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The Respiratory and Cardiovascular Changes Associated with the Emersion Response of *Carcinus maenas* (L.) during Environmental Hypoxia, at Three Different Temperatures*

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Summary. 1. When exposed to progressive hypoxia in shallow seawater, *Carcinus maenas* partially emerged into air and aerated its branchial chambers by reversing the direction of their irrigation. Emersion took place at a mean P_I, O_2 of 18 mm Hg at 6°C, 21 mm Hg at 12°C and 59 mm Hg at 17°C.

2. At low oxygen tensions submerged crabs underwent a progressive bradycardia. Heart rate first became significantly lower than the rate in normoxia below a P_I, O_2 of 30 mm Hg at 6°C, 40 mm Hg at 12°C and below 60 mm Hg at 17°C. The proportion of total time spent irrigating the gills in a reversed direction increased in hypoxic seawater ($P_I, O_2 < 50$ mm Hg), but respiratory rate was unchanged.

3. Emersion into air always occurred during a reversal of irrigation and was accompanied by prolonged reversals, with consequent aeration of the branchial chambers, and by an immediate and maintained tachycardia back towards the rate in normoxic seawater. Crabs emerging into a hypoxic atmosphere ($P_{O_2} < 10$ mm Hg) showed neither a maintained reversal of irrigation nor a maintained tachycardia.

4. The oxygen tension of the postbranchial blood (P_a, O_2) was 94 mm Hg in crabs submerged in normoxic seawater (P_I, O_2 146 mm Hg) at 12°C. During progressive hypoxia P_a, O_2 fell in direct proportion to the drop in P_I, O_2. Emersion caused no significant increase in P_a, O_2.

5. The mean oxygen content of postbranchial blood (C_a, O_2) was 0.96 vol.-% at a P_I, O_2 of 145 mm Hg. C_a, O_2 fell to 0.19 vol.-% in submerged crabs at a mean P_I, O_2 of 25 mm Hg but rose to 0.45 vol.-% following 10 min emersion into air at a mean P_I, O_2 of 22 mm Hg.

6. The results provide evidence of a respiratory role for the emersion response and also of an adaptive role for the high affinity of the blood pigment in *Carcinus*.

Introduction

The shore crab *Carcinus maenas* (L.), as its common name implies, inhabits the littoral zone around the British coast. In the Summer months it is commonly found stranded on the shore at low tide either in air or

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in small pools of water (Naylor, 1962). In either of these circumstances internal or environmental hypoxia are potential problems. Bohn (1897) and Taylor and Butler (1973) have described how *Carcinus* emerges into air by raising itself onto the back of its abdomen in shallow hypoxic seawater and reverses the direction of its scaphognathite beat, causing air to enter the branchial chambers via the normally exhalent openings around the mouth. Borradaile (1922) first postulated that this behaviour may enable the crab to obtain adequate oxygen during exposure to stagnant conditions.

The respiratory and cardiovascular physiology of *Carcinus* has been the subject of a number of investigations. Arudpragasam and Naylor (1964a, b) described the regular reversals of gill irrigation in *Carcinus* and also its responses to hypoxia and hypercapnia in terms of its oxygen consumption, ventilation volume, percentage utilisation of oxygen and respiratory frequency. Hughes, Knights and Scammell (1969) described the patterns of water flow, water pressures and oxygen tensions in the branchial chambers of *Carcinus*, Ahsanullah and Newell (1971) described some factors effecting its heart rate, Truchot (1971) studied the oxygen affinity of its blood and Wallace (1972) measured rates of O_2 consumption in air and water. The present investigation describes the behaviour, measurements of heart rate, branchial chamber pressures, blood oxygen tension and blood oxygen content in *Carcinus* during progressive environmental hypoxia, at three environmental temperatures, and includes a closer study of the response first described by Bohn (1897).

Material and Methods

The present report contains observations on 169 male and female shore crabs, *Carcinus maenas* (L.), weighing between 30 and 130 g. The animals were obtained from the Laboratories of the Marine Biological Association, Plymouth, and were held in aquaria supplied with recirculated seawater at 6°C, 12°C or 17°C for at least two weeks prior to experiments. This time was allowed to enable the animals to acclimate to each of the experimental temperatures and to lose any tidal rhythms of activity (Arudpragasam and Naylor, 1964b; Ahsanullah and Newell, 1971). The stage in the moulting cycle was not determined but all the crabs used had a hardened carapace and it is likely that they were in either intermoult or terminal anecdysis.

For the preliminary work on the behaviour of unrestrained animals, groups of 6 crabs were placed on a perforated platform in a "Perspex" tank 26 × 26 × 19 cm. In this tank the crabs were allowed to move freely in 5 l of seawater, and were covered to a depth of approximately 5 cm. The water in the tank was continuously pumped up to a glass column through which air or nitrogen was bubbled, then ran by gravity feed back into the tank. The oxygen tension of the water in the experimental tank was monitored by means of an oxygen electrode (Radiometer type E5046 with the Acid-Base Analyser PHM71) inserted above the platform. During experiments the water was rendered hypoxic by bubbling nitrogen through the gas exchange column at a gradually increased rate so that the P_{O_2} of the water in the tank fell from air saturation levels down to below 10 mm Hg over a period

of approximately 45 min. In a few experiments the tank was covered with a lid which could be rendered airtight by means of a water-seal, a stream of nitrogen or air was then blown through side tubes above water level to vary the P_{O_2} of the atmosphere in the tank. The tank was inserted into a constant temperature water-bath, and water from this bath was passed through a cooling coil in the experimental tank. Water temperature was maintained within $\pm 1°C$ of the experimental temperature of 6°C, 12°C or 17°C throughout the series of experiments. The more detailed observations of heart-rate, branchial chamber pressures and blood oxygen tension and content were conducted on individual crabs in the same experimental tank. Each crab was first exposed to progressive hypoxia in deep seawater (approximately 12 cm) from which it was unable to emerge. The water-level was then reduced to 5 cm and the crab exposed to hypoxic seawater from which it was able to emerge. In this way a direct comparison was obtained of the measured variables from crabs submerged in and after emersion from hypoxic seawater.

Water pressures in the branchial chambers of the crabs were measured via a flexible nylon cannula (Portex flex 3) approximately 20 cm long, one end of which was inserted through a hole drilled in the ventral wall of the branchiostegite behind the Milne-Edward's opening at the base of the cheliped. This positioned it in the epibranchial chamber over the midregion of gill 6 (see Hughes *et al.*, 1969). The other end of the cannula was attached to a S.E. Laboratories pressure transducer (type SE4-81). The complete recording system had a natural frequency of 50 Hz and damping was 20% of critical.

