

RESPIRATION AND ADAPTATION TO THE TERRESTRIAL HABITAT IN THE LAND HERMIT CRAB *COENOBITA CLYPEATUS*

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SUMMARY

The frequencies of heart (f_H) and scaphognathite (ventilatory = f_{sc}) pumping, and responses to hypoxia, hypercapnia and wetting (simulated rain), as well as oxygen consumption (\dot{M}_{O_2}), pre- and postbranchial haemolymph oxygen tension (P_{O_2}), oxygen content (C_{O_2}), carbon dioxide content (C_{CO_2}) and pH were measured in adult land crabs *Coenobita clypeatus*. There was a large increase in f_{sc} in response to both hypoxia and wetting but a smaller increase in response to even severe hypercapnia. Some evidence suggests that ventilation via the scaphognathites may have been supplemented by a second (branchiostegal) pump when ventilatory requirement was high. f_H was less responsive to either hypoxia or hypercapnia, but decreased with severe exposure to either. Haemolymph oxygen tensions were low ($P_{a,O_2} = 14$, $P_{v,O_2} = 8$) but haemocyanin oxygen affinity was high *in vivo* ($P_{50} = 10$ torr at 23 °C) and postbranchial haemocyanin was 60-80% saturated. Oxygen content was also high allowing adequate oxygen release to the tissues despite the low oxygen tensions. $\Delta P_{50}/\Delta t = 0.37$ torr/ Δ °C, $\log \Delta P_{50}/\Delta pH = -0.84$ torr/pH unit, both determined *in vitro* were lower than literature values for marine and littoral species. As in other terrestrial species, C_{CO_2} and P_{CO_2} (calculated) were high, as were both bicarbonate and non-bicarbonate buffering capacities. Water loss was less (0.08% body weight.h⁻¹) in *Coenobita* than in other terrestrial crustaceans, this resulting from the protection of the adopted shell. Results obtained from *Coenobita* are compared with those from other terrestrial and littoral crabs to illustrate the influence of the adopted shell on the degree of modification needed to enter terrestrial habitats.

INTRODUCTION

The mechanisms of physiological compensation to environmental change exhibited by terrestrial decapodan crustaceans are of particular interest in understanding the evolution of terrestrial life. These animals, which approached the terrestrial habitat from the sea via the littoral zone, would be expected to show somewhat different

solutions to the problems posed by a terrestrial existence than those exhibited by animals which approached the land from fresh waters. Unfortunately, physiological compensation to environmental change in the terrestrial decapods has been relatively poorly studied and is not well understood. Interest in the respiratory physiology of these animals, however, has shown a recent revival. Burnett (1978) describes ventilatory responses to hypoxia in *Ocypode quadrata*, and ventilatory responses to both hypoxia and hypercapnia have been quantified for the anomuran *Birgus latro* and for several species of brachyurans by Cameron & Mecklenburg (1973) and Cameron (1975). The respiratory properties of haemocyanin have been studied in *Gecarcinus* (Redmond, 1968), *Birgus* (Cameron & Mecklenburg, 1973) and *Ocypode* (Burnett, 1978).

Although the above animals do not stray far from the littoral zone and are dependent on the sea, at least for reproduction and early development, they are nonetheless completely exposed to the terrestrial environment as adults. The terrestrial hermit crabs *Coenobita* sp. differ in that they retain the adopted gastropod shell even as adults. This shell can serve as a reservoir for fresh or sea-water stores, protects the body surface from desiccation, and offers considerable thermal insulation. In short, the adopted shell may have allowed these animals to enter the terrestrial habitat with less modification than the more exposed forms. To test this hypothesis the present study examines ventilation, oxygen uptake and oxygen and carbon dioxide transport with particular emphasis on the potential effects of temperature and desiccation. The results are compared with those from other littoral and terrestrial crustaceans in order to assess the influence of the adopted shell, and its associated water store on the evolution of terrestrial respiration.

MATERIALS AND METHODS

Twelve adult *Coenobita clypeatus* (Herbst) 1791 (identified using Bright, 1966) of either sex, and varying from 20–40 g mass without their adopted shells, were obtained from the West Indies via commercial suppliers. The animals were housed at 23 ± 2 °C in 10 gal aquaria on 3–4 in. of fine sand into which they often burrowed. They were supplied regularly with fresh water and sliced apple and other fruits.

Several techniques were tried in attempts to record heart and scaphognathite rates from unrestrained animals residing normally in the shell. A major problem of keeping electrodes and cannulae in place while allowing the animals unimpeded movement back and forth within the shell was circumvented by cutting (using a diamond saw on a temporarily vacated shell) a groove from the shell rim back along 50% of the length of the 1st spiral (Fig. 1). The groove, 2–3 mm wide, allowed electrodes and cannulae to move freely with the animal as it moved in and out of the shell aperture, prevented the animals from snagging the electrodes with their legs, and thus allowed a reasonably long effective life of electrodes and cannulae. A second problem, that of visualizing the current site, or state, of an electrode or cannula on the animal when inside the shell was never solved, but the final location of electrodes and cannulae was always checked by removing the animals from the shell at the end of an experiment.

In six animals the frequency of heart beat (f_H) was detected by the impedance change between fine (0.075 mm), insulated, stainless steel wire, electrodes implanted

