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EFFECT OF MAINTAINED HYPOXIC EXPOSURE ON THE CRAYFISH *ORCONECTES RUSTICUS*

I. VENTILATORY, ACID-BASE AND CARDIOVASCULAR ADJUSTMENTS

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SUMMARY

Oxygen uptake in the crayfish *Orconectes rusticus* was maintained at normoxic levels throughout 6 days exposure to an ambient oxygen tension of 45–55 torr. This was attributed to compensatory responses of the ventilatory and cardiovascular pumps. The first 72 h of hypoxic exposure were characterized by a transitory 3-fold increase in both scaphognathite rate and cardiac output above initial normoxic values. During the latter 72 h of hypoxic exposure both scaphognathite pumping and cardiac output were significantly below maximum values but were maintained above initial normoxic levels. Thus, as reflected by the increased convection requirements for both water and haemolymph, the increased scaphognathite and heart activity served to increase oxygen delivery to both the branchial chambers and the tissues. Additionally, the increased branchial ventilation and gill perfusion facilitated removal of dissolved carbon dioxide from the haemolymph, effecting a respiratory alkalosis. The increased haemolymph pH elevated the oxygen affinity of the haemocyanin via the Bohr affect, enabling a greater volume of oxygen to be picked up at the gills by the haemocyanin despite the reduced pressure gradient.

INTRODUCTION

In decapod crustaceans short-term exposure to either progressively declining P_{O_2} or maintained hypoxia results in an increased frequency of scaphognathite pumping and therefore ventilation volume (Thomas, 1954; Wolvekamp & Waterman, 1960; Larimer & Gold, 1961; McMahon & Wilkens, 1975; Burnett, 1979; Wheatly & Taylor, 1981; McMahon & Wilkes, in preparation). Two decapods, *Orconectes virilis* (McMahon, Burggren & Wilkens, 1974) and *Homarus vulgaris* (Butler, Taylor & McMahon, 1978) have been subjected to longer-term (10 and 4 days respectively) exposure to maintained hypoxia (45–55 torr), while Burnett & Johansen (1981) maintained *Carcinus maenas* under more severe hypoxia (25–30 torr) for 72 h. In these extended studies a ventilatory response similar to that observed in the short term was observed initially but peak ventilatory levels were established within 6–24 h after which pumping rates gradually declined. In both *O. virilis* (McMahon *et al.* 1974)

and *H. vulgaris* (Butler *et al.* 1978) ventilatory pumping decreased to reach a new steady level significantly below peak values within 72 h of hypoxic exposure. The new plateau levels were still significantly elevated over normoxic values and were maintained for up to 10 days of maintained hypoxia. McMahon *et al.* (1974) noted that in *O. virilis* oxygen consumption was maintained despite this decline in ventilation volume and reasoned that internal changes allowing increased efficiency of oxygen transfer across the gills must have occurred. These authors were unable to assess the nature of these changes but Butler *et al.* (1978) and McMahon, Butler & Taylor, (1978*a*) showed utilization of venous reserve oxygen and increase in haemocyanin oxygen affinity, in the larger *H. vulgaris*, both of which helped to maintain the normoxic rate of oxygen uptake from the oxygen depleted water. In *H. vulgaris* the increased oxygen affinity resulted from a state of alkalosis effected initially from hyperventilation and associated CO_2 washout. This was later replaced by a metabolic alkalosis which developed as ventilation fell.

Although the ventilatory responses to long-term hypoxia are similar in *O. virilis* and *H. vulgaris*, internal compensatory responses to hypoxia in freshwater decapods have not been examined. The present study examines the ventilation, oxygen uptake, haemolymph oxygen status, cardiovascular responses and haemolymph acid-base status in a fresh water crayfish, *Orconectes rusticus*, during long-term (6 days) steady-state hypoxic exposure. *O. rusticus* was used in this study since it has been shown to inhabit a wide variety of conditions where long term hypoxia may be commonplace (Berrill, 1978).

