

AERIAL GAS EXCHANGE IN AUSTRALIAN FRESHWATER/LAND CRABS OF THE GENUS HOLTHUISANA

By PETER GREENAWAY

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

H. H. TAYLOR

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

AND J. BONAVENTURA

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

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SUMMARY

Holthuisana valentula Riek and *H. agassizi* (Rathbun) both ventilate their lungs by lateral oscillations of the thoracic walls within the branchial chambers. Air enters and leaves the lungs via the prebranchial apertures and the Milne-Edwards apertures. At rest, active ventilation in *H. transversa* was low ($0.95 \text{ ml g}^{-1} \text{ h}^{-1}$) and a high diffusional component was evident. After disturbance, \dot{V}_A rose to $45.4 \text{ ml g}^{-1} \text{ h}^{-1}$ and % extraction was low (1.1%). Ventilation increased during moderate hypercapnia and marked hypoxia. $\dot{M}\text{O}_2$ was high after disturbance ($2.95 \mu\text{mol g}^{-1} \text{ h}^{-1}$) but declined to very low levels at rest ($0.45 \mu\text{mol g}^{-1} \text{ h}^{-1}$). The haemocyanin had a relatively low affinity for oxygen during air-breathing ($P_{50} = 13 \text{ Torr}$) but it was saturated with oxygen at normal $\dot{P}\text{O}_2$ (56.1 Torr). CCO_2 in the haemolymph (13 mmol l^{-1}) was characteristic of air-breathing crabs. The respiratory performances of *H. transversa* in air and water are compared.

INTRODUCTION

Many groups of marine brachyurans have amphibious or terrestrial species, in particular the Ocypodidae (*Ocypode*, *Uca*), Grapsidae (*Sesarma*, *Leptograpsus*, *Goniopsis*, *Aratus*) and Mictyridae (*Mictyris*), whilst members of the Gecarcinidae are highly terrestrial (*Gecarcinus*, *Cardisoma*). Additionally, members of all three groups of freshwater crabs (Potamoidea, Parathelphusoidea and Pseudothelphusoidea [Bott, 1969, 1970]) show strong tendencies towards an amphibious or terrestrial mode of life, particularly in areas with distinct wet and dry seasons. Many amphibious crabs are not well adapted for air-breathing and their branchial chambers retain water which may be recirculated through the gills (Alexander & Ewer, 1969; Wood & Randall,

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1981a). Most crabs that are active out of water have lungs formed by expansion of the anterior portions of the branchial chambers, – e.g. *Ocypode*, *Gecarcinus* (Bliss, 1968), *Holthuisana* (Taylor & Greenaway, 1979) and various pseudoscorpionid crabs (Diaz & Rodriguez, 1977).

Very few studies have been made on gas exchange in terrestrial crabs but a general pattern is apparent. Ventilation during air-breathing in crabs of marine origin is effected in the same manner as water-breathing, i.e., air enters the branchial chambers via the Milne-Edwards apertures, moves through or around the gills, through the lungs and exits via the prebranchial apertures. Ventilation is powered by the scaphognathites (Cameron & Mecklenburg, 1973; Cameron, 1975; Bliss, 1968). The freshwater/land crab *Holthuisana transversa* breathes air by a tidal mechanism involving lateral oscillations of the thoracic walls within the branchial chambers and cessation of scaphognathite activity (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979). A similar mechanism was reported for *Uca una* (Jobert, 1876).

In terrestrial decapods ventilation rates are high with a low extraction of oxygen (Cameron & Mecklenburg, 1973; Cameron, 1975; O'Mahoney, 1977; McMahan & Burggren, 1979). In the few brachyurans which have been studied, and in the anomuran *Birgus*, carbon dioxide provided the stimulus for ventilation, although in another anomuran, *Coenobita*, oxygen controlled the ventilation rate (Cameron & Mecklenburg, 1973; Cameron, 1975; McMahan & Burggren, 1979).

The few studies on the oxygen-carrying characteristics of the haemolymph of terrestrial decapods have provided very variable data. The carrying capacity is generally low, but variable between species. The affinity varies from low to very high, and this is reflected by the oxygen tension of post-branchial haemolymph (Mangum, 1980; Taylor & Davies, 1981). These parameters appear to be linked closely to the particular habitat and mode of life of individual species and generalizations are difficult to make. In common with other terrestrial animals, the PCO_2 and CCO_2 of the haemolymph are elevated relative to aquatic species (McMahan & Burggren, 1979).

The Australian freshwater/land crab *Holthuisana transversa* is an extreme example of a bimodally breathing animal and can live permanently in air or in water, as can several other species of the genus. The accompanying paper examines gas exchange during water-breathing (Greenaway, Bonaventura & Taylor, 1983) and compares gas exchange with that of primarily aquatic species. In this study gas exchange in air is examined in *H. transversa*, and to a lesser extent in *H. valentula* and *H. agassizi*, and compared with gas exchange in water and in other air-breathing crabs.

MATERIALS AND METHODS

Holthuisana (Austrothelphusa) transversa (von Martens) were collected from Bourke, Gulargambone and Lightning Ridge in Western N.S.W., whilst *Holthuisana valentula* Riek were collected near Coen in the Cape York Peninsula, N. Queensland and *Holthuisana agassizi* (Rathbun) from the Cooktown area of N. Queensland. All three species were maintained in the laboratory at 25°C as described previously (Greenaway & MacMillen, 1978).

The rate of oxygen consumption was measured at 25°C in a closed respirometer system. Crabs which had been breathing air were exercised for 1 min and then sealed

to plastic, screw-top containers of 70–80 ml capacity. After various intervals, an air sample was withdrawn in a 10 ml plastic syringe and the air in the container was renewed by blowing fresh air through, prior to the commencement of a new period of measurement. Three-way Teflon taps on the respirometers and syringes allowed samples to be taken without contamination by room air. The PO_2 of air samples was measured as described below.

