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THE RESPIRATION OF *CANCER PAGURUS* UNDER NORMOXIC AND HYPOXIC CONDITIONS

BY S. M. BRADFORD AND A. C. TAYLOR
Department of Zoology, University of Glasgow, Glasgow, G12 8QQ

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SUMMARY

The respiration of *Cancer pagurus* under normoxic conditions and its respiratory responses to hypoxia are described. Respiration of quiescent crabs is characterized by a rhythmic pattern of ventilation and cardiac activity in which periods of apnoea and bradycardia of approximately 5 min duration alternate with longer periods of active ventilation and cardiac activity. The significance of this rhythmic ventilatory behaviour is discussed and evidence is presented to account for this behaviour in terms of allowing energy savings to be made during periods of inactivity. During a ventilatory pause the P_{O_2} of the post-branchial blood falls from its normal level of 94 ± 5 torr to only 24 ± 3 torr. The blood of *Cancer* provides a store of oxygen which is used during pausing to maintain aerobic metabolism. Anaerobic metabolism does not appear to contribute significantly to energy production during these periods since no increase in the blood lactate concentration was recorded.

Cancer haemocyanin has a high oxygen affinity (P_{50} = 5–10 torr) and exhibits a large, positive Bohr shift ($\Delta \log P_{50}/pH = -1{\cdot}18$). However, under normal conditions the pigment has only a minor role in supplying oxygen to the tissues, since over 91 % is carried in solution.

Cancer pagurus exhibits quite a high degree of respiratory independence and is able to maintain its rate of oxygen consumption approximately constant over a wide range of ambient oxygen tension, down to a P_{O_2} of 60–80 torr, below which it declines. Similarly there was little change in heart rate during hypoxia until a P_{O_2} of 20–40 torr was reached below which it also declined sharply. Oxygen consumption during hypoxia was maintained primarily as a result of an increase in ventilation volume and oxygen extraction. During hypoxia the P_{O_2} of both the pre- and post-branchial blood declined and resulted in a reduction in the P_{O_2} gradient across the respiratory surface (ΔP_{O_2}). Oxygen uptake during hypoxia was facilitated, however, by an increase in the transfer factor (T_{O_2}).

INTRODUCTION

The respiratory physiology of *Cancer pagurus* has been the subject of a number of studies. Ansell (1973) described a daily rhythm of oxygen consumption and heart rate in this species and studied the effects of starvation on active and resting metabolic rates as well as its effect on this daily rhythm. Aldrich (1975) found a 20-day rhythm of oxygen consumption and also investigated the effects of feeding and handling on

this rhythm. More recently Bottoms (1977) and Burnett & Bridges (1981) have described the existence of short term rhythmic patterns of respiration in *Cancer pagurus* in which periods of ventilation of the branchial chambers alternate with periods of apnoea and bradycardia.

Although these studies have made important contributions to our knowledge of the respiratory physiology of this species, a comprehensive study of respiration in *C. pagurus* comparable to those of Johansen, Lenfant & Mecklenburg (1970) and McDonald, Wood & McMahon (1980) on *Cancer magister* has not been attempted. As part of a wider study of the respiratory physiology of *Cancer pagurus*, the present study is an attempt to provide a more complete description of the respiration of *C. pagurus* under normoxic conditions and of its respiratory responses during exposure to hypoxic conditions.

MATERIALS AND METHODS

The study was carried out on male *Cancer pagurus* (L.) obtained from the University Marine Biological Station at Millport, Isle of Cumbrae. The animals, weighing between 250–450 g, were kept in a recirculating sea-water aquarium at a temperature of 10 ± 1 °C and salinity of 32‰ and were subject to a 12 h light–dark regime. The animals were fed fresh mussels once a week but were starved for at least 2 days prior to use in experiments, since the nutritional state has been shown to have a pronounced effect on the rate of oxygen consumption (Ansell, 1973; Aldrich, 1975). All experiments were carried out at 10 °C.

Respiratory measurements

The respiratory characteristics of *C. pagurus* were studied using the method of Arudpragasm & Naylor (1964) as modified by Taylor (1976). Exhalant water was collected by placing a balloon mask over the anterior of the animal with a plastic mask underneath to prevent interference with the mouthparts and antennae. The balloon was sealed to the carapace using cyanoacrylate adhesive. Ventilation volume ($\dot{V}_\omega$) was measured by collecting the exhalant water in a measuring cylinder for a known period of time.

The P_{O_2} of both inhalant and exhalant water was measured by slowly siphoning water past an oxygen electrode (Radiometer E5046) enclosed in a thermostatted cell at 10 °C. Oxygen consumption ($\dot{M}_{O_2}$) was calculated from the ventilation volume and the difference in oxygen content of the inhalant and exhalant water, using the Fick principle.

Heart and scaphognathite rates were recorded using the impedance technique (Taylor, 1976). Fine silver-wire electrodes were inserted through small holes drilled in the carapace on either side of the heart and, in the case of the scaphognathites, through the ventral surface of the branchial chamber lateral to the 3rd maxillipeds (Ansell, 1973). The electrodes were held in place using cyanoacrylate adhesive.

The responses of *Cancer* to hypoxia were studied by controlling the P_{O_2} of the inhalant water. This was achieved by bubbling gas mixture of varying O_2 : N_2 ratios through the water reservoir, the gas mixtures being supplied by a gas mixing apparatus.

Blood–gas analysis

Post-branchial blood samples (0·5 ml) were obtained from unrestrained animals acclimated to the desired P_{O_2}. Prior to the experiment a small hole was drilled through the carapace, dorsal to the heart, and was covered with a rubber cap using cyanoacrylate adhesive. The animals were then left undisturbed for 24 h prior to sampling. Samples were taken by inserting the hypodermic needle of a syringe through the rubber cap into the pericardial cavity. This method allowed blood samples to be taken without loss of blood and with minimal disturbance to the animals. Pre-branchial blood samples were taken directly into a syringe via the arthrodial membrane at the bases of the pereiopods. Blood sampling from animals in the respirometers proved more difficult and only post-branchial blood could be obtained without gross disturbance to the animals. Attempts were made to obtain blood samples using indwelling catheters but this proved difficult due to problems in positioning the catheters and with blood clotting in them once in place.

