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THE EFFECT OF PROGRESSIVE HYPOXIA ON HEART RATE, VENTILATION, RESPIRATORY GAS EXCHANGE AND ACID-BASE STATUS IN THE CRAYFISH *AUSTROPOTAMOBIUS PALLIPES*

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SUMMARY

1. Male and female crayfish, *Austropotamobius pallipes* (Lereboullet) maintained a constant rate of O_2 uptake ($\dot{M}_{O_2}$) during a progressive reduction in ambient O_2 tension down to a critical tension (P_c) of 40 mmHg at 15 °C.

2. Heart rate (f_H) slowed progressively during hypoxia but blood flow was maintained by an increase in cardiac stroke volume.

3. At a P_{I,O_2} of 50 mmHg the rate of ventilation ($\dot{V}_w$) had increased to 2·8 times the normoxic value. This was effected by a doubling in respiratory frequency (f_R) accompanied by increases in both the mean amplitude of the pressure pulse and the mean hydrostatic pressure recorded in the branchial chambers. The effectiveness of removal of O_2 from the water ventilating the gills was maintained (E_w), and there is evidence that the ability of the respiratory surface to transfer O_2 (T_{O_2}) improved during hypoxia.

4. The hyperventilation enhanced CO_2 elimination so that CO_2 tensions were halved and respiratory alkalosis occurred in the haemolymph.

5. This alkalosis had the effect of increasing the affinity of the blood pigment for O_2, reducing the half saturation pressure (P_{50}) from 8 to 4·5 mmHg. Thus despite reductions in pre- and postbranchial O_2 tensions by 50% the arteriovenous O_2 content difference was maintained and the effectiveness of removal of O_2 into the blood (E_b) remained high in moderately hypoxic water (P_{O_2} 63 ± 1 mmHg).

6. At P_{I,O_2} levels below 40 mmHg, when crayfish were denied access to air, they were unable to sustain the hyperventilation. Haemolymph O_2 content and the $a-v$ O_2 content difference decreased, and as the haemolymph was no longer saturated with O_2 on its passage through the gills, E_b was reduced. As a consequence of these changes $\dot{M}_{O_2}$ fell.

7. Under these conditions a partial switch to anaerobic metabolism occurred, with lactic acid accumulating in the haemolymph. The resultant metabolic acidosis partially offset the respiratory alkalosis.

INTRODUCTION

In a previous report it was established that *Austropotamobius pallipes* (Lereboullet), the common British crayfish, will migrate from hypoxic water into air when the ambient oxygen tension is lowered to a mean level of 42 ± 5 mmHg (Taylor &

Wheatly, 1980). A complete analysis of respiratory gas exchange and acid–b[redacted] status has been presented for animals subjected to short- and long-term aerial exposure in comparison with settled crayfish submerged in normoxic water (see Taylor & Wheatly, 1980, 1981). However, as the behavioural migration into air was from a hypoxic environment, and water samples collected by divers from the refuges inhabited by crayfish indicated that the animals may routinely encounter moderate hypoxia (Wheatly, unpublished observations), it was considered useful to measure respiratory gas exchange and blood gas variables during progressive hypoxia and to relate them to the changes associated with the migration into air.

The response of animals to varying levels of induced hypoxia has been the subject of numerous investigations. Hill (1976) in a review of the field stated that as the environmental O_2 tension falls animals can either experience a continuous reduction in O_2 uptake (i.e. conform to the change) or maintain O_2 uptake at the normoxic level down to low O_2 tensions (i.e. regulate against the change). In crustaceans it has been recorded that O_2 uptake varies with the available O_2 over a wide range of tensions in *Homarus americanus* (Amberson, Mayerson & Scott, 1924), *Homarus vulgaris* (Thomas, 1953) *Procambarus simulans* (Larimer & Gold, 1961) and *Orconectes immunis* (Wiens & Armitage, 1961): i.e. they are O_2 conformers. Other authors maintain that species such as *Cancer magister* (Johansen, Lenfant & Mecklenberg, 1970), *Panulirus interruptus* (Winget, 1969) and *Carcinus maenas* (Taylor, Butler & Al-Wassia, 1977*b*) can regulate O_2 uptake down to low levels of P_{O_2}, i.e. they are O_2 regulators.

However, the situation is complicated by a number of factors such as acclimation temperature and activity levels. Thus a species may conform at high environmental temperatures but regulate at lower temperatures (Spitzer, Marvin & Heath, 1969; Butler & Taylor, 1975). Likewise Thomas (1954) observed conformation in restrained lobsters whereas Spoek (1974) at the same temperature found regulation down to tensions as low as 30 mmHg. The degree of regulation depends also on the duration of hypoxia and the rate at which it is induced (McMahon, Burggren & Wilkens, 1974; Butler, Taylor & McMahon, 1978).

Ventilatory flow rate ($\dot{V}_w$) of aquatic animals is very much influenced by variations in water oxygenation. Fox & Johnson (1934) and Lindroth (1938) have observed in crustaceans that f_H is inversely related to oxygenation levels. Enhanced $\dot{V}_w$ during hypoxia has been measured directly by Arudpragasam & Naylor (1964), Taylor, Butler & Al-Wassia (1977*b*), Butler *et al.* (1978) and McMahon *et al.* (1974).

Similarly, perfusion of the respiratory organs with blood or haemolymph may vary during hypoxia. Heart rate (f_H) is generally either maintained constant (e.g. *Homarus* – Butler *et al.* 1978) or exhibits a bradycardia (e.g. *Carcinus* – Taylor, Butler & Sherlock, 1973). A reduction in f_H does not necessarily indicate reduced blood flow, however, as in some fish the perfusion volume is maintained despite the bradycardia by an increase in stroke volume (Holeton & Randall, 1967; Short, Taylor & Butler, 1979) and a similar mechanism was suggested to account for an apparent increase in perfusion during aerial exposure in *Carcinus* (Taylor & Butler, 1978).

The responses to hypoxia, which are primarily aimed at maintaining adequate oxygenation of the blood, will inevitably lead to changes in the acid–base balan[redacted]

d this approach to the response is more recent and innovative. Dejours & Beekenkamp (1977), Truchot (1975) and McMahon, Butler & Taylor (1978) have all studied the effect of various levels of hypoxia on acid–base status in decapodan crustaceans. The general observation is that the hyperventilation, which serves to maintain O_2 supply from depleted water, also enhances CO_2 excretion at the gill surface, effecting a reduction in CO_2 tension and a concomitant alkalosis. The reverse situation is found in conditions of hyperoxia (Dejours & Beekenkamp, 1977).