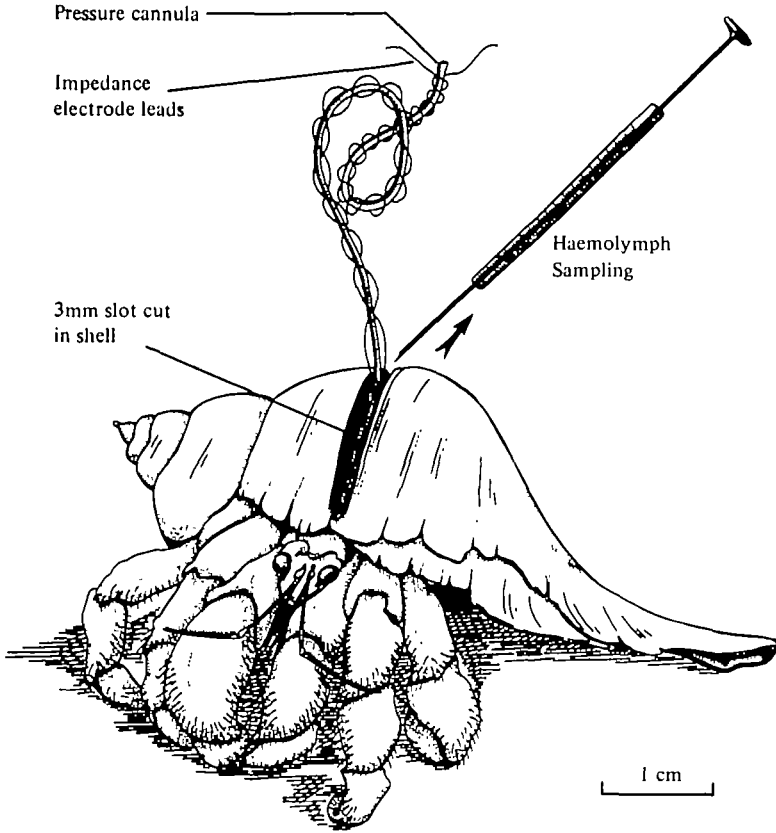


Fig. 1. Drawing of *C. clypeatus* partially emerged from the adopted shell showing slot cut in shell to allow movement of catheter and electrodes and access for postbranchial haemolymph sampling.

through the branchiostegite to lie above the pericardium on either side of the heart. Impedance changes between similar electrodes inserted into the animal's tissue above and below the scaphognathites (two animals), or placed in the scaphognathite channel (four animals), detected the intermittent rhythmic activity of the scaphognathite. These impedance changes were amplified by Biocom 2991 impedance converters and displayed on a Brush/Gould 2600 oscillograph. In three animals pressure changes in the branchial cavities were recorded by a Hewlett-Packard 270 differential air-pressure transducer connected to the branchial cavity via a fine polyethylene (Pez0) cannula. This cannula was heat moulded to conform to the animal's contours and then placed so that its open end looped up into the branchial cavity posteriolaterally. Once in place the cannula was secured to the animal's carapace dorsally with cyanoacrylate glue and the distal end passed out through the slit in the shell (Fig. 1). Impedance electrodes placed anteriorly recorded many movements of anterior appendages which were not necessarily respiratory in nature, but in two animals simultaneous recording of both branchial pressure and impedance waveforms allowed identification of a characteristic impedance signal from which scaphognathite activity could be quantified. In all cases implantation of electrodes and cannulae occurred on

animals anaesthetized by intra-abdominal injection of 70 mg/kg. Xylazine (Rompun) and then removed from the shell. This anaesthetic dose induced complete flaccidity for only 5–10 min but complete recovery often took 5–6 h. Rate measurements were not made for at least 24 h following anaesthesia.

Oxygen consumption (\dot{M}_{O_2}) measurements were carried out on animals in 500 ml capacity glass respirometers held at the desired temperature in a temperature-controlled bath. Between measurement periods the respirometers were supplied with a constant flow of air at 3–5 ml/min. For measurements of oxygen consumption, however, the respirometer taps were closed for periods of 1–2 h. At the end of this time 60 ml of the air in the respirometer was displaced by an attached syringe, through the measurement cell of a Servomex O.A. 150 paramagnetic O_2 analyser, capable of detecting changes in O_2 concentration as low as 0.02%. Oxygen content in the air of the respirometers was normally maintained above 20% but was occasionally depleted as low as 19% when animals were active in the respirometers. No CO_2 absorbent was used, and thus carbon dioxide levels rose to 1–2%. Although this degree of hypercapnia may have produced slight hyperventilation (Table 1), it was assumed to have had very little effect on oxygen consumption, as these animals are markedly resistant to even severe hypercapnia (see results). All animals were placed in the respirometer 24 h before readings commenced and at least ten oxygen consumption measurements on each of five animals were taken over the subsequent 24–48 h.

Haemolymph was sampled anaerobically from the five largest animals by hypodermic puncture through sites prepared at least 2 days previously. Prebranchial haemolymph was sampled from the anterior abdominal cavity via a hole drilled in the adopted shell above the soft abdomen. Postbranchial haemolymph was sampled through a small hole drilled through the carapace above the pericardium lateral to the heart. The original hole did not penetrate the epidermis, and was sealed with latex rubber sheet and marked so that it was visible through the slot cut in the shell (Fig. 1). These procedures allowed sampling of haemolymph from animals which invariably withdrew into the shell as the operators approached. Sampling, however, was difficult, and these small animals rarely yielded more than 200 μ l per sample. Nonetheless this small sample usually allowed measurement of oxygen tension (P_{O_2}), oxygen content (C_{O_2}), pH and carbon dioxide content (C_{CO_2}), but the blood used for C_{O_2} and C_{CO_2} measurements had at times previously been used to monitor pH and P_{O_2} respectively. In all cases great care was taken to avoid contamination with air.

Haemolymph samples were collected in ice-cold 1.0 or 0.25 ml syringes and maintained on ice for the few minutes preceding analysis. P_{O_2} and pH were measured with Radiometer microelectrodes mounted in cuvettes which were thermostatically controlled at the experimental temperature. CO_2 was measured on 40 μ l samples injected into a Lex- O_2 -Con oxygen analyser with technique modified as in McMahon *et al.* (1978b). C_{CO_2} was measured on 40 μ l samples by the method of Cameron (1971). P_{CO_2} was calculated from pH and C_{CO_2} using the Henderson–Hasselbalch equation in the form

$$pH = \frac{pK'_1 + \log [CO_2] - (\alpha CO_2 \cdot P_{CO_2})}{\alpha CO_2 \cdot P_{CO_2}}$$

Bicarbonate [HCO_3^-] and carbonate [CO_3^{2-}] concentrations were calculated from the Henderson-Hasselbalch equations in the form:

$$\text{pH} = \frac{\text{p}K'_1 + \log [\text{HCO}_3^-]}{\alpha\text{CO}_2 \cdot P_{\text{CO}_2}} = \frac{\text{p}K'_2 + \log [\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}.$$

The constants $\text{p}K'_1$, $\text{p}K'_2$ and αCO_2 were estimated at appropriate temperature and ionic content using the nomograms prepared for *Carcinus* haemolymph by Truchot (1976a).