The P_{O_2} and pH values of the blood samples were measured using a Radiometer oxygen electrode (E5046) and a Radiometer capillary pH electrode (G299A) with a K172 reference electrode, both being housed in thermostatted cells at 10 °C. The total oxygen content of the blood (C_{O_2}) was determined using the technique of Bridges, Bicudo & Lykkeboe (1979). The lactate content of some post-branchial blood samples was measured using the method of Gutmann & Wahlefeld (1974).

The P_{CO_2} of pre- and post-branchial blood was determined by the Astrup method. Samples were tonometered in a Radiometer BMS II at 10 °C with gas mixtures, supplied by a gas-mixing apparatus, and the pH of the blood determined. *In vivo* P_{CO_2} values were obtained for each animal by interpolation from a graph of log P_{CO_2} *v.* pH, using the *in vivo* pH values previously determined.

The concentration of bicarbonate [HCO_3^-] and carbonate [CO_3^{2-}] ions in the blood was calculated from the pH and P_{CO_2} values using the Henderson–Hasselbalch equations in the forms

$$pK_1 = pH - \log \frac{[HCO_3^-]}{\alpha CO_2 . P_{CO_2}}, \quad pK_2 = pH - \log \frac{[CO_3^{2-}]}{[HCO_3^-]}$$

Values for the constants pK_1, pK_2 and αCO_2 were not determined for *Cancer* but were derived from the alignment nomograms given by Truchot (1976) for *Carcinus maenas*. As a result the values for [HCO_3^-] and [CO_3^{2-}] may be subject to slight error. The total carbon dioxide content of the blood (C_{CO_2}) was calculated as follows:

$$C_{CO_2} = [HCO_3^-] + [CO_3^{2-}] + [CO_2 \text{ diss}],$$

where [CO_2 diss] = dissolved CO_2 in the blood = $\alpha CO_2 \times P_{CO_2}$.

Oxygen equilibrium curves were determined on fresh blood samples which had been filtered through cheesecloth to remove coagulated proteins. The samples were tonometered at 10 °C in the Radiometer BMS II with gas mixtures supplied by the gas-mixing apparatus. The total oxygen content of 20 μl of blood was then determined using the technique previously described. To determine the extent of the Bohr effect the pH of the blood was varied by altering the P_{CO_2} of the gas mixture being supplied.

The pH of the blood sample was then determined at or near the P_{50}. P_{50} values of the blood at each P_{CO_2} were calculated from the Hill plot.

The notation and the respiratory indices used to characterise different components of the respiratory system are those detailed by McMahon, McDonald & Wood (1979) and by Taylor & Spencer Davies (1981). Values presented in both text and tables are means ± S.D.

RESULTS

Respiration measurements

A typical pattern of respiration exhibited by an individual animal throughout a 10 h period in the respirometer is shown in Fig. 1. Initially oxygen consumption ($\dot{M}_{O_2}$) was high but declined throughout the first 4 h to a lower, more constant level which represented the $\dot{M}_{O_2}$ of the animal when inactive. At intervals during this 4 h period the animal exhibited periods of activity during which $\dot{M}_{O_2}$ increased markedly to a level 3–4 times that recorded during quiescence.

Recordings of ventilation volume ($\dot{V}_\omega$) and oxygen extraction ($Extr_\omega$) made simultaneously with those of $\dot{M}_{O_2}$ showed that variations in the rate of oxygen consumption were more closely related to changes in ventilation volume than to changes in oxygen extraction. During periods of activity, the pronounced increase in $\dot{M}_{O_2}$ was associated with a concurrent increase in $\dot{V}_\omega$. Oxygen extraction, however, was generally low (between 6–12%) and increased only slightly during activity. In contrast to the variation in the rate of $\dot{M}_{O_2}$ recorded during this initial period the heart showed only minor fluctuations and was relatively unaffected by periods of activity.

During the quiescent phase all animals exhibited bilateral pumping usually with one scaphognathite beating at a slightly higher rate than the other. Reversals of beat were also observed but these were not always bilaterally synchronous. Both of these features have been observed in a number of other crabs and appear to be a characteristic of decapod respiration (Ansell, 1973; Arudpragasm & Naylor, 1964, 1966; Bottoms, 1977; McMahon & Wilkens, 1972, 1975, 1977).

Once the resting phase of respiration became fully established, quiescent animals in well-aerated water often exhibited a spontaneous rhythmic pattern of respiration characterized by periods of cardiac arrest and apnoea (Fig. 1). The rhythm of alternating periods of ventilation and apnoea was very regular with periods of apnoea of 5–7 min duration occurring approximately every 30 min, although some variation in this pattern was observed between individuals. Periods of apnoea and cardiac arrest are commonly termed ventilatory pauses (McMahon & Wilkens, 1972) and were only observed in completely quiescent animals. The rhythm would often continue for several hours until the animals became active, either spontaneously or by being disturbed.

Fig. 2 shows recordings of both scaphognathites and of the heart from an animal during a ventilatory pause of 5·5 min duration. The onset of the pause occurred suddenly and was marked by almost simultaneous cessation of beating of both the heart and the scaphognathites. During each period of apnoea there was a complete cessation of scaphognathite activity but occasional single heart beats were recorded, being observed most frequently as occurring in bursts towards the end of the pause