Systems used elsewhere to monitor the rates of ventilation and $\dot{M}O_2$ of air-breathing crabs (Cameron & Mecklenburg, 1973; Cameron, 1975) could not be used with *Holthuisana* which breathes tidally. The system used in these experiments monitored pressure changes in a closed respirometer due to the ventilation of one lung; the other lung was isolated and connected to the outside air. This was necessary as no pressure changes could otherwise be detected because inhalation in one lung coincided exactly with exhalation in the other; ventilation was achieved by lateral displacement of the thoracic walls due to the transfer of haemolymph (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979). Pressure changes, due to unilateral ventilation, in the respirometers (1l or 2l) were measured with Statham PM 15E TC differential pressure transducers (operating range ± 25 mm water) and recorded on a Gilson ICT 2H Duograph. Because this system was exceedingly sensitive to temperature fluctuations, the respirometer and thermobarometer were immersed in a waterbath at 25 °C. The thermobarometer effectively eliminated pressure fluctuations on the open side of the pressure transducer. Recordings were calibrated by injecting known volumes of air into the respirometer and monitoring the resultant changes in pressure. The right lung was isolated from the respirometer as follows. A small hole was drilled in the carapace over the anterolateral margin of the lung. A red-hot needle was inserted into the hole to perforate the lining of the lung and cauterize the wound. A glass cannula (1 mm i.d.) was fixed in position in the hole with a quick setting epoxy glue and a length of fine vinyl tubing was attached. The other openings of the lung were then sealed with soft petroleum grease dispensed through a hypodermic syringe and 18 gauge needle. This procedure effectively sealed and isolated the right lung and the vinyl tubing was led out of the respirometer and connected to its own gas reservoir at ambient pressure. The crabs were left several days to recover from cannulation before the lung was sealed and the animal was placed in the respirometer. Initial records, made immediately after setting a crab up in the apparatus, indicated a very high ventilation rate but a slow stable pattern was established within a few hours. The effects of hypoxia and hypercapnia were studied on crabs in this resting condition by changing the composition of the gas respired. Gas mixtures were prepared by mixing N_2 and air (hypoxia) or CO_2 and air (hypercapnia) in appropriate amounts using flowmeters and bottled gases. The composition of the gases was checked periodically with a blood gas analyser. The gases were humidified and their temperatures were equilibrated with the waterbath before they were passed to the respirometers.

Samples of haemolymph for the determination of PO_2 , PCO_2 , pH and HCO_3^- concentration were taken from the pericardial cavity or the ventral thoracic sinuses of resting crabs at 25 °C. The methods of sampling and of analysis are described by Greenaway *et al.* (1983).

Oxygen-binding curves were determined in the presence of CO_2 at physiological using a modification of the spectrophotometric method used earlier (Greenaway

et al. 1983). This involved filling the tonometers with 1.3% CO₂ in nitrogen atmospheric pressure and injecting increments of oxygen to give a series of different PO₂ values at an essentially constant PCO₂ of 9.9 Torr. Oxygen affinity and cooperativity of the haemocyanin were determined from Hill plots of percentage saturation against PO₂.

Symbols used were as defined by Déjours (1981). Mean values are given $\pm 1 \times$ S.E.M.

RESULTS

Oxygen consumption

The $\dot{M}O_2$ declined rapidly after disturbance and reached a fairly stable level after about 5 h. A much slower decline in $\dot{M}O_2$ over a long time period was also apparent (Fig. 1). A similar pattern of $\dot{M}O_2$ was observed in water-breathing crabs following disturbance (Greenaway *et al.* 1983). The resting value of $\dot{M}O_2$ ($0.45 \mu\text{mol g}^{-1} \text{h}^{-1}$, 150 h after disturbance) was similar to that reported previously for crabs in artificial burrows (MacMillen & Greenaway, 1978).

Ventilation mechanisms

The mechanism of ventilation in *H. valentula* and *H. agassizi* was examined to enable comparison with previous studies on *H. transversa* (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979). Ventilation was caused by lateral oscillations of the antero-dorsal regions of the thoracic walls similar to those described for *H. transversa*. The points of entry and exit of air from the lungs were identified by placing

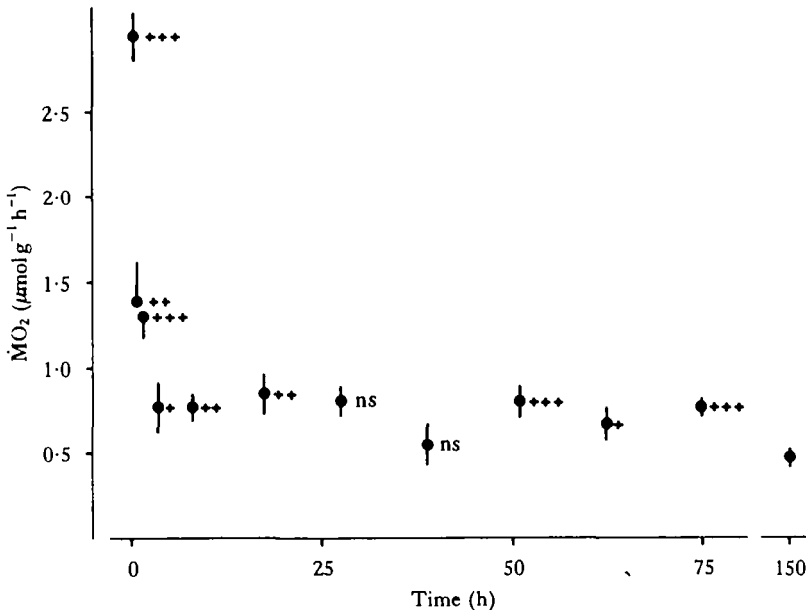


Fig. 1. Oxygen consumption following exercise. Vertical bars indicate S.E.M., $n = 5$. The crosses represent the significance of differences between values of $\dot{M}O_2$ at each time interval and $\dot{M}O_2$ at 150 h (paired t test) (+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$). ns = not significant.

ms of dilute detergent across all possible orifices. Air entered and left the lungs in a tidal manner by means of the Milne-Edwards apertures and the two prebranchial openings. These two routes were of approximately equal importance, whereas in *H. transversa* only the prebranchial apertures allowed passage of air to and from the lungs (Greenaway & Taylor, 1976).

The two branchial chambers were potentially connected posteriorly in all three species of *Holthuisana* studied. The posterior margin of the carapace overlaid the first abdominal segment and abutted against a raised cuticular ridge which formed the posterior limitation of this segment. Narrow, posterior extensions of the branchial chambers opened into the space between the ventral surface of the carapace and the dorsal surface of the first abdominal segment. Normally, this connection did not allow detectable air movement between the lungs but at maximal rates of \dot{V}_A in the experimental apparatus such movements were recorded, occasionally for *H. transversa* and frequently for the other two species. At each exhalation some air passed into the 'isolated' lung and was lost to the exterior. This resulted in a stepwise decrease in pressure within the respirometer at each breath and was clearly visible on the recordings.