Oxygen equilibrium curves were plotted both from the *in vivo* blood samples and from *in vitro* results from a pooled sample of haemolymph (0.6 ml of prebranchial haemolymph taken from the abdomen of each of five animals anaesthetized and removed from the shell as described above). The haemolymph clot was broken down by gentle homogenation in a tissue blender, then removed by centrifugation. The haemolymph was then halved and one half equilibrated with an appropriate CO_2 - N_2 gas mixture, the other with an equivalent amount of CO_2 in air. Oxygen equilibrium curves were then determined by a method based on that of Lenfant & Johansen (1965), where percentage saturation of haemocyanin was varied by taking appropriate ratios of oxygenated to deoxygenated serum anaerobically into a glass syringe and mixing the contents thoroughly using a mercury drop. Oxygen tension was then measured on the resultant mixture.

Oxygen equilibrium curves were determined at 15, 20, 25 and 30 °C (P_{CO_2} held constant at 6 torr) and at 3, 6, and 9 torr P_{CO_2} (temperature held constant at 25 °C). pH and C_{CO_2} were measured on aerated and deoxygenated sera at each temperature and P_{CO_2} level. These latter data were used to determine the CO_2 dissociation curve and buffering capacity of haemolymph.

To assess the effects of enforced dehydration on haemolymph volume and concentration 5 animals were weighed daily for several weeks before, and after, a 7-day period when access to water was denied.

Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and Cu^{2+} levels were determined on pooled haemolymph using a Jarrell Ash 850 Atomic Absorption Spectrophotometer. Cl^- levels were measured using a chloride sensitive electrode (Orion 94-17A).

Significance levels where quoted were determined using a Students *t* test (1-tailed).

RESULTS

The relationship between changes in impedance recorded between electrodes spanning each scaphognathite channel together with a recording of pressure changes in the left branchial cavity is shown in Fig. 2A. The branchial pressure trace shows a rhythmic biphasic pressure fluctuation, the frequency of which corresponds exactly with that of the ipsilateral scaphognathite impedance record. Normally both upward and downward movements of the scaphognathite cause equivalent pressure changes and probably move equivalent amounts of air. The pressure fluctuations are small ($< 0.5 \text{ mm H}_2\text{O}$) as might be expected from an air-breathing system, but the pressure waveforms are essentially similar to those recorded from aquatic decapodan crustaceans (Hughes, Knights & Scammel, 1969; McMahan & Wilkens, 1975, 1977). Occasional large sub-ambient pressure pulses were noted in the pressure records

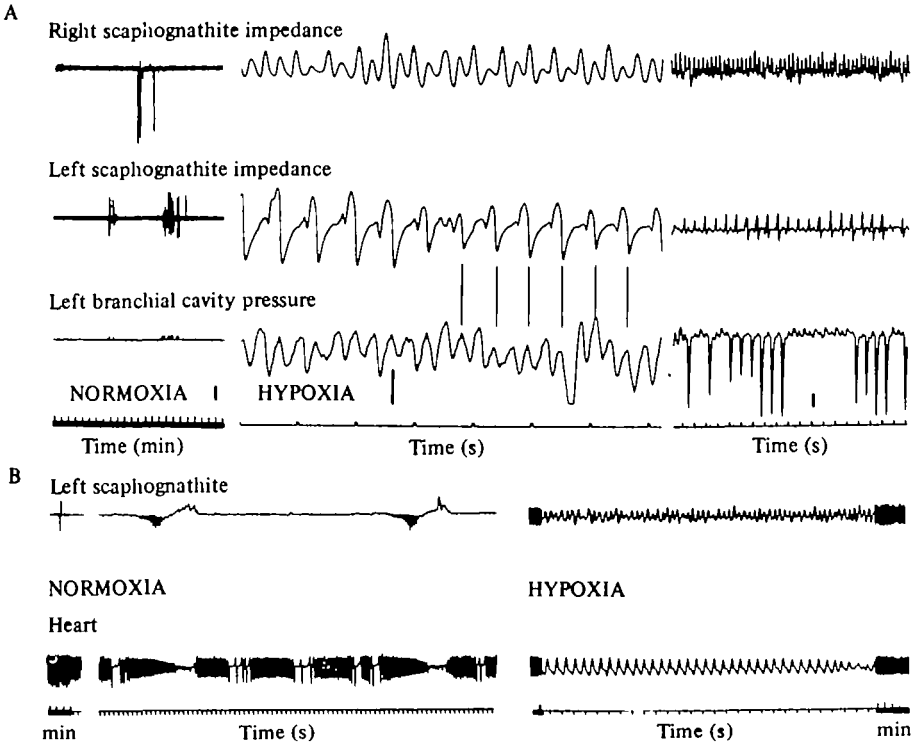


Fig. 2. (A) Impedance waveforms recorded from both left and right scaphognathite channels simultaneously with left branchial pressure demonstrating the close correlation between ipsilateral scaphognathite impedance and pressure waveforms, and a marked increase in scaphognathite pumping in response to hypoxia ($P_{I, O_2} < 20$ torr). During hypoxia the branchial pressure waveform shows large sub-ambient pressure pulses occurring intermittently, but always in a particular phase of the scaphognathite cycle. (B) Changes in heart and scaphognathite rates and patterns in *Coenobita* breathing air and a hypoxic gas mixture ($P_{O_2} < 20$ torr). Vertical bars = 0.1 cm H₂O.

(Fig. 2A). Although these occurred irregularly, they always occurred within a particular portion of the ipsilateral scaphognathite cycle. No change was noted in the scaphognathite impedance waveform, however (Fig. 2A) and these pulses are probably not caused by the action of the scaphognathite alone but more probably result from movement of the flexible walls of the posterior branchiostegites. Bursts of such 'branchiostegal' pumping movements were observed in animals whilst removed from the shell but pressures were not recorded under these conditions. Movements of the branchial chamber walls were not visible inside the adopted shell and thus these movements cannot be conclusively linked to the pressure pulses observed. Movements of the animals within the shell and also locomotor movements could also be detected at times on both pressure and impedance records.