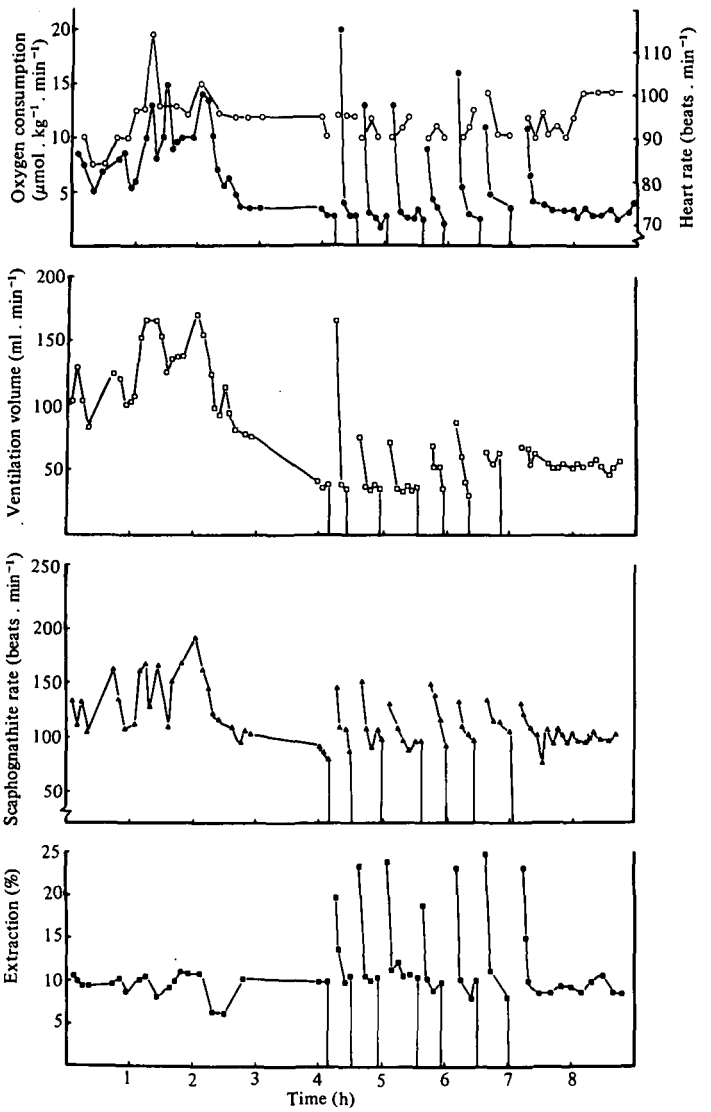

Fig. 1. Simultaneous recordings of oxygen consumption (●), heart rate (○), ventilation volume (□), scaphognathite rate (▲) and % extraction (■) from an individual *Cancer pagurus* over a 9 h period during which the animal exhibited rhythmic pausing behaviour for approximately 3 h.

Following the onset of pumping at the end of the pause, the heart rate returned to pre-pause levels almost immediately, and, in most cases, no 'overshoot' in heart rate was recorded. In contrast, both scaphognathites exhibited slightly elevated rates of beating lasting approximately 2 min before returning to the rates shown immediately prior to the pause. This elevation in the rate of scaphognathite beating was correlated with an increase in ventilation volume ($\dot{V}_\omega$) during this period (Fig. 1).

At the onset of pumping, very low values of P_{e,O_2} were recorded, resulting in a pronounced increase in the calculated values of oxygen extraction. However, it is

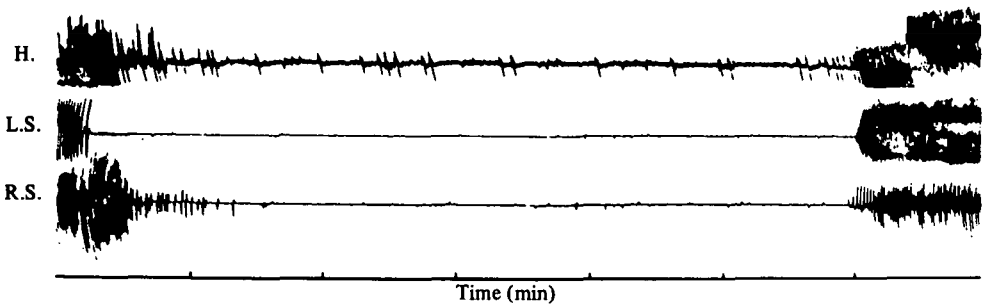

Fig. 2. Simultaneous recordings of heart rate (H), left scaphognathite (L.S.) and right scaphognathite (R.S.) before, during and after a ventilatory pause of 5 min duration.

unlikely that this represented a true increase in oxygen extraction by the animal during the post-pause period, but was merely due to a 'flushing-out' of oxygen depleted water from the branchial chambers. During periods of apnoea, oxygen will continue to be taken up from the water remaining in the branchial chambers as long as a diffusion gradient is maintained across the gills. Following the resumption of pumping activity, low values of P_{e,O_2} will be recorded whilst this water is flushed from the branchial chambers. The increased ventilatory activity seen immediately after the onset of pumping appears to correspond with this 'flush-out'. Both Extr_w and $\dot{V}_\omega$ returned to prepause levels after about 4–5 min.

It would appear therefore that although there was some rise in $\dot{M}_{O_2}$ due to the increase in $\dot{V}_\omega$, the calculated values of $\dot{M}_{O_2}$ in the first minute after a pause are probably in excess of the actual rate of oxygen consumption of the animal during this period.

The rhythmic pausing behaviour shown by this animal (Fig. 1) continued for a period of 3 h but ceased when the animal exhibited a period of locomotor activity. Although all respiratory parameters returned to quiescent levels within an hour of this period of activity, the rhythmicity was not observed again, although the animal was monitored for a further 2 h. Ventilatory pauses were observed in almost all animals studied yet it was impossible to predict exactly when the onset of this rhythm would occur.

Blood–gas analysis

In animals that were inactive but that were not exhibiting pausing behaviour, the P_{O_2} of the post-branchial blood was 94 ± 5 torr ($N = 18$), while pre-branchial values of 23 ± 3 torr ($N = 10$) were recorded. Blood samples from pausing animals were difficult to obtain since the animals became disturbed and the rhythm was generally lost.

The procedure adopted was to allow the animals to establish a rhythm and then remove post-branchial samples at a set time of 5 min into a pause. This method was used not only to enable blood samples to be collected after a constant time, but since the average pause duration was approximately 5 min the values obtained should also be representative of the blood P_{O_2} of animals at the end of a typical pause.