It must also be appreciated that in the face of exposure to severe hypoxia, crustaceans may possess the facility to maintain metabolic rate by switching to anaerobic metabolism. This has been substantiated by Butler *et al.* (1978) and Spoek (1974).

The aim of the present investigation was to make a complete assessment of respiratory and cardiovascular physiology, blood gas analysis and the relative performance of the gas-exchange system during short-term progressive hypoxia in the crayfish *A. pallipes*, in order to assess the respiratory performance of the submerged animal prior to the migration into air described by Taylor & Wheatly (1980).

MATERIALS AND METHODS

The present report contains observations on 35 specimens of the common British crayfish *Austropotamobius pallipes* (Lereboullet) of either sex. The animals were collected and maintained as previously described (Taylor & Wheatly, 1980, 1981). Ventilation, heart rate and blood gas variables were measured using a series of techniques described in earlier reports.

Rate of oxygen consumption ($\dot{M}_{O_2}$) of crayfish during progressive hypoxia was measured using the same continuous-flow respirometer as described by Taylor *et al.* (1977*a*). Rate of gill ventilation ($\dot{V}_w$) was measured as described by Butler *et al.* (1978) by positioning an open-ended mask in front of both exhalant openings to enable sampling of the O_2 tension of mixed expired water, which together with the P_{O_2} of the inspired water and rate of O_2 consumption enables the calculation of branchial ventilation via the Fick principle. Respiratory frequency (f_R) was recorded as fluctuations in the hydrostatic pressure associated with ventilation by the scaphognathite (see Taylor *et al.* 1973; Butler *et al.* 1978), and heart rate (f_H) was measured as the electrocardiogram by means of a pair of stainless steel wires inserted through the carapace above the heart (cf. Taylor *et al.* 1977*a*). Samples of prebranchial haemolymph were withdrawn from the base of a walking leg and postbranchial samples were withdrawn from the pericardial sinus surrounding the heart (Butler *et al.* 1978), and were used for the measurement of oxygen tension (P_{v,O_2}, P_{a,O_2}) oxygen content (C_{v,O_2}, C_{a,O_2}) total CO_2 (Σ_{v,CO_2}, Σ_{a,CO_2}) and pH (pH_v, pH_a) using standard techniques (Taylor & Wheatly, 1979). Values for CO_2 tension (P_{v,CO_2}, P_{a,CO_2}) were calculated from the measured values for Σ_{CO_2} and pH via the Henderson–Hasselbalch equation as described by McMahon *et al.* (1978). Haemolymph lactate levels were measured on samples of mixed pre- and postbranchial haemolymph (Taylor *et al.* 1977*b*).

Respiratory and cardiovascular variables were measured with progressive reduction O_2 tension from settled normoxic levels, measured after 18 h in the experimental chamber (Taylor & Wheatly, 1980), down to approximately 30 mmHg, reducing the

inspired O_2 tension in 7–8 steps of 15–30 mmHg at 90 min intervals, since it proved impossible to obtain specific values for P_{I,O_2}. Values of any variable at 10 mmHg intervals of $P_{I\ O_2}$ between 140 mmHg and 30 mmHg were then obtained for each animal by linear interpolation on O_2 dependency curves for individual animals, and this enabled the construction of a mean curve for the effect of progressive hypoxia on the chosen variable (cf. Butler & Taylor, 1975).

Blood gas analysis was performed on two groups of animals at O_2 tensions either above or just below the critical O_2 tension (P_c) for O_2 uptake. These have been compared with the settled normoxic blood gas values previously obtained (Taylor & Wheatly, 1980).

The relative performance of the respiratory gas exchange system of the crayfish during progressive hypoxia has been assessed using the established criteria recently described by Short *et al.* (1979). In this final analysis, the values for the respiratory and cardiovascular variables under normoxic conditions, and at the two levels of hypoxia at which blood gas analysis was performed, were interpolated from the average curves. Thus these derived indices have been computed on mean values and cannot be accompanied by any indication of variability.

Directly measured variables are expressed throughout as mean values ± S.E.M. with the number of observations in parentheses (n). Differences between mean values were subjected to Student's t test and significant differences assigned at a confidence level of 95%

RESULTS

(1) *The respiratory and cardiovascular changes associated with progressive hypoxia*

(*a*) *Rate of oxygen uptake* ($\dot{M}_{O_2}$)

The rate of oxygen uptake ($\dot{M}_{O_2}$) was measured for 7 animals of mean mass 61·6 ± 3·7 g (range 49–80 g) at progressively reduced O_2 tensions and is illustrated, together with other respiratory and cardiovascular variables, in Fig. 1. The mean settled level of O_2 uptake for these animals was 11·2 ± 1·6 μmol kg^{-1} min^{-1}. As inspired O_2 tension was progressively reduced there was a slight, though insignificant rise in the O_2 uptake down to a level of 70 mmHg. Further reduction caused a drop in $\dot{M}_{O_2}$ below 50 mmHg, which became significantly different from the settled normoxic value at a P_{I,O_2} of 40 mmHg, by which time it had fallen to 44% of its initial value. Animals were consistent in their degree of variability about the mean value throughout the experiment.

(*b*) *Rate of branchial ventilation* ($\dot{V}_w$)

The rate of branchial ventilation of these same 7 animals settled in normoxic water was 96 ± 9 ml kg^{-1} min^{-1}. As the P_{I,O_2} was progressively lowered, rate of branchial ventilation increased, until by 80 mmHg the level was significantly raised to almost double the settled level. With further progressive hypoxia, the branchial ventilation rate continued to rise until it reached a maximum level at 50 mmHg of 266 ± 48 ml kg^{-1} min^{-1}, which was almost a threefold increase above the settled level. This increase, however, was not sustained with further reduction in P_{I,O_2}, and at values below 50 mmHg there was a progressive reduction in the rate of branchial ventilation returning it to the normoxic level (Fig. 1).

