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RESPIRATORY AND CIRCULATORY RESPONSES TO HYPOXIA IN THE LOBSTER *HOMARUS AMERICANUS*

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SUMMARY

Contrary to previous reports, oxygen consumption is maintained over a wide range of external oxygen tensions in the lobster *Homarus americanus*. In animals acclimated to the experimental conditions this response is mediated by increased branchial pumping, increased effectiveness of oxygen uptake by the gills and an increased contribution by the respiratory pigment to the oxygen delivered to the tissues. Circulatory blood oxygen levels are generally high in lobsters resting in well-aerated water. Mechanisms for detection of hypoxia and possible control mechanisms are discussed.

INTRODUCTION

Functional analysis of aquatic respiratory systems depends on a detailed knowledge of the structure and diffusion capabilities of the respiratory surface, the relationship between water and blood flow at the gill level and quantitative measurement of gas transport by the blood system. Although the morphology of decapod crustacean gill systems has been extensively studied (Huxley, 1896; Bock, 1925; Fisher, 1972), functional studies have followed more slowly and have produced some contradictory data.

The mechanics of the scaphognathite suction pump which powers a continuous flow of water across the gill surfaces has been described for the brachyuran *Carcinus maenas* (Hughes, Knights & Scammel, 1969) and the macruran *Homarus americanus* (Wilkens & McMahon, 1972) and for two crayfish species (Larimer, 1961, 1964; McMahon, Burggren & Wilkens, 1974). The pumping mechanism seems essentially similar in all species studied, although the flow patterns of water over the gills differ markedly between the brachyuran and macruran types (Hughes *et al.* 1969; Fisher, 1972; Burggren *et al.* 1975).

The diffusion characteristics of the gill surface and respiratory properties of the blood have also received recent attention. Here some confusion arises from the literature, as earlier workers (Redmond, 1955; Larimer, 1964) reported very low circulating blood oxygen tensions and suggested that the poor diffusion capabilities of the gill surface require the maintenance of a large oxygen tension gradient between blood and branchial water in order to provide adequate oxygen exchange, while more

recent reports (Johansen, Lenfant & Mecklenburg (1970) in *Cancer magister*; McMahon & Wilkens (1972) in *Homarus americanus*; Taylor & Butler (1973) in *Carcinus maenas*) have measured high postbranchial blood oxygen tensions in animals resting in well-aerated water, thus indicating good saturation of the blood with a lower diffusion gradient across the gill surface.

Some controversy also occurs as to the responses of these animals to hypoxia. Earlier reports indicated that some species conform to a hypoxic environment such that oxygen consumption decreased linearly in response to a progressive decrease in external oxygen tension (Amberson, Mayerson & Scott (1924) for *Homarus americanus*; Thomas (1954) for *H. vulgaris*; Weins & Armitage (1969) for *Orconectes* spp). More recent reports, however, show considerable ability to regulate oxygen consumption in a hypoxic environment (Thompson & Pritchard (1969) for *Upogebia* and *Callianassa*; Johansen *et al.* (1970) for *Cancer magister*; McMahon *et al.* (1974) for the crayfish *Orconectes virilis*). Such marked differences between animals with an essentially similar respiratory apparatus are interesting and prompted the authors to re-examine the respiratory performance of *Homarus americanus* at several ambient oxygen levels.

MATERIALS AND METHODS

Lobsters (*Homarus americanus*) were mostly obtained from commercial dealers but a few specimens were collected by divers from a recently seeded area off the west coast of Vancouver Island. Animals were acclimated to aquarium conditions of fresh running sea water at 12–15 °C and 70–100 mmHg P_{O_2} for at least 1 week before experiments commenced. During experiments the animals were contained in a perspex chamber modified from a design used by Larimer (1961). Inhalant and exhalant respiratory water streams were separated by inserting the cephalothorax through a snug-fitting sheet-rubber partition to a level just anterior to the bases of the chelae. To facilitate this operation the chelae were usually removed at the autotomy plane 1 or more days prior to experimental use. The membrane divided the experimental tank into two chambers and allowed measurement of the volume of water passing over the gills as described by McMahon *et al.* (1974). The animals were usually held in position solely by a thread attached to the rostrum and tied to an external support. Other strictures were rarely needed but occasionally the telson of the animal was held lightly to prevent tail flips and consequent wave action damaging to apparatus and records.

Fresh sea water at 12–15 °C (daily experimental variation 0·5 °C) flowed through a gas exchange column and into the posterior compartment at 700–1000 ml/min. The oxygen tension of this water could be varied during its passage through the column by exposure to a counter current stream of nitrogen or air. Regulation of gas flow rates allowed the incoming water to be held at any desired oxygen tension. This method of deoxygenation could also have rendered the water hypocarbic. However, the extreme difficulties involved in the measurement of carbon dioxide levels in either water or blood precluded investigation of this parameter in the present study.

The rates of scaphognathite beating were extrapolated from hydrostatic pressure recordings from both branchial chambers, and heart rates from electrical signals

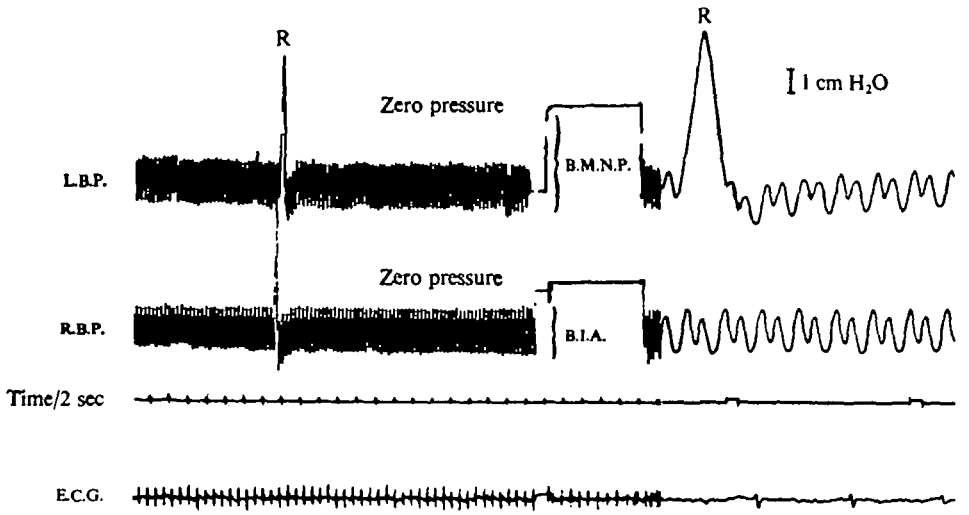

Fig. 1. Heart rate (E.C.G.) and branchial cavity pressures recorded simultaneously from a lobster following introduction into the experimental chamber. L.B.P., R.B.P., left and right branchial pressures respectively. B.I.A., peak-to-peak amplitude of the pressure waveform (indicating force of scaphognathite beat); B.M.N.P., maintained negative pressure in the branchial cavity (indicating force powering suction flow over the gills). Zero pressure = ambient pressure at cannula tip level. R, A single reversed scaphognathite beat in one or both cavities.

recorded from the pericardial cavity, as described by McMahon & Wilkens (1972). The level of negative pressure (BMNP) maintained in the branchial cavity was also measured (Fig. 1) as an indication of the force developed by the scaphognathite pumps. Exhalant water drawn out of the branchial cavities by the action of the scaphognathites passed out of a stand pipe in the anterior chamber. Branchial water flow was determined either by collection of the volume outflow per minute, or, over longer periods, by a calibrated recording siphon. Exhalant water samples (P_{E,O_2}) were drawn into a 1 ml glass syringe via a cannula positioned within 1 mm of one exhalant aperture. Inhalant water samples (P_{I,O_2}) were taken simultaneously from a point closely adjacent to the inhalant apertures between limb bases. In both cases a volume greater than the cannula and dead space volume was taken and rejected immediately prior to sampling.