The mean P_{O_2} value of the post-branchial blood samples taken after this period

was 24 ± 3 torr ($N = 8$). Further blood samples were taken 1 min and 5 min after pumping had become re-established and in these samples the mean P_{a,O_2} had risen to 35 and 96 torr respectively. Thus the blood is rapidly re-oxygenated following the commencement of ventilatory activity, with the P_{O_2} of the post-branchial blood returning to normal levels within 5 min.

The mean lactate concentration in the post-branchial blood of inactive, but non-pausing animals in normoxic sea water was $1{\cdot}12 \pm 0{\cdot}2$ μmol. ml^{-1} ($N = 28$) and that of the blood of animals after a pause of 5 min duration was $1{\cdot}09 \pm 0{\cdot}17$ μmol. ml^{-1} ($N = 10$). The difference between these means was not found to be significant ($P > 0{\cdot}001$).

The ventilatory and circulatory characteristics of *Cancer* under normoxic conditions are presented in Table 1. The mean oxygen-carrying capacity of the blood (dissolved + bound) was $0{\cdot}62$ μmol.ml^{-1}. At the high oxygen tensions prevailing in the post-branchial blood under normoxic conditions, the blood returning to the heart was fully saturated with a total oxygen content of $0{\cdot}54$ μmol.ml^{-1}. Despite the lower values of P_{v,O_2}, the pre-branchial blood was 93% saturated, with an oxygen content of $0{\cdot}40$ μmol.ml^{-1}. As a result, under normoxic conditions only 9% ($0{\cdot}012$ μmol.ml^{-1}) of the oxygen given up to the tissues was carried by the pigment whereas 91% ($0{\cdot}128$ μmol.ml^{-1}) was carried in solution.

Cardiac output was calculated from the rate of oxygen consumption and the difference in oxygen contents of the pre- and post-branchial blood by the Fick principle. In a 390 g animal cardiac output was 18 ml.min^{-1} (45 ml.kg^{-1}.min^{-1}) and with a mean heart rate of 77 beats.min^{-1} the stroke volume of the heart was $0{\cdot}23$ ml.beat^{-1}.

Oxygen dissociation curves determined at three P_{CO_2} levels are shown in Fig. 3. For the *in vivo* range of pH values (7·74–7·95) the *in vitro* P_{50} was between 5·5 and 9·8 torr, which is comparable to P_{50} values reported by Burnett & Bridges (1981) for *C. pagurus* and by McMahon *et al.* (1979) for *C. magister*. The blood of *C. pagurus* exhibits a large normal Bohr effect. The slope of the line ΔP_{50} *v.* pH for the pH range 7·4–8·0 was $-1{\cdot}18$. The value is comparable to the values of $-0{\cdot}95$ and $-1{\cdot}0$ obtained for this species by Burnett & Bridges (1981) and by Truchot (1971) respectively.

The relationship between log P_{CO_2} and pH forthe blood of *Cancer* is shown in Fig. 4. No Haldane effect was detected. The *in vivo* values for the pH of pre- and post-branchial blood of *Cancer* and the total CO_2 content (C_{CO_2}), P_{CO_2} and concentrations of bicarbonate [HCO_3^-] and carbonate [CO_3^{2-}] ions are given in Table 3.

Hypoxia

The responses of *Cancer pagurus* to hypoxia were investigated in quiescent animals exhibiting resting levels of respiration but which were not showing the rhythmic ventilatory pausing behaviour. Under conditions of declining P_{O_2}, *Cancer* showed a high degree of respiratory independence, being able to maintain the rate of oxygen consumption approximately constant down to a critical oxygen tension (P_c), of between 60–80 torr. Below the P_c oxygen consumption decreased rapidly (Fig. 5).

In quiescent animals the initial response to a reduction in ambient P_{O_2} was a sharp increase in $\dot{M}_{O_2}$ to a level more usually correlated with an active animal, although no

Table 1. *Respiratory and circulatory parameters of* Cancer pagurus *at* 10 °C (Values are means ± S.D.; n = 10–30)

Oxygen consumption

$\dot{M}_{O_2}$ (μmol.min^{-1})* = 2·03 ± 0·08

Ventilation

$\dot{V}_w$ (ml.min^{-1})* = 63·6 ± 5·3
f_{sc} (beats.min^{-1})* = 81·5 ± 4·6
P_{i,O_2} (torr) = 155
P_{e,O_2} (torr) = 135
Extr$_w$ (%) = 13·0

Circulation	P_{O_2} (torr)	C_{O_2} (μmol.ml^{-1})	S_{O_2} (%)	$\dot{V}$b* (ml.min^{-1})	$f$heart* (beats.min^{-1})	Sv* (ml.beat^{-1})	Blood oxygen† capacity (μmol.ml^{-1})
Post-branchial	94 ± 5	0·54 ± 0·01	100	17·9	77 ± 3	0·23	0·62
Pre-branchial	23 ± 3	0·40 ± 0·01	93				

Respiratory indices

E_w (%)	E_b (%)	ΔP_{O_2} (torr)	T_{O_2}* (μmol.min^{-1}.torr^{-1})	$\dot{V}_w/\dot{M}_{O_2}$* (ml.μmol^{-1})	$\dot{V}_b/\dot{M}_{O_2}$* (ml.μmol^{-1})	$\dot{V}_w/\dot{V}_b$	C_w/C_b
15·2	63·6	86·5	0·029	28·0	7·2	3·9	2·0

* Values for a 390 g animal. † Oxygen-carrying capacity at P_{O_2} 158 torr.