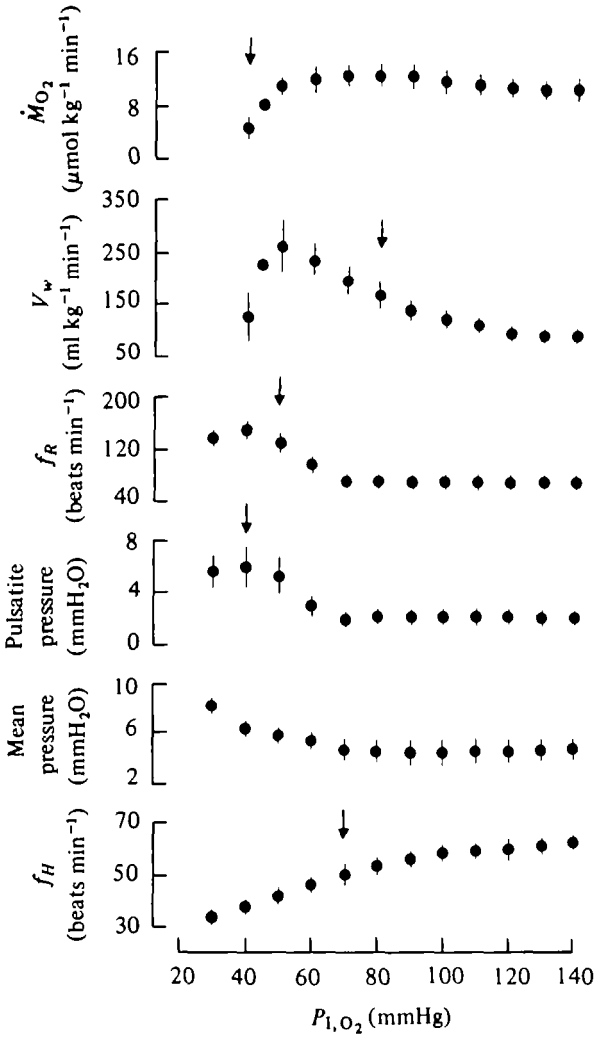

Fig. 1. Changes in the mean values (±S.E. of mean) of rate of oxygen consumption ($\dot{M}_{O_2}$), ventilation rate ($\dot{V}_w$), rate of scaphognathite movements (f_R), pulsatile pressure and mean hydrostatic pressure generated in the branchial chambers by the action of the scaphognathite, and heart rate (f_H), from a crayfish submerged in water at 15 °C which was rendered progressively hypoxic in a series of 8 steps over a 12 h period. The arrows indicate the hypoxic P_{O_2} value at which the measured mean varied significantly from the mean level at a P_{O_2} of 140 mmHg ($P < 0.05$).

(*c*) *Respiratory frequency and pressures generated in the branchial chamber*

The observed changes in rate of branchial ventilation during progressive hypoxia were reflected in measurements made on 6 animals of mass 40·4 ± 3·8 g (range 29·8–52·3 g) with respect to f_R and the pressures generated in the branchial chambers (Fig. 1).

The settled f_R was 74 ± 6 beats min^{-1}. During progressive hypoxia f_R stayed relatively constant until an inspired O_2 tension of 70 mmHg and thereafter began to rise. It first reached a level significantly above the settled rate at 50 mmHg and peaked at a P_{I,O_2} of 40 mmHg, by which time the rate was 152 ± 13(6) which constituted a doubling of the initial rate. Further reduction in O_2 tension evoked a

slight fall in the mean f_R, which however, remained significantly elevated in comparison with the normoxic level.

Concomitant with this observed rise in f_R at declining O_2 tensions were increases in both the mean amplitude of the pressure pulse and the mean pressure recorded in the branchial chambers. Under normoxic conditions the pulsatile pressure was $2{\cdot}10 \pm 0{\cdot}44$ mmH_2O, which remained relatively constant with P_{O_2} reductions down to 70 mmHg. A gradual increase was then observed which first became significant at a P_{I,O_2} of 40 mmHg and remained elevated with further reductions in P_{O_2} at three times the initial value.

The mean pressure recorded in the branchial chamber under normoxic conditions was $4{\cdot}68 \pm 0{\cdot}85$ mmH_2O below ambient hydrostatic pressure. Like pulsatile pressure, this was maintained down to 70 mmHg, and thereafter increased in magnitude down to 30 mmHg, when the level recorded was double that in settled animals. However, despite this apparent increase in mean pressure, animals exhibited such variability about the mean level that at no point was the increase significant.

(*d*) *Heart rate* (f_H)

Measurements of f_H were made on 6 animals of mass $37{\cdot}7 \pm 3{\cdot}4$ g (range 24·8–45·0 g). The mean settled level recorded during normoxia was 63 ± 3 beats min^{-1}. This decreased at a fairly constant rate down to an inspired O_2 tension of about 90 mmHg, at which point the rate of induction of the bradycardia increased. The level first became significantly reduced at a P_{I,O_2} of 70 mmHg, when it was 80% of its initial value. Heart rate continued to decrease proportionately until at 30 mmHg it was reduced to half its initial value (Fig. 1).

The values for these variables at P_{I,O_2} values of 145, 63 and 36 mmHg, which were the mean P_{I,O_2} levels at which blood gas analysis was conducted, have been interpolated from the appropriate graphs and are given, together with the relevant blood gas values, in Table 1.

(2) *Blood gas analysis and determination of acid–base status during progressive hypoxia*

Having ascertained that the critical O_2 tension for maintenance of O_2 uptake was 40 mmHg, it was decided to investigate blood gas analysis below and above this critical level. Experiments were therefore conducted on 8 animals of mean mass 37·9 g (range 25·5–48·0 g) at a mean P_{I,O_2} of 63 ± 1 mmHg, by which stage rate of branchial ventilation would be elevated and cardiac frequency reduced; and on another 8 animals of mean mass 30·1 g (range 14·5–43·4 g) at a mean P_{I,O_2} of 36 ± 2 mmHg, by which stage $\dot{M}_{O_2}$, $\dot{V}_w$, f_R and f_H were all reduced, indicating that respiratory gas exchange may be limited. These values are compared with the normoxic values previously obtained from settled submerged animals by Taylor & Wheatly (1980) in Table 1).

(*a*) O_2 *tension and content*

A progressive reduction in inspired O_2 tension caused a progressive reduction in the tension of both post- and prebranchial haemolymph. These variations are indicated along with O_2 content values and the arteriovenous O_2 ($a-v\,O_2$) conte

Table 1. *Values for directly measured and derived variables of respiratory gas exchange and acid–base balance from crayfish settled in normoxic water and under conditions of environmental hypoxia at mean inspired* O_2 *tensions of* 63 ± 1 *and* 36 ± 2 mmHg.

(Most are mean values ± S.E. of mean with number of observations in parentheses. [a] denotes significant differences from normoxic values ($P < 0.05$); [b] denotes interpolation from mean O_2 dependency curves; [c] denotes calculation from mean values.