Blood oxygen tensions were determined from samples taken into 0·5 ml syringes either from the pericardium to one side of the heart (postbranchial blood; P_{a,O_2}) or from the ventral thoracic sinus (prebranchial blood; P_{v,O_2}). Sampling by means of a catheter into either location was impracticable due to rapid clotting which blocked all cannulae within minutes. Attempts to monitor blood pressure were similarly confounded. Blood samples were analysed immediately to prevent clotting in the analyser. Both blood and sea-water oxygen tensions were measured using a Radiometer BMS 3 blood gas analyser calibrated with humidified gases of known oxygen content and also with equilibrated water samples. The analyser was maintained at the same temperature as the experimental sea water.

Oxygen dissociation curves were determined on fresh defibrinated blood by a method similar to that of Riggs (1951). No apparatus was available on site to determine oxygen saturation, and Redmond's (1955) figures have been used in this study.

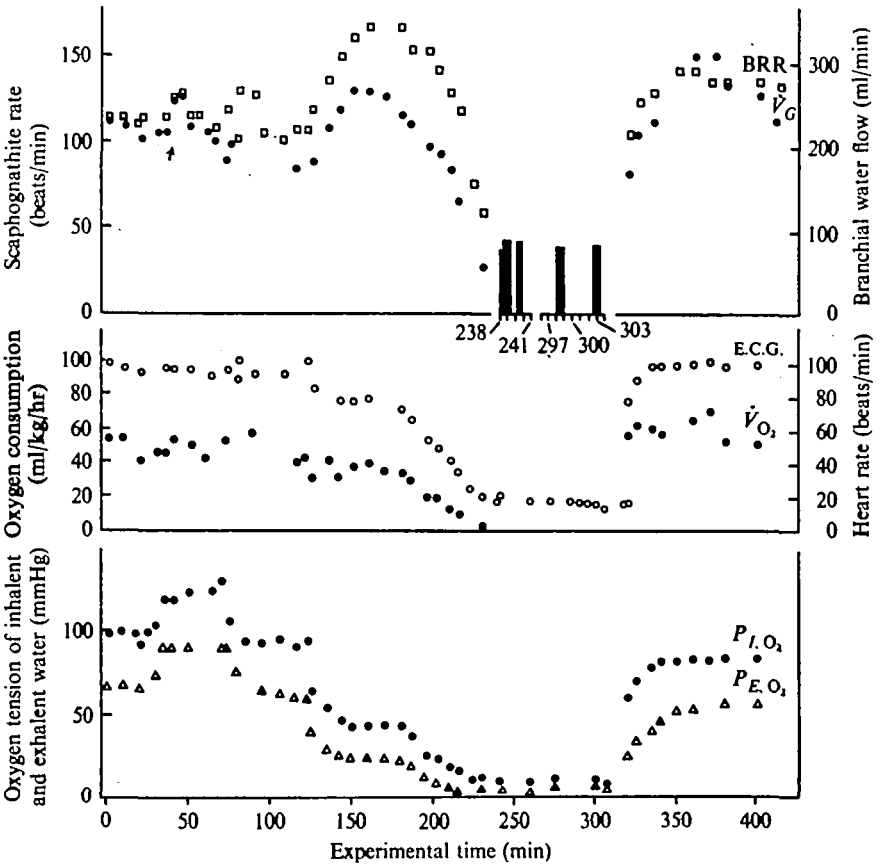

Fig. 2. A typical hypoxic stress experiment showing respiratory and cardiac parameters plotted against time. Between 238–303 min the scale is expanded in the upper graph to show the frequency and duration of periods of intermittent branchial pumping typical of prolonged exposure to severe hypoxia. At 310 min the ambient oxygen tension was allowed to rise to normoxic levels. P_{I,O_2} = inhalant oxygen tension = ●. P_{E,O_2} = exhalant oxygen tension = △. $\dot{V}_G$ = branchial water flow (ml/kg/mm) = ●. $\dot{V}_{O_2}$ = oxygen consumption (ml/kg/h) = ●. BRR = scaphognathite rate (beats/min) = □. E.C.G. = heart rate (beats/min) = ○. The arrow in the upper graph denotes a period of slight struggling.

Oxygen dissociation curves for the calculations of Table 3 were determined at pH 7·50, this being the mean pH of samples of both pre- and postbranchial blood taken at all oxygen ranges. Frequent measurement of pH during experiments was difficult as we could not prevent clotting in the capillary pH electrode. No attempt was made to calculate a 'true' dissociation curve, i.e. an intermediate between curves plotted at pre- and postbranchial carbon dioxide tensions, as we could not accurately measure the extremely low carbon dioxide tensions in the blood.

RESULTS

Fig. 1 shows typical pressure recordings and the electrocardiogram from an experimental lobster at the start of an experiment. Regular beating of both scaphognathites pumps water out of the branchial cavities anteriorly and creates a maintained negative pressure which draws ambient water into the branchial chamber and over

Table 1. *Respiratory and circulatory response to hypoxia in short term experiments (200 min or less)*

Ambient oxygen tension range (mmHg)	BRR (bt/min)	BMNP (cm H_2O)	$\dot{V}_G$ (ml/kg/mm)	Ut (%)	$\dot{V}_{O_2}$ (ml/kg/min)	E.C.G. (bt/min)
150 → 100	159	2·0	1163	22	1·20	96
90 → 70	166	1·8	1046	24	0·88	82
50 → 30	149	1·4	842	26	0·53	47
20 → 10	71	0·6	410	44	0·15	30
30 → 50	104	(1·0)	(649)	(73)	0·98	(36)
70 → 90	130	1·4	713	(43)	1·10	(70)
100 → 150	135	1·45	916	22	1·63	90

BRR = scaphognathite rate. BMNP = maintained negative pressure in the right branchial cavity. $\dot{V}_G$ = volume of water pumped through both branchial cavities. Ut = Utilization of oxygen expressed as the percentage of oxygen content removed from the branchial water flow.

$\dot{V}_{O_2}$ = oxygen uptake from the branchial water expressed as ml O_2/kg body wt/min. E.C.G. = electrocardiogram.