Heart rate was monitored as the E.C.G. by means of a pair of fine, varnished copper wires, bared at the tips, inserted through small holes in the carapace above the heart and secured to the crab with epoxy resin (Holts' "Cataloy"). Both the E.C.G. and the branchial chamber pressures were displayed on a Devices M4 direct writing recorder with rectilinear coordinates.

During experiments the crab irrigated its branchial chambers with water from the experimental tank. The measured P_{O_2} of this water was therefore designated the P_I, O_2 (i.e. the oxygen tension of the inspired water). Samples of post-branchial blood were withdrawn from the pericardium and the measured oxygen tensions, designated the P_a, O_2 (i.e. oxygen tension of arterial blood) and oxygen contents, designated the C_a, O_2 (i.e. oxygen content of arterial blood) were related to P_I, O_2.

Blood samples were obtained from the pericardium by drilling a small hole through the carapace at a position just behind the posterior margin of the heart in the mid-line. Care was taken to leave the hypodermis intact. A short length (approximately 7 mm) of glass tubing approximately 1 mm in internal diameter, which was flamed to smooth the ends and siliconised with "Siliclad" (Clay Adams Inc) was mounted in epoxy-resin above this hole. The hypodermis was then broken with a sharpened hypodermic needle inserted down the glass tube, which allowed blood to escape from the pericardial cavity. A length of nylon tubing (Portex flex 3) was inserted into the glass tube, which it fitted tightly, and connected to a siliconised, glass syringe. Blood samples of approximately 0.3 ml were withdrawn slowly into the syringe. A rubber diaphragm was then placed over the end of the syringe and two 20 µl subsamples were withdrawn by inserting the needle of a high-precision syringe (Hamilton Co. Inc. California) through this diaphragm. These subsamples were transferred immediately to a Lex-O_2-Con oxygen analyser (Lexington Instruments Corp. Waltham Mass.) which automatically measured the total oxygen content of the blood in vol.-% (ml O_2/100 ml of blood). The remainder of the sample (approximately 0.2 ml) was transferred immediately to a glass cuvette containing a Radiometer oxygen electrode, which was surrounded by a water-jacket at the experimental temperature (see Butler and Taylor, 1971).

When performed with care, the complete procedure for taking blood samples had no observable effect upon the measured variables. The crab was left for at least 15 min before another sample was taken, and a maximum of 3 samples was withdrawn from each crab. It proved extremely difficult to extract more than 3 samples from an individual *Carcinus* and accordingly the simultaneous monitoring of the oxygen tension and content of venous blood proved impossible. It was considered, however, that the present report was of value without a complete investigation of all aspects of the respiratory exchange of the crab.

The values for blood oxygen content obtained by the automatic technique were compared with measurements using the Van Slyke apparatus (Van Slyke and Neill, 1924). This latter technique required at least 1 ml of blood for each measurement which often entailed pooling samples from 2 or 3 crabs. The measurements using the two techniques are shown in Table 3, there was no significant difference between them. This demonstrated the accuracy of the automatic technique which, because it required relatively small blood samples, allowed some degree of repetitive sampling from each crab.

Measurements of P_a,O_2 are plotted against P_I,O_2 and subjected to regression analysis, C_a,O_2 is tabulated and plotted against P_a,O_2. All other measured variables are plotted against time or P_I,O_2 as individual observations or as mean values $\pm$ SE of mean. Numerical values in the text are also given, wherever possible, as means $\pm$ SE of mean with the number of observations (n) in parentheses. Student's t-test was used to test the significance of any difference between two mean values and in the present report the word "significant" means at the 95% confidence level ($P < 0.05$). The term normoxia is used to denote the control conditions in the experiments i.e. aerated seawater ($P_I,O_2 > 130$ mm Hg) at either 6°C, 12°C or 17°C.

Results

a) The Emersion Response

In this initial series of experiments 78 unrestrained and intact crabs were observed in groups of 6 in the experimental tank. Water temperature was controlled within 1°C of 6°C, 12°C or 17°C and the water surface was in contact with air at room temperature. The crabs were placed in aerated water for one hour prior to the onset of hypoxia. The behaviour of *Carcinus*, both in normoxia and as the water became progressively more hypoxic is described elsewhere (Taylor and Butler, 1973). Briefly, the activity of the crabs increased in hypoxic seawater and they eventually straightened their walking legs and raised the chelipeds and front of the body sufficiently to bring the exhalent openings of the branchial chambers above the water-surface. They then irrigated the branchial chambers in the reversed direction, causing air to bubble through the water surrounding the gills and to stream from the normally exhalent Milne-Edward's openings at the bases of the chelipeds. This whole response was designated "emersion".

The P_I,O_2 when each crab emerged was noted and tabulated against the sex and weight of the crab. The results are summarised in Table 1. The P_I,O_2 at emersion was significantly higher at 17°C than at either

Table 1. *Carcinus maenas* (L.). Mean P_I, O_2 at emersion from hypoxic seawater

Temperature range	No of crabs observed	Mean wt. (g)	No of crabs emerging	Mean P_I, O_2 (mm Hg) at emersion
6°C (5–7°C)	18	45.9 ± 3.4	16	18 ± 3
12°C (10.5–12.5°C)	30	49.7 ± 3.1	27	21 ± 3
17°C (16°–19°C)	30	41.1 ± 2.4	28	59 ± 6

12°C or 6°C. At each temperature a small number of crabs failed to emerge from hypoxic seawater. The response was shown equally by males and females and there was no observable relationship between weight of crab and P_I, O_2 at emersion.

Once a crab had emerged from hypoxic seawater it remained almost continuously emersed as long as the P_I, O_2 was low. When the P_I, O_2 was increased rapidly by reaeration the crab immediately ceased the emersion response. The walking legs were relaxed, the chelipeds lowered and the crab submerged. Having expelled a stream of bubbles from the exhalent openings the crab then resumed normal irrigation of the branchial chambers with seawater.

b) Branchial Chamber Pressures and Heart Rate

The following results were obtained from experiments on a total of 69 crabs with a mean weight of 69.7 ± 3 g. They were held individually in the experimental tank, supplied with a continuous flow of aerated seawater, whilst values for all the measured variables in normoxia were obtained. The water was then rendered progressively hypoxic and the crabs were allowed to emerge from it.