Simultaneous recordings were also made of impedance changes resulting from heart activity (Fig. 2B). In quiescent, normoxic animals the heart beats continually but with a regularly occurring arrhythmia. Bursts of scaphognathite pumping are apparently accompanied by suppression of heart activity (see normoxic portion of Fig. 2B).

Table 1. Frequency of heart (H) and scaphognathite (sc) pumping recorded(Figures given are means \pm S.E.M. (n) observations on (3) animals.)

P_{O_2} range (torr)	f_{sc} (beats.min ⁻¹)	f_H (beats.min ⁻¹)	n
(A) Recorded simultaneously at three levels of progressive oxygen depletion.			
140 = air	1.5 \pm 0.4	120 \pm 4	(17)
80-120	8.6 \pm 1.0	126 \pm 3	(10)
20-80	30.5 \pm 5	121 \pm 3	(55)
< 20	92 \pm 8.5	83 \pm 5	(18)
(B) Recorded at three levels of progressive hypercapnic exposure.			
0.2 = air	< 1	108 \pm 4	(27)
7-33	4 \pm 1.5	120 \pm 3	(9)
33-99	16 \pm 4	121 \pm 2	(8)
> 120	22 \pm 10	119 \pm 2	(11)

Responses to hypoxia

Responses of the heart and scaphognathite pumps to progressive hypoxia were studied in five experiments on three animals. Animals breathing room air demonstrated intermittent bursts of scaphognathite pumping. These could involve one or both scaphognathites, and were of variable frequency and duration (Fig. 2). Mean heart and scaphognathite frequencies at three levels of hypoxia are presented in Table 1A. In all animals increased frequency of scaphognathite pumping occurred as the ambient oxygen tension fell, but in moderate hypoxia (P_{I,O_2} = 50-120 torr) pumping was still intermittent and irregular. Below P_{O_2} 20-50 torr, however, scaphognathite pumping became more or less continuous as seen in the latter (hypoxic) portions of Fig. 2A and B. The incidence of high amplitude, sub-ambient pressure pulses increased dramatically during severe hypoxic exposure (Fig. 2A).

The frequency of heart pumping remained relatively constant as P_{I,O_2} decreased to approximately 20 torr. Between P_{I,O_2} 20-120 torr cardiac arrhythmia occurred less often, perhaps resulting in a slight overall increase in frequency. Below P_{I,O_2} = 20 torr the heart pumped continuously (Fig. 2B) but at a reduced frequency (Table 1).

Responses to hypercapnia

Six experiments on four *Coenobita* demonstrated a remarkable tolerance to hypercapnia. In one case 50% carbon dioxide in air did not cause anaesthesia over a 2 h exposure. The animals clearly detected high concentrations of CO₂ since after 10-20 min exposure at 5-10% CO₂ they became active, presumably seeking pure air. However, in three animals tested, relatively little effect was seen on heart rates. A small tachycardia, again associated with the loss of the cardiac arrhythmia, occurred initially (Table 1b). Increased scaphognathite pumping frequency was also noted (Table 1b). In both cases the changes observed could have resulted directly from hypercapnia or could be attributed to the resultant increased activity.

All animals tested showed increased frequency of scaphognathite pumping (Fig. 2A), and occasionally tachycardia, as ambient CO₂ levels decreased following severe (> 20% CO₂) hypercapnia. The responses were of variable intensity and duration but consisted of one or more periods of intense ventilation often accompanied by

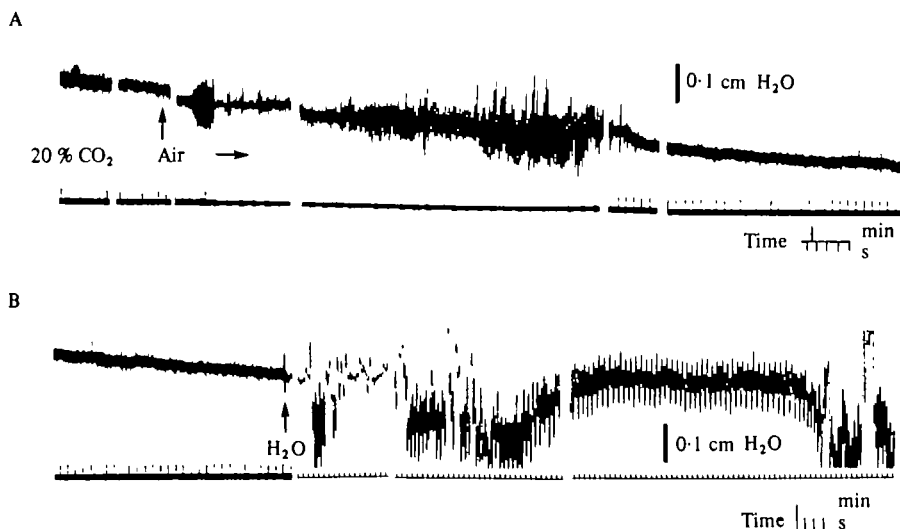


Fig. 3. (A) Branchial pressure recorded from *C. clypeatus* during and immediately following exposure to 20% CO₂ in air, showing increased incidence of scaphognathite pumping following hypercapnia. (B) Branchial pressure recorded from *C. clypeatus* at rest and after sprinkling with water, showing increase in f_{sc} and in incidence of large sub-ambient pressure pulses.

increased heart pumping. Hyperventilation following severe CO₂ exposure might last for 1–2 h before pre-hypercapnic rates were re-established.

Distinct hyperventilation was invariably seen in response to wetting the animals by lightly sprinkling their shells with water. This caused an immediate and often sustained increase in activity which often led to drinking. The incidence and duration of bouts of scaphognathite pumping increased markedly (Fig. 3 B), and a large number of high-amplitude (branchiostegal) pumping movements were also seen. It is not possible at this time to separate the effects of wetting from the resultant activity. Any increase in activity is always associated with increased scaphognathite pumping but the increases are very variable and have not been recorded over sufficiently long periods to be quantified here.

Under the conditions tested sudden movements or vibrations caused the animals to retract rapidly into their shells. Apart from the movement disturbance of the pressure record, no definite 'withdrawal' responses could be observed. The heart and scaphognathite pumping frequencies given for normoxic animals (Table 1) are invariably those of animals sequestered in the shell. At room temperature (23 ± 2 °C) the animals in fact rarely moved unless wetted, or exposed to severe hypoxic or hypercapnic stimuli.