The data above are averaged from experiments (six on four animals) characterized by short periods of adjustments to the experimental chamber and exposure to rapidly changing oxygen tensions. Data in parentheses are where $n = 5$ or less data points.

The last three lines are from animals recovering from hypoxia.

the gills. Single reversed scaphognathite beats occur periodically, causing a brief positive pressure pulse in one or both branchial cavities. The form and function of these reversed beats and an analysis of forward beating has been described elsewhere (Wilkens & McMahon, 1972). Sudden rate changes were seen in response to slight struggling (Fig. 2) or during occasional 'pauses' (periods of simultaneous inhibition of both cardiac and scaphognathite pumps). These 'pauses' are often seen in animals resting in aerated water and have been discussed in detail elsewhere (McMahon & Wilkens, 1972).

The water in our holding facilities was never fully saturated with air and during the course of this experimental series varied from 70 to 100 mmHg ambient oxygen tension, with tensions usually in the range 90–100 mmHg. As these were the levels to which our animals were acclimated, initial readings in the experimental chamber were usually made in the range 90–100 mmHg and the animals were subsequently exposed to aerated water 120–150 mmHg and/or oxygen depleted water.

An initial series of experiments was performed to determine the tolerance levels of *Homarus americanus* to hypoxia and to estimate the tensions at which hypoxic responses might occur. In these experiments recordings commenced shortly after the animal had been placed in the experimental chamber and the animals were exposed to progressively deepening hypoxia over a period of 1–2 h. In these experiments heart rates declined steadily with decrease in ambient oxygen tension (Table 1) but the response of the scaphognathite pumping system to moderate hypoxia was variable. Below 50 mmHg all animals showed depression of scaphognathite rate, but between 50 and 100 mmHg some animals exhibited maintained or even slightly increased rates. Calculation of the oxygen consumption of animals during these short experiments demonstrated a progressive (but non-linear) decrease in consumption with reduction of ambient oxygen tension as had been reported by Amberson *et al.* (1924).

Table 2. *Respiratory and circulatory responses to hypoxia in longer experiments* 300+ *min*

Ambient oxygen tension range (mmHg)	BRR (bt/min)	BMNP (cm H_2O)	$\dot{V}_G$ (ml/kg/min)	*Ut* (%)	ΔP_G mmHg	T_{O_2} ($\dot{V}_{O_2}$/min/$\dot{V}_G$)	*Ew* (%)	$\dot{V}_{O_2}$ (ml/kg/min)	E.C.G. (bt/min)
100 ← 150	101	1·5	487	23	67	0·007	27	0·50	92
70 ← 90	116	1·8	595	29	39	0·013	38	0·50	84
30 ← 50	137	2·3	666	41	15	0·032	59	0·47	50
10 ← 20	88	1·1	394	43	5	0·030	80	0·13	29
30 → 50	141	(2·2)	(563)	(44)	—	—	—	0·47	36
70 → 90	126	2·3	641	37	—	—	—	0·88	70
100 → 150	103	1·7	579	32	—	—	—	0·87	90

Symbols as in Fig. 1 plus:

ΔP_G = average tension difference between inspired water and prebranchial blood in mmHg.

T_{O_2} = transfer factor (diffusing capacity of the gills for O_2) expressed as mlO_2/min/kg/mmHg = $\dot{V}_{O_2}/\Delta P_G$.

Ew = effectiveness (%) of oxygen transfer from water to blood = $[(P_{I,O_2}-P_{B,O_2})/(P_{I,O_2}-P_{V,O_2})] \times 100$.

The data above are averaged from experiments (nine on six animals) characterized by ≏ 2 h periods of adjustment to the experimental chamber and exposure to several maintained levels of oxygen depletion. Data in parentheses are where $n = 5$ or less data points.

The last three lines are from animals recovering from hypoxia.

During the course of this first experimental series we observed that pumping rates in both heart and scaphognathite systems were very high (Fig. 1) immediately following insertion into the experimental chamber, presumably due to handling and insertion of cannulae and electrodes, but decreased steadily reaching a relatively stable level only after 1–3 h. Thus in the second series of experiments animals were allowed to acclimate to the experimental chamber for at least 2 h before data were collected. Also in these experiments the animals were exposed to maintained oxygen levels for periods of 40–120 min to allow more time for hypoxic responses to develop.

Table 2 and Fig. 2 show the results of this series of longer experiments. The ranges are chosen for ease of comparison with Table 1 and do not indicate fluctuations in oxygen tension, which was maintained at set levels within these ranges. Under these experimental conditions pronounced responses to hypoxia were observed in each experiment. Decrease of ambient oxygen tension to a critical level of 30–40 mmHg P_{O_2} caused a progressive increase in the rate of scaphognathite pumping, greater negative pressure in the branchial cavities and increased branchial water flow. The average oxygen pressure gradient between inhalant water and prebranchial blood (ΔP_G) decreased proportionately over the range 150–30 mmHg ambient oxygen tension but both the transfer factor for oxygen (T_{O_2}) and the effectiveness of oxygen uptake from water (% *Ew*) increased progressively. These latter factors, together with the increased branchial water flow, were partially responsible for the maintained oxygen consumption seen over this range.

Prebranchial (P_v) and postbranchial (P_a) blood was sampled at each oxygen level. Relatively few venous samples were taken as they were difficult to obtain without disturbing the animals and also to avoid substantial depletion of the animals' circulating haemocyanin. The results obtained from six experiments in which blood samples were taken are presented in Table 3. The oxygen content of the blood was

Table 3. *Oxygen transport at four ranges of ambient oxygen tension*

Ambient oxygen tension range P_{O_2}	P_{a,O_2} (mmHg)	P_{v,O_2} (mmHg)	P_{O_2} difference $a-v$ (mmHg)	S_{a,O_2} (%)	S_{v,O_2} (%)	C_{a,O_2} (vol. %)	C_{v,O_2} (vol. %)	Total O_2 delivered to tissues (vol. %)	O_2 delivered by Hcy (vol. %)	O_2 delivered from solution (vol. %)	$\dot{Q}$ (ml/min)	$\dot{V}_{O_2}$ (ml/kg/mm)	E.C.G. (bt/min)
100–150	69	18	51	100	93	0·86	0·80	0·248	0·06	0·188	60·5	0·50	92
70–90	42	17	25	100	91	0·86	0·78	0·173	0·08	0·093	88·2	0·50	84
30–50	22	12	10	96	75	0·82	0·64	0·217	0·18	0·037	59·9	0·47	50
10–20	8	7	1	32	24	0·275	0·206	0·073	0·069	0·004	54·8	0·13	29

$S_a . S_{v,O_2}$ = percentage oxygen saturation of post- and prebranchial blood calculated from the dissociation curve at pH = 7·50 and 15 °C.
$C_a . C_{v,O_2}$ = oxygen content of post and prebranchial blood based on haemocyanin oxygen capacity of 0·86 vol. % = 100 % saturation.
$\dot{Q}$ = cardiac output in ml/min calculated using the Fick principle.
P_{a,O_2} = postbranchial blood oxygen tension.
P_{v,O_2} = prebranchial blood oxygen tension.
Hcy = haemocyanin.