Ventilation rate in resting crabs

The rate and pattern of ventilation were studied in resting crabs which had been in the experimental apparatus for at least 24 h. In small crabs (approx. 10 g), the ventilatory movements were extremely small and infrequent and some individuals ceased breathing altogether (Figs 2, 5 trace E), whilst in larger animals a more regular pattern was evident with bursts of ventilatory movements alternating with long periods of apnoea (Fig. 2). Each crab showed an individual pattern.

The frequency of breathing and V_T for the left lung were determined from recordings for crabs which had been left undisturbed for at least 20 h. These data were used to calculate \dot{V}_A for both lungs assuming that V_T was the same in both. \dot{V}_A in these crabs (Table 1) was much lower than that found in crabs following disturbance (Table

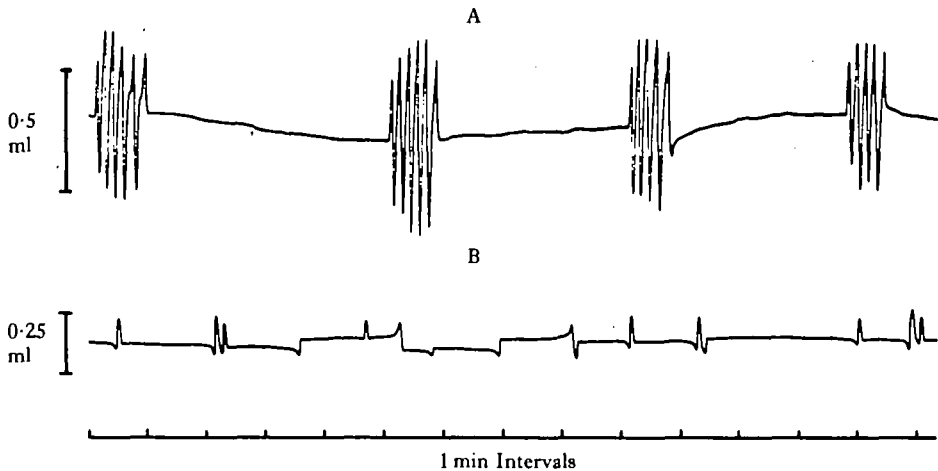


Fig. 2. Sample traces showing the pattern of ventilation in resting crabs. A, a large crab (30 g) B, small crab (13.6 g). Records are for the left lung only.

Table 1. Tidal volume (\bar{V}_T , ml), rate of ventilation (\bar{V}_A , ml g⁻¹ h⁻¹) and oxygen extraction (% Ext) in *H. transversa* at rest

Crab	Weight (g)	Period of rest	\bar{V}_T	Breathing frequency (cycles/h)	\bar{V}_A	Estimated MO ₂ *	% Ext	\bar{V}_A/MO_2
657	11.99	20 h	0.064	61.5	0.659	1.27	20.6	0.52
		54 h	0.033	12.0	0.067	1.27	202.2	0.05
731	13.57	71 h	0.107	50.0	0.789	0.81	11.0	0.97
775	13.80	10 days	0.034	109.5	0.539	0.49	9.7	1.10
741	13.97	71 h	0.057	14.0	0.114	0.85	79.3	0.14
765	18.70	24 h	0.160	117.6	2.010	0.74	3.9	2.71
748	25.44	72 h	0.139	72.0	0.785	0.50	6.8	1.57
809	30.40	20 h	0.307	130.3	2.630	0.47	1.9	5.57
$\bar{x} \pm \text{s.e.m.}$					0.95	0.80		1.58
					± 0.32	± 0.115		± 0.646

* Estimated from data in Fig. 2 and corrected for body weight using data of McMillen & Greenway (1978). \bar{V}_T data are for left lung only.

2) and percentage extraction (% Ext, [$\text{MO}_2/\text{amount O}_2$ inspired] 100) was higher in all cases. The high values for % Ext seen in some crabs (Table 1) indicated a large diffusional supplement to active ventilation. This was particularly obvious in crab 657 where ventilation could, at maximum, have supplied only half the oxygen consumed. The shortfall must have been supplied by diffusion.

Response to hypoxia

The ventilatory responses to low levels of oxygen in inspired air were examined by exposing resting crabs to air, 10% O₂, 5% O₂, 2.5% O₂ and in some cases zero oxygen (N₂). In a gas mixture containing 10% O₂ (75 Torr), many crabs showed a small enhancement of the frequency of ventilatory movements but others showed no response. In 5% O₂ (38 Torr) all of the crabs which were examined showed a marked increase in frequency of breathing movements and \bar{V}_T . Maximal stimulation of \bar{V}_A occurred in 2.5% O₂ (19 Torr) and in N₂ (Fig. 3). Thus *H. transversa* showed a clear response to hypoxia but only after the level of oxygen dropped well below 10% (= 75 Torr). In an atmosphere of N₂ the scaphognathites of some crabs began beating causing small amplitude traces superimposed on the much larger traces caused by tidal ventilation.

Response to hypercapnia

Crabs in a resting state were exposed to successively higher levels of CO₂ in their air supply. In some individuals, a small increase in amplitude and frequency of ventilatory movements was observed when the level of CO₂ in their air was increased to 1% but all crabs showed a significant response to 2.5% CO₂ (19 Torr). Levels were generally maximal in air containing 5% or more CO₂ (38 Torr) (Fig. 4). A marked response to hypercapnia, therefore, was evident after moderate changes in PCO₂ of the inspired air.