α) Normoxia. Fig. 1 illustrates the pattern of water pressure variations recorded from the epibranchial spaces of *Carcinus* in normoxia. During forward irrigation of the branchial chambers a mean negative pressure was recorded in the epibranchial space. Periodically the irrigation current was reversed, water entering the branchial chambers by the normally exhalent openings and leaving by the normally inhalent openings around the legs (Arudpragasam and Naylor, 1964a). As the current reversed an abrupt change to a mean positive pressure was recorded in the epibranchial space (cf. Hughes *et al.*, 1969). During both forward and reversed irrigation the recordings of branchial chamber pressure were pulsatile, with a complex waveform reflecting the activity of the scaphognathite. Immediately following a reversal the return to

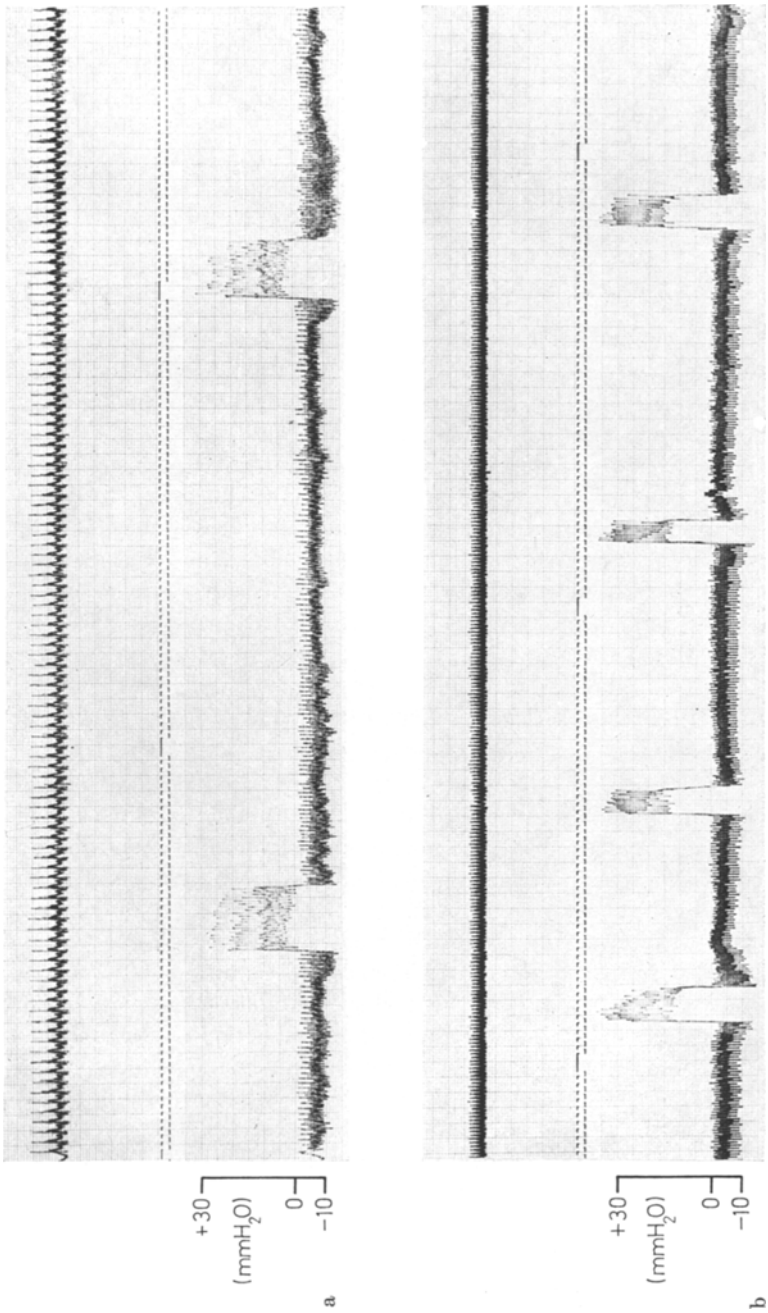

Fig. 1a and b. E.C.G. and branchial chamber pressures recorded from *C. maenas* in normoxic seawater at (a) 6°C and (b) 17°C. The traces are, in each case, from the top downwards, e.c.g.; time-base in sec; hydrostatic pressure in the left branchial chamber

forward irrigation was often accompanied by strongly negative pressures with a higher frequency than normal.

Table 2 lists values for all the measured variables in normoxia. Both heart rate and respiratory frequency increased with temperature (see Fig. 1). Although there was an indication that the amplitude of the water pressure measured in the branchial chambers increased with increasing temperature, in the present investigation this trend was not significant. The frequency of reversals of the irrigation current was significantly higher at 17°C than at 6°C. The reversals at 6°C were, however, typically of longer duration than those measured at 17°C (see Fig. 1). The percentage of total time spent irrigating the branchial chambers in a reversed direction was, therefore similar at the two extreme temperatures (i.e. between 5% and 6%).

Simultaneous recordings from the right and left branchial chambers of *Carcinus* revealed that a whole series of reversals may occur at the same time on both sides of the animal, though the onset and cessation of the reversals was not always exactly synchronous. Often, however, the two scaphognathites acted independently, sometimes reversing the current on one side only, or ceasing to pump completely on one side and not on the other. This confirms the observations made by Borradaile (1922) who noted that the scaphognathites in *Carcinus* can work independently.

The heart rate of *Carcinus* in aerated seawater was sometimes very variable, often over short periods of time. In some crabs it was apparently unaffected by the reversal of irrigation in normoxic conditions (see Fig. 1). In others, however, the heart missed a beat at the onset of each reversal and sometimes slowed or even stopped beating for the duration of each reversal. Occasionally heart rate increased during a reversal of the irrigation current.

In response to any disturbance, and often apparently quite spontaneously, the shore crab ceased to irrigate its branchial chambers. The pressure in the epibranchial chamber immediately became non-pulsatile and equal to the surrounding hydrostatic pressure (i.e. zero pressure). On cessation of irrigation, cardiac frequency slowed or the heart ceased to beat altogether. On resumption of irrigation the heart immediately began to beat again at its original rate. McMahon and Wilkens (1972) have recently described a similar phenomenon in the lobster, *Homarus americanus*.