Variable	Normoxic water, P_{I,O_2} 145 mmHg	Hypoxic water, $P_{I\,O_2}$ 63 ± 1 mmHg	Hypoxic water, $P_{I\,O_2}$ 36 ± 2 mmHg
Directly measured variables			
M_{O_2} (μmol kg^{-1} min^{-1})	11·2[b]	12·7[b]	4·9[b]
f_H (beats min^{-1})	63[b]	48[b]	36[b]
f_R (beats min^{-1})	74[b]	88[b]	148[b]
P_{I,O_2} (mmHg)	145[b]	63[b]	36[b]
$P_{E\,O_2}$ (mmHg)	88[c]	36[c]	17[c]
P_{a,O_2} (mmHg)	$33 \pm 5(5)$	$13 \pm 2(8)$[a]	$8 \pm 1(6)$[a]
P_{v,O_2} (mmHg)	$17 \pm 2(5)$	$6 \pm 2(8)$[a]	$4 \pm 1(4)$[a]
C_{a,O_2} (mmol l^{-1})	$0{\cdot}33 \pm 0{\cdot}04(9)$	$0{\cdot}37 \pm 0{\cdot}02(9)$	$0{\cdot}17 \pm 0{\cdot}03(6)$[a]
C_{v,O_2} (mmol l^{-1})	$0{\cdot}18 \pm 0{\cdot}03(9)$	$0{\cdot}19 \pm 0{\cdot}02(8)$	$0{\cdot}09 \pm 0{\cdot}01(8)$[a]
pH_a	$7{\cdot}896 \pm 0{\cdot}024(9)$	$7{\cdot}964 \pm 0{\cdot}034(8)$	$7{\cdot}982 \pm 0{\cdot}027(8)$[a]
pH_v	$7{\cdot}861 \pm 0{\cdot}011(9)$	$7{\cdot}927 \pm 0{\cdot}033(8)$	$7{\cdot}949 \pm 0{\cdot}049(8)$
Σ_{a,CO_2} (mmol l^{-1})	$7{\cdot}10 \pm 0{\cdot}40(9)$	$4{\cdot}90 \pm 0{\cdot}49(8)$[a]	$4{\cdot}23 \pm 0{\cdot}49(8)$[a]
Σ_{v,CO_2} (mmol l^{-1})	$7{\cdot}68 \pm 0{\cdot}56(9)$	$5{\cdot}08 \pm 0{\cdot}57(8)$[a]	$4{\cdot}45 \pm 0{\cdot}52(8)$[a]
Lactate (mmol l^{-1})	$0{\cdot}40 \pm 0{\cdot}56(6)$	$0{\cdot}49 \pm 0{\cdot}03(7)$	$0{\cdot}97 \pm 0{\cdot}13(8)$[a]
Derived via the Henderson–Hasselbalch equation			
P_{a,CO_2} (mmHg)	$3{\cdot}03 \pm 0{\cdot}24(9)$	$1{\cdot}73 \pm 0{\cdot}07(8)$[a]	$1{\cdot}45 \pm 0{\cdot}15(8)$[a]
P_{v,CO_2} (mmHg)	$3{\cdot}53 \pm 0{\cdot}28(9)$	$1{\cdot}95 \pm 14(8)$[a]	$1{\cdot}67 \pm 19(8)$[a]
Derived via the Fick equation			
$\dot{V}_w$ (ml kg^{-1} min^{-1})	96[b]	230[b]	128[b]
$\dot{V}_b$ ml kg^{-1} min^{-1})	74[c]	71[c]	58[c]
Resp. stroke vol. (ml kg^{-1})	1·30[c]	2·61[c]	0·86[c]
Card. stroke vol. (ml. kg^{-1})	1·17[c]	1·48[c]	1·61[c]

difference in Fig. 2. At a P_{I,O_2} of 63 ± 1 mmHg both arterial and venous tensions were significantly reduced to less than half the normoxic values. By 36 ± 2 mmHg a further significant reduction had occurred, so that post- and prebranchial levels had reached 23 and 20% respectively of their settled normoxic levels, which reflects the percentage drop in O_2 tension of the inspired water that has occurred by this stage.

This situation is not mirrored in the O_2 content values. At a mean P_{I,O_2} of 63 mmHg both C_{a,O_2} and C_{v,O_2} were somewhat elevated, though not significantly ($P = 0{\cdot}2$–$0{\cdot}1$ and $0{\cdot}8$–$0{\cdot}7$ respectively) with the net result that at this level of hypoxia the $a-v$ O_2 content difference was maintained above the settled normoxic level. This may provide for the slight elevation in $\dot{M}_{O_2}$ at this point. However, at a P_{I,O_2} of 36 ± 2 mmHg, the marked reduction in P_{a,O_2}, $\dot{V}_w$, and concomitantly $\dot{M}_{O_2}$, was reflected in marked reductions in C_{a,O_2} and C_{v,O_2} to 54 and 52% of their settled normoxic levels ($P < 0{\cdot}001$ in both cases). This resulted ultimately in a reduction in $a-v$ O_2 content difference to 0·2 vol %, which is a reduction of 40% from the settled normoxic value, and proves to be just significant.

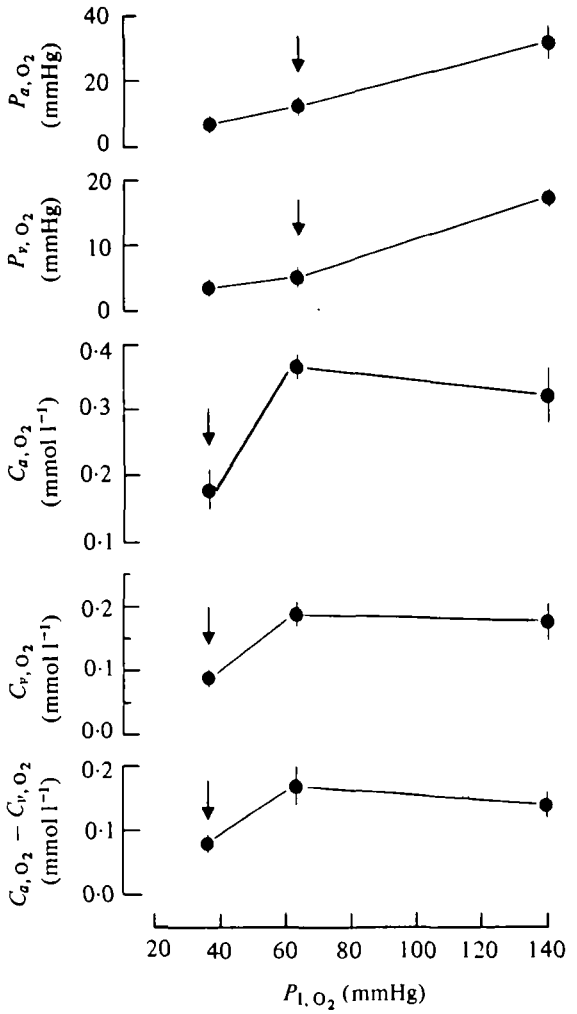

Fig. 2. Changes in the mean values (± S.E. of mean) of oxygen tension (P_{a,O_2} and P_{v,O_2}), oxygen content (C_{a,O_2} and C_{v,O_2}) and oxygen content difference ($C_{a,O_2} - C_{v,O_2}$), of postbranchial and prebranchial haemolymph from crayfish submerged in normoxic water and following 3 h exposure to 2 levels of hypoxia (63 ± 1 mmHg or 36 ± 2 mmHg). The arrows indicate the P_{O_2} at which the variable was significantly reduced from the normoxic level ($P < 0{\cdot}05$).