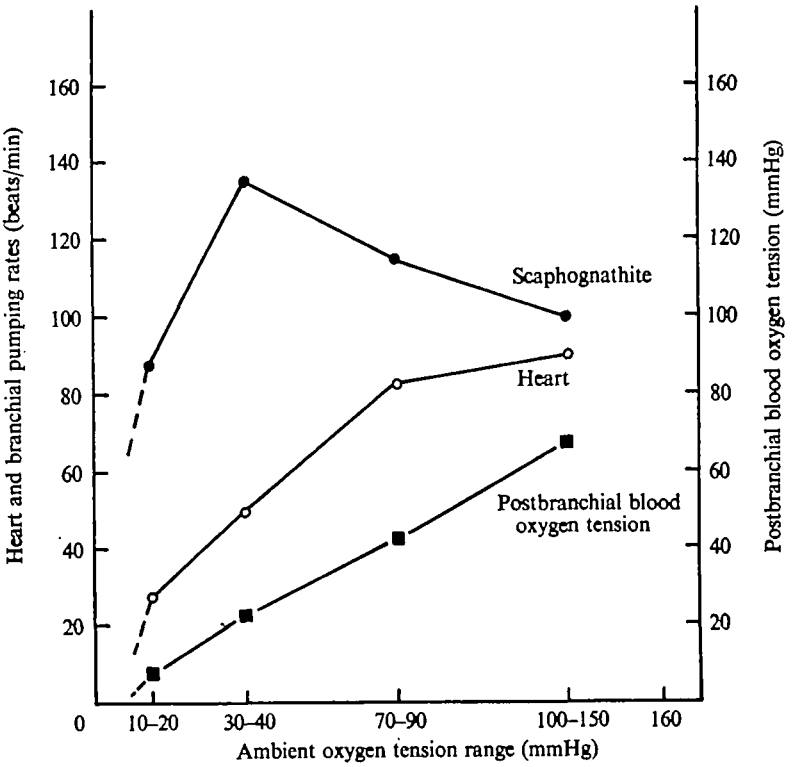

Fig. 3. Scaphognathite and heart-beat rates, and postbranchial blood oxygen tension plotted at four ranges of external oxygen tension. Data from the longer-term experimental series (Tables 2, 3).

extrapolated from a dissociation curve plotted at pH 7·5 and 15 °C and from Redmond's (1955) figures for the oxygen capacity of *Homarus* blood.

The oxygen tensions of both prebranchial and postbranchial blood taken from animals resting in well-aerated water are considerably higher than those reported previously for this species (Redmond, 1955, 1968). As lobster haemocyanin has a relatively high affinity for oxygen (P_{50} = 10 mmHg at pH 7·5 and 15 °C) these values show that both pre- and postbranchial blood were both virtually saturated with oxygen at these higher ambient oxygen tension levels. Under these conditions very little (24%) of the oxygen delivered to the tissues was released from the haemocyanin, but the large difference in oxygen tension between pre- and postbranchial blood allowed substantial release (76% of total) of the oxygen dissolved in the haemolymph.

As the ambient oxygen tension decreased, both prebranchial and postbranchial blood oxygen tensions and the difference between these blood oxygen tensions decreased proportionately, thus reducing the volume of oxygen displaced from solution to the tissues. However, the reduction in oxygen tensions (particularly of prebranchial blood) allowed progressively greater release of oxygen from the haemocyanin. Table 3 and Fig. 7 demonstrate that over the range 30–150 mmHg ambient P_{O_2} oxygen supply to the tissues could be maintained by such increased participation of the haemocyanin molecule.

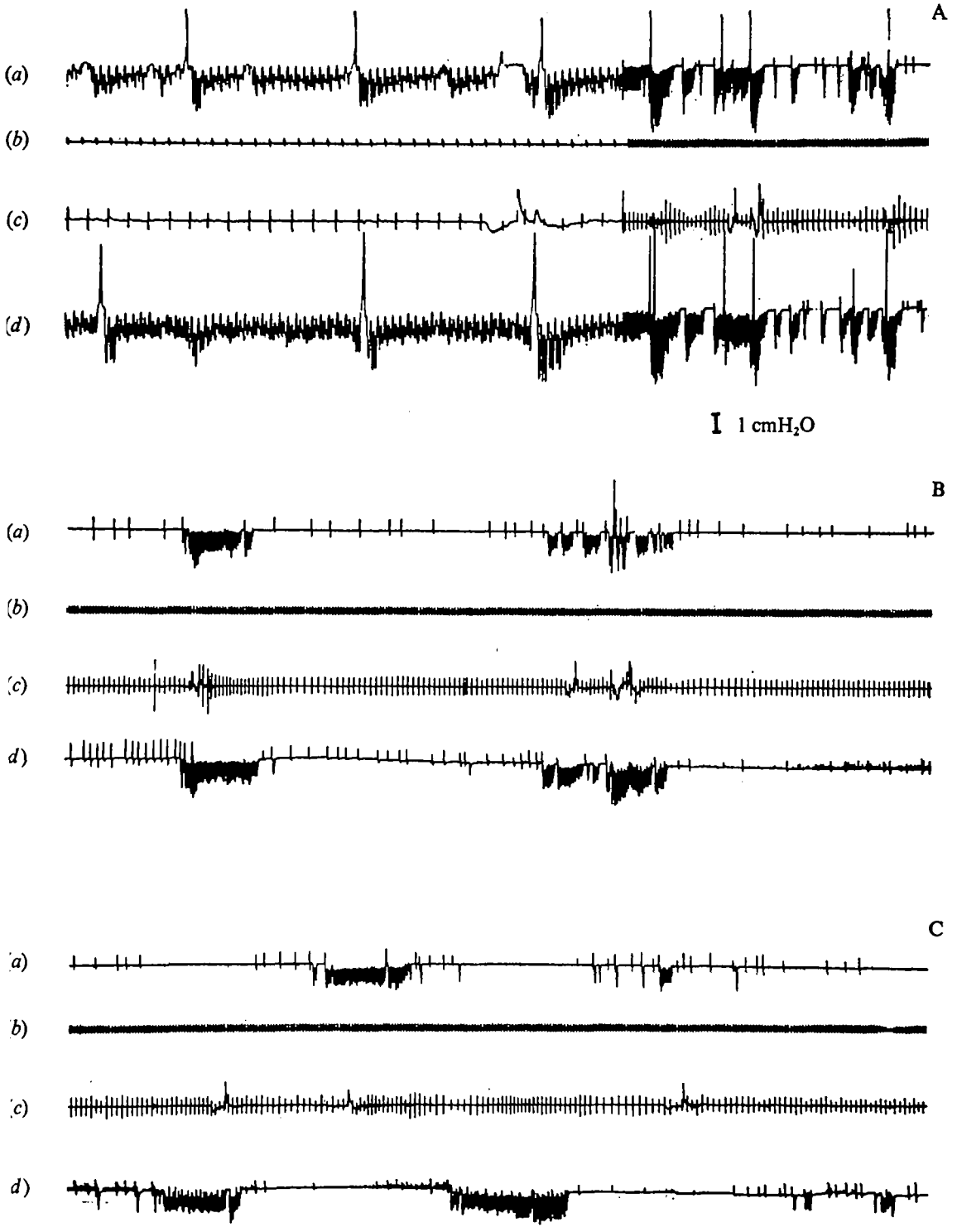

Fig. 4. Respiratory and cardiac pumping rates recorded at 10 min (A) and 40 min (B, C) of a 60 min exposure to an oxygen tension of 9–12 mmHg. (*a*, *d*) Left and right branchial pressures. (*c*) E.C.G. (*b*) Time marker at 2 sec intervals. A shows beginning of intermittent breathing, B and C show simultaneous and alternating scaphognathite activity respectively. During apnoeic periods pressure levels are equivalent to zero pressure.