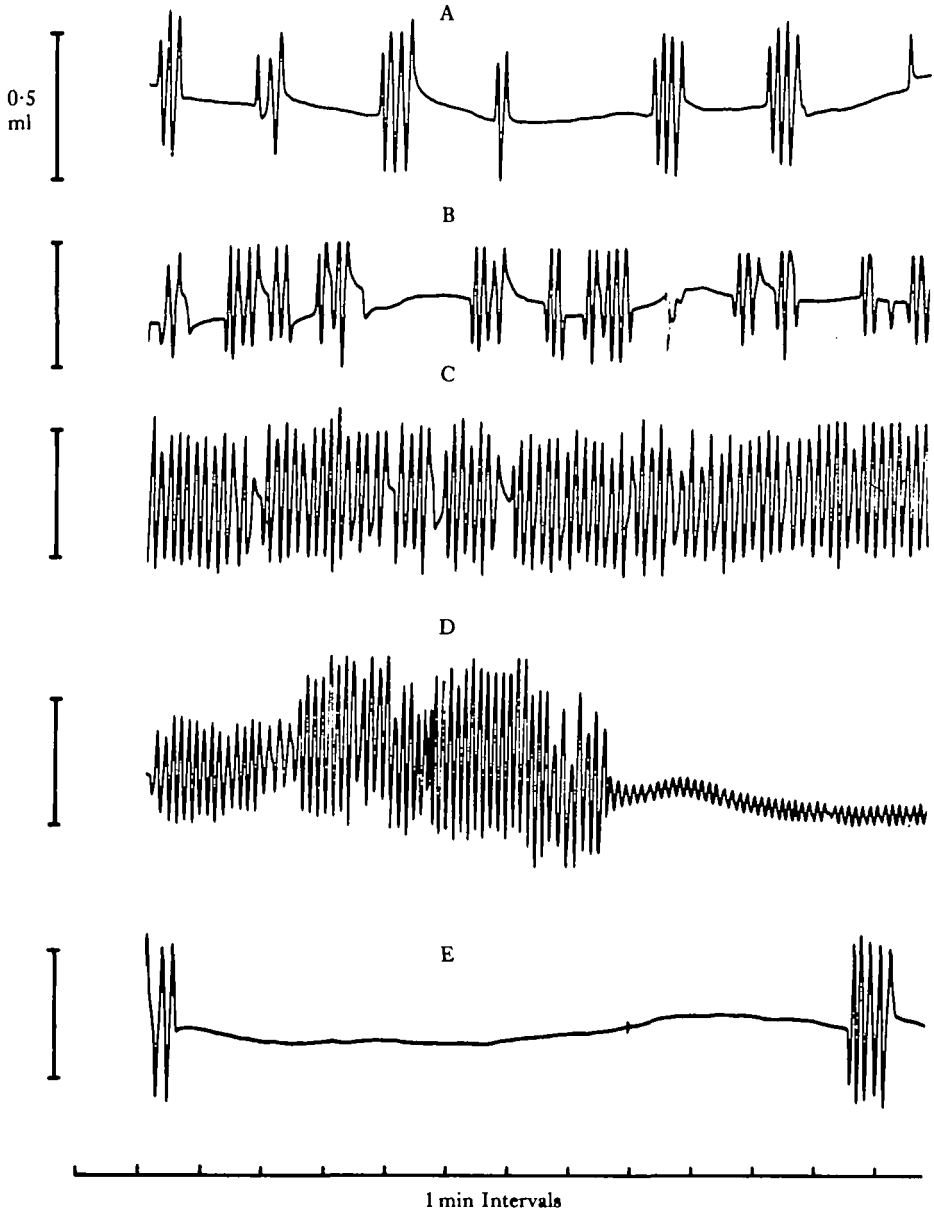


Fig. 3. Sample traces showing ventilatory responses to hypoxia in *H. transversa*. A = air, B = 10.5% O₂, C = 5% O₂, D = 2.5% O₂, E = air. Records are for the left lung.

The effect of disturbance

The effect of disturbance on V_T and the frequency of ventilatory movements were determined by recording these parameters immediately after crabs had been placed in the experimental apparatus. The crabs had struggled extensively whilst the lung was sealed and were considered to be maximally stimulated. However, there was an unavoidable delay of several minutes before recordings could be begun and it is probable that some decline in \dot{V}_A occurred during this time. A series of sample traces

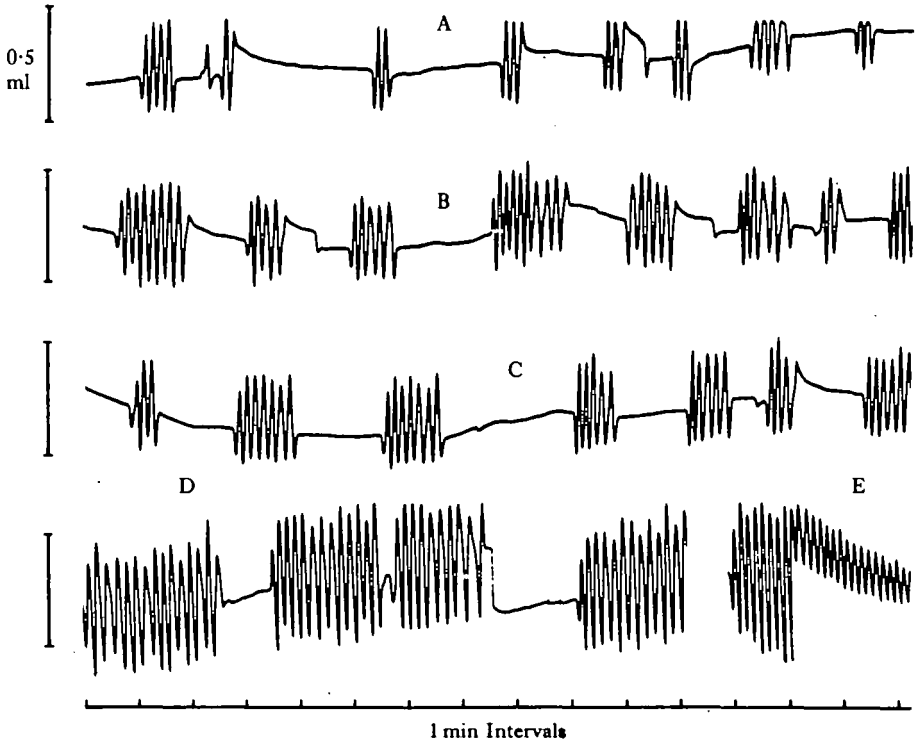


Fig. 4. Sample traces showing ventilatory responses to hypercapnea in *Holthuisana*. A = air, B = 1% CO₂ in air, C = 2.5% CO₂ in air, D = 5% CO₂ in air, E = 10% CO₂ in air.