β) Hypoxia. Fig. 2 illustrates the changes in the measured variables from submerged crabs during a progressive reduction in P_I, O_2. During progressive hypoxia there was no significant change in respiratory frequency at all three temperatures. Hypoxia affected both the frequency and duration of the periods of reversed irrigation. There was a significant

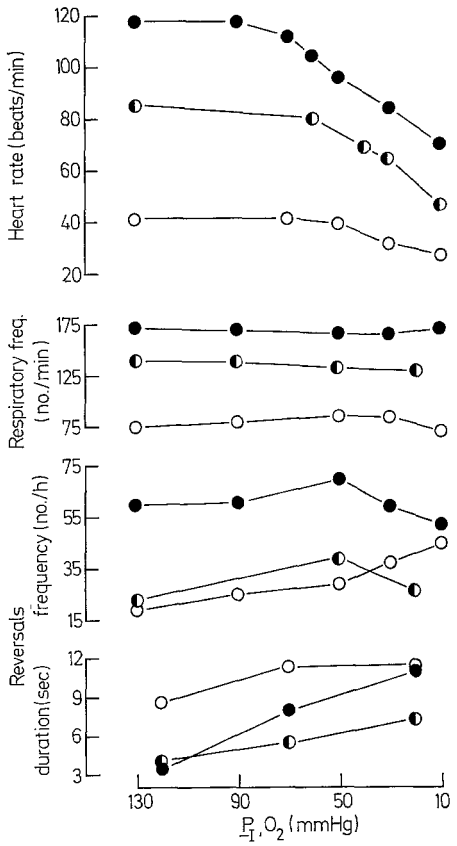

Fig. 2. Changes in the heart rate and patterns of irrigation of the branchial chambers in *Carcinus* during progressive hypoxia at 6°C (○), 12°C (◐) and 17°C (●). The traces are from the top downwards, mean heart rate, respiratory frequency, the frequency of the periods of reversed irrigation, the duration of the periods of reversed irrigation

increase in reversal rate, above the rate in normoxia, at a P_I, O_2 of 50 mm Hg at 6°C and 12°C and the duration had increased significantly above the normoxic level at a P_I, O_2 of 70 mm Hg, at all three temperatures. The combined effect of these two changes was a marked increase from approximately 5% up to approximately 15% in the proportion of total time which the crabs spent irrigating their branchial chambers in a reversed direction, when the P_I, O_2 fell from normoxic levels down to 20 mm Hg.

During progressive hypoxia, submerged crabs underwent a developing bradycardia, until at a P_I, O_2 of 10 mm Hg heart rate was approximately

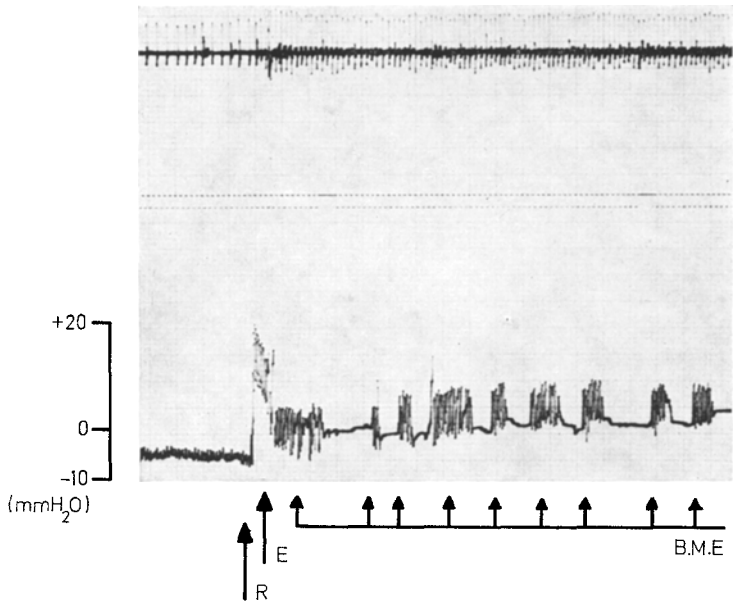

Fig. 3. (*C. maenas*, ♂, 58 g, 12° C) E.C.G. and branchial chamber pressures during emersion into air from hypoxic seawater, *R* reversal of irrigation; *E* emersion; *B.M.E.* periods when bubbles of air were streaming from the Milne-Edward's openings. Time-base sec

60% of the normoxic level at all three temperatures (Fig. 2). The P_I, O_2 for the onset of a significant bradycardia varied at the three temperatures, When mean heart rate was measured at 5 mm Hg intervals of P_I, O_2 during progressive hypoxia it was found to have first decreased significantly ($P < 0.05$) from the normoxic rate at 60 mm Hg at 17° C, at 40 mm Hg at 12° C and at 30 mm Hg at 6° C.

γ) Emersion into Air from Hypoxic Seawater. The rise in the frequency and duration of reversals with progressive reduction in P_I, O_2 usually culminated in the crab emerging from the water and bubbling air through its branchial chambers in a reversed direction. Emersion always took place during a reversal of irrigation and the pressure recordings during emersion differed from those recorded in submerged crabs. There were wide fluctuations in pressure, with occasional rapid switches from positive to negative pressure and periods of zero pressure when the scaphognathite was not pumping. The periods when streams of bubbles were observed emerging from the Milne-Edward's openings coincided with positive pressure fluctuations in the epibranchial spaces which were of relatively low frequency, but had a large amplitude (see Fig. 3). The

Table 2. Mean values of measured variables $\pm$ S.E. of mean, from unrestrained *Carcinus meanas* (L.) in aerated seawater (P_I, $O_2 > 130$ mm Hg)

Acclimation temperature	6° C ($n = 11$) (5–7° C	12° C ($n = 35$) (10.5–12.5)	17° C ($n = 23$) (16–19)
Heart rate (beats/min)	41 ± 1.7	85 ± 2.8	118 ± 3.8
Respiratory frequency (no./min)	74 ± 9.8	141 ± 14.7	172 ± 8.4
Branchial chamber pressures			
(a) *during forward irrigation*			
median pressure (mm H_2O)	-6.4 ± 0.7	-10 ± 0.9	-8.5 ± 1.2
pulse pressure (mm H_2O)	7.0 ± 0.8	5.1 ± 0.7	8.0 ± 1.4
(b) *during reversed irrigation*			
median pressure (mm H_2O)	11.4 ± 1.2	15.2 ± 1.5	14.7 ± 2.3
pulse pressure (mm H_2O)	12.3 ± 1.1	14.5 ± 2.7	16.7 ± 2.1
Frequency of reversal of irrigation (reversals/h)	19 ± 2.4	22.7 ± 2.3	60 ± 5.8
Duration of each reversal (sec)	8.7 ± 0.8	4.1 ± 0.2	3.5 ± 0.3

ease with which the scaphognathite pumps air into and out of the branchial chambers and is able to bubble air through water contained in the chambers, indicates that it must form air-tight seals with the upper and lower walls of the exhalent canal (Borradaile, 1922) as it undergoes its sinusoidal pumping action.

Water samples withdrawn from the branchial chambers of two crabs during emersion from hypoxic seawater at 12° C demonstrated that the accompanying aeration being performed by the animal was successful in raising the P_{O_2} of the water contained in the branchial chambers to between 40–60 mm Hg when the P_I, O_2 had fallen to approximately 10 mm Hg.