No increase occurred in heart rate in response to these moderate hypoxic levels. Indeed heart rate decreased progressively as the oxygen tension was reduced, as in the shorter experimental series. Fig. 3 shows the markedly different rate responses of heart and scaphognathite pumps in acclimated animals exposed to hypoxia. At ambient oxygen levels below 50 mmHg P_{O_2}, rhythmic pumping was often seen in the swimmerets; perhaps this increases local oxygen supply or is part of a general locomotor response to low oxygen exposure. No definite correlation was observed between the frequency of reversed pumping strokes and ambient oxygen level but the actual incidence of reversals was so variable as to preclude definite analysis. Sequences of maintained reversed pumping commonly seen in crabs were not observed from *Homarus*.

Below 30 mmHg ambient oxygen tension both heart and scaphognathite rates declined sharply (Figs. 2, 3), and below 10–20 mmHg both rates became very slow and irregular (Fig. 4). Fig. 4 shows recordings of branchial pressures and cardiac rates during a 60 min exposure to an ambient P_{O_2} level of 9–12 mmHg. On initial exposure both scaphognathite and cardiac beat rates became very slow (Fig. 4) and the pressure developed in the branchial cavity was reduced considerably. Very little water was pumped over the gills at this level. With prolonged exposure, periods of apnoea were seen (Figs. 2, 4B–C). The duration of these periods of apnoea increased with continuous exposure and eventually reached several minutes duration (Fig. 2). Heart rate was irregular at this time (Fig. 4), falling to very low levels (12–14/min) during apnoea and usually increasing slightly during periods of scaphognathite activity. Cardiac arrest was not seen during apnoea; these periods are apparently different from the 'pauses' described earlier, which are only seen in well-aerated conditions and involve simultaneous inhibition of both pumping systems.

Between apnoeic periods low-level pumping activity occurred. This could occur simultaneously in both scaphognathites (Fig. 4A, B) or more usually an alternating pattern was observed in which scaphognathite beating occurred on one side while the other was apnoeic (Fig. 4C). Increase in heart rate occurred whether one or both branchial pumps were working (Fig. 4B, C). It is of interest to note that a minimum beat frequency occurs in the scaphognathite system (Table 4). Scaphognathite beating below 48 beats/min was rarely seen in these animals.

During exposure to these very low O_2 tensions, scaphognathite pumping became ineffectual. Even when one or both pumps were active, very small negative pressures were generated in the branchial cavities and branchial water flow was less than 10 ml/min. Although the percentage utilization of oxygen from the water remained relatively high (45%), very little oxygen was actually obtained and blood oxygen tensions and the tension difference between prebranchial and postbranchial blood fell to low levels. Under these conditions little oxygen was transferred to the tissues and the animals rapidly became anoxic.

As soon as prolonged apnoea was seen on both sides, air-saturated water was immediately run into the chamber. Under these conditions an extremely rapid recovery was seen (Fig. 2). Dramatic increases in the frequency of both scaphognathite and heart beat and in the volume of water pumped through the branchial cavities occurred in 1–2 min, and prehypoxic levels were reached or exceeded within 10 min. It may be that in these experiments the rate of recovery was limited by the rate at

Table 4. *Minimum rates of scaphognathite beating observed during severe hypoxia*

(Successive trials of any animal were separated by at least 2 days and on two occasions by a week. The oxygen tension given is that level at which periods of apnoea were seen alternating with periods of low level pumping.)

Lobster no.	Trial	Minimum rate (beats/min)	P_{O_2} (mmHg) ambient
1	1	51–0	8–10
—	2	48–0	10
—	3	48–0	10
2	1	51–0	—
3	1	48–0	9
—	2	54–0	9
—	3	54–0	9
4	1	36–0	9–11
5	1	54–0	11
6	1	60–0	12
7	1	60–0	6
8	1	48–0	8
9	1	54	5
10	1	60	6

Minimum rate shows minimum rate during low level pumping. In apnoeic periods pumping rate falls immediately to zero. In two cases, 9 and 10, apnoeic periods were not recorded.

which the water in the experimental system could be replaced. In order to study further the rate of recovery, we injected small volumes of aerated sea water into one branchial cavity of animals that had been held for over 60 min in water at 10 mmHg P_{O_2} and were showing very little spontaneous pumping activity. Fig. 5 shows the results of such an experiment. Very little oxygen was needed to start the recovery responses as injections of 5–10 ml caused significant changes in both heart and branchial pumping systems. The response time of the recovery system was relatively fast as injections of 30 ml or more of sea water caused marked changes in both heart and branchial pumping in 20–30 sec. Increase in heart rate preceded increase in scaphognathite rate by approximately 10 sec. The addition of such small amounts of oxygen to the system caused substantial recovery, with the heart rate reaching 75% of the prehypoxic level within 60 sec after injection. Such responses, however, declined rapidly as branchial pumping brought more deoxygenated water into the branchial cavities. Control injections of similar volumes of oxygen-depleted water produced no increase in either heart or scaphognathite beat frequency.

Animals recovering from severe hypoxic exposure often exhibited scaphognathite beat frequencies and branchial water flows above those measured in previous normoxic and hypoxic recordings. Percentage utilizations of up to 75% were observed and oxygen consumption figures reached levels substantially higher than those recorded from the same animal prior to hypoxic exposure (Fig. 2; Tables 1, and 2). This latter observation may be partly explained by the high levels of pumping activity occurring at this time but may also indicate the repayment of an oxygen debt incurred during the hypoxic period. Over a period of 1–4 h scaphognathite pumping rates and oxygen consumption declined at a rate dependent on the length and severity of the previous hypoxic period.