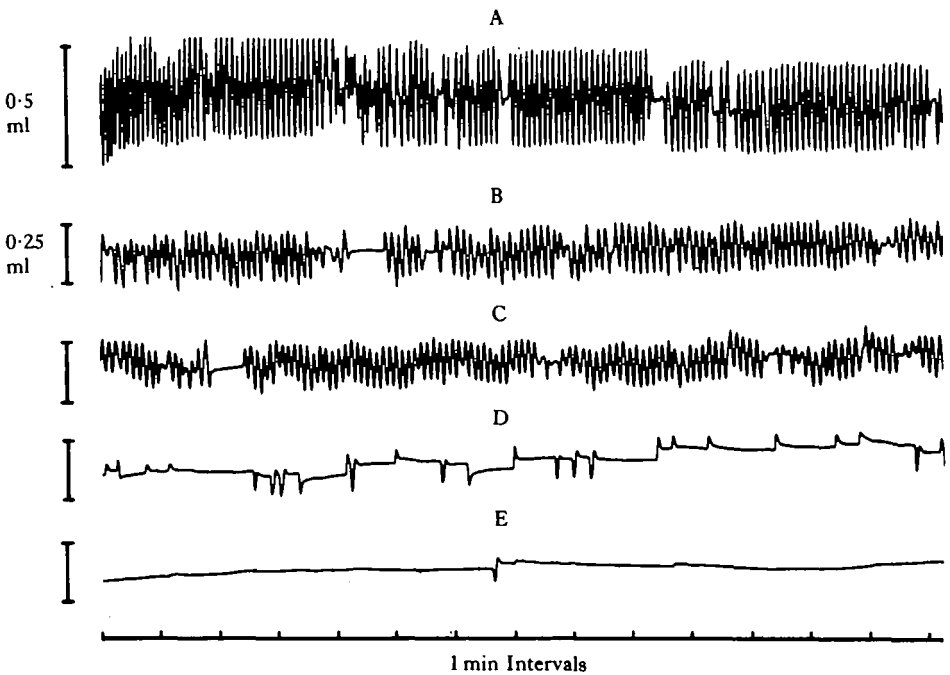


Fig. 5. Sample traces showing ventilation during recovery from exercise in *H. transversa*. Period A, 0-14 min; B, 34-50 min; C, 1 h; D, 18.5 h; E, 43 h after exercise.

show the pattern of ventilation at increasing time intervals after disturbance are included (Fig. 5). The V_T and frequency of breathing were measured from the traces during the period of maximal ventilation (Table 2). The frequency was somewhat lower than the maximum previously recorded by direct visual counting during disturbance (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979) and this indicated that the highest rates of ventilation measured here were lower than the highest rates possible. This was presumably due to the delay between completion of disturbance and the commencement of recording. Certainly $\dot{M}O_2$ fell rapidly after disturbance (Fig. 1). The V_T during the period of maximal ventilation was linearly related to the body weight of the crabs ($V_T = 0.023$ body weight + 0.039 ml, $P < 0.001$); data are for one lung only. Based on this equation, V_T in *H. agassizi* (0.22 ml, 16 g crab) and *H. valentula* (0.289 ml, 17.4 g crab) were well below the values for *H. transversa* of similar size.

In the 30 min period immediately following disturbance, $\dot{M}O_2$ was $2.95 \mu\text{mol g}^{-1} \text{h}^{-1}$ and \dot{V}_A was $28.3 \text{ ml g}^{-1} \text{h}^{-1}$ (Fig. 1, Table 2). These values yielded a figure of 1.1% for extraction of oxygen from inspired air in this post-disturbance period. The pattern of ventilation observed after disturbance in crabs corresponded well with the data for post disturbance $\dot{M}O_2$ (Fig. 1). The initially high \dot{V}_A , associated with rapid and continuous breathing, declined quickly giving way to periods of apnoea which became more frequent and of longer duration until the resting pattern of ventilation was established after several hours.

Blood-gas levels and oxygen binding

The \bar{P}_aO_2 was relatively high in resting crabs (*H. transversa* 56.1 ± 8.9 Torr, *H. agassizi* 48.4 ± 11.2 Torr, *H. valentula* 74.6 ± 8.4 Torr) and no significant differences were found between species. Some individuals of each species had P_aO_2 values close to 100 Torr which indicated the potential for a high degree of saturation at the lung. \bar{P}_vO_2 was very low in *H. transversa* (7.4 ± 0.5 Torr). Data were not available for the other species. After 5 min of exercise, \bar{P}_aO_2 in *H. transversa* was 76.6 ± 9.1 Torr but this increase was not significant ($P > 0.1$). The $\bar{P}O_2$ levels in the haemolymph of other air-breathing decapods are shown in Table 3 for comparison.

The pH of arterialized haemolymph (7.41 ± 0.028) was significantly higher than that of venous haemolymph (7.32 ± 0.022) ($0.02 < P < 0.05$) in agreement with the measured \bar{P}_aCO_2 (9.6 ± 0.94 Torr) and \bar{P}_vCO_2 (11.3 ± 0.79 Torr). The measured

Table 2. Ventilation rate (\dot{V}_A , $\text{ml g}^{-1} \text{h}^{-1} \pm \text{S.E.M. [n]}$) and breathing frequency (cycles per min) after exercise in three species of *Holthuisana*

Species	\dot{V}_A	Max. \dot{V}_A	Frequency	
			Mean	Max.
<i>H. transversa</i>	28.3 ± 2.4 (13)	45.4	9.1 ± 0.4 (13)	11.8
<i>H. agassizi</i>	10.2 (1)	—	7.9 (1)	—
<i>H. valentula</i>	15.1 (1)	—	7.6 (1)	—

$\bar{P}\text{CO}_2$ of the haemolymph was higher than that found in *H. transversa* breathing water or in other aquatic crabs (Greenaway *et al.* 1983). However, measured $\bar{P}_a\text{CO}_2$ may be unreliable. Preferential loss of CO_2 from the dissolved CO_2 pool during gas exchange would result in low $P_a\text{CO}_2$ and a disequilibrium between CO_2 and HCO_3^- in arterialized haemolymph. During the time required for $P\text{CO}_2$ determination, equilibrium would be restored and $P_a\text{CO}_2$ elevated above its initial value. Without knowledge of $P\text{CO}_2$ in the lung and the relative losses of CO_2 from the dissolved CO_2 and HCO_3^- pools it is not possible to comment on the accuracy of the measured $\bar{P}_a\text{CO}_2$. However, it is likely to err on the high side. Values for $C_a\text{CO}_2$ ($13.05 \text{ mmol l}^{-1}$) and $C_v\text{CO}_2$ ($13.17 \text{ mmol l}^{-1}$) indicated a loss of about 1% of the CO_2 , carried by venous haemolymph, during gas exchange. The concentrations of bicarbonate in arterIALIZED and venous haemolymph (12.66 ± 0.66 and $12.71 \pm 0.96 \text{ mmol l}^{-1}$) and the CCO_2 in the haemolymph were characteristic of air-breathing crabs (Table 3).