Crabs emerging into air from hypoxic seawater characteristically showed a tachycardia. The measured heart rates from 9 crabs which showed emersion responses at 12° C were 58 ± 5 beats/min (18) in the minute prior to emersion (this is a highly significant ($P < 0.001$) reduction in heart rate from the level in normoxia) and 81 ± 2 beats/min (18) immediately after emerging into air. The tachycardia on emersion is, therefore, an increase in heart rate back towards the rate for crabs in a normoxic environment (see Table 2). These crabs, which had E.C.G. electrodes and the pressure cannula inserted into them, first emerged from the hypoxic seawater at a P_I, O_2 of 19 ± 3 mm Hg (9) which is not significantly different from the value obtained with unrestrained and intact crabs at 12° C. The changes in heart rate associated with emersion into air from hypoxic seawater at 17° C, are listed in Table 3.

Table 3. The heart rate and oxygen tension and content of the post-branchial blood in *Carcinus maenas* (L.) at 17°C, in normoxia and in hypoxic seawater before and after emersion into air. (Values are means ± S.E. of mean, figures in parentheses denote number of observations)

	P_I, O_2 (mm Hg)	P_a, O_2 (mm Hg)	C_a, O_2 (vol.-%)	Heart rate (beats/min)
Normoxia				
(a) using Van Slyke apparatus	140 ± 3 (10)	—	1.04 ± 0.10 (10)	—
(b) using Lex-O_2-Con	145 ± 2 (8)	82 ± 6 (7)	0.96 ± 0.06 (8)	130 ± 8 (7)
Hypoxia				
(a) submerged crabs	25 ± 1 (8)	10 ± 1 (5)	0.19 ± 0.04 (8)	59 ± 6 (7)
(b) following 10 min continuous emersion into air	22 ± 2 (7)	12 ± 1 (6)	0.45 ± 0.05 (7)	99 ± 8 (6)

Often the change in heart rate on emersion was immediate, with the tachycardia developing within the time interval between two consecutive heart beats. This sudden increase in heart rate was synchronous with the crab emerging from the water surface and not with the onset of the reversal of irrigation which always heralded the emersion response (see Fig. 3).

The pattern of changes in heart rate on emersion and consequent submersion into hypoxic seawater is illustrated in Fig. 4a. The tachycardia was maintained as long as the crab stayed emerged from the hypoxic seawater and reverse irrigated its branchial chambers with air. If the crab resubmerged into hypoxic seawater its heart rate fell immediately. At the conclusion of the experiment, when the seawater was reaerated, the crab shown an immediate tachycardia which occurred before the oxygen electrode recorded the return to normoxia in the experimental tank.

δ) Emersion into a Hypoxic Atmosphere. A further series of experiments was carried out on 16 crabs held individually in the larger experimental chamber at 12°C with the airtight lid enclosing a hypoxic atmosphere in which the P_{O_2} was less than 10 mm Hg. The shallow seawater surrounding the crab was rendered progressively hypoxic. Of the 16 crabs observed, 8 failed to emerge into the hypoxic atmosphere when the water was made hypoxic. The other 8 showed a total of 49 separate emersion responses, 25 associated with an initial tachycardia, 22 with no change in heart rate and 2 with a bradycardia. In contrast to the response on emersion into air, emersion into a hypoxic atmosphere did not produce a maintained tachycardia. Whenever the heart rate did rise on emersion

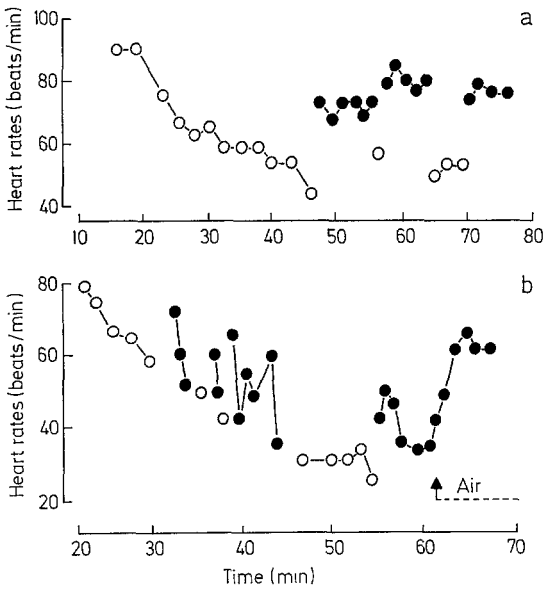

Fig. 4a and b. (*C. maenas*, 12°C) Changes in heart rate associated with progressive hypoxia and subsequent emersion into (a) air and (b) a hypoxic atmosphere ($P_{O_2} < 10$ mm Hg). In (b) the hypoxic atmosphere was replaced by air, this was followed by an immediate and maintained tachycardia. (Heart rate of submerged crabs ○, heart rate during emersion ●)

it characteristically decreased again towards the rate in hypoxic seawater, whilst the crab remained emersed (see Fig. 4b).

The patterns of behaviour and measured variations in branchial chamber pressures were also affected by exposure to a hypoxic atmosphere. Emersion was accompanied by a rapid alternation of the direction of irrigation with bubbles appearing alternately from the inhalent and exhalent openings. When, during an emersion, the hypoxic atmosphere was replaced by air, then the crab switched immediately to the behaviour and pattern of pressure variations associated with emersion into air. The reversal of irrigation with consequent aeration of the branchial chamber was maintained and the heart underwent a rapid and maintained tachycardia (see Fig. 4b).

c) Oxygen Tension and Content of the Postbranchial Blood

Samples of postbranchial blood were withdrawn from the pericardial cavities of 46 unrestrained crabs, submerged both in normoxic seawater and at various measured levels of hypoxia at 12°C. The oxygen tension of these samples was measured and they are plotted against P_I, O_2 in