Table 2. *Ventilatory and circulatory characteristics of* Cancer pagurus *during hypoxia*

P_{i,O_2} (torr)	Extr$_w$ (%)	$\dot{V}_w$ (ml.min^{-1})	$\dot{M}_{O_2}$ (μmol.min^{-1})	P_{a,O_2} (torr)	P_{v,O_2} (torr)	C_{a,O_2} (μmol.ml^{-1})	C_{v,O_2} (μmol.ml^{-1})	$\dot{V}_b$ (ml.min^{-1})	E_b (%)	E_w (%)	P_{O_2} (torr)	T_{O_2} (μmol.min^{-1} torr^{-1})	C_w/C_b	$\dot{V}_w/\dot{V}_b$
155	13	70	2·5	94	23	0·54	0·40	17·9	63·6	15·2	86·5	0·029	2·0	3·9
140	15	130	4·9	65	20	0·50	0·38	40·8	57·1	17·5	89·5	0·055	1·6	3·2
120	20	140	5·9	42	16	0·48	0·37	53·6	61·1	23·1	79·0	0·075	1·2	2·6
100	25	150	6·6	33	12	0·46	0·33	50·8	68·4	28·4	64·0	0·103	1·2	2·9
80	30	158	7·0	14	7	0·38	0·21	41·2	68·0	32·9	48·5	0·144	1·5	3·8
60	38	170	7·0	10	6	0·31	0·17	50·0	46·7	42·6	41·0	0·171	1·2	3·4
40	45	116	3·8	8	5	0·26	0·13	31·7	42·9	51·4	28·2	0·136	1·0	3·7
30	40	60	1·3	6	4	0·18	0·09	14·4	30·0	46·1	19·7	0·066	1·3	4·2

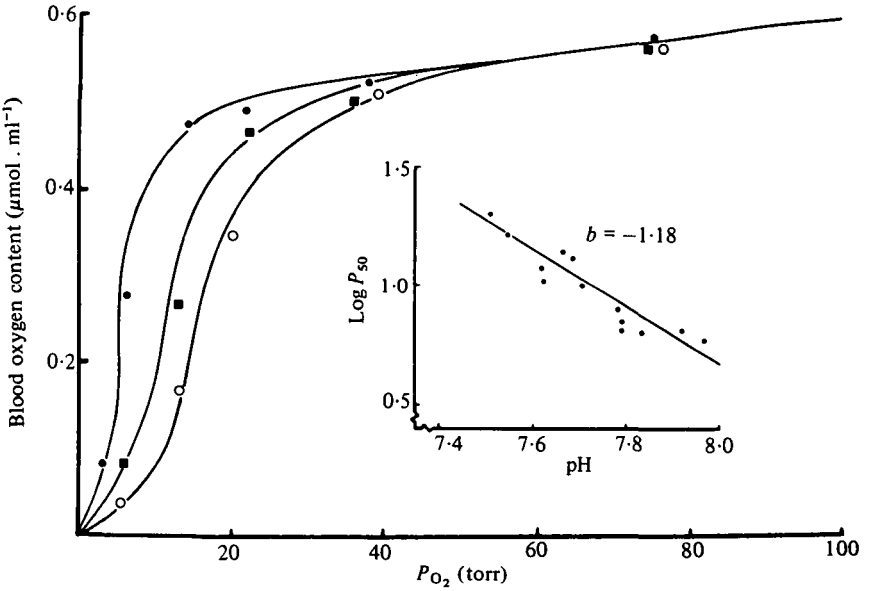

Fig. 3. Oxygen dissociation curves for whole blood of *Cancer pagurus* at three pH values, obtained by tonometering at three P_{CO_2} levels at 10 °C. (● = P_{CO_2} 3·8 torr, pH 7·94; ■ = P_{CO_2} 7·6 torr, pH 7·69; ○ = P_{CO_2} 11·4 torr, pH 7·44). Insert: plot of P_{50} *v.* pH to show the Bohr shift.

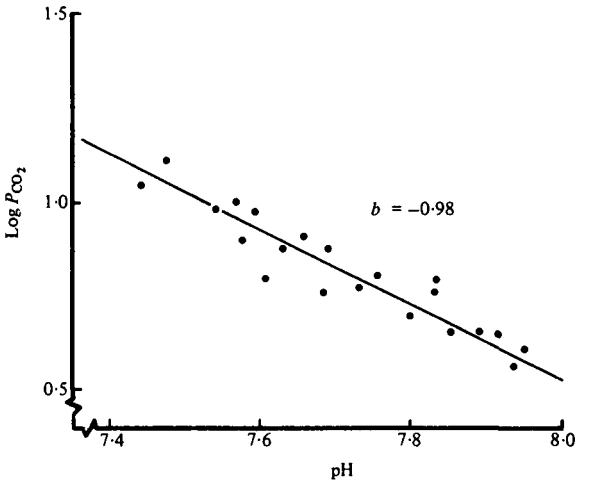

Fig. 4. The pH of the blood of *Cancer pagurus* as a function of P_{CO_2} at 10 °C.

Table 3. *The pH, carbon dioxide and lactate content of the blood of* Cancer pagurus *at* 10 °C

	P_{CO_2} (torr)	C_{CO_2} (μmol.ml^{-1})	[HCO_3^-] (μequiv.ml^{-1})	[CO_3^{2-}] (μequiv.ml^{-1})	pH	Lactate (μmol.ml^{-1})
Post-branchial	4·78 ± 0·6	17·99	17·20	0·52	7·85 ± 0·05	1·12 ± 2·0
Pre-branchial	5·49 ± 0·4	18·01	17·24	0·45	7·79 ± 0·04	