Oxygen-binding characteristics of whole haemolymph were determined at two pH values, spanning the measured range of pH in the haemolymph, in the presence of CO_2 (9.9 Torr). The pigment was 50% saturated with oxygen at about 13 Torr PO_2 and approached full oxygenation at 35 Torr PO_2 (Fig. 6). Cooperativity between haemocyanin units was high ($n_{50} = 2-3$). At the measured $\bar{P}_a\text{O}_2$ the haemocyanin of *H. transversa* was fully saturated with oxygen, whilst at $\bar{P}_v\text{O}_2$ the pigment was 30% saturated indicating a moderate venous oxygen reserve. The affinity of the pigment was lower at 9.9 Torr PCO_2 than that measured in the absence of CO_2 but at the same pH (Greenaway *et al.* 1983). Clearly the affinity of the haemocyanin was affected by PCO_2 as well as pH. Data for other air-breathing decapods are shown in Table 3.

The oxygen content of the haemolymph was not measured in air-breathing crabs but can be approximated from earlier data on oxygen content and % saturation of haemocyanin (Greenaway *et al.* 1983). Thus at $\bar{P}_a\text{O}_2$ (56.1 Torr) the haemolymph

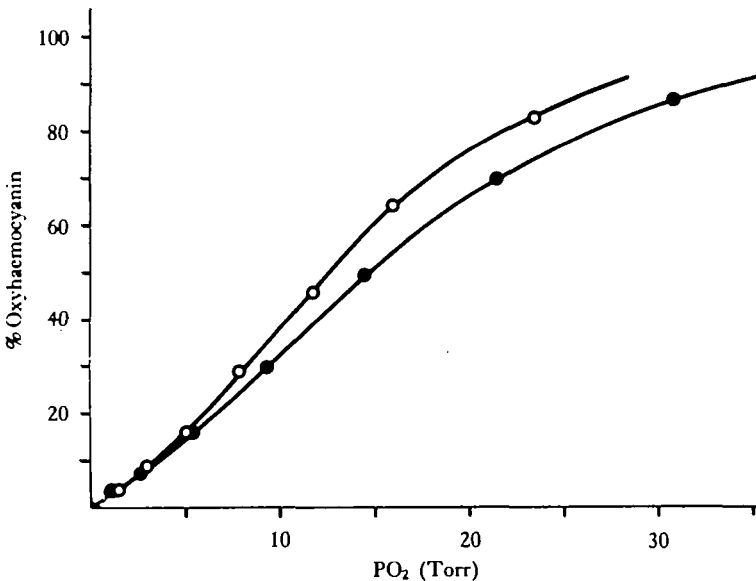


Fig. 6. Oxygen-binding curves for the haemocyanin of male *H. transversa* at a PCO_2 of 9.9 Torr. Open circles (pH 7.59), closed circles (pH 7.3).

could carry $437 \mu\text{mol O}_2 \text{ l}^{-1}$ of which 19% would be in solution. This was lower than the oxygen content at $P_a\text{O}_2$ in *Gecarcinus* ($630 \mu\text{mol l}^{-1}$) and much lower than in *Ocypode quadrata* ($1093 \mu\text{mol l}^{-1}$) (Table 3).

DISCUSSION

Characteristically, decapods use the scaphognathites for ventilation both in water and in air. Within the genus *Holthuisana* they provide the motive power for water-breathing but are replaced by tidal ventilation, involving oscillatory movements of the thoracic walls, when the crabs leave water. At present there is virtually no information on ventilation in air by other freshwater crabs, although in the pseudoscorpion, *Trichodactylus petropolitanus*, the scaphognathites cease beating on emersion (Valente, 1948). Further studies are needed to determine the breathing mechanism in other groups of freshwater crabs.

The tidal ventilatory system may possess certain advantages over the unidirectional flow system using scaphognathites. Tidal breathing in *H. transversa* renews a very large proportion of the air in the lungs at each breathing cycle, thus ensuring a very short diffusion distance between tidal air and the respiratory epithelium. In *Birgus* and the gecarcinid crabs, inspired air is reported to move through the centre of the lung, resulting in a much longer diffusion pathway to the lung epithelium (Cameron & Mecklenburg, 1973; Cameron, 1975). This situation seemed to permit a relatively lower $\dot{V}_A/\dot{M}\text{O}_2$ in *H. transversa* than in other resting crabs (Table 3), but great differences in resting patterns existed between individuals of *H. transversa*, and the smaller crabs appeared to rely largely on diffusion (Figs 2, 5 trace E). At high \dot{V}_A , caused by disturbance, the maximum \dot{V}_A recorded for *H. transversa* was $45.4 \text{ ml g}^{-1} \text{ h}^{-1}$. Higher values were considered likely. This was much higher than the maximum of $22\text{--}28 \text{ ml g}^{-1} \text{ h}^{-1}$ reported for *Cardisoma* (Herreid, Lee & Shah, 1979) but lower than the tenfold increase over resting \dot{V}_A reported by Cameron (1975) for both *Cardisoma* and *Gecarcinus*. Thus the tidal method of ventilation has a capacity for air movement similar to the mechanism found in other air-breathing crabs. However, the % Ext of oxygen at high \dot{V}_A (1.1%) was much lower than in *Cardisoma* (3.8%) (Herreid, Lee & Shah, 1979). This was reflected in the higher air convection requirement of $9.6 \text{ ml air}/\mu\text{mol O}_2$ (Table 3). At rest, \dot{V}_A for *H. transversa* was considerably lower than for other air-breathing crabs, reflecting the lower resting $\dot{M}\text{O}_2$ and perhaps also a greater reliance on diffusional gas renewal in the lungs.

Comparison of the energetic cost of tidal and scaphognathite ventilation cannot be made directly but it is important to consider the factors involved. The scaphognathites moved air directly in an apparently efficient manner (Cameron & Mecklenburg, 1973; Cameron, 1975). The movement of air in *H. transversa* was indirect, caused by lateral movement of similar volumes of haemolymph within the haemocoel, which due to its higher viscosity and density involved a potentially greater expenditure of energy. However, the apparent cost of tidal ventilation may well be reduced by the inertia of the moving haemolymph and by elastic recoil of the thoracic walls and their musculature, especially at high \dot{V}_A . Additionally, the distance haemolymph must travel is short whereas air must move over longer distances in scaphognathite ventilation and must also pass through the resistance of the gills.