Oxygen consumption

Oxygen consumption (\dot{M}_{O_2}) was measured at 25 °C. \dot{M}_{O_2} levels measured from quiescent animals ($0.0205 \text{ mmol. O}_2 \cdot \text{kg}^{-1} \text{ min}^{-1}$) correspond closely with those for other air-breathing decapods including *Birgus* (Cameron & Mecklenburg, 1973), *Gecarcinus* and *Cardisoma* (Cameron, 1975) and *Ocyropde quadrata* (Burnett, 1978). Activity was noted occasionally in all animals but measurements from active animals

are not included in the value given above. The \dot{M}_{O_2} rates from active animals, however, show that oxygen consumption can be increased by a factor of at least 5 over the mean inactive levels. The \dot{M}_{O_2} values shown here were from animals of 20–40 g mass and fit closely the relationship between \dot{M}_{O_2} and body mass presented for *Coenobita brevimanus* by Burggren (1975).

In vivo measurements

Mean P_{O_2} , C_{O_2} , C_{CO_2} and pH values for both post- and prebranchial haemolymph samples from 5 animals are presented in Table 2. Very low oxygen tensions were measured in both postbranchial ($P_{a,O_2} = 14$) and prebranchial ($P_{v,O_2} = 8$) haemolymph. However, postbranchial oxygen contents were extremely high when compared with other crustaceans ($C_{a,O_2} = 1.34 \text{ mmol.l}^{-1}$) and even at these low oxygen tensions haemolymph gained $0.85 \text{ mmol.O}_2.\text{l}^{-1}$ in transit through the gills.

Cardiac output was estimated from mean oxygen consumption and mean pre- and postbranchial haemolymph oxygen contents using the Fick principle. The oxygen consumption value quoted above was for resting animals; a condition not exactly matched during haemolymph sampling in the present study. Our animals were not active in the locomotor sense but equally were not 'resting', being disturbed by handling and presumably performing isometric work to maintain position within the shell. For this reason the value for \dot{M}_{O_2} used in the Fick calculations ($0.0324 \text{ mmol.kg}^{-1}.\text{min}^{-1}$) represents a mean value for all measurements (i.e. active as well as inactive animals) as the authors feel this value may more accurately represent the oxygen consumption range exhibited by our animals at the time of sampling. Used in the Fick calculation this value yields a cardiac output estimate of $38 \text{ ml.kg}^{-1}.\text{min}^{-1}$. The estimated haemolymph convection requirement was thus $1.17 \text{ l.mmol}^{-1}.\text{O}_2$ and the efficiency of oxygen extraction by haemolymph ($E_b = \dot{M}_{O_2}/\dot{V}_b.C_{a,O_2}$) was 0.64.

The *in vivo* values for P_{O_2} and C_{O_2} (Table 2) were plotted in the form of an oxygen equilibrium curve (Fig. 4A). Despite the extremely low oxygen tensions measured (see Table 2) postbranchial haemolymph was 60–80% saturated and prebranchial haemolymph 20–40% saturated with oxygen, allowing 40–60% of the haemolymph oxygen content to be released to the tissues under these conditions.

In vitro haemolymph oxygen and carbon dioxide binding

One day subsequent to the *in vivo* sampling described above, 3 ml haemolymph (approx. 0.6 ml from each animal) were drawn from the same five animals. Complete oxygen equilibrium curves determined at P_{CO_2} 3, 6 and 9 torr are shown in Fig. 4B. At the sampling temperature of 25°C and at $P_{CO_2} = 6$ torr (equivalent to *in vivo* levels), *in vitro* P_{50} was 5.5 torr, pH_{50} was 7.711 and the slope of the line $\Delta P_{50}/\text{pH}$ was -0.84 ($\Delta P_{50}/\text{pH}$ unit = 16 torr). With P_{CO_2} held constant and temperature varying over the range $15\text{--}35^\circ\text{C}$, $\Delta P_{50}/\Delta t$ equalled $0.037 \text{ torr}/^\circ\text{C}$.

The values for *in vitro* oxygen affinity are higher than that obtained at similar temperature *in vivo* on the same animals a day earlier. *In vitro* haemolymph oxygen content was also lower by 25–30%. These discrepancies were rationalized as follows. In the course of *in vivo* sampling slots were drilled in the animals' shells. These allowed loss of fluid stores from the shell. To compensate for this and to prevent desiccation the animals were placed in 0.5 cm of fresh water overnight. They clearly

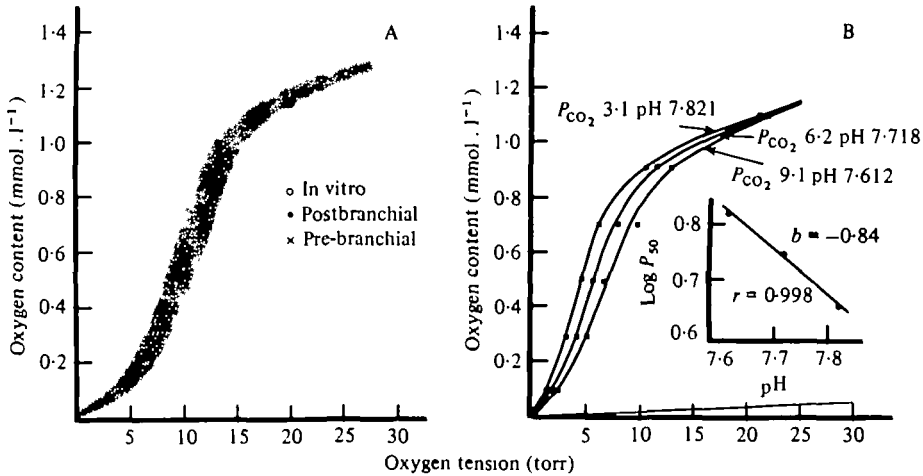


Fig. 4. (A) *In vivo* oxygen equilibrium curve for *Coenobita* haemolymph. Data (from five animals) normalized to constant (average) haemolymph oxygen capacity. Stippled area drawn by eye. (B) Oxygen equilibrium curves determined *in vitro* for pooled (five animals) *Coenobita* serum. Inset figure shows the relationship between ΔP_{50} and pH. Individual pH values included were measured at 50% oxygen saturation of serum. $t = 24.5^\circ \text{C}$.

absorbed the fresh water in quantity and thus underwent considerable haemodilution, which could have been responsible for the observed changes in both oxygen content and oxygen affinity (see Discussion).