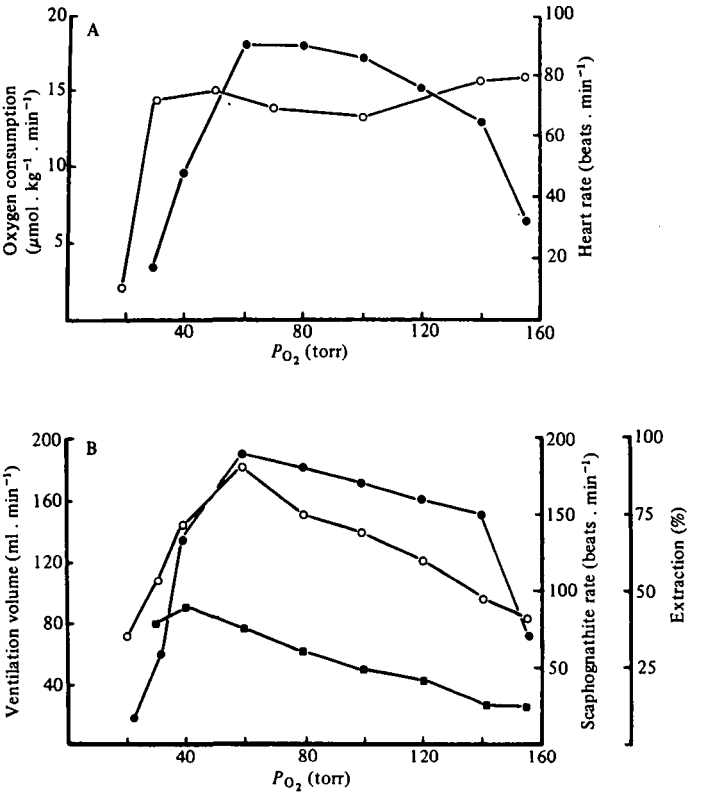

Fig. 5. Simultaneous recordings of (A) oxygen consumption (●) and heart rate (○) and (B) ventilation volume (●), scaphognathite rate (○) and % extraction (■) of *Cancer pagurus* during declining ambient oxygen tension.

overt activity was displayed. Further reduction in ambient P_{O_2} resulted in $\dot{M}_{O_2}$ remaining approximately constant over a range of P_{O_2} until the P_c was reached, below which $\dot{M}_{O_2}$ declined sharply.

During hypoxia, the heart rate remained fairly constant but fell abruptly as the oxygen tension was reduced below a P_{O_2} of 20–40 torr. This critical pressure was rather lower than the P_c of 60–80 torr recorded for $\dot{M}_{O_2}$.

As in other decapods, a high correlation was observed between ventilation volume ($\dot{V}_\omega$) and scaphognathite rate (f_{sc}) (McMahon & Wilkens, 1977; McDonald *et al.* 1980). During hypoxia both increased progressively reaching a maximum at a P_{O_2} of 60–80 torr below which both declined rapidly. Oxygen extraction also increased during hypoxia from a value of approximately 13 % under normoxic conditions to a maximum of 45 % at a P_{O_2} of 40 torr (Table 2).

The mean values for the P_{O_2} of the pre- and post-branchial blood of *Cancer* at different ambient oxygen tensions are shown in Fig. 6. During hypoxia, a marked reduction in the P_{O_2} of the post-branchial blood (P_{a,O_2}) was observed. The P_{O_2} of the pre-branchial blood (P_{v,O_2}) also declined but the reduction was less pronounced. Under normoxic conditions, 91 % of the O_2 delivered to the tissues was carried in physical solution, but as the ambient oxygen tension declined the proportion delivered from haemocyanin became progressively larger (Fig. 7).

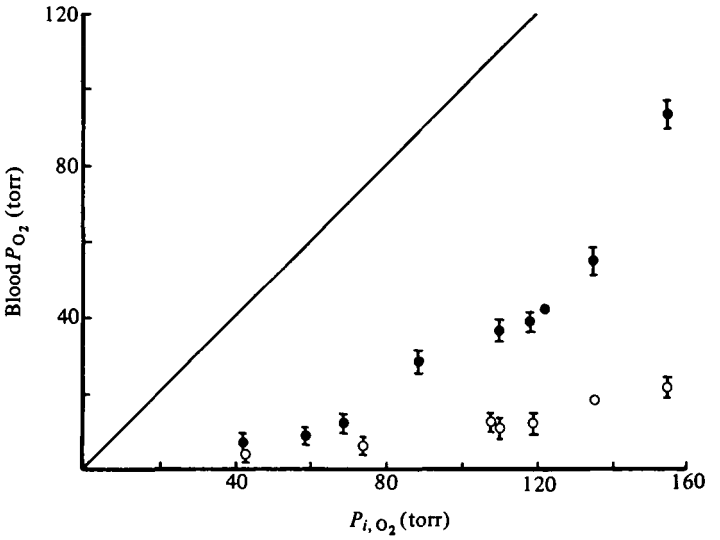

Fig. 6. Oxygen tension of the pre- (○) and post-branchial (●) of *Cancer pagurus* measured at different ambient oxygen tensions. Points are means ± S.D.

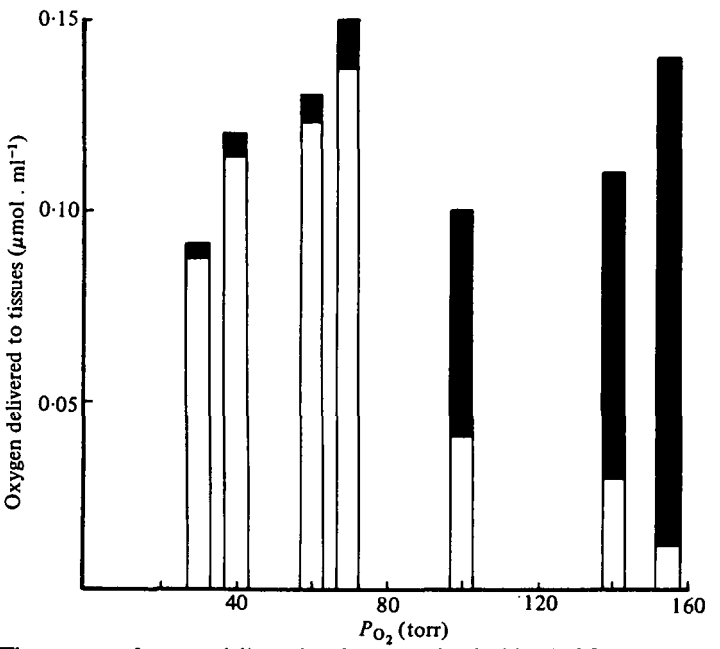

Fig. 7. The amount of oxygen delivered to the tissues by the blood of *Cancer pagurus* at different ambient oxygen tensions. Proportion from haemocyanin (□) and from solution (■).

Under conditions of declining oxygen tension, there was an initial increase in cardiac output ($\dot{V}_b$) corresponding to the increase in $\dot{M}_{O_2}$, but further reduction of the ambient P_{O_2} resulted in cardiac output remaining approximately constant over a range of P_{O_2}, until the P_c was reached, below which $\dot{V}_b$ declined (Table 2). Since the heart rate showed little variation during this period, the initial increase in cardiac output appears to have been due almost entirely to an increase in the stroke volume of the heart (S_v) from 0·2 to 0·7 ml.beat^{-1}.