(*b*) *Acid–base status*

The acid–base status during progressive hypoxia is summarized for post- and prebranchial haemolymph in a HCO_3^-/pH diagram (Fig. 3) and individual post-branchial variables are shown with progressive hypoxia in Fig. 4. Presumably because of the resultant hyperventilation, hypoxia was associated with significant reductions in CO_2 tension. At a P_{I,O_2} of 63 mmHg the P_{CO_2} values in pre- and post-branchial haemolymph were reduced to 56% of their normoxic values, and with a further reduction in P_{I,O_2} to 36 mmHg they showed a further slight reduction to 48% of their initial values. The total CO_2 content of the haemolymph was significantly lowered. Examination of levels of $[HCO_3^-]$, $[CO_3^{2-}]$ and dissolved CO_2 indicate that reductions occurred in all three moieties during progressive hypoxia. $[HCO_3^-]$ le[illegible]

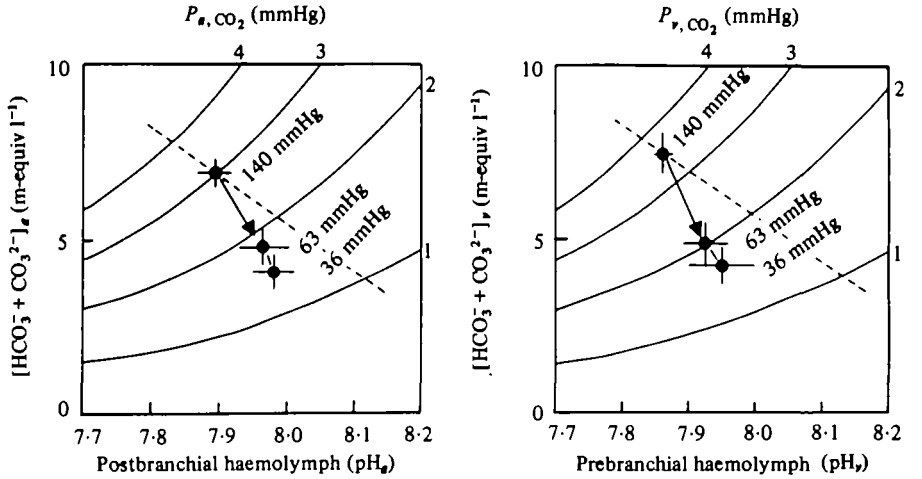

Fig. 3. Variation in mean pH and $[HCO_3^- + CO_3^{2-}]$ levels (±S.E. of mean) in the post-branchial and prebranchial haemolymph of crayfish following exposure to the 3 levels of oxygen tension indicated beside each point. The thin continuous lines are isopleths for P_{CO_2} levels over the range of variation observed. The oblique broken line traces the buffer line calculated from two *in vitro* pH and $[HCO_3^-]$ measurements at different P_{CO_2} levels.

were reduced by 40% and dissolved CO_2 by 53%, both reductions being significant. $[CO_3^{2-}]$ also exhibited a slight reduction to about 74% of its initial level. These changes in CO_2 levels were associated with a significant respiratory alkalosis in the haemolymph (see Table 1 and Fig. 3).

(*c*) *Lactic acid accumulation*

The possibility that the crayfish resorts to anaerobiosis under conditions of hypoxia was investigated on these same two groups of animals at mean P_{I,O_2} of 63 ± 1 and 36 ± 2 mmHg. Lactic acid concentration in settled normoxic conditions was 0·40 ± 0·05 mmol l^{-1}. By the time the ambient O_2 tension had been lowered to 63 mmHg there was a slight though insignificant rise in circulating lactic acid to 0·49 ± 0·03 mmol l^{-1}. However, with a further reduction in P_{I,O_2} to 36 mmHg the lactate level rose to 2·4 times its initial normoxic value, which was a significant increase. It is possible that a metabolic acidosis arising from the accumulation of lactate during hypoxia may counteract the respiratory alkalosis which results from hyperventilation, thus explaining the deviation of the measured values from the buffer line for CO_2 on Fig. 3.

(3) *The effect of progressive hypoxia on the* O_2 *combining properties of the haemolymph*

The effect of the alkalosis experienced in the haemolymph on the O_2-combining properties of the blood pigment was assessed by construction of an *in vivo* O_2 equilibrium curve. For this purpose post- and prebranchial values were combined for both levels of hypoxia since statistical analysis demonstrated that the pH levels were not significantly different and that the mean pH of the combined data was 7·956 ± 0·018 (n = 23). The O_2 content was related to O_2 tension for each individual sample of haemolymph and the resultant *in vivo* curve is illustrated (Fig. 5). The half saturation pressure (P_{50}) of this curve was 4·5 mmHg, which represents an increase in O_2

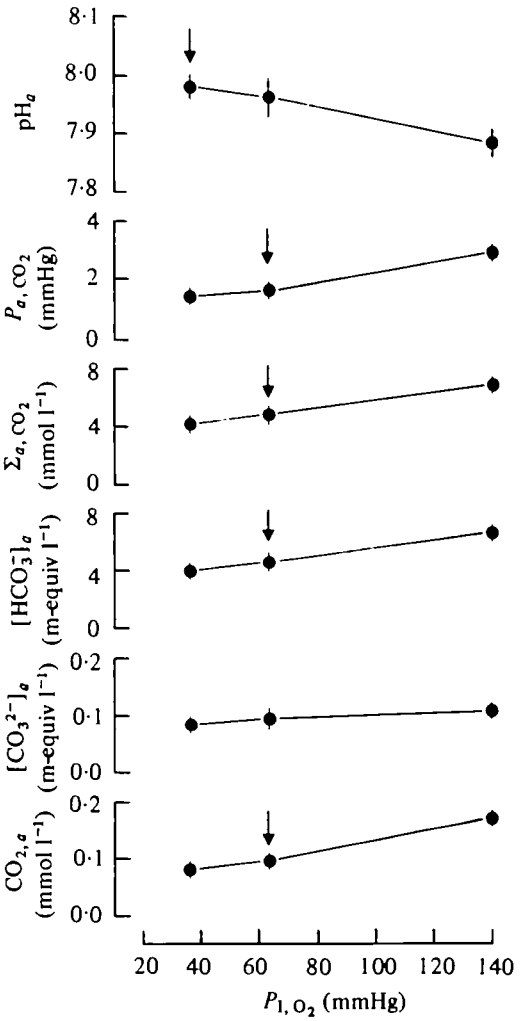

Fig. 4. Changes in the mean values (±S.E. of mean) of the factors governing acid–base status in the post branchial haemolymph of crayfish exposed to 3 levels of oxygen tension. The traces are, from above downwards: pH_a; P_{a,CO_2}, Σ_{aCO_2} (total CO_2 measured by acidifying samples of haemolymph); $[HCO_3^-]_a$ (concentration of bicarbonate ions); $[CO_3^{2-}]_a$ (concentration of carbonate ions) and $CO_{2,a}$ (dissolved CO_2). The arrows indicate the P_{O_2} at which the variable deviated significantly from the mean normoxic value.

