectes, Holthuisana* was an oxygen conformer at rest but was capable of increasing $\dot{V}_w$ (5 times) and $\dot{M}O_2$ (at least 3 times) during exercise.

In the aquatic crabs studied, haemocyanin generally carried most of the oxygen transported by the haemolymph (Mangum, 1980). Although the P_aO_2 maintained in aerated water differed widely between species of crabs, the haemocyanin was nearly saturated with oxygen in each case and P_aO_2 largely reflected the P_{95} of the pigment (Mangum, 1980). In *Holthuisana*, $\bar{P}_aO_2$ (17·8 Torr) was lower than that found in any other aquatic crab and lower than that found in freshwater crayfishes e.g. *Astacus*

Table 2. *Mean values for* $\dot{M}O_2$ ($\mu mol\,g^{-1}\,h^{-1}$), $\dot{V}_w$ ($ml\,g^{-1}\,h^{-1}$), $\dot{V}_w/\dot{M}O_2$ (*ml water/* μ*mol* O_2) *and % Ext by water-breathing crabs in air-saturated water*

	T_a °C	Weight (g)	$\dot{V}_w$	$\dot{M}O_2$	% Ext	$\dot{V}_w/\dot{M}O_2$	Author
Holthuisana transversa	25	15–30	15·6	1·65	46·4	9·45	This study
Libinia emarginata	25	60–235	34·5	3·13	44·5	11·02	Burnett (1979)
Carcinus maenas	25	30	—	4·21	—	—	Taylor & Wheatly (1979)
	18	52·8	34·7	1·86	33·3	18·66	Taylor, Butler & Al-Wassia (1977)
Callinectes sapidus	25	165–297	27·5	2·60	55·0	10·6	Batterton & Cameron (1978)
Cancer magister	8	262–960	17·3	1·47	34·0	11·77	McDonald, Wood & McMahon (1980)
	9	650–1150	37·4	1·63	16·0	22·9	Johansen *et al.* (1970)

leptodactylus (28 Torr) and *Austropotamobius pallipes* (33 Torr) (Angersbach Decker, 1978; Wheatly & Taylor, 1981). However, given the affinity of the haemocyanin at normoxic pH, near-saturation was achieved at $\tilde{P}_aO_2$ and the pigment was responsible for more than 90 % of oxygen transport. Removal of oxygen by the tissues was quite small, and a relatively large venous reserve was present in the resting crab.

The very low P_aO_2 requires some further comment because a substantial gradient of PO_2 existed between post-branchial haemolymph (17·8 Torr) and water leaving the gills (71·5 Torr). The simplest explanation was that % Ext from the water actually passing over the gill lamellae was very high (and the gradient of PO_2 much lower) but much of the water bypassed the gills so that the measured extraction was much lower. The low $\dot{V}_w$ may be seen as minimizing energy expenditure on ventilation as it was adequate to permit a high level of saturation of the haemocyanin, which carried most of the oxygen used, and higher energy expenditure on $\dot{V}_w$ would not have correspondingly increased oxygen content. Additionally, the affinity of the haemocyanin would enable saturation of the respiratory pigment at the low ambient PO_2 which the crabs may frequently encounter in their water-filled burrows and in the warm, shallow temporary pools which they inhabit. In shallow water, crabs were frequently observed to augment water breathing by taking air into the branchial chambers and this probably represented a normal respiratory pattern in shallow water. This behaviour elevated P_aO_2 and would act to increase delivery of oxygen to the tissues. The PCO_2 of the haemolymph of aquatic decapods is believed to be controlled largely by physical factors. Thus the high solubility of CO_2 and the low oxygen content of water would ensure that the PCO_2 of exhaled water would not exceed about 5 Torr at 25 °C and 100 % extraction of oxygen (Rahn, 1966). In the absence of a significant barrier to diffusion of CO_2 across the gill, P_aCO_2 should be close to that of exhaled water and indeed this has been demonstrated in certain fish, cephalopods and crabs (Rahn, 1966). In *Holthuisana* the maximum P_aCO_2 should have been about 2·5 Torr, given the mean % Ext measured, and the measured value was clearly erroneous as discussed above. Using an estimate of 2·5 Torr for P_aCO_2, about 40 % of total CO_2 loss at the gills originated in the dissolved CO_2 pool and 60 % from the bicarbonate pool.