Table 3. Values for blood gas tensions (Torr), total gas content (mmol l^{-1}), oxygen affinity of haemocyanin (P_{50} , Torr), oxygen consumption ($\mu\text{mol g}^{-1} \text{h}^{-1}$), ventilation rate ($\text{ml g}^{-1} \text{h}^{-1}$) and air convection requirement ($\text{ml air} / \mu\text{mol O}_2$) in air breathing crabs at rest

Species	P_aO_2		C_tO_2 at		P_{50}	P_aCO_2	P_vCO_2	P_aCO_2	CCO_2		\dot{V}_A	MO_2	\dot{V}_A/MO_2	Temp. °C	Source
	P_aO_2	P_vO_2	P_aO_2	P_vO_2					a	v					
<i>Holthuisana transversa</i>	56.1	7.4	437	13	13	9.6	11.3	13.05	13.17	0.95	0.80	1.6	25	This study	
										28.3†	2.95†	9.6†	25		
<i>Cecarcinus lateralis</i>	80.5	25.6	630	17-20	7.6*	9.1*	8.7	9.1	12.0	24.6	2.06	5.8	25	Taylor & Davies (1981)	
											2.23	11.0	24-26	Cameron (1975)	
<i>Cardisoma guanhumi</i>	—	—	—	7.3	—	15.6	—	—	10.2	10.2	2.6	3.9	24-26	O'Mahoney (1977), Young (1972), Cameron (1975)	
										7.2	2.23	3.2	24-27	Herreid, O'Mahoney & Shah (1979)	
										22-28†	7.14†	3.1-4.0†	24-27	Herreid, Lee & Shah (1979)	
<i>Cardisoma carnifex</i>	67	11	810	13.5	11-15	13.4-16	19	19	4.2*	4.2*	2.31	1.8	25	Wood & Randall (1981a, b), Burggren & McMahon (1981)	
<i>Gecarcoidea lalandi</i>	43	17	—	—	—	—	11.9	12.5	8.5	8.5	2.87	2.96	27-30	Cameron & Mecklenburg (1973)	
<i>Ocyropsis quadrata</i>	20	10	1093	10	—	—	—	—	—	—	2.32	—	25	Burnett (1979)	
<i>Birgus latro</i>	78	15	—	14.5	6.2	9.2	14.1	14.0	3.4	3.4	1.45	2.35	27-30	Cameron & Mecklenburg (1973), Burggren & McMahon (1981)	
			730	21	—	—	—	—	—	—	—	—	—		
<i>Coenobita clypeatus</i>	14	8	1340	10	4.1*	6.8*	11.7	13.0	—	—	1.23	—	23	McMahon & Burggren (1979)	

* Data for only two specimens.

† Calculated value.

‡ Post-disturbance value.

It is interesting that several decapods with scaphognathite ventilation have developed accessory ventilatory mechanisms. Thus in *Coenobita clypeatus* pressure changes caused by movements of the flexible branchiostegal walls aid ventilation (McMahon & Burggren, 1979) whilst similar but smaller movements are reported in *Birgus* (Cameron & Mecklenburg, 1973). In *Aratus pisoni*, a tree-climbing grapsoid crab, ventilation is aided by carapace movements (Carter, 1931). Lateral oscillations of the thoracic walls are reported to be responsible for ventilation in *Uca una* during air-breathing (Jobert, 1876). With the possible exception of *Uca* these mechanisms play only a minor role in ventilation, compared to the tidal mechanism of *Holthuisana*, but again suggest some selective advantage to types of aerial ventilation which do not use scaphognathites.

The \dot{V}_A was sensitive to both the PO_2 and PCO_2 of inspired air in *H. transversa*. The rise in PCO_2 required to stimulate \dot{V}_A was much smaller than the fall in PO_2 needed to produce a similar effect. Given a respiratory exchange ratio of approx. 1, it is clear that PCO_2 will normally control \dot{V}_A . This is also the case in other air-breathing decapods (Cameron & Mecklenburg, 1973; Cameron, 1975), with the exception of *Coenobita clypeatus* (McMahon & Burggren, 1979). Recent reports on *Birgus* suggest that stimulation of ventilation by elevated PCO_2 in the inspired air is mediated via pH or $[HCO_3^-]$ of the haemolymph rather than by direct action of CO_2 (Smatresk & Cameron, 1981). In water, *H. transversa* was sensitive to PO_2 (Greenaway *et al.* 1983) a situation common to aquatic crabs. Development of air-breathing, therefore, has been associated with the retention of the usual oxygen-sensitive control of scaphognathite activity in water and the acquisition of a new control mechanism for tidal air-breathing primarily influenced by carbon dioxide.

The haemolymph of both *Ocypode* and *Coenobita* is characterized by high C_aO_2 , high oxygen affinity and low P_aO_2 , whilst the other species examined have lower C_aO_2 , low oxygen affinity and high P_aO_2 (Table 3). These two groups may represent two different strategies of air-breathing. It would be interesting to compare air convection requirements of the groups but no data are available for \dot{V}_A in *Coenobita* or *Ocypode*.

Many terrestrial decapods show a high degree of independence from permanent water and need only a supply of 'drinking' water, e.g. *Gecarcinus*, *Gecarcoidea*, some species of *Ocypode* and the anomurans *Birgus* and *Coenobita clypeatus* (Bliss, 1968; Cameron, 1981). *Cardisoma guanhumi*, however, requires water in its burrow and *C. carnifex* carries water in its branchial chambers (Herreid & Gifford, 1963; Wood & Randall, 1981a). *H. transversa* behaves rather differently and although well suited to terrestrial life (Greenaway & MacMillen, 1978; Taylor & Greenaway, 1979; Greenaway, 1980) is normally active only under wet or aquatic conditions owing to the severity of its near-desert environment at other times. This behaviour pattern is largely shared by *H. valentula* and *H. agassizi* from monsoonal regions of Australia and probably by many other freshwater crabs from monsoonal regions of Asia (McCann, 1938). Clearly *H. transversa* must be able to cope well with both air and water-breathing and it is informative to compare gas exchange in the two media by examination of the data provided by Greenaway *et al.* (1983). The crucial point of comparison is the $\dot{M}O_2$. The capacity for oxygen uptake (maximum $\dot{M}O_2$) was very similar in both media as were the air/water convection requirements. Clearly then,

maximum performance of the tidal ventilatory system and lungs in air was similar that of scaphognathite ventilation and gills in water. $\dot{M}O_2$, however, was lower than in other crabs in both media. \dot{C}_aO_2 was lower in water-breathing crabs and, applying the Fick principle, cardiac output must have been greater in water-breathing crabs. At rest (24 h undisturbed) $\dot{M}O_2$ in water-breathing crabs was higher than in air-breathing crabs; this is probably a reflection of normal behaviour patterns since in the field crabs were active in water and quiescent in air. In the predominantly aquatic crab *Carcinus*, $\dot{M}O_2$ was also similar in air and water (Taylor & Butler, 1978) but as data were for resting crabs this again may reflect oxygen requirements in the two conditions rather than the capacity of the system to breathe air and water.