affinity of the pigment when compared with the P_{50} of 8 mmHg, measured at the pH of the haemolymph from animals in normoxic water (Taylor & Wheatly, 1981).

(4) *Relative performance of the respiratory gas exchange system during progressive hypoxia*

The derived indices describing respiratory gas exchange during progressive hypoxia in the crayfish are given in Table 2. Again the values have been calculated for the two levels of hypoxia which were induced, that is 63 ± 1 mmHg and 36 ± 2 mmHg, and are compared with normoxic values.

It has already been noted that $\dot{V}_w$ increased considerably during hypoxia. Down to a P_{I,O_2} of 63 mmHg this was manifested by increases in f_R and stroke volum

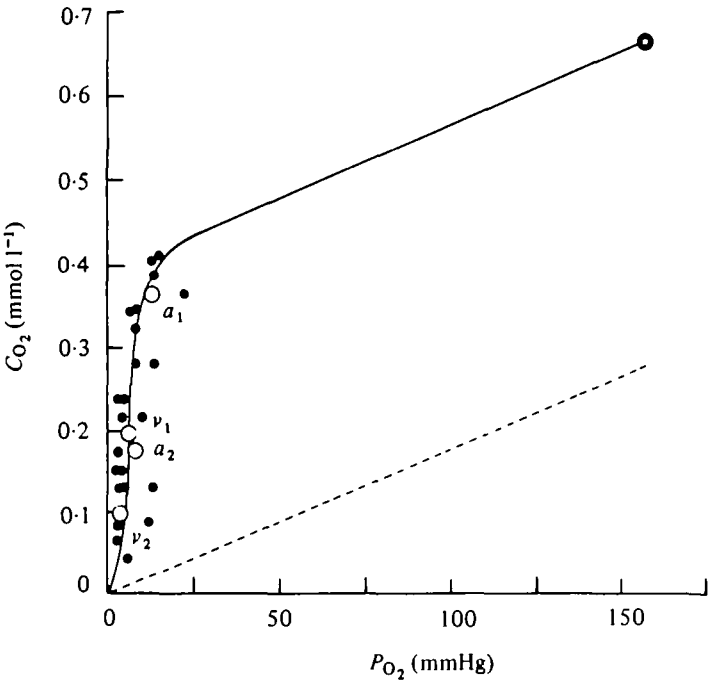

Fig. 5. The *in vivo* oxygen equilibrium curve for crayfish haemolymph collected from animals exposed to two levels of oxygen tension. The filled symbols are individual measurements, the open symbols denote mean O_2 tension and content values from postbranchial (a_1 and a_2) and prebranchial (v_1 and v_2) haemolymph samples taken from animals exposed to mean P_{I,O_2} levels of 63 ± 1 mmHg (a_1 and v_1) and 36 mmHg (a_2 and v_2). The starred symbol is a value for haemolymph equilibrated *in vitro* with a gas mixture having a P_{O_2} of 152 mmHg. The broken line indicates the dissolved O_2 content of the haemolymph at 15 °C.

Table 2. *The derived indices describing respiratory gas exchange in the crayfish submerged in normoxic water and at two levels of hypoxia at* 15 °C

The derivation and possible significance of these indices have recently been described by Dejours (1975) and Short *et al.* (1979).

P_{I,O_2} (mmHg)	$\dot{V}_w$ (ml kg^{-1} min^{-1})	$\dot{V}_b$ (ml kg^{-1} min^{-1})	$\dot{V}_w/\dot{V}_b$	$\dot{V}_w/\dot{M}_{O_2}$ (ml μ-mol^{-1})	$\dot{V}_b/\dot{M}_{O_2}$ (ml μ-mol^{-1})	Extraction (%)	E_w (%)	E_b (%)	$C_{a.O_2} - C_{v,O_2}$ (m-moll^{-1})	ΔPg (mmHg)	T_{O_2} (μ-mol kg^{-1} min^{-1} $mmHg^{-1}$)
Normoxia 140 mmHg	96	74	1·30	8·57	6·61	39·3	44·6	70·0	0·15	91·7	0·122
Hypoxia 3 ± 1 mmHg	230	71	3·24	18·11	5·59	42·9	47·5	56·3	0·18	39·9	0·318
Hypoxia 6 ± 2 mmHg	128	58	2·21	26·12	11·84	52·2	58·7	22·7	0·08	20·6	0·238

Thereafter $\dot{V}_w$ decreased slightly, though to a level still above the normoxic level. Although f_R was further elevated at this time it was accompanied by a reduction in respiratory stroke volume. Rate of perfusion ($\dot{V}_b$) appeared to remain constant at least until 63 mmHg as calculated from the maintained levels of $\dot{M}_{O_2}$ and $a - vO_2$ content difference. This was accomplished by an increase in cardiac stroke volume, since f_H was markedly reduced at this stage. Further reductions in P_{I,O_2}, however,

caused a decrease in $\dot{V}_b$, and cardiac stroke volume declined with progressive bradycardia. The outcome of these observed changes was that ventilation/perfusion ratio increased during hypoxia. At 63 mmHg this was by virtue of an elevation in $\dot{V}_w$. At 36 mmHg the ratio was reduced, but still higher than the normoxic value, despite the reduction in $\dot{V}_w$, by virtue of a proportionate reduction in $\dot{V}_b$.

The convection requirement for water increased during hypoxia. At moderately hypoxic levels the convection requirement increased because $\dot{M}_{O_2}$ was maintained despite the reduction in O_2 availability by virtue of the increase in $\dot{V}_w$. It rose still further at severely hypoxic levels despite the drop in $\dot{V}_w$ because $\dot{M}_{O_2}$ had similarly decreased. The convection requirement for the haemolymph was about 6·6 under normoxic conditions. During exposure to moderate hypoxia, this decreased slightly by virtue of the maintained level of $\dot{M}_{O_2}$. With further reduction in O_2 tension, however, this value doubled, since $\dot{M}_{O_2}$ experienced a larger proportional reduction than $\dot{V}_b$.