Dissolved CO_2 is lost passively across the gill epithelium, but the bicarbonate must be exchanged for a counterion or be converted to CO_2 (Cameron, 1979). The uncatalysed conversion of HCO_3^- to CO_2 is too slow to contribute significantly to loss of CO_2 during the residence time of haemolymph in the gills (Aldridge & Cameron, 1979) and, given the low fluxes of ions in *Holthuisana*, adequate excretion of HCO_3^- by ion exchange alone seems unlikely. It follows that most of the HCO_3^- lost must firstly be dehydrated by carbonic anhydrase, which is reported to be present in the gill epithelium of crabs (Burnett, Woodson, Rietow & Vilich, 1981; Maren, 1967; Aldridge, 1977). The pH of the haemolymph of *Holthuisana* was lower than values recorded for marine crabs at similar temperatures (Mangum & Schick, 1972; Aldridge & Cameron, 1979; Taylor & Wheatly, 1979) and lower than those found in freshwater crayfish which are more similar in haemolymph chemistry (Angersbach & Decker, 1978; Wheatly & Taylor, 1981).

Gas exchange by *Holthuisana* in water was characterized by very low $\dot{V}_w$ and P_aO_2 values and low $\dot{M}O_2$. Resting $\dot{M}O_2$, although lower than in other aquatic crabs, w

gher than in *Holthuisana* breathing air. However, behaviour patterns differ in air and water; *Holthuisana* is normally inactive and $\dot{M}O_2$ very low in air in order to conserve food reserves (MacMillen & Greenaway, 1978) whilst in water the crab forages actively. Maximum $\dot{M}O_2$ values were similar in both media. *Holthuisana* was evidently successful at gas exchange in both media although $\dot{M}O_2$ in absolute terms was low in both (Greenaway, *et al.* 1983).

We are grateful to G. Ferruzzi, S. Tesh, T. Brouwer, G. Godette and D. Hair for technical assistance. K. Schmidt-Nielsen and Barbara Grubb made the measurements of oxygen content possible. The work was supported by the Australian Research Grants Committee through grant no. D17615225R to P. Greenaway and by grants from the U.S. National Institute of Health (HL15460) and N.S.F. (PCM79–06462).

REFERENCES

ALDRIDGE, J. B. (1977). Structure and respiratory function in the gills of the blue crab *Callinectes sapidus* (Rathbun). M.A. thesis, University of Texas at Austin.

ALDRIDGE, J. B. & CAMERON, J. N. (1979). CO_2 exchange in the blue crab, *Callinectes sapidus* (Rathbun). *J. exp. Zool.* **207**, 321–328.

ANGERSBACH, D. & DECKER, H. (1978). Oxygen transport in crayfish blood; effect of thermal acclimation and short term fluctuations related to ventilation and cardiac performance. *J. comp. Physiol.* **123**, 105–112.

ARUDPRAGASAM, K. D. & NAYLOR, E. (1964). Gill ventilation volumes, oxygen consumption and respiratory rhythms in *Carcinus maenas* (L.) *J. exp. Biol.* **41**, 309–321.

BATTERTON, C. V. & CAMERON, J. N. (1978). Characteristics of resting ventilation and response to hypoxia, hypercapnia and emersion in the blue crab *Callinectes sapidus* (Rathbun). *J. exp. Zool.* **203**, 403–418.

BONAVENTURA, J., BRUNORI, M., WILSON, M. T., MARTIN, J. P., GARLICK, R. L. & DAVIS, B. J. (1979). Properties of hemocyanins isolated from Amazon River arthropods and molluscs. *Comp. Biochem. Physiol.* **62** A, 251–256.

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**, 289–299.

BURNETT, L. E., WOODSON, P. B. J., RIETOW, M. G. & VILICH, V. C. (1981). Crab gill intra-epithelial carbonic anhydrase plays a major role in haemolymph CO_2 and chloride ion regulation. *J. exp. Biol.* **92**, 243–254.

CAMERON, J. N. (1979). Excretion of CO_2 in water-breathing animals – A short review. *Mar. Biol. Lett.* **1**, 3–13.

DEJOURS, P. (1981). *Principles of comparative Respiratory Physiology*, Second Edition. Amsterdam: Elsevier/North Holland Biomedical Press.

GREENAWAY, P. (1981). The fate of glomerular filtration markers injected into the haemolymph of the amphibious crab *Holthuisana transversa*. *J. exp. Biol.* **91**, 339–347.

GREENAWAY, P. & MACMILLEN, R. E. (1978). Salt and water balance in the terrestrial phase of the inland crab *Holthuisana* (*Austrothelphusa*) *transversa* Martens (Parathelphusoidea: Sundathelphusidae). *Physiol. Zoöl.* **51**, 217–229.

GREENAWAY, P. & TAYLOR, H. H. (1976). Aerial gas exchange in Australian arid-zone crab, *Parathelphusa transversa* von Martens. *Nature, Lond.* **262**, 711–713.