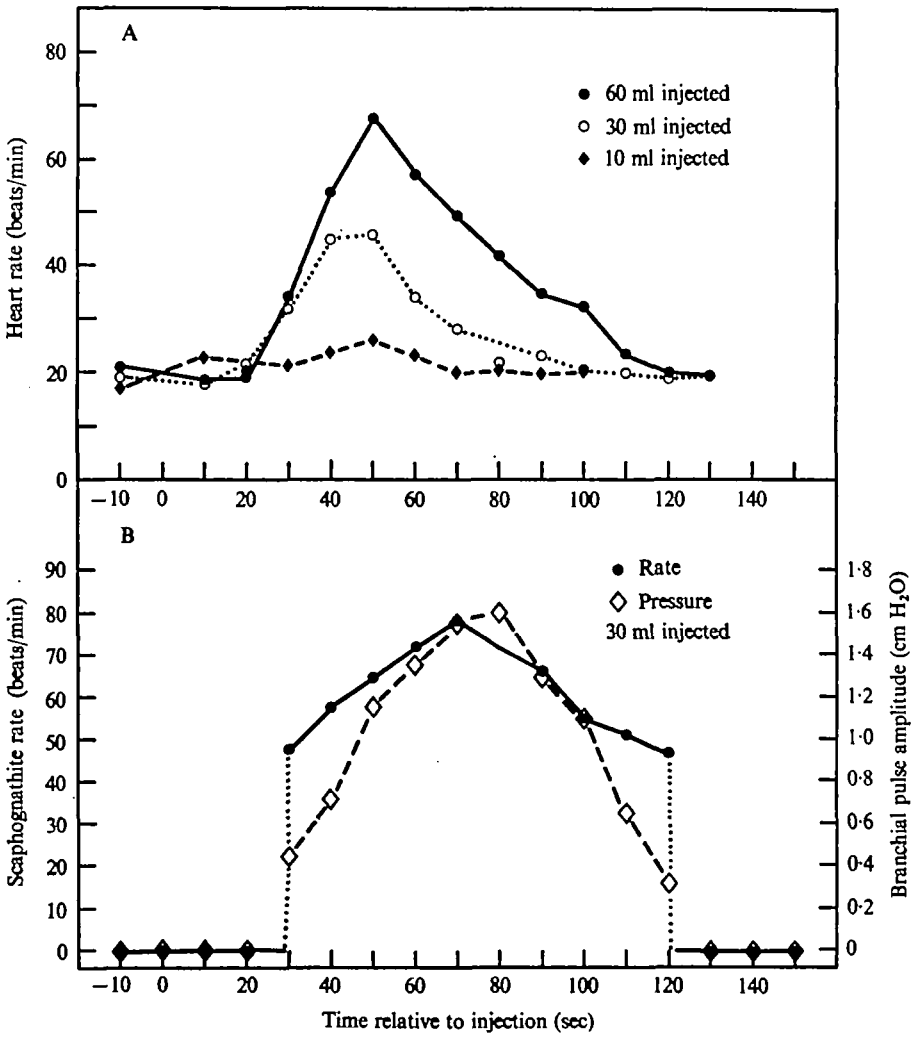

Fig. 5. Responses of one scaphognathite and the heart to injection of aerated sea water into the opposite branchial cavity in a hypoxic lobster. (A) The elevation of heart rate associated with injection of from 10–60 ml aerated water. (B) The elevation of scaphognathite beat rate and amplitude of pressure pulse (BPA) in response to a 30 ml injection.

DISCUSSION

After a 2 h period of acclimation to the experimental chamber, lobsters invariably responded to hypoxic stress. Scaphognathite beat frequency increased, causing the development of a greater negative pressure in, and increased water flow through, the branchial chambers. The effectiveness of oxygen uptake across the gills ($Ew\%$) also increased with hypoxia but the maintenance of oxygen consumption at prehypoxic levels was largely dependent on the increased rate of scaphognathite activity. This increased branchial pumping must itself increase the animal's oxygen demand and a critical external oxygen tension must occur below which the additional scaphognathite activity consumes more oxygen than it gains. In our animals this level may

have been reached at 30–40 mmHg P_{O_2}. Below this level insufficient oxygen was available, and both respiratory and heart rates declined rapidly. Due to the high energy cost, increased branchial pumping is probably only useful as a short-term response to hypoxic conditions enabling the animal to combat short natural hypoxic periods or allowing time for other adaptive mechanisms to develop. McMahon *et al.* (1974) have investigated the responses to one week of hypoxic exposure in the freshwater crayfish *Orconectes virilis* and show that the high levels of branchial water flow which are developed initially decline steadily over 7–10 days of maintained hypoxia. Presumably, other adaptive mechanisms, which increase either the effectiveness of oxygen uptake or metabolic efficiency, develop during this period. Similar effects could occur in *Homarus americanus* but long-term hypoxic exposure would seem unlikely in the natural habitat of this lobster and thus chronic effects were not determined in this study.

During exposure to ambient oxygen tensions below 30–40 mmHg both branchial and cardiac pumping rates decrease markedly (Fig. 3; Table 1) and become irregular (Fig. 4). Death results from prolonged exposure but most animals could survive exposures of 1–2 h. Unless the hypoxia had caused cellular damage, recovery was very rapid once aerated water was reintroduced, and within minutes heart and branchial pumping rates had reached or exceeded prehypoxic levels (Fig. 2). Very high values for oxygen consumption were observed for several hours following hypoxia, suggesting the repayment of an 'oxygen debt' incurred during the hypoxic period.

The results presented here do not support the conclusions reported previously in the literature for either *Homarus americanus* (Amberson *et al.* 1924) or *Homarus vulgaris* (Thomas, 1954). These authors reported no modification of branchial pumping in response to hypoxia and showed a linear decrease in oxygen consumption with falling oxygen concentration. Larimer & Gold (1961) examined hypoxic responses in the crayfish *Procambarus simulans* and reported decreased oxygen consumption on hypoxic exposure despite evident increased branchial water flow. Allowing oxygen uptake and thus metabolic rate to fluctuate with, or conform to, environmental levels is perhaps to be expected of smaller or less complex invertebrate forms (Mangum & van Winkle, 1973), but total conformation seems surprising in these larger decapod Crustacea. In fact many recent studies (Thompson & Pritchard, 1969; Johansen *et al.* 1970; Winget, 1971; McMahon *et al.* 1974) on other decapod species and the present study on *Homarus* (Fig. 6) all indicate a considerable ability to regulate oxygen consumption under hypoxic conditions. Taylor, Butler & Sherlock (1973) observed no increase in scaphognathite rate of hypoxic exposed shore crabs (*Carcinus maenas*) but also showed that these animals emerge and pump air into the branchial cavities by reversed scaphognathite pumping in response to exposure to hypoxic water. Bosworth, O'Brien & Amberson (1936) may have anticipated one of the causes of this disparity. These authors, while commenting on earlier work by Amberson *et al.* (1924), note that 'a strict proportionality between oxygen tension and oxygen consumption is only valid where the animals are allowed to reduce the oxygen tension at a rapid rate' and also note that some increase in scaphognathite rate is observed in hypoxic exposed *Homarus americanus*. In the present study some experiments were of short duration and characterized by short acclimation to the experimental chamber followed by a fairly rapid decrease to low oxygen tensions.