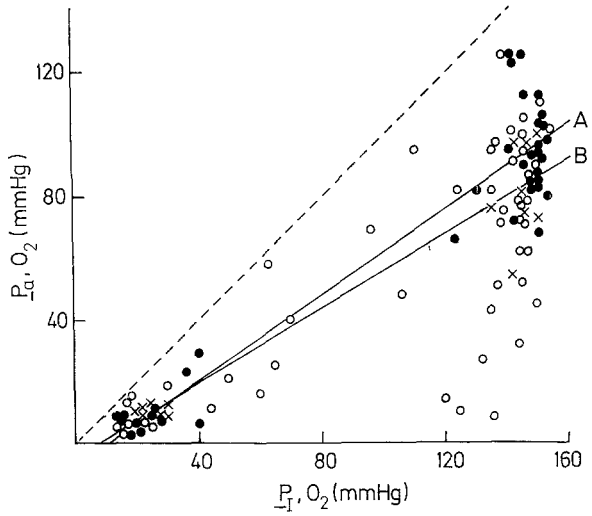

Fig. 5. Effect of progressive hypoxia on the oxygen tension of the post-branchial blood from 46 *C. maenas* submerged in seawater. • Blood samples from crabs which were observed to have a regular heart beat and were irrigating their branchial chambers. The regression line (A) is through these values only. ○ Samples from crabs in which heart rate and branchial chamber pressures were not monitored. The regression line (B) was derived from all the values for crabs at 12° C. × Samples from crabs submerged in seawater at 17° C. Equation for regression lines is $y = \hat{a} + \hat{b}(x - \bar{x})$ where for (A) $a = 66$, $b = 0.68$ (± 0.04), $\bar{x} = 107$ ($n = 40$); and for (B) $a = 59$, $b = 0.61$ (± 0.04), $\bar{x} = 107$ ($n = 83$)

Fig. 5. In 27 of these crabs the heart rate and branchial chamber pressures were monitored during the withdrawal of blood samples. In those crabs which were actively irrigating their branchial chambers and had a steady heart beat, the mean P_a, O_2 was 94 ± 3.0 mm Hg (27) at a mean P_I, O_2 of 146 ± 1 mm Hg (27). Blood samples were withdrawn from 3 of the crabs, submerged in normoxic seawater (mean P_I, O_2 143 mm Hg), after they had ceased to irrigate their gills for short periods. The P_a, O_2 was very much reduced to approximately 15 mm Hg. Combining all measurements of P_a, O_2 at P_I, O_2 values above 120 mm Hg, which included many taken from crabs in which heart rate and branchial chamber pressures were not monitored, gave a significantly lower mean P_a, O_2 of 81 ± 4 mm Hg at a mean P_I, O_2 of 144 ± 1 mm Hg (54). The low P_a, O_2 of some samples withdrawn from crabs in normoxia, and in which branchial chamber pressure and heart rate were not monitored, indicates that they had temporarily ceased to irrigate their branchial chambers whilst their blood was being withdrawn.

Blood samples were taken from 27 crabs which had spontaneously emerged from shallow hypoxic seawater at 12°C, and were bubbling air through their branchial chambers. The mean P_a, O_2 of these samples was 9 ± 2 mm Hg (27) at a mean P_I, O_2 of 15 ± 1 mm Hg. Interpolation on the regression line for variation in P_a, O_2 with P_I, O_2 in submerged crabs (Fig. 5) gives a P_a, O_2 of approximately 4 mm Hg at a P_I, O_2 of 15 mm Hg. It seems, therefore, that the spontaneous emersion response, which occurred at a low P_I, O_2 at 12°C, caused only a small increase in P_a, O_2, despite the consequent aeration of the branchial chambers. This observation was investigated further under more controlled conditions with crabs acclimated to 17°C, which characteristically emerge at a higher P_I, O_2.

Blood was withdrawn from 15 large *Carcinus* (mean weight 76.7 ± 3.6 g), which had previously shown a clear emersion response, whilst they were submerged in normoxic seawater 12 cm deep, at 17°C. This water was then rendered hypoxic down to a P_I, O_2 of approximately 25 mm Hg, by which time each crab was unsuccessfully attempting to emerge, and more samples were taken. The depth of the hypoxic water was then reduced to 5 cm, when each crab immediately emerged into air. Following 10 min continuous emersion another blood sample was taken. The oxygen tension and content of these samples were measured and the mean values are listed in Table 3.

The mean P_a, O_2 in normoxia was not significantly different from that obtained with crabs at 12°C, and during hypoxia P_a, O_2 fell to similar low values. Consequently, the P_a, O_2 values equivalent to measured levels of P_I, O_2 at 12°C and 17°C may be estimated by interpolation on line A in Fig. 5.

Following emersion the P_a, O_2 of each crab rose by between 1 and 5 mm Hg. The mean P_a, O_2 values before and after emersion were not, however, significantly different in the statistical sense ($P > 0.05$).

The C_a, O_2 values from crabs submerged in normoxic seawater were closely similar using two very different techniques for their determination (see Table 3). The oxygen content of postbranchial blood decreased to a low level in crabs submerged in hypoxic seawater (P_I, O_2 25 mm Hg) but rose significantly ($P < 0.001$), to approximately half the saturation value, following 10 min emersion into air from hypoxic seawater (P_I, O_2 22 mm Hg). The relationship between the P_a, O_2 and C_a, O_2 values of each of these blood samples, together with their means, is illustrated in Fig. 6, which also compares the equivalent oxygen content of seawater at the same temperature (17°C) and over the same range of oxygen tensions.

When 3 crabs were removed from aerated seawater at 12°C into room air they shown a marked decrease in P_a, O_2 from approximately

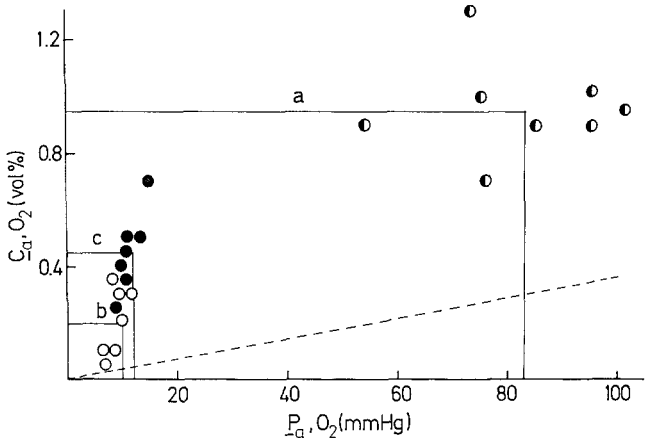

Fig. 6. The relationship between the oxygen content (C_a, O_2) and oxygen tension (P_a, O_2) of postbranchial blood samples withdrawn from crabs submerged in normoxic seawater (◐), submerged in hypoxic seawater (○) and after 10 min emersion into air from hypoxic seawater (●) at 17°C. The vertical and horizontal lines labelled a, b and c indicate the respective mean values for C_a, O_2 and P_a, O_2, and the divided line indicates the approximate oxygen content of seawater at 17°C over the range of P_{O_2} values on the diagram

90 mm Hg down to 17 mm Hg after less than 15 min exposure. Despite this marked reduction in P_a, O_2, heart rate in *Carcinus* did not change during aerial exposure.