The reduction in the P_{O_2} difference between the pre- and post-branchial blood was largely responsible for the decline in the mean P_{O_2} gradient across the respiratory surfaces (ΔP_{O_2}) during hypoxia (Table 2). The reduction in ΔP_{O_2} resulted in an increase in the calculated value for the transfer factor (T_{O_2}) (Randall, Holeton & Stevens, 1967; Taylor & Spencer Davies, 1981). Only small variations in the capacity rate ratio (C_w/C_b) (Hughes & Shelton, 1962; Mangum, 1977) were observed during hypoxia.

DISCUSSION

Rhythmic patterns of respiration have been described in a number of decapod Crustacea. Persistent tidal and circadian rhythms of ventilatory activity and oxygen consumption have been recorded in *Carcinus maenas* (Arudpragasm & Naylor, 1964; Aldrich, 1979), *Cancer pagurus* (Ansell, 1973) and the fiddler crabs *Uca pugnax* and *U. pugilator* (Brown, Bennet & Webb, 1954). In recent studies, however, rhythmic patterns of shorter duration have been noted (Ansell, 1973; Bottoms, 1977; McMahon & Wilkens, 1977; Schembri, 1979*a*; Burnett & Bridges, 1981). These studies have shown that in a number of species the ventilatory activity of resting, undisturbed animals is regularly interrupted by periods of apnoea accompanied by bradycardia or cardiac arrest. The duration of these ventilatory pauses and of the time spent actively ventilating varies both between species and between individuals. Schembri (1979*a*) reported that resting *Ebalia tuberosa* exhibit periods of ventilatory pumping for several minutes alternating with longer periods of apnoea, while McMahon & Wilkens (1977) reported pauses of approximately 10 min duration occurring twice hourly in resting *Cancer productus*. Ansell (1973) recorded 'intermittent ventilation' in *Cancer pagurus*. Burnett & Bridges (1981) reported that *C. pagurus* exhibits pausing behaviour for 40% of the time during quiescence, while in this study individuals of the same species paused for about 20% of the time.

The functional significance of ventilatory pausing behaviour is not fully understood. McMahon & Wilkens (1972, 1977) and Wilkens *et al.* (1974) have suggested that pausing may perform a defensive role concealing animals from predators which may be able to detect electrical or pressure gradients associated with normal heart and scaphognathite beating, since ventilatory pauses could be induced in the laboratory by a variety of external stimuli (Wilkens, Wilkens & McMahon, 1974; Cumberlidge & Uglow, 1977). Such ventilatory pauses, however, were generally of short duration, usually 30–60 s, and are not comparable to the longer rhythmic pauses observed in many animals. It is unlikely that these rhythmic ventilatory pauses have a defensive role since the onset of this behaviour appeared to be spontaneous and was not readily correlated with any outside disturbance (McMahon & Wilkens, 1972, 1977). Indeed, during the present study it was frequently observed that any disturbance or stimulation of the animal during a period of rhythmic pausing behaviour generally resulted in an abrupt return to uninterrupted ventilatory activity.

A more satisfactory explanation for these long-term pauses, particularly when found to occur rhythmically, is that they may enable the animal to make a saving of metabolic energy during periods of quiescence by reducing the energetic cost of pumping both water and blood (Bottoms, 1977; McMahon & Wilkens, 19[illegible] McDonald *et al.* 1980; Burnett & Bridges, 1981).

During a pause, the animals must rely on oxygen stored in the blood and as a result the P_{O_2} of the post-branchial blood declines during a ventilatory pause. Burnett & Bridges (1981) made estimates of oxygen consumption during a pause in two specimens of *Cancer pagurus* from the decline in blood O_2 content and haemolymph volume. They obtained values of between 2·3–7·0 μmol.kg^{-1}.min^{-1}, indicating a fall to between 50 and 84% of the mean pre-pause $\dot{M}_{O_2}$ value of 14·6 μmol.kg^{-1}.min^{-1}. Similar calculations carried out on data obtained during the present study gave a mean value for the rate of oxygen consumption during a ventilatory pause of 3·4 μmol.kg^{-1}.min^{-1} for animals of 300–400 g weight, whereas the mean oxygen consumption measured immediately before the onset of pausing was 4·3 ± 1·9 μmol.kg^{-1}.min^{-1} (N = 17). Thus $\dot{M}_{O_2}$ during pausing may fall by up to 30% of pre-pause levels. The calculated rates of oxygen consumption during a ventilatory pause are likely to be subject to considerable error in both studies since the blood volume of the animals was not determined but a value of 30% of the body weight was used (Gleeson & Zubkoff, 1977). Nevertheless, these studies demonstrate that there is a considerable reduction in oxygen consumption during a ventilatory pause. The greater reduction in $\dot{M}_{O_2}$ during pausing found by Burnett & Bridges (1981) may be due to the higher pre-pause oxygen consumption that they recorded and the fact that their animals exhibited pausing behaviour for between 40 and 50% of the time whereas in this study animals generally paused for about 15–20% of the time. Since the duration of a pause is likely to depend on the store of oxygen in the blood and on the rate at which this is utilized, the greater the reduction in oxygen demand, the longer the pause will be able to continue.

These calculations demonstrate that the animals are able to maintain a reduced rate of aerobic metabolism throughout the ventilatory pause and that anaerobic metabolism is unlikely to contribute significantly to energy production during this time. This is confirmed by the lack of accumulation of lactate in the blood of *Cancer* during a pause, indicating that anaerobic metabolism, at least involving conventional biochemical pathways, is unimportant during ventilatory pausing.

As in previous studies of pausing behaviour in decapods (McMahon & Wilkens, 1972; McDonald *et al.* 1980), a transient increase in $\dot{M}_{O_2}$ was recorded immediately following a pause. This appears to represent the repayment of an 'oxygen debt' incurred, not as the result of any anaerobic metabolism during the pause, but because the stores of oxygen within the animal that are used during a pause must be replenished when ventilation resumes.