METHODS AND MATERIALS

Orconectes rusticus, ranging from 10 to 25 g body mass, were collected from the Kawartha Lakes region of Southern Ontario and shipped by air to the University of Calgary (elevation *c.* 1048 m), where this work was conducted. In order to allow complete recovery from shipping, crayfish were maintained for a minimum of 2 weeks at the experimental temperature of 15 °C in running, dechlorinated water prior to experimentation. During the holding period crayfish were fed a diet of smelt, Tetramin or pellet trout-food three times weekly. Animals were not fed during the 6-day hypoxic period.

In order to avoid anomalies in scaphognathite and heart recordings by sampling disturbance, and deleterious consequences of excessive haemolymph loss, the experiments were run in three series, each using different animals. In series I ($n = 8$) oxygen consumption ($\dot{M}_{O_2}$), and both scaphognathite rate and heart rate ($f_{\bar{w}}$, f_H) were measured. In series II ($n = 8$) postbranchial haemolymph pH and total CO_2 (C_{CO_2}) were measured. In series III ($n = 12$) post- and prebranchial haemolymph oxygen pressure and content (P_{a,O_2}, P_{v,O_2}, C_{a,O_2}, C_{v,O_2}) together with f_H were measured. The experimental protocol in each series consisted of measurements at 24 h prior to (P_{I,O_2} = 120–130 torr), during, and 24 h after 6 days hypoxic exposure (P_{I,O_2} = 45–55 torr), thus each animal served as its own control.

Hypoxic water was obtained by passage through an oxygen stripping column in which oxygen in the incoming water was partially replaced by a counter-current flow of nitrogen. The flow rate of nitrogen was adjusted at the tank to maintain a constant

level of hypoxia. All animals were allowed 24–48 h recovery after manipulation and handling prior to initial normoxic haemolymph sampling and subsequent hypoxic exposure. During experiments tactile and auditory disturbances were minimized and visual disturbance was reduced by use of opaque containers with dark plastic coverings.

Postbranchial haemolymph samples were obtained by the following method: A 23-gauge needle fitted to an ice-cold 250 μl syringe was inserted through a previously drilled and covered (dental dam and cyanoacrylate cement) sampling port into the pericardial cavity. The combined blood and hydrostatic pressure served to force the haemolymph up the barrel of the open syringe. Prebranchial haemolymph samples were obtained similarly from the ventral abdominal sinus. It was not possible to predrill or cover prebranchial sampling ports but bleeding from this sampling site was not apparent. Animals were briefly restrained but not removed from the water during the process. Samples were taken within 10–45 s to minimize stress resulting from restraint. Sampling was aborted if 45 s was exceeded. In order to extend the haemolymph clotting time samples were placed immediately on ice during the 5 min required to complete subsequent measurements. As frequent sampling has been shown to induce acidosis in crustaceans (Truchot 1975; McMahon *et al.* 1978*a*) the sampling frequency did not exceed once per 48 h, and in animals which were to be sampled repetitively no single sample exceeded 250 μl (representing $\approx$ 6% blood volume in a 15 g crayfish).

Oxygen consumption ($\dot{M}_{O_2}$) was measured on individual animals placed in 0·5 l opaque (plastic) flow-through respirometers. The rate of water flow was adjusted so that the drop in P_{O_2} across the respirometer was limited to 5–10 torr. Inflow and outflow P_{O_2} was measured using an oxygen electrode (Radiometer E5046) thermostatted to 15 °C and displayed on a Radiometer Acid-Base Analyzer (PHM 71, 72 or 73). $\dot{M}_{O_2}$ was calculated from:

$$\dot{M}_{O_2} = \frac{(\Delta P_{O_2})\,(\alpha_{O_2})\,(\text{water flow rate, l.min}^{-1})}{\text{W}} \times 1000 \text{ (mmol O}_2\text{.kg}^{-1}\text{.min}^{-1}\text{)}$$

measured as per Cameron

The $\dot{M}_{O_2}$ value for each crayfish mean of 5–10 determinations of ΔP_{O_2} taken over a period of 1–