Discussion

In normoxia, the mean values of respiratory frequency, the frequency and duration of the periods of reversed irrigation and the pressures in the epibranchial spaces during forward and reversed irrigation all fell within the ranges given for *Carcinus* by Hughes *et al.* (1969).

Between 6°C and 17°C heart rate had a Q_{10} of 2.6 and respiratory frequency had a Q_{10} of 2.2. This Q_{10} for heart rate is higher than that measured over a similar temperature range by Ahsanullah and Newell (1971). These marked increases in heart rate and respiratory frequency with acclimation temperature are probably an expression of an overall increase in the activity and, therefore, oxygen requirements of the crabs.

During progressive hypoxia, respiratory frequency did not change significantly from the rate in normoxia at either temperature, even at very low oxygen tensions. This agrees with the early observation by Johnson (1936) who inferred that *Carcinus* did not, therefore, show any respiratory adaptation to hypoxia. Arudpragasam and Naylor (1964b)

described an increase in irrigation volume in *Carcinus* in response to hypoxia which was directly proportional to an increase in reversal frequency. The same authors reported a decrease in reversal frequency during stagnation (i.e. hypoxia plus hypercapnia) which they attributed mainly to the accumulation of carbon dioxide. In the present investigation, prior to the emersion response, the mean frequency and duration of the periods of reversed irrigation increased in unrestrained crabs. Crabs which were exposed to hypoxia in deep seawater from which they could not emerge did, however, show an eventual decrease in reversal frequency at low values of P_I, O_2, particularly at 17°C (see Fig. 2). The initial increase in the proportion of total time which unrestrained crabs spent irrigating their gills in the reversed direction, as the surrounding water became progressively more hypoxic, may be regarded as evidence that the reversals subserve a respiratory role as well as clearing the gills of clogging detritus (cf. Bohn, 1897). Arudpragasam and Naylor (1964a) believed that the current reversals may serve to irrigate a poorly ventilated space dorsal to the posterior gills in the branchial chambers and indeed Johansen *et al.* (1970) cited evidence for a "large ventilation dead-space" in the branchial chambers of *Cancer magister*. Nevertheless, any conclusions on the respiratory role of the current reversals, which would be expected to disrupt the counter-current flow between water and blood at the respiratory surfaces, are dependant upon further measurements of patterns of water flow and oxygen tensions in the branchial chambers (see Hughes *et al.*, 1969).

During progressive hypoxia it was noted that although heart rate remained independant of P_I, O_2 down to relatively low levels, *Carcinus* showed a progressive bradycardia at low oxygen tensions. The P_I, O_2 at which heart rate first fell significantly below the rate in normoxia varied from 30 mm Hg at 6°C to 40 mm Hg at 12°C (P_a, O_2 approximately 20 mm Hg) and to 60 mm Hg (P_a, O_2 approximately 34 mm Hg) at 17°C (see Fig. 5). This influence of acclimation temperature on the threshold of oxygen tension for bradycardia during progressive hypoxia was reflected in the relatively high P_I, O_2 of 59 mm Hg at which *Carcinus* emerged from hypoxic seawater when acclimated to 17°C (see Table 1). *Carcinus* has been reported to maintain its rate of oxygen consumption independent of P_I, O_2 down to low tensions (Henze, 1910; Arudpragasam and Naylor, 1964b), though a fresh investigation is required to determine a precise critical oxygen tension (see Prosser and Brown, 1961) for oxygen consumption in this species. The different oxygen tensions at which *Carcinus* first showed a significant bradycardia and finally emerged from shallow seawater during progressive environmental hypoxia at 6°C, 12°C and 17°C may relate to the critical oxygen tensions for oxygen uptake in this species.

During exposure to hypoxia in shallow seawater, *Carcinus* typically emerged into air. Emersion was accompanied by prolonged reversals of the irrigation current which caused air to bubble through the water contained in the branchial chambers, with a consequent local increase in oxygen tension. On emersion into air there was an immediate and maintained tachycardia, with heart rate increasing towards the normoxic level. Although the volume flow of blood ($\dot{Q}$) was not measured in the present study, this marked tachycardia implies an increased blood flow and may represent an increase in oxygen consumption above the rate for crabs submerged in hypoxic seawater. The possibility of a respiratory role for the emersion response is supported by the changed pattern of responses of crabs exposed to progressive hypoxia with a hypoxic atmosphere above the water surface. Under these conditions *Carcinus* seemed less inclined to emerge and on emersion the branchial chambers were irrigated alternately in a forward and reversed direction and the heart showed at best only a transient tachycardia. The replacement of the hypoxic atmosphere with air led to an immediate establishment of the typical emersion response. There is considerable circumstantial evidence for the involvement of oxygen receptors in the initiation of these responses, but further discussion of this point must await direct investigations of their nervous control.

When *Carcinus* was irrigating and perfusing its gills with aerated water at 12°C the oxygen tension of its postbranchial blood was high (P_a, O_2 94 mm Hg at a P_I, O_2 of 146 mm Hg). This value is very similar to other aquatic water-breathing animals such as the trout (Holeton and Randall, 1967), dogfish (Butler and Taylor, 1971) and the dogfish and stingray (Cameron *et al.*, 1971). The relationship between P_I, O_2 and P_a, O_2 during progressive hypoxia is also similar to that measured on the dogfish at 12°C (Butler and Taylor, 1971). The measurements of blood oxygen tension in crabs are at variance with the remarkably low values previously reported for aquatic decapod crustaceans by Redmond (1955, 1968). Recently, however, Johansen *et al.* (1970) measured very similar oxygen tensions in *Cancer magister* (P_a, O_2 91 mm Hg at a P_I, O_2 of 132 mm Hg). In their thorough study of respiratory exchange in this large crab they adequately discussed the significance of their findings in the light of Redmond's theories on the role of haemocyanin, and effectively discounted the existence of large diffusion gradients consequent upon the supposed existence of barriers to diffusion at the gills of decapod crustaceans.

Johansen *et al.* (1970) emphasised the importance of proper conditions for blood sampling in their investigation. Redmond (1955) took large blood samples from animals whilst holding them out of water. The present study indicated that aerial exposure produces rapid internal

hypoxia in *Carcinus* due possibly to the collapse of the gills. This, rather than any specific difference, may account for the discrepancy between the measured P_a, O_2 in the present investigation, where the crabs remained immersed in seawater during blood sampling, and the low values reported for other species of crustaceans by Redmond (1955). Some preliminary observations on the lobster *Homarus vulgaris* in our laboratory, using the same technique of blood sampling as described above, indicated that P_a, O_2 is approximately 90 mm Hg (C_a, O_2 0.6 vol.-%) and P_v, O_2 approximately 35 mm Hg (C_v, O_2 0.3 vol.-%) in unrestrained animals irrigating their gills with aerated seawater at 11 °C. McMahon and Wilkens (1972) reported a P_a, O_2 of between 50–60 mm Hg in *H. americanus*. Redmond (1955), however, reported P_a, O_2 values of 10 mm Hg for this species.