It was unusual, in view of the hyperventilation observed, to record an initial maintenance and then increase in the percentage extraction of O_2 from the water ventilating the gills at severely hypoxic levels. Measurements showed that the effectiveness of removal of O_2 from the water (E_w) was maintained at 63 mmHg but increased at lower inspired O_2 tensions. This may be partially explained by consideration of changes in the ability of the respiratory surface to transfer O_2. It can be seen from Table 2 that the transfer factor (T_{O_2}) increased during progressive hypoxia as $\dot{M}_{O_2}$ was maintained against reductions in the mean pressure gradient for diffusion of O_2 across the respiratory surface.

The same was not true of the effectiveness of uptake of O_2 into the blood (E_b) which exhibited a reduction with progressive hypoxia. It had decreased somewhat at 63 mmHg but the significant reduction occurred at 36 mmHg, when it had fallen to half of its normoxic level, despite the increase in the O_2 affinity of the haemocyanin. At this stage the O_2 tensions recorded in the haemolymph had fallen below the P_{satn} for the pigment (Fig. 5) and $a-v$ O_2 content difference could no longer be maintained. Thus the drop in E_b and also $\dot{V}_b$ explain the eventual reduction in O_2 uptake, despite the observation that the ability of the gas exchange surface to transfer O_2, and thus E_w, had improved.

DISCUSSION

The present investigation has demonstrated that respiratory gas exchange and acid–base balance in the freshwater crayfish are greatly influenced by variations in the degree of water oxygenation. It was observed that $\dot{M}_{O_2}$ can be maintained down to an inspired O_2 tension of 50 mmHg at 15 °C. Thereafter $\dot{M}_{O_2}$, and presumably long-term survival, is severely limited by further reduction in P_{O_2}. McMahon *et al.* (1974) noted that in the crayfish *Orconectes virilis* death resulted from prolonged exposure to O_2 tensions below 30 mmHg. This may explain the reported observation that crayfish submerged in shallow water over a shelving bed of gravel respond to a progressive reduction in ambient O_2 by migrating into air (Taylor & Wheatly, 1980). This occurred at a mean P_{I,O_2} of 42 ± 5 mmHg at 15 °C and enabled them to maintain their O_2 uptake at submerged normoxic levels by utilization of the aerial environment as a source of oxygen.

The response of water-breathing animals to environmental hypoxia is generally to increase branchial ventilation, and this was observed in the crayfish. The ventilatory requirement for water is often inversely proportional to the level of water oxygenation (Dejours, Garey & Rahn, 1970); this holds for conditions of hyperoxia as well as hypoxia (Dejours & Beekenkamp, 1977). Crayfish, like shore crabs (Taylor *et al.* 1977*b*), exhibit the capability of increasing $\dot{V}_w$ by as much as 2·5–3·0 times the resting level during hypoxia. Butler *et al.* (1978) recorded an increase of 1·2 times in lobsters and McMahon *et al.* (1974) recorded an increase of 5 times in the crayfish *Oronectes*. $\dot{V}_w$ was enhanced in the crayfish by increasing the overall index of ventilatory activity (cf. Dejours & Beekenkamp, 1977): f_R, stroke volume and the force of the scaphognathite beat all contributed to the increase in $\dot{V}_w$. McMahon *et al* (1974) recorded a similar response of *Orconectes virilis* to long-term exposure to moderate hypoxia where respiratory frequency and amplitude increased respectively by 1·5 and 3 times the normoxic levels, and there was a 1·3-fold increase in the level of hydrostatic pressure in the branchial chambers.

The present investigation revealed, however, that ventilatory compensations cannot be made when the animals are exposed to more severely hypoxic conditions at P_{I,O_2} levels below 50 mmHg. It may well be that hyperventilation is expensive in terms of energy costs, and it was observed in the present investigation that $\dot{M}_{O_2}$ rose as branchial ventilation increased. Presumably, should branchial ventilation be increased beyond a certain limit, the energy cost of ventilation outweighs any respiratory advantage to the animal (Jones, 1971). At this point the animal reduces the level of branchial ventilation at the cost of reduced O_2 uptake. (McMahon *et al.* (1978) found a similar response in *Homarus vulgaris* when exposure to the same level of hypoxia continued for long periods of time, though it was not accompanied by a reduction in $\dot{M}_{O_2}$. There is a possibility that changes may occur in the circulatory system during hypoxia, such as improved perfusion of the gills by opening of previously poorly perfused regions, which could explain the increased value for T_{O_2} during hypoxia and may reduce the requirement for hyperventilation (cf. Randall, 1970).

The situation for the O_2 dependency of heart rate did not follow the pattern evident so far in the other variables. Cardiac frequency decreased approximately in proportion with inspired O_2 tension, reaching a critical level at 70 mmHg. Taylor *et al.* (1973) recorded a similar bradycardia in the shore crab with a comparable P_c value. Butler *et al.* (1978) observed a maintenance of f_H in response to hypoxia in the lobster, although cardiac pauses, which are a feature of normal perfusion in this species, disappeared. Therefore it is incorrect to conclude that f_H was unaffected. McMahon *et al.* (1974) conversely recorded a 50% increase in f_H in *Orconectes*. However, they considered that this may have been an indirect response arising from the increased muscular effort involved in large increases in branchial pumping.

The critical O_2 tension (P_c), below which $\dot{M}_{O_2}$ decreased with further reduction in P_{I,O_2}, is the point at which the gas-exchange system fails to continue to provide sufficient O_2 to maintain the normoxic level of aerobic metabolism. The compensatory adjustments that occur during moderate hypoxia represent an attempt to maintain $\dot{M}_{O_2}$ at the normoxic level by holding arterial O_2 tension at a level close to the shoulder of the O_2 equilibrium curve of the blood pigment, as indicated by the values on Fig. 5. The arterial and venous O_2 tensions recorded are markedly reduced from their

normoxic values. However, the respiratory alkalosis arising from hyperventilation has the effect of shifting the O_2 equilibrium curve to the left, thus increasing the affinity of the blood for O_2. At an inspired O_2 tension of 63 mmHg the P_{a,O_2} was 13 mmHg, which provides for 94% saturation of the pigment. However, the further reduction of P_{I,O_2} to 36 mmHg reduced P_{a,O_2} to 8 mmHg and caused postbranchial haemolymph to be only 55% saturated (Fig. 5). Therefore an increase in O_2 affinity of the pigment enhances saturation at reduced tensions when compared with pre-hypoxic levels so that content values can be maintained down to moderate levels of hypoxia, but O_2 content eventually falls to critically low levels as the level of hypoxia decreases. Reductions to this level result in reductions in E_b since the pigment is no longer saturated during its passage through the gills, $a-v$ O_2 content difference is reduced and $\dot{M}_{O_2}$ unavoidably decreases. The failure to maintain O_2 uptake during severe hypoxia is, therefore, the consequence of a drastic reduction in E_b.