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REFERENCES

- ALEXANDER, S. & EWER, D. F. (1969). A comparative study of some aspects of the biology and ecology of *Sesarma ctenate* Ort. and *Cyclograpsus punctatus* M. Edw. with additional observations on *Sesarma meinerti* de Man. *Zool. Afr.* **4**, 1–35.
- BLISS, D. E. (1968). Transition from water to land in decapod crustaceans. *Am. Zool.* **8**, 355–392.
- BOTT, R. (1969). Die Süsswasserkrabben Süd-Amerikas und ihre Stammesgeschichte. Eine Revision der Trichodactylidae und der Pseudothelphusidae Oestliche der Anden (Crustacea, Decapoda). *Abh. senckenb. naturforsch. Ges.* **518**, 1–94.
- BOTT, R. (1970). Die Süswasserkrabben von Europa, Asien, Australien, und ihre Stammesgeschichte. Eine Revision der Potamoidea und der Parathelphusoidea (Crustacea, Decapoda). *Abh. senckenb. naturforsch. Ges.* No. **526**, 1–338.
- BURNETT, L. E. (1979). The effects of environmental oxygen levels on the respiratory function of haemocyanin in the crabs *Libinia emarginata* and *Ocyrode quadrata*. *J. exp. Zool.* **210**, 289–299.
- BURGGREN, W. W. & McMAHON, B. R. (1981). Hemolymph oxygen transport, acid-base status, and hydromineral regulation during dehydration in three terrestrial crabs, *Cardisoma*, *Birgus*, and *Coenobita*. *J. exp. Zool.* **218**, 53–64.
- CAMERON, J. N. (1975). Aerial gas exchange in the terrestrial *Brachyura Gecarcinus lateralis* and *Cardisoma guanhumi*. *Comp. Biochem. Physiol.* **52A**, 129–134.
- CAMERON, J. N. (1981). Brief introduction to the land crabs of the Palau Islands. Stages in the transition to air breathing. *J. exp. Zool.* **218**, 1–5.
- CAMERON, J. N. & MECKLENBURG, T. A. (1973). Aerial gas exchange in the coconut crab *Birgus latro* with some notes on *Gecarcoidea lalandii*. *Resp. Physiol.* **19**, 245–261.
- CARTER, G. S. (1931). Aquatic and aerial respiration in animals. *Biol. Rev.* **6**, 1–35.
- DEJOURS, P. (1981). *Principles of comparative respiratory Physiology*, Second edition. Amsterdam: Elsevier/North Holland Biomedical Press.
- DIAZ, H. & RODRIGUEZ, G. (1977). The branchial chamber in terrestrial crabs: a comparative study. *Biol. Bull. mar. biol. Lab., Woods Hole* **153**, 485–504.
- GREENAWAY, P. (1980). Water balance and urine production in the Australian arid-zone crab, *Holthuisana transversa*. *J. exp. Biol.* **87**, 237–46.
- GREENAWAY, P. & MACMILLEN, R. E. (1978). Salt water balance in the terrestrial phase of the inland crab *Holthuisana (Austrothelphusa) transversa* Martens. (Parathelphusoidea: Sundathelphusidae). *Physiol. Zool.* **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., BONAVENTURA, J. & TAYLOR, H. H. (1983). Aquatic gas exchange in the freshwater/land crab *Holthuisana transversa*. *J. exp. Biol.* **103**, 225–236.
- HERREID, C. F. & GIFFORD, C. A. (1963). The burrow habitat of the land crab, *Cardisoma guanhumi* (Latreille). *Ecology* **44**, 773–775.
- HERREID, C. F., LEE, L. W. & SHAH, G. M. (1979). Respiration and heart rate in exercising land crabs. *Res. Physiol.* **37**, 109–120.

- BRREID, C. F., O'MAHONEY, P. M. & SHAH, G. M. (1979). Cardiac and respiratory response to hypoxia in the land-crab *Cardisoma guanhumi*. *Comp. Biochem. Physiol.* **63A**, 145–152.
- JOBERT, M. (1876). Recherche sur l'appareil respiratoire et le mode de respiration de certaines Crustacés Brachyures (crabes terrestres). *Annls. Sci. nat. Paris Ser. Zool. Biol. Anim.* **4**, 1–5.
- MCCANN, C. (1938). Notes on the common land crab, *Paratelphusa (Barytelphusa) guerini* (M. Eds.) of Salsette Island. *J. Bombay nat. Hist.* **30**, 531–542.
- MCMAHON, B. R. & BURGGREN, W. W. (1979). Respiration and adaptation to the terrestrial habitat in the land hermit crab *Coenobita clypeatus*. *J. exp. Biol.* **79**, 285–281.
- MACMILLEN, R. E. & GREENAWAY, P. (1978). Adjustments of energy and water metabolism to drought in an Australian arid-zone crab. *Physiol. Zool.* **51**, 231–240.
- MANGUM, C. P. (1980). Respiratory function of the haemocyanins. *Am. Zool.* **20**, 19–38.
- O'MAHONEY, P. M. (1977). Respiration and acid-base balance in Brachyura decapod crustaceans: The transition from water to land. Ph.D. thesis, State University of New York, Buffalo.
- SMATRESK, N. J. & CAMERON, J. N. (1981). Post-exercise acid-base balance and ventilatory control in *Birgus latro*, the coconut crab. *J. exp. Zool.* **218**, 75–82.
- TAYLOR, E. W. & BUTLER, P. J. (1978). Aquatic and aerial respiration in the shore crab, *Carcinus maenas* (L.) acclimated to 15°C. *J. comp. Physiol.* **127**, 315–323.
- TAYLOR, A. C. & DAVIES, P. S. (1981). Respiration in the land crab, *Gecarcinus lateralis*. *J. exp. Biol.* **93**, 197–208.
- 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.
- VALENTE, D. (1948). Mecanismo da respiração *Trychodactylus petropolitanus* (Goeldi). *Bol. Fac. Filos. Cienc. Let. Univ. Sao Paulo Ser. Zool.* **13**, 259–316.
- WOOD, C. M. & RANDALL, D. J. (1981a). Oxygen and carbon dioxide exchange during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 7–22.
- WOOD, C. M. & RANDALL, D. J. (1981b). Haemolymph gas transport, acid-base regulation and anaerobic metabolism during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 23–35.
- YOUNG, R. E. (1972). The physiological ecology of haemocyanin in some selected crabs. II. The characteristics of haemocyanin in relation to terrestriality. *J. exp. mar. Biol. Ecol.* **10**, 193–206.