The present results also demonstrate that it is essential to monitor the activity of the heart and scaphognathite during blood sampling as the apparently spontaneous propensity of the shore crab to cease the irrigation and perfusion of its gills causes a rapid drop in P_a, O_2. It may be argued that taking blood from crabs without monitoring heart rate and respiratory frequency represents a random sampling procedure which, because it involves taking some samples when the crab has spontaneously ceased to irrigate and perfuse its gills gives a significantly lower mean P_a, O_2 (P_a, O_2 81 ± 4 at a P_I, O_2 of 144 ± 1 mm Hg) which may be closer to the routine relationship between blood and water tensions in normal crabs. Nevertheless, this lower value is still considerably higher than those obtained by Redmond (1955).

During progressive hypoxia the P_a, O_2 of submerged crabs decreased in direct proportion to the reduction in P_I, O_2 (see Fig. 5). On emersion into air, the crabs observed at 17 °C showed a small increase in P_a, O_2 which, because of individual variation and the limitations imposed by the measuring techniques, was not statistically significant ($P > 0.05$).

During hypoxia the oxygen content of the blood decreased markedly in submerged crabs. On emersion, however, C_a, O_2 increased up to approximately half saturation (see Table 3). This marked increase in C_a, O_2 on emersion seems to conflict with the absence of a significant increase in P_a, O_2. Inspection of Fig. 6 indicates that the relationship between these *in vivo* measurements of P_a, O_2 and C_a, O_2 resembles an oxygen equilibrium curve for a blood pigment having a relatively high affinity for oxygen (k in Hill's approximation—see Jones, 1972), with a P_{50} of approximately 12 mm Hg, and a relatively steep slope over its functional range (n in Hill's approximation). Thus, the marked increase in C_a, O_2 on emersion, because it occurs around the P_{50} value for the pigment, where its buffering action for oxygen is highest, is accompanied by a relatively small increase in P_a, O_2. This conclusion is supported by the similarity of

the present results with the *in vitro* and *in vivo* measurements of the oxygen binding properties of *Carcinus* blood, made by Truchot (1971, 1973). Truchot (1971) obtained *in vitro* P_{50} values between 6 and 14 mm Hg over the pH range 7.98 to 7.39, at 15°C.

Further examination of Fig. 6, and the results given by Truchot (1971), indicates that the PSATN for *Carcinus* blood lies between 30–40 mm Hg at 17°C. Unlike mammalian blood, this value corresponds neither to the oxygen tension at the respiratory exchange surface in normoxia, nor to the P_a, O_2 value of arterialised blood. Johansen *et al.* (1970) were unable to suggest any adaptive pattern related to behaviour or habitat in the relative oxygen affinity of the blood in decapod crustaceans. In *Carcinus* it seems that during normoxia, although the presence of the blood pigment raises the carrying capacity of the blood above the equivalent value for seawater at the same P_a, O_2 (see Fig. 6), the functional range of the pigment is only likely to be fully utilised during internal hypoxia.

The present investigation reveals two circumstances, both leading to internal hypoxia, when the functional range of the blood pigment in *Carcinus* may have an adaptive significance. Firstly, the aeration of the branchial chambers during emersion which, although it caused only a small increase in P_a, O_2 was, by virtue of the relative values of k and n for the blood pigment, accompanied by a marked increase in oxygen content of the blood. As this increase was probably accompanied by a rise in blood flow, consequent upon the tachycardia on emersion, the supply of oxygen to the tissues is likely to have been increased significantly following emersion into air from hypoxic seawater. Secondly, there were periods when *Carcinus* in normoxic seawater ceased to irrigate and perfuse its gills, causing a precipitate fall in P_a, O_2 (see Fig. 5). This behaviour may enable the crab to conserve energy, and Johansen *et al.* (1970) discussed the probability that the energy cost of breathing is high in decapod crustaceans because of their relatively high irrigation rates. It is possible that the relatively high affinity of the crab's blood pigment for oxygen enables it to continue to transport much of its available oxygen to the tissues down to the low P_a, O_2 values reached during spontaneous cessation of irrigation and perfusion of the respiratory surfaces.

The PSATN value for *Carcinus* blood of between 30–40 mm Hg at 17°C corresponds to the P_a, O_2 at which *Carcinus* first showed a significant bradycardia when submerged during progressive hypoxia and to the mean P_a, O_2 when unrestrained crabs emerged from shallow hypoxic seawater (P_I, O_2 approximately 60 mm Hg, equivalent to a P_a, O_2 of approximately 35 mm Hg, see Fig. 5). Though it is only possible to speculate on the physiological basis for this relationship, it may be

significant that Randall and Smith (1967) noted a similar relationship between the P_I, O_2 at which the trout showed an initial bradycardia, and the P_a, O_2 required to desaturate its blood.

The present investigation supported the observation made by Ahsanullah and Newell (1971) that heart rate in *Carcinus* remains high when the crab is removed from water. The majority of water-breathing animals, including even some fish species which can survive prolonged aerial exposure, show a pronounced bradycardia when held in air (Satchell, 1971). *Carcinus* is, therefore, atypical in this respect. Ahsanullah and Newell (1971) believed that the absence of a bradycardia during aerial exposure indicated an ability to maintain "aerobic metabolism by air-breathing." This may be so, but Wallace (1972) reported a reduced rate of oxygen consumption in *Carcinus* when held in air, which increased when the crabs were submerged in seawater, and in the present study the P_a, O_2 was observed to fall rapidly to low levels after the crabs were removed from water. Nevertheless, it is possible that the maintenance of blood flow during aerial exposure allows some gas exchange to continue over the respiratory surfaces, or alternatively it may enable the crab to utilise all the available oxygen from its blood pigment as the P_a, O_2 falls to low levels. *Carcinus* shows a great ability to survive for long periods out of water and the P_{50} value for its blood pigment, though low, is higher than the measured values from many other decapod crustaceans. Young (1973) considered a relatively high P_{50} value to be one adaptation to air-breathing in land crabs. On the basis of the present investigation, however, *Carcinus* cannot be regarded as a semi-terrestrial, facultative air-breather. Its respiratory physiology seems adapted to enable it to survive periods of environmental hypoxia by taking advantage of a blood pigment with a relatively high affinity for oxygen, which has a particular function in relation to the emersion response.

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