The occurrence of haemodilution was confirmed by preliminary analysis of the ionic content of pooled haemolymph used in the preparation of the oxygen equilibrium curves. This analysis showed an average 20% reduction in Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} and Cl^- concentrations when compared with a similar pooled sample taken previously.

In order to ascertain whether such changes in haemolymph concentration might occur under 'natural' conditions, a group of five animals (without shell slots) were weighed daily for periods of 1 month prior to, and following, a 7-day period over which access to water was denied. Spontaneous variation in body weight rose as high as 12.5% ($\bar{x} = 10.5 \pm 2.0$ S.D.) in animals with free access to water, while in animals which were allowed water following a 7-day 'dry' period, increases of up to 22% ($\bar{x} = 20.2 \pm 1.3$ S.D.) were observed. Assuming that water comprises 70% of the animal this would mean that body volume varies 15% (15 ± 3 S.D.) with free access to water and greater than 30% (30 ± 2 S.D.) under conditions of rehydration following periods of dehydration. Assuming some protection of the cellular environment actual changes in haemolymph volume, and hence ion and haemocyanin concentrations, may occur naturally in this animal.

Carbon dioxide dissociation curves determined over the P_{CO_2} range 1–13 torr are shown in Fig. 5(a). Values for both oxygenated and deoxygenated *Coenobita* sera are included as no significant Haldane effect could be ascertained. pH was also measured at each P_{CO_2} level. Fig. 5(b) shows the relationship between bicarbonate and pH. The slope of this line gives the non-bicarbonate buffering capacity (15.99 m-equiv. HCO_3^- /pH unit) of *Coenobita* haemolymph.

Table 2. Post- and prebranchial haemolymph oxygen, carbon dioxide and pH values sampled from five *Coenobita* in room air at P_{i,O_2} 139-141 torr ($\bar{x} \pm$ S.E.)

	P_{O_2} (torr)	C_{O_2} mmol.l ⁻¹	pH	C_{CO_2} mmol.l ⁻¹	P_{CO_2} calc (torr)	HCO_3^- calc (m-equiv.l ⁻¹)	CO_3^{2-} calc (m-equiv.l ⁻¹)	CO_2 calc (mmol.l ⁻¹)
Postbranchial haemolymph	13.7 ± 0.09	1.34 ± 0.22	7.84 ± 0.08	11.7 ± 0.6	4.1	11.05	0.46	0.16
Prebranchial haemolymph	8.4 ± 1.1	0.49 ± 0.18	7.656 ± 0.12	13.0 ± 0.6	6.8	12.40	0.33	0.27
Postbranchial prebranchial difference	5.3	0.85	—	-1.3	-2.7	-1.35	0.15	-0.11

Table 3. Comparative oxygen, carbon dioxide and acid-base data for crabs from sub- to supralittoral habitats

Habitat	Temp. range (°C)	Oxygen affinity			$C_{H_2O}^{max} O_2$ (mmol.l ⁻¹)	$[HCO_3^-]$ (m-equiv. l ⁻¹)	CO_2 capacitance ΔC_{CO_2} (mmol. l. P_{CO_2})	Buffer value (β) ΔHCO_3^- (m-equiv/ pH unit)	Source
		$\Delta P_{50}/pH$ (torr)	$\Delta P_{50}/\Delta t$ (torr)	$\Delta P_{50}/\Delta t$ (torr)					
Cancer magister	7-17	30	1.0	—	—	2.5*	4	McDonald (1977)*, McMahon <i>et al.</i> (1978)	
Carcinus maenas	10-30	20 ¹	0.65 ¹	3.9 ³	3.9 ³	1.4-3.2 ³	7 ³	¹ Truchot (1975 a), ³ Truchot (1976b)	
Coenobita clypeatus	15-35	16	0.37	11.7	11.7	0.6	16	Present study	
Birgus latro	—	—	—	14.1	14.1	0.4	16	Cameron & Mecklenburg (1973)	
Gecarcinus	—	12	—	—	—	—	—	Redmond (1968)	

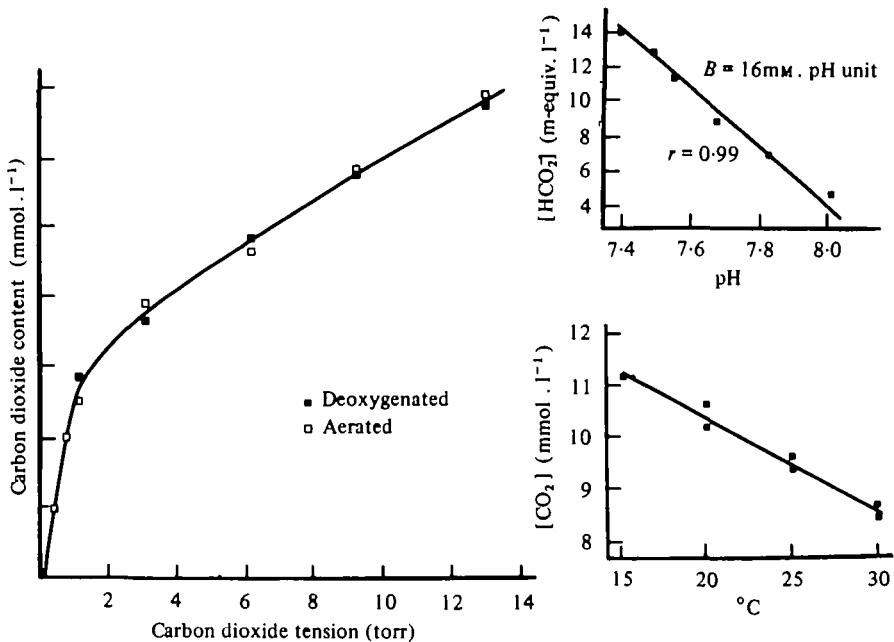


Fig. 5. (A) CO₂ dissociation curve for *Coenobita* serum pooled from five animals. Data for oxygenated and deoxygenated sera included. (B) Relationship between HCO₃⁻ content and pH in *Coenobita* serum. Slope of the line indicates non-bicarbonate buffering capacity, $\beta = 16$ m-equiv HCO₃⁻/pH unit. (C) Effects of temperature on haemolymph pH *in vitro*. P_{CO_2} held constant at 6.2 torr throughout.