Average scaphognathite rate heart rates (f_H) were measured using an impedance technique of Ansell (1973). Teflon-coated stainless-steel electrodes (0·114 of insulation for 1–2 mm, were inserted horizontally for about drilled in the carapace over the heart and scaphognathite regio te and held firmly in place with dental dam and cyanoacrylate cement. Impedance changes resulting from movement of heart and scaphognathites were amplified by Biocom 2991 Impedance Convertors and displayed on an oscillographic recorder (Harvard Apparatus). The accuracy of the impedance method in $f_{\bar{H}}$ determinations for crayfish has been confirmed by simultaneous pressure/impedance recordings on *Procambarus clarki* by Hassall (1979).

Ventilation volume ($\dot{V}_{vol}$) for *O. rusticus* during normoxia and 6 days hypoxic exposure was calculated from an empirically determined relationship between ventilation volume and scaphognathite rate, i.e. stroke volume, established for the closely related

species *O. virilis* by Burggren & McMahon (in preparation). This relationship was measured for *O. virilis* during normoxia, initial (0–48 h) and long-term (72–168 h) exposure to the same level of hypoxia as used in the present study (50–60 torr). A similar relationship has been reported for the crayfish *P. clarki* (Hassall, 1979; Hassall & McMahon, 1980) as well as for brachyurans (Batterton & Cameron, 1978; Cumberlidge & Uglow, 1977; McDonald, Wood & McMahon, 1980). A strong general relationship between scaphognathite stroke volume and body mass for a variety of decapod crustaceans is reported by McMahon & Wilkens (1982). Direct measurement of ventilation volume was not possible in these unrestrained *O. rusticus* in the present study, but since (i) the size and architecture of the branchial chambers and scaphognathite channels are essentially similar in both species, and (ii) the rate of oxygen uptake and scaphognathite beating respectively for the two species are in excellent qualitative and quantitative agreement during normoxia and hypoxia (McMahon *et al.* 1974), the pre-existing relationship for *O. virilis* was assumed to apply to *O. rusticus*. In the present study, calculated ventilation volume for *O. rusticus* incorporates the transitory changes in scaphognathite stroke volume observed in *O. virilis* on entry to hypoxic water (Burggren & McMahon, in prep.).

Direct measurement of stroke volume, i.e. by use of a mask and electromagnetic flow probe, as utilized for crustaceans by Johansen, Lenfant & Mecklenburg (1970), McDonald *et al.* (1980) and others was not feasible, in these small, freshwater animals. In fact several advantages accrue from the indirect method utilized. Firstly, the animals were tethered but not restrained, allowing them to behave normally. Marked effects of restraint on patterns of scaphognathite pumping are described for *P. clarki* by Hassall (1979). Secondly, in these relatively long-term experiments the use of a mask would have meant 2 weeks of sensory and food deprivation. Effects of starvation and sensory deprivation on respiratory and cardiac patterns in crustaceans are discussed by Ansell (1973) and Florey & Kriebel (1974).

Haemolymph pH (40 μl) and P_{O_2} (100 μl) were measured with Radiometer electrodes (G229A liquid junction reference electrode, E5046 electrode respectively) thermostatted to 15 °C. The pH electrode was calibrated to within $\pm 0{\cdot}005$ pH units with Radiometer Precision Buffers, corrected for temperature. The oxygen electrode was calibrated with nitrogen gas and air saturated distilled water at 15 °C. Total CO_2 (C_{CO_2}) was measured as per Cameron (1971), using a Radiometer E5036 P_{CO_2} electrode standardized with 15 μl of a 30 mM-$NaHCO_3$ solution before and after each measurement. All electrode responses were displayed on a Radiometer Acid-base Analyzer (PHM 71, 72 or 73). Haemolymph P_{CO_2} was calculated from an empirically determined relationship between pH, C_{CO_2} and P_{CO_2} developed by Wilkes, deFur & McMahon (1980). Haemolymph oxygen content (C_{O_2}) was measured on 40 μl of sample using a Lex-O_2-Con Oxygen Analyzer (Lexington Inst.) as per McMahon *et al.* (1978*b*). Oxygen equilibrium curves were plotted both from *in vivo* measurements of P_{O_2} and C_{O_2} and measurements obtained *in vitro* as described in detail by Wilkes & McMahon (1982). Accurate gas mixtures for oxygen equilibrium curves and calibration of elctrodes were delivered by Wöstoff gas mixing pumps.