Hyperventilation, whilst serving to maintain the O_2 supply from depleted water as discussed above, has a far reaching effect on acid–base balance, since facilitation of CO_2 excretion into the water ventilating the gills causes a reduction in P_{CO_2} and thus an alkalosis in the haemolymph. Pre- and postbranchial haemolymph exhibited an increase in pH of 0·1 unit. Similar changes in pH were recorded by MacMahon *et al.* (1978) during long-term hypoxic stress in the lobster, and by Truchot (1975) in the shore crab. Environmental conditions of hyperoxia have the reverse effect, that is reducing pH and increasing P_{CO_2}, and again the disturbance is of ventilatory origin (Dejours *et al.* 1970; Dejours & Beekenkamp, 1977). The halving of CO_2 tension values observed in the present investigation with hyperventilation agrees likewise with McMahon *et al.* (1978) and Truchot (1975). McMahon *et al.* (1978) also recorded a reduction in blood buffer consequent to the reduction in P_{CO_2}, but Truchot (1975) reported that the level of $[HCO_3^- + CO_3^{2-}]$ remained unchanged.

It was found, however, by McMahon *et al.* (1978) that during long-term hypoxic exposure the hyperventilation was not maintained because it was metabolically expensive. Thus P_{CO_2} rose so that by 18 h of hypoxic exposure it had reached pre-hypoxic levels. The pH however remained elevated due to a substantial rise in haemolymph bicarbonate concentration. The relatively short duration of the experiments in the present investigation did not allow us to determine whether this mechanism operated in the crayfish.

The respiratory alkalosis observed during hypoxia was partially offset by a metabolic acidosis consequent upon the accumulation of lactate, though this was insufficient to explain the whole of the deviation of the mean values from the buffer line in Fig. 3, as the measured lactate level at a P_{O_2} of 36 mmHg is equivalent to an acidosis of only 0·025 pH units using the analysis described by Taylor & Wheatly (1981). It has been found by Butler & Taylor (1975) in dogfish, Holeton & Randall (1967) in trout, and Eddy (1974) in tench that blood pH is unaltered during hypoxia. In these cases it was felt that large increases in blood lactate counteract any tendency towards alkalosis resulting from a rise in $\dot{V}_w$ (Dejours, 1973). The lactate accumulation observed in the crayfish, although significant, is small in comparison with these more active species, and by virtue of its reduced effect on blood pH does not completely counteract the respiratory alkalosis, which alters the characteristics of the O_2 dissociation curve to the animal's advantage during hypoxia.

The crayfish can maintain aerobic metabolism down to an inspired O_2 tension of 63 mmHg. The animals hyperventilate, $\dot{V}_b$ is maintained, and thus an increase in the ventilation perfusion ratio is observed, which is generally the physiological response of aquatic gill-breathing animals to hypoxia (Taylor *et al.* 1977*b*). Despite this increase in $\dot{V}_w$ the percentage of available O_2 extracted on passage through the gills remains approximately constant. A similar result was obtained in the crayfish *Procambarus* by Larimer (1961), and the shore crab (Taylor *et al.* 1977*b*). This was true also of *Homarus*, since Butler *et al*, (1978) recorded that percentage extraction was unchanged during hypoxia. A. C. Taylor (1976) working on *Carcinus* reported that percentage extraction from water decreased during hypoxia, which is generally the case in fish (Hughes & Shelton, 1962), though the crabs may have been disturbed in these experiments, as was suggested by Taylor *et al.* (1977*b*). The convection requirement for the water increases by virtue of hyperventilation: this agrees with the observation of Dejours *et al.* (1970) that the ventilatory requirement is inversely related to the water oxygenation.

When more severe hypoxia was induced, the ventilation perfusion ratio fell slightly though it was still maintained above the pre-hypoxic level. Percentage extraction and E_w exhibited an increase at this stage. The only explanation for this and the previous observation that percentage extraction and E_w are maintained during hyperventilation in hypoxic water is that the respiratory surface improves its capabilities to transfer O_2, as described for the trout by Holeton & Randall (1967). This conclusion is borne out by the values for transfer factor in the crayfish, which increased during hypoxia, because $\dot{M}_{O_2}$ was maintained in spite of a reduction in mean pressure for diffusion of O_2. Both the convection requirement for the haemolymph and E_b are maintained relatively constant in moderate hypoxia.

When these observed responses to environmental hypoxia are viewed in the context of the overall behavioural responses of the animal, the respiratory role of the migration into air emerges. Firstly we see that at P_{O_2} levels below 50 mmHg, $\dot{M}_{O_2}$ will eventually become limited, and that migration of the animal into air anticipates this problem and serves to maintain $\dot{M}_{O_2}$ and f_H at their pre-hypoxic levels (Taylor & Wheatly, 1980). When this migration occurs blood pH will be alkalotic due to the effects of hyperventilation, and thus the extent of the change to relative acidosis inherent in the initial period of air breathing must be greater than was described by Taylor & Wheatly (1981). In air, ventilation rate ($\dot{V}_{air}$) was only 5% of normoxic $\dot{V}_w$ or approximately 2% of hypoxic $\dot{V}_w$, so that the saving on the energy cost of ventilation associated with migration into air will be large.

This attempt to describe the physiological response of the crayfish *Autropotamobius pallipes* to progressive hypoxia thus gives credibility to the behavioural migration on to land, which occurs at a P_{O_2} close to the P_c for $\dot{M}_{O_2}$, and thus enables the animal to avoid a reduction in metabolic rate during hypoxia. When denied access to air at P_{I,O_2} levels below 40 mmHg, the crayfish resorts to anaerobiosis and lactic acid accumulates in the haemolymph. Using the simple assumptions described by Taylor & Wheatly (1981) it is possible to deduce that ATP production by lactic fermentation replaces approximately 10% of that lost due to the reduction in $\dot{M}_{O_2}$ below 40 mmHg. It seems possible that some other acid-end product of anaerobiosis may accumulate in the crayfish during hypoxia to make up this shortfall and also to explain the

marked deviation from the buffer line in Fig. 3. A similar choice between anaerobiosis or the exploitation of air as a source of oxygen when submerged in hypoxic water is presented by the emersion response in the shore crab, *Carcinus maenas* (Taylor & Butler, 1973; Taylor *et al.* 1977*b*).

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