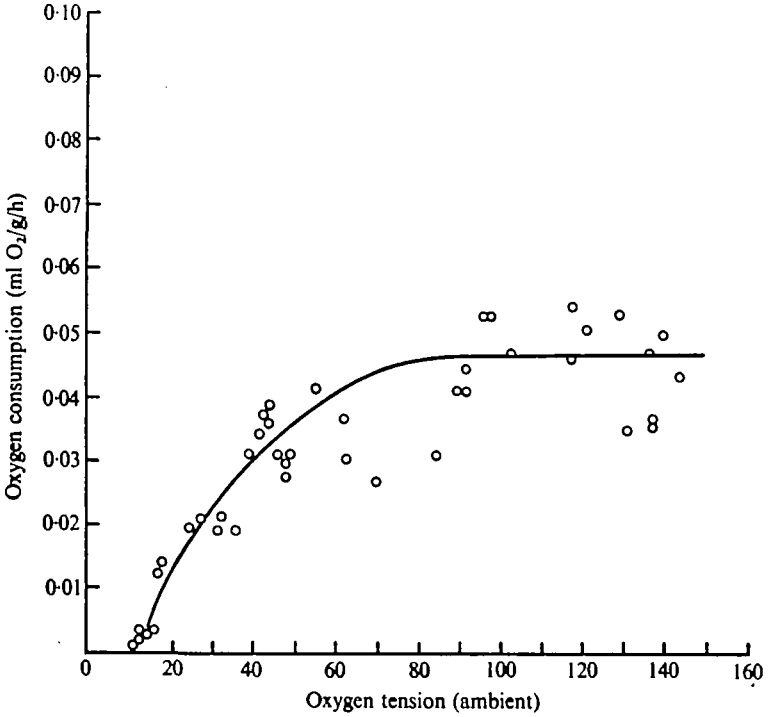

Fig. 6. The relationship between oxygen consumption and ambient oxygen tension in a typical experiment (lobster no. 3, Expt 1) from longer experimental series (Table 2).

Under these conditions little or no increase in scaphognathite frequency occurred and oxygen consumption decreased progressively with decrease in ambient oxygen tension (Table 1). A later series of experiments was designed with 2 or more hours acclimation preceding exposure to controlled oxygen levels. In these experiments increased scaphognathite activity and increased branchial water flow were routinely observed and oxygen consumption was held constant over a wide range of ambient oxygen tensions (Table 2, Fig. 6).

This evidence suggests that the degree of regulation observed experimentally in these decapod species may have been largely determined by the conditions of the experiment. If hypoxic stress is initiated on animals already highly stressed following experimental manipulation, they may already be showing almost maximal branchial pumping which may mask any compensatory response to hypoxia. Moreover, as the experiment progresses their branchial pumping performance and oxygen consumption may steadily decrease with acclimation. Should this also be superimposed on a sudden hypoxic experience, the resulting decrease in oxygen consumption could wrongly be attributed to hypoxia. The hypoxic treatments of Thomas (1954) and Larimer & Gold (1961) may have been too short or the rate of depletion too rapid to allow the development of true hypoxic responses and thus may not have shown the true regulatory ability of the species studied.

Redmond's (1955, 1968) extensive studies on the oxygen transport potential of the pigment haemocyanin are widely quoted and have contributed largely to the belief that the crustacean gill has poor characteristics for oxygen uptake and that

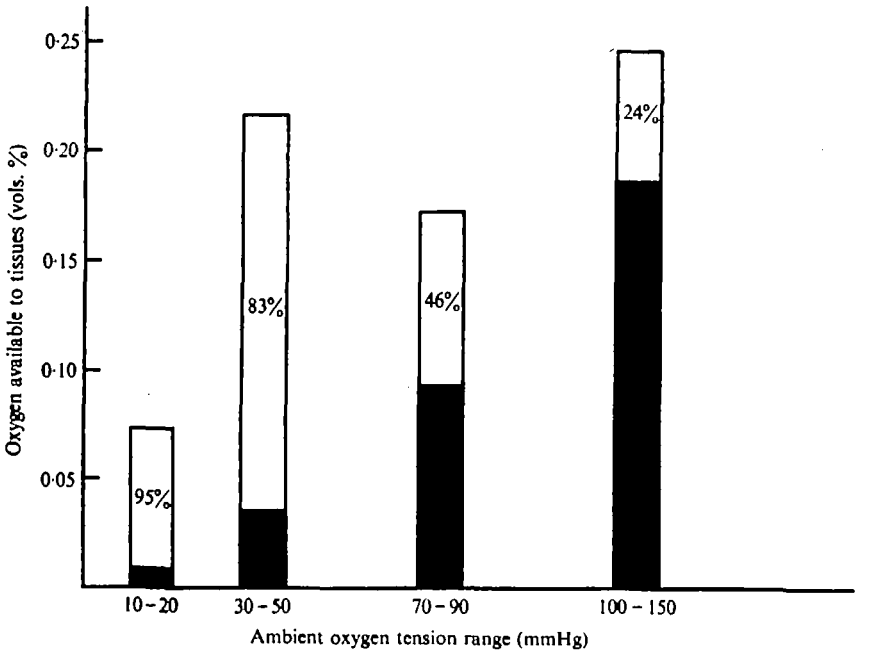

Fig. 7. Proportions of oxygen delivered to tissues of haemocyanin at four ranges of environmental oxygen tension. □, From Hcy; ■, from solution.

the blood of several large decapod species is normally of very low oxygen tension and largely unsaturated with oxygen even in well-aerated water. Larimer (1964) finds evidence to support this view in his studies of the freshwater crayfish *Procambarus*. Redmond (1955, 1968) concluded that under these conditions of low oxygen tension and saturation very little oxygen (0·01 vols %) is delivered to the tissues from simple solution while the majority (0·26 vols %) is delivered by the haemocyanin. He thus deduced that under normal conditions most of the oxygen delivered to the tissues was carried via the haemocyanin molecule. This author also stated that a low blood oxygen tension would serve to facilitate diffusion of oxygen across the gill surface by maintaining a large gradient between circulating blood and water.

This classical picture, however, is not supported by the results of Johansen *et al.* (1970) and Taylor & Butler (1973) for two species of crab nor those of McMahon & Wilkens (1972) and the present study on *Homarus americanus*. These authors find that postbranchial blood is well saturated and of high oxygen tension in well-aerated water, and the level of saturation decreases only during exercise or under hypoxic stress. We must concur with Johansen's (1970) conclusion that Redmond's animals were rendered hypoxic during the sampling process or were in poor physiological condition.