The rhythmic ventilatory behaviour was observed in nearly all experimental animals and appears to represent the normal ventilatory pattern, not only in quiescent *Cancer pagurus* but also in a number of other decapods. In many other aspects of its respiratory physiology, *Cancer pagurus* exhibits features that are gradually becoming established as characteristic of the majority of decapod Crustacea.

The P_{O_2} of the post-branchial blood was 94 torr, which is similar to the values of 90–95 and 118 torr obtained by Burnett & Bridges (1981) and by Bottoms (1977) respectively in their studies of this species. The oxygen-carrying capacity of the blood does vary greatly amongst decapods. The highest carrying capacities are normally in terrestrial crabs (Taylor & Spencer Davies, 1981), but even among

aquatic crabs there can be considerable variation (0·44–1·12 μmol.ml^{-1}) (Mangum 1980). The carrying capacity of the blood of *Cancer* in the present study (0·62 μmol.ml^{-1}) falls in the middle of the range and is similar to the values found by Burnett & Bridges (1981) for this species.

A characteristic of most aquatic animals is the existence of low partial pressures of carbon dioxide in the blood, due to the high solubility of carbon dioxide in water, which results in a given volume of gas having a lower partial pressure than it would in air (Rahn, 1966; Howell *et al.* 1973). Crabs are no exception to this rule and therefore the blood of aquatic crabs generally has a lower P_{CO_2} than does the blood terrestrial crabs (see Taylor & Spencer Davies, 1981). In accordance with this relationship, the blood of *Cancer pagurus* was found to have a low P_{CO_2}, although not as low as the values previously recorded in some other aquatic crabs (Taylor & Butler, 1978; Batterton & Cameron, 1978).

It is now well established that, in general, decapod crustacea are capable of exhibiting a high degree of respiratory independence during hypoxia, i.e. they are able to maintain their rate of oxygen consumption independent of the ambient oxygen tension over a wide range of P_{O_2}. It now appears that some of the early studies, which reported that in some species the rate of oxygen consumption was dependent on the ambient P_{O_2}, were carried out on animals which were either active or disturbed. It has since been demonstrated that the level of metabolic activity has a direct effect on the degree of respiratory independence exhibited by an animal; respiratory independence is greatest when metabolic rates are low (Ansell, 1973; Taylor, 1976). Consequently, active animals are unlikely to show respiratory independence under hypoxic conditions. However, even when adequate precautions have been taken to ensure that the animals are not disturbed or active, some species, e.g. *Ebalia tuberosa* (Schembri, 1979*b*), still show only a very limited ability to maintain respiratory independence.

Cancer pagurus exhibits quite a high degree of respiratory independence and is able to maintain its rate of oxygen consumption approximately constant down to a P_{O_2} of 60–80 torr (see also Ansell, 1973). The initial increase in $\dot{M}_{O_2}$ during hypoxia was probably due to the fact that the animals, which had been left undisturbed in the respirometer for several hours, were very quiescent at the start of the experiment and exhibited very low rates of $\dot{M}_{O_2}$. The initial reduction in ambient P_{O_2} resulted in the arousal of the animal from this quiescent state with a concurrent increase in metabolic activity to a level intermediate between that of a completely quiescent animal and that exhibited when fully active. An initial increase in $\dot{M}_{O_2}$ by *Carcinus maenas* during hypoxia has also been reported (Arudpragasm & Naylor, 1964).

Under conditions of declining P_{O_2} the rate of oxygen consumption was maintained independent of ambient P_{O_2}, primarily as a result of the increase in ventilatory activity, since both the rate of beating of the scaphognathites and the ventilation volume increased progressively until very low oxygen tensions were reached, below which both declined. An increase in ventilation volume is a common respiratory response of decapods, and of other aquatic animals, to hypoxia (Arudpragasm & Naylor, 1964; Taylor, 1976; Batterton & Cameron, 1978; Burnett, 1979). Such a response helps to maintain the supply of oxygen to the respiratory surfaces despite the reduced oxygen content of the medium. However, an increase in ventilatory activity will itself increase the O_2 demand of the animal until eventually the oxygen supply

sufficient only to meet the energy requirements of the ventilatory pump. It is at this point, which corresponds to the critical oxygen tension (P_c), that respiratory independence can no longer be maintained.

During hypoxia the transfer factor (T_{O_2}) and the effectiveness of oxygen uptake from the water (E_w%) both increased progressively. The increase in ventilation volume and in E_w% resulted in the maintenance of the rate of oxygen consumption under these conditions. McMahon & Wilkens (1975) recorded a similar increase in both these factors in *Homarus americanus* during hypoxia, although in other species the response has been more variable (Taylor, 1976; Burnett, 1979).

The increase in E_w% and T_{O_2} during hypoxia presumably resulted from the reduction in the P_{O_2} of the pre-branchial blood, permitting an increase in oxygen binding at the respiratory surfaces. There was, however, little variation in the $C_{a,O_2} - C_{v,O_2}$ difference under these conditions, which resulted in the cardiac output, calculated by the Fick principle, remaining unchanged during hypoxia except for an initial increase associated with the slight increase in $\dot{M}_{O_2}$ at the start of the experiment. Similarly, there was little change in the efficiency of extraction of oxygen from the blood by the tissues (E_b%) as P_{O_2} declined. There was, however, an increase in importance of the respiratory pigment in supplying oxygen to the tissues. In non-active crabs under normoxic conditions the respiratory pigment has only a minor role in supplying oxygen to the tissues; the pigment provides only 9% of the oxygen supplied to the tissues. During hypoxia, however, the decrease in blood oxygen tension results in the pre-branchial blood haemocyanin entering the steep part of the oxygen dissociation curve, causing a much greater release of oxygen from the pigment. As a result there is a reduction in the capacity rate ratio (C_w/C_b) (Hughes & Shelton, 1962; Mangum, 1977) from a value of 2 under normoxic conditions to values closer to 1 during hypoxia indicating that the effectiveness of oxygen transfer is increased under these conditions which allows the animal to maintain respiratory independence over a wide range of ambient oxygen tension.

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