GREENAWAY, P., TAYLOR, H. H. & BONAVENTURA, J. (1983). Aerial gas exchange in Australian freshwater/land crabs of the genus *Holthuisana*. *J. exp. Biol.* **103**, 237–251.

HUGHES, G. M., KNIGHTS, B. & SCAMMELL, C. A. (1969). The distribution of PO_2 and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab *Carcinus maenas* L. *J. exp. Biol.* **51**, 203–220.

JOHANSEN, K., LENFANT, C. & MECKLENBURG, T. A. (1970). Respiration in the crab, *Cancer magister*. *Z. vergl. Physiol.* **70**, 1–19.

KOTAIAH, K. & RAJABAI, B. S. (1975). Starvation stress on the metabolism of the tropical freshwater crab *Paratelphusa hydrodromus* (Herbst), with reference to size, sex and sudden changes of temperature. *Indian J. exp. Biol.* **13**, 180–184.

LARIMER, J. L. (1961). Measurement of ventilation volume in decapod crustaceans. *Physiol. Zoöl.* **34**, 158–166.

MCDONALD, D. G., WOOD, C. M. & MCMAHON, B. R. (1980). Ventilation and oxygen consumption in the ungeness crab, *Cancer magister*. *J. exp. Zool.* **213**, 123–136.

McMAHON, B. R., McDONALD, D. G. & WOOD, C. M. (1979). Ventilation, oxygen uptake and haemolymph oxygen transport, following enforced exhausting activity in the Dungeness crab *Cancer magister*. *J. exp. Biol.* **80**, 271–285.

McMAHON, B. R., SINCLAIR, F., HASSALL, C. D., DEFUR, P. L. & WILKES, P. R. H. (1978). Ventilation and control of acid-base status during temperature acclimation in the crab *Cancer magister*. *J. comp. Physiol.* **128** B, 109–116.

McMAHON, B. R. & WILKENS, J. L. (1977). Periodic respiratory and circulatory performance in the red rock crab *Cancer productus*. *J. exp. Zool.* **202**, 363–374.

MACMILLEN, R. E. & GREENAWAY, P. (1978). Adjustments of energy and water metabolism to drought in an Australian arid-zone crab. *Physiol. Zoöl.* **51**, 231–240.

MANGUM, C. P. (1980). Respiratory function of the haemocyanins. *Am. Zool.* **20**, 19–38.

MANGUM, C. P. & SCHICK, J. M. (1972). The pH of the body fluids of marine invertebrates. *Comp. Biochem. Physiol.* **42A**, 693–697.

MAREN, T. H. (1967). Carbonic anhydrase in the animal kingdom. *Fed. Proc. Fedn. Am. Socs exp. Biol.* **26**, 1097–1103.

RAHN, H. (1966). Aquatic gas exchange theory. *Resp. Physiol.* **1**, 1–12.

RAHN, H. & HOWELL, B. J. (1976). Bimodal gas exchange. In *Respiration of Amphibious Vertebrates*, (ed. G. M. Hughes). London: Academic Press.

RIGGS, A. & WOLBACH, R. A. (1956). Sulphydryl groups and the structure of haemoglobin. *J. gen. Physiol.* **39**, 585–605.

TAYLOR, A. C. (1976). The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. exp. Biol.* **65**, 309–322.

TAYLOR, E. W., BUTLER, P. J. & AL-WASSIA, A. (1977). The effect of a decrease in salinity on respiration, osmoregulation and activity in the shore crab, *Carcinus maenas* (L.) at different acclimation temperatures. *J. comp. Physiol.* **119**, 155–170.

TAYLOR, E. W. & WHEATLY, M. G. (1979). The behaviour and respiratory physiology of the shore crab, *Carcinus maenas* (L.) at moderately high temperatures. *J. comp. Physiol.* **130**, 309–316.

TAYLOR, H. H. & GREENAWAY, P. (1979). The structure of the gills and lungs of the arid-zone crab, *Holthuisana* (*Austrothelphusa*) *transversa* (Brachyura: Sundathelphusidae) including observations on arterial vessels within the gills. *J. Zool., Lond.* **189**, 359–384.

TRUCHOT, J. P. (1976). Carbon dioxide combining properties of the blood of the shore crab *Carcinus maenas* (L.): carbon dioxide solubility coefficient and carbonic acid dissociation constants. *J. exp. Biol.* **64**, 45–57.

WHEATLY, M. G. & TAYLOR, E. W. (1981). The effect of progressive hypoxia on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. *J. exp. Biol.* **92**, 125–41.