DISCUSSION

Most coenobitid crabs differ from the more frequently studied *Birgus latro* in that the adults continue to inhabit molluscan shells, which protect them from the rigours of the terrestrial habitat. In many species, i.e. *C. clypeatus* (present study) and *C. brevimanus* (Gross, 1964), a store of fluid is maintained in the shell. This fluid, which is roughly isosmotic with haemolymph (Gross, 1964), may be pumped into the gill cavities (Bliss, 1968), serving to humidify the gills and to provide a reservoir of water and of salts for ionoregulation. Harms (1932) reports that *C. clupeatus* (here assumed to be synonymous with *C. clypeatus*) can be adapted to live without the shell, but that this occurs rarely in the natural habitat. Normally then, the animals live in a portable fluid incubator. This discussion will examine the modifications involved in this mode of life, as compared with that of *Birgus* and the terrestrial brachyurans such as *Gecarcinus*, to ascertain whether *Coenobita* exhibits a lesser degree of adaptation to the terrestrial habitat.

In the terrestrial crabs *Birgus* (Cameron & Mecklenburg, 1973), *Gecarcinus* and *Cardisoma* (Cameron, 1975) there is only a modest increase in f_{80} in response to hypoxia, but a marked increase occurs in response to even moderate hypercapnia. These ventilatory responses are typical of terrestrial animals. Since air is a respiratory medium rich in oxygen and rarely hypoxic, there are few selection pressures to maintain a sensitive ventilatory feedback system responding primarily to hypoxia. On the other hand, terrestrial animals have elevated blood P_{CO_2} levels due to difficulties inherent in

aerial CO₂ extraction from gills or lungs, and have evolved a CO₂-sensitive control over ventilation to minimize acid-base disturbances. In *Coenobita* increase in f_{sc} occurred routinely in response to hypoxia, but less markedly in response to hypercapnia, with definite increase occurring only at non-physiological levels of CO₂. Such responses more closely resemble those of marine Crustacea (McMahon & Wilkens, 1975, 1977; Butler, Taylor & McMahon, 1978; Jouve & Truchot, 1978; McMahon, Butler & Taylor, 1978a; Batterton & Cameron, 1978; Burnett, 1978), than the terrestrial forms. Marine organisms rarely, if ever, encounter hypercapnia, and may be unable to regulate carbon dioxide levels and acid-base balance through changes in gill ventilation, consequently succumbing to quite low levels of CO₂. Although the ventilatory sensitivity of *C. clypeatus* to the two respiratory gases is perhaps more similar to that of aquatic forms, this anomuran has a clearly elevated blood P_{CO_2} and bicarbonate concentration relative to sublittoral or littoral crabs (Tables 2, 3), and apparently has evolved the tolerance of high CO₂ levels more characteristic of terrestrial forms. The marked resistance of *C. clypeatus* to hypercapnia is demonstrated by the failure of long exposures to 50% CO₂ to cause anaesthesia. That the gas does penetrate gills is suggested by the marked increase in scaphognathite pumping which occurs to 'blow off' CO₂ when this severe hypercapnic exposure is terminated. The extent of, and mechanisms involved in, this CO₂ tolerance, however, remain to be fully elucidated.

Increase in scaphognathite pumping, whether resulting from hypoxia, hypercapnia or wetting, is usually associated with a high incidence of subambient pressure pulses in the branchial cavity (Fig. 2A) which could also increase ventilation. Although clearly related to scaphognathite pumping, these pulses may not arise from scaphognathite action directly, but may be associated with pumping movements of the branchiostegite, which were first noted by Harms (1932). The intermittent occurrence of these pressure pulses in a fixed relationship within the scaphognathite cycle is strongly reminiscent of the situation described for reversed scaphognathite pumping in *Homarus americanus* by Wilkens & McMahon (1972). *Homarus* clearly shows a large negative-going pressure wave-form associated with contraction of the epimeral retractor muscles at a fixed point in each reversed scaphognathite beat. Similar muscle systems have been described for *C. clypeatus* (Harms, 1932) and apparently served to inflate the posterior part of the branchial cavity. It is thus possible that the negative-going pressure pulses occurring in *C. clypeatus* are homologous with the reversed pumping of aquatic macrurans. In *Coenobita*, branchiostegal pumping presumably forcefully ventilates the gills and perhaps also serves to ventilate the shell air space. Its greatly increased occurrence in animals which have been wetted (i.e. in simulated rain) may suggest an accessory role: that of pumping external water back through the branchial cavity and into the shell. Reversed flow of water, i.e. entering the exhalant opening anteriorly would be a necessity here, but a reversed flow of air would also be of advantage to an animal most of whose time is spent retracted into the shell, as this would allow fresh external air to enter the branchial cavity anteriorly. Movements of the carapace were also noted in *Birgus* by Cameron & Mecklenburg (1973) but apparently had little or no influence on air flow in this species.