Thomas (1954) observed increased percentage oxygen utilization in hypoxic exposed *Homarus vulgaris* and (in the absence of increased branchial pumping) suggested that 'some change in the efficiency of the blood circulation is the main compensatory response to changes in the availability of oxygen'. In our experiments animals resting in aerated water (P_{O_2} 100–150 mmHg) had oxygen tensions of 51–96 mmHg for postbranchial and 11–27 mmHg for prebranchial blood. Calculations

show that 0·248 vol. % oxygen were carried to the tissues, of which 76% was carried in simple solution while 24% was carried by the respiratory pigment. Table 3 and Fig. 7 show that as external oxygen tension falls, blood oxygen tensions decrease proportionately, forcing the prebranchial blood haemocyanin into the steeply descending portion of its oxygen dissociation curve, thus causing a much greater release of bound oxygen to the tissues. Thus the contribution of oxygen carried and released by the pigment increased from 25% at ambient P_{O_2} 100–150 mmHg to 46% at 70–90 mmHg and 83% at 30–40 mmHg (Fig. 7). This increased participation by the haemocyanin contributes to the maintenance of an adequate oxygen uptake and supply to the tissues at low external oxygen levels. Blood samples withdrawn from animals exposed to ambient oxygen tensions of 10–20 mmHg P_{O_2} revealed average prebranchial and postbranchial blood oxygen tensions of 7 and 8 mmHg respectively. Under these conditions, 95% of the oxygen delivered to the tissues is carried by haemocyanin but due to the small oxygen tension difference between prebranchial blood the amount of oxygen delivered is very much reduced. Consequently the majority of animals succumbed to hypoxia at these low levels. These blood-sampling experiments support the contention of Johansen *et al.* (1970) that the blood of these decapod crustacea is normally well saturated with oxygen in aerated external conditions, but they also support and extend Redmond's (1955) conclusions that, as the oxygen tension of the blood falls, so the oxygen-carrying contribution of the respiratory pigment increases, until at low levels it accounts for most of the oxygen exchanged. An essentially similar depression of the internal oxygen levels and use of the haemocyanin oxygen reserve capacity is well demonstrated by Johansen *et al.* (1970) in exercised *Cancer magister*. Percentage oxygen extraction from the branchial water flow was also increased during hypoxia in *H. americanus*. The effectiveness of oxygen extraction from the branchial water (% *Ew*) and the transfer factor for oxygen both increased almost proportionately with the degree of oxygen depletion. This is presumably due to the increased oxygen binding at the gill site which occurs as the circulating blood oxygen tension falls.

The variation in total oxygen delivered to the tissues (Fig. 7) over the range 30–150 mmHg ambient oxygen tension may be in part due to our limited sampling procedures, but calculation (Fick principle) of cardiac output figures shows that compensatory changes in this parameter might largely equalize oxygen delivery to the tissues above 30–40 mmHg. The cardiac output figures, calculated from our data, are very high when compared with those of *Cancer magister* (Johansen *et al.* 1970) and with the few figures available for other invertebrate animals (Prosser, 1973). However, the oxygen-carrying capacity of *Homarus* blood (1·31 vol. %; Redmond, 1955) is much lower than that measured for *Cancer* (3·3 vol. %) and this may necessitate a greater cardiac output as the oxygen uptake levels are very similar in both species. While high, the cardiac output figures may not be unrealistic as the average stroke volume calculated for animals in well-aerated water (0·65 ml) is apparently within the capabilities of the hearts of 300–500 g lobsters. Blatchford (1971) estimates the cardiac volume of a 220 g *Carcinus* as 0·31 ml.

With the above exceptions, respiratory performance values presented here for *Homarus* agree well with those published for *Cancer* (Johansen *et al.* 1970). Branchial water flow values are slightly lower per unit weight than for *Cancer*. This could be

due to the damping effect of our experimental system as compared to the low-resistance flow-meter method used by Johansen, or it could be due to the fact that only values for acclimated *Homarus* are used here. Prior to acclimation and during hypoxic responses, branchial water flow exceeded the values from normoxic *Cancer*. The percentage utilization of oxygen from the branchial water was slightly higher than from *Cancer*, as might be expected from a slightly lower flow rate. Extremely high (up to 80%) oxygen extraction was observed from rapid branchial water flow during recovery from hypoxia. This no doubt reflects the large diffusion gradient between water and blood, but may also indicate either that the large dead space ventilation indicated for two species of crabs (Hughes *et al.* 1969; Johansen *et al.* 1970) is less evident in the different branchial structures of *Homarus*, or that dead space can be reduced in stress conditions to achieve more efficient gaseous exchange. Oxygen extractions of up to 70% have been recorded from low-oxygen-acclimated *Orconectes* (McMahon *et al.* 1974), and it is possible that increased blood perfusion of the gills or modification of water flow patterns can occur to increase gas exchange effectiveness within the branchial chamber.

Little is known of crustacean oxygen and carbon dioxide monitoring systems. Larimer (1964) speculates that an internal sensor monitors the oxygen tension of postbranchial blood and may control both heart and ventilation rate by feedback inhibition. Alternatively, Page (1973) and Crabtree & Page (1974) confirm the presence of oxygen-sensitive elements on the gills of *Limulus polyphemus*, and preliminary work in our laboratory indicates that receptors in the gills of *Orconectes* may also increase their firing rates in hypoxic situations. These 'external' systems could also effect respiratory and branchial pumping reflexly. However, Chalazonitis (1963) has shown that both hypoxia and hypercapnia can directly increase the bursting rate of pacemaker cells in *Aplysia*. As both cardiac (Maynard, 1960) and scaphognathite beat (Mendelsohn, 1971) are controlled by pacemaker systems in decapod crustaceans, we suggest that the levels of oxygen and carbon dioxide in the postbranchial blood may act directly on the pacemaker systems so as to adjust branchial and cardiac pumping systems to environmental change. The presence of oxygen-sensitive receptors at the gill surface could also be important in allowing rapid sensing of the respiratory environment. The increase in both cardiac and branchial pumps in response to injection of small quantities of aerated water (Fig. 5) has a latency of 20–30 sec and a duration of 40–100 sec, depending on the amount of oxygen added. These time courses would agree more closely with an internal receptor relying on circulation of the internal body fluids than with a nervous reflex mediated by surface receptors. Routinely, the response occurred first in the heart followed by the branchial pump. This would be logical if the effect was directly on the pacemaker cells since oxygenated blood must reach the cardiac ganglion before being pumped ventrally to the suboesophageal ganglion.

However the response is mediated, it is clear that both the heart and branchial pumping systems in *Homarus americanus* respond to changes in the ambient oxygen level. Decreased oxygen causes increased pumping in the branchial system but heart rate falls progressively as the oxygen tension decreases. McMahon & Wilkens (1972) in *Homarus* and Wilkens, Wilkens & McMahon (1974) in *Cancer magister* have shown evidence of highly correlated heart and scaphognathite beating during pausing and

startle responses, but clearly they can respond independently to developing hypoxia (Fig. 3). The cardiac pacemaker system may lack the capacity for increased performance in hypoxia and thus the heart rate conforms metabolically to its ambient oxygen tension. These different responses may contribute to the maintenance of oxygen transport in hypoxic situations by increasing the effectiveness of gaseous exchange at the gill surface.

Unlike previously published reports the present study shows that *Homarus americanus* can maintain oxygen consumption at moderate levels of reduced ambient oxygen. This regulation involves increased branchial water flow, increased effectiveness of oxygen uptake and increased participation in oxygen transport by haemocyanin. The respiratory performance levels and capabilities of *Homarus americanus* are very similar to those reported recently for the brachyuran *Cancer magister*.

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