In accordance with the primary reliance of *Coenobita* on aerial gas exchange, *in vivo* haemolymph oxygen, carbon dioxide and pH levels fall within the range of those reported for other terrestrial crustaceans (see Table 4). Terrestrial crabs usually

possess more haemocyanin of a higher affinity than that of marine species. The reasons for this are not fully clear but Redmond (1968) suggested that the reduced gill area (and also thickened respiratory surface), which are characteristic of the gills of land crabs (Pearse, 1929; Gray, 1957; Bliss, 1968) and which primarily serve to reduce water loss by evaporation, result in a diffusion, rather than perfusion, limited gas exchanger. Compensation for this occurs by perfusing the gills with a high affinity pigment that can be fully oxygen-saturated at low oxygen tension. It is also clear that animals which ventilate intermittently would further reduce ventilatory water loss if they could almost completely deplete the oxygen stored in the branchial cavity gas. In a non-counter-current system such as we assume to occur (at least between scaphognathite bursts) in these land crabs this can only be achieved by the presence of an extremely high affinity haemocyanin. A high oxygen capacity in the haemolymph decreases the haemolymph convection requirement, increases the residence time in the gills thus facilitating O_2 and CO_2 exchange, and also facilitates oxygen uptake by maintaining a high oxygen gradient across the gills. In conjunction with the large haemolymph volume of the crustacean open circulatory system, the high haemolymph oxygen capacity also provides a considerable oxygen store. Assuming a haemolymph volume of 30% (Alspach, 1972) and prebranchial haemolymph oxygen content of 0.49 mmol.l^{-1} a 30 g *Coenobita* has a maximum venous oxygen reserve sufficient to last for 7 min at resting M_{O_2} levels. This could be important in animals which burrow extensively and could also be useful during sporadic immersions into water where oxygen availability and oxygen uptake across the gills may both be considerably reduced. A final reason for the high oxygen affinity is that the animals must occasionally contend with ambient temperatures of 40°C or more. In order for the pigment to be useful under these circumstances it must either be temperature-insensitive or must have a very high affinity at 20°C . In a burrowing animal subject to fluctuations in ambient carbon dioxide levels a similar rationale might apply for a decreased Bohr effect. The data of Table 3 suggest reduced $\Delta P_{50}/\Delta \text{pH}$ ratios for *Coenobita* and other air-breathing animals when compared with littoral and marine species, but the data in this table were not determined under identical conditions and thus allow only qualitative comparison. It is interesting to note that in *Birgus latro*, Cameron & Mecklenberg (1973) measured markedly lower oxygen affinity and higher circulating oxygen tensions than those seen in *Coenobita* or normally found in other terrestrial crabs. *Birgus* has an extremely well-developed 'lung' (Harms, 1932) which presumably allows enhanced oxygen uptake in this species.

A linear relationship has been demonstrated between protein (\equiv imidazole) buffering capacity and haemolymph oxygen capacity in two crab species; *Carcinus maenas* (Truchot, 1976*b*) and *Cancer magister* (McDonald, 1977). This relationship is supported and extended by the much higher oxygen capacity ($1-1.5 \text{ mmol.O}_2.\text{l}^{-1}$) and non bicarbonate buffer capacity ($\beta = 16 \text{ m-equiv HCO}_3$) measured in *Coenobita*. P_{CO_2} levels are high in terrestrial species (see earlier discussion) and compensation for this respiratory acidosis may occur by an increase in haemolymph bicarbonate. Manipulation of haemolymph bicarbonate levels has been shown to play a major role in compensatory responses to environmental variation in Crustacea, i.e. in response to hypoxia (Truchot, 1975*a, b*; McMahon *et al.* 1978*a*); temperature variation (McMahon *et al.* 1978*b*); emersion and immersion (Truchot, 1975*c*) and recovery from exercise (McMahon, McDonald & Wood, 1977). Table 3 shows that bicarbonate

levels increase with increasing terrestrial exposure in littoral crabs as was suggested by Kokubo (1930) for fish species. Thus increase in both bicarbonate and non-bicarbonate buffering capacities may be important in compensatory responses of terrestrial crabs such as *Coenobita* which are necessarily exposed to greater variability in their environment.

Desiccation is possibly the most important environmental stress encountered by the terrestrial decapod Crustacea. The highly terrestrial *Gecarcinus* may lose 0.23% body wt. h⁻¹ when deprived of water, reaching a lethal 30% body water loss in 4 days (Bliss, 1966; Harris, 1977). Other terrestrial forms lose water more rapidly, e.g. *Sudanautes* 0.30%, *Ocyropde* 0.63% body wt. h⁻¹ (Lutz, 1969). Rates of water loss are markedly lower (0.08% body wt. h⁻¹) in *Coenobita* demonstrating the efficacy of the adopted shell in reducing water loss. Nonetheless up to 15% water loss occurs in a 7-day 'dry' period and body water and haemolymph ion and haemocyanin concentrations may vary as much as 25–30% following rehydration. These changes pose particular problems for the respiratory system. Haemodilution decreases oxygen content (at constant P_{O_2}), increases the blood convection requirement, and thus reduces the efficiency of oxygen uptake across the gills. Our *in vitro* studies demonstrated that oxygen affinity increased with haemodilution, perhaps to compensate for these effects. A similar increase in oxygen affinity of haemocyanin was observed on dilution of the medium and thus of the haemolymph in *Carcinus* (Truchot, 1975a) and in the Xiphosuran *Limulus polyphemus* (Mangum *et al.* 1976) but the mechanisms involved remain obscure.

Carbon dioxide dissociation curves for *Coenobita* are similar to that described for *Birgus latro* (Cameron & Mecklenburg, 1973) and for those from the essentially submarine and littoral brachyurans *Cancer magister* (McDonald, 1977) and *Carcinus maenas* (Truchot, 1976b), except that carbon dioxide contents are higher, and CO_2 capacitance ($\Delta C_{CO_2}/\Delta P_{CO_2}$) lower in terrestrial forms, when compared over each species' physiological range (Table 3). This can be correlated with the very low, stable, P_{CO_2} levels found in aquatic forms and may also be correlated with the tendency of aquatic decapods to regulate acid-base status in response to environmental change by change of CO_2 content rather than CO_2 tension (McMahon *et al.* 1978a, b). In contrast, air-breathing forms have the ability to regulate P_{CO_2} tension while holding CO_2 content relatively constant.

Comparison of the respiratory physiology of *Coenobita clypeatus* with that of *Birgus latro*; of the more fully terrestrial Brachyura *Gecarcinus* and *Cardisoma*, and of the sublittoral and littoral species, indicates that *Coenobita* does show a high degree of terrestrial adaptation, as might be expected in a species often found considerable distances from the sea. However, in some features, including a predominantly hypoxic, rather than hypercapnic ventilatory drive, and a relatively large Bohr shift (compared to terrestrial forms), it has apparently retained some aquatic tendencies. These may be related to the animal's ability to carry some of the aquatic environment with it within the confines of the adopted shell. Retention of the shell is also important in the reduction of water loss which is lower in *Coenobita* than in other terrestrial decapod species.

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