All data are from non-moulting crayfish and are presented as mean values $\pm$ 1 S.E.M. Values are reported significantly different when $P < 0{\cdot}05$ as calculated by Student's two-tailed t test.

Measured f_H, f_w, P_{a,O_2}, P_{v,O_2}, C_{a,O_2}, C_{v,O_2} and $\dot{M}_{O_2}$ for *O. rusticus* were used to derive a number of expressions which reflect cardiovascular performance and the role of haemocyanin both in oxygen uptake at the gills and delivery to the tissues. As a number of the measurements used in these calculations are mean values of samples obtained either at different times from the same group or from separate groups of crayfish, these expressions, as well as those derived from ventilation volume, are meant to serve as estimates only.

CALCULATIONS

Cardiac output (Fick principle) (ml haemolymph.kg^{-1}.min^{-1})	$\dot{V}_b = (1000)\,(\dot{M}_{O_2})/C_{a,O_2} - C_{v,O_2})$
Cardiac stroke volume (ml.stroke^{-1}) ($\dot{V}_b$ corrected to 15 g)	$S_v = \dot{V}_b/f_H$
Volume of oxygen carried by haemocyanin (mmol.l^{-1})	$C_{O_2}^{Hcy} = C_{O_2} - (\alpha O_2)(P_{O_2})$
Saturation of Haemocyanin (%)	$C_{O_2}^{Hcy}/C_{max}^{Hcy}O_2 \times 100$
Amount of oxygen delivered to tissues (a–v difference) mmol O_2.l^{-1}	$C_{a,O_2} - C_{v,O_2}$
Extraction of oxygen from the water (%)	$\mathrm{Ext}_w = \dfrac{\dot{M}_{O_2}}{(C_{I,O_2})\,(\dot{V}_w)} \times 100\,(\%)$
Extraction of oxygen from the haemolymph (%)	$\mathrm{Ext}_b = \dfrac{C_{a,O_2} - C_{v,O_2}}{C_{a,O_2}} \times 100\,(\%)$
Ventilation/perfusion ratio	$\dot{V}_w/\dot{V}_b$
Convection requirement for water (l.mmol O_2^{-1})	$\dot{V}_w/\dot{M}_{O_2}$
Convection requirement for haemolymph (l.mmol O_2^{-1})	$\dot{V}_b/\dot{M}_{O_2}$
Mean $\Delta P_{G,O_2}$ gradient across the gills (torr)	$\Delta P_{G,O_2} = \dfrac{(P_{I,O_2}) + (P_{E,O_2})}{2} - \dfrac{(P_{a,O_2}) + (P_{v,O_2})}{2}$
Transfer factor (mM O_2.kg^{-1}.min^{-1}.torr^{-1}) (calculated as per Wood *et al.* 1979)	$T_{O_2} = \Sigma_{10}\,\Delta T_{O_2} = \dfrac{\dot{M}_{O_2}}{10} - \Sigma_{10}\Delta P_{G,O_2}$
C_{E,O_2} (μM–O_2)	$(C_{I,O_2}) - \left(\dfrac{\%\ \mathrm{Ext}_w}{100}\right).(C_{I_2,O_2})$
P_{E,O_2} (torr)	$C_{E,O_2}/\alpha_{O_2}$
Quantity of oxygen delivered to the gills (μmol O_2.kg^{-1}.min^{-1})	$(C_{I,O_2})\,(\dot{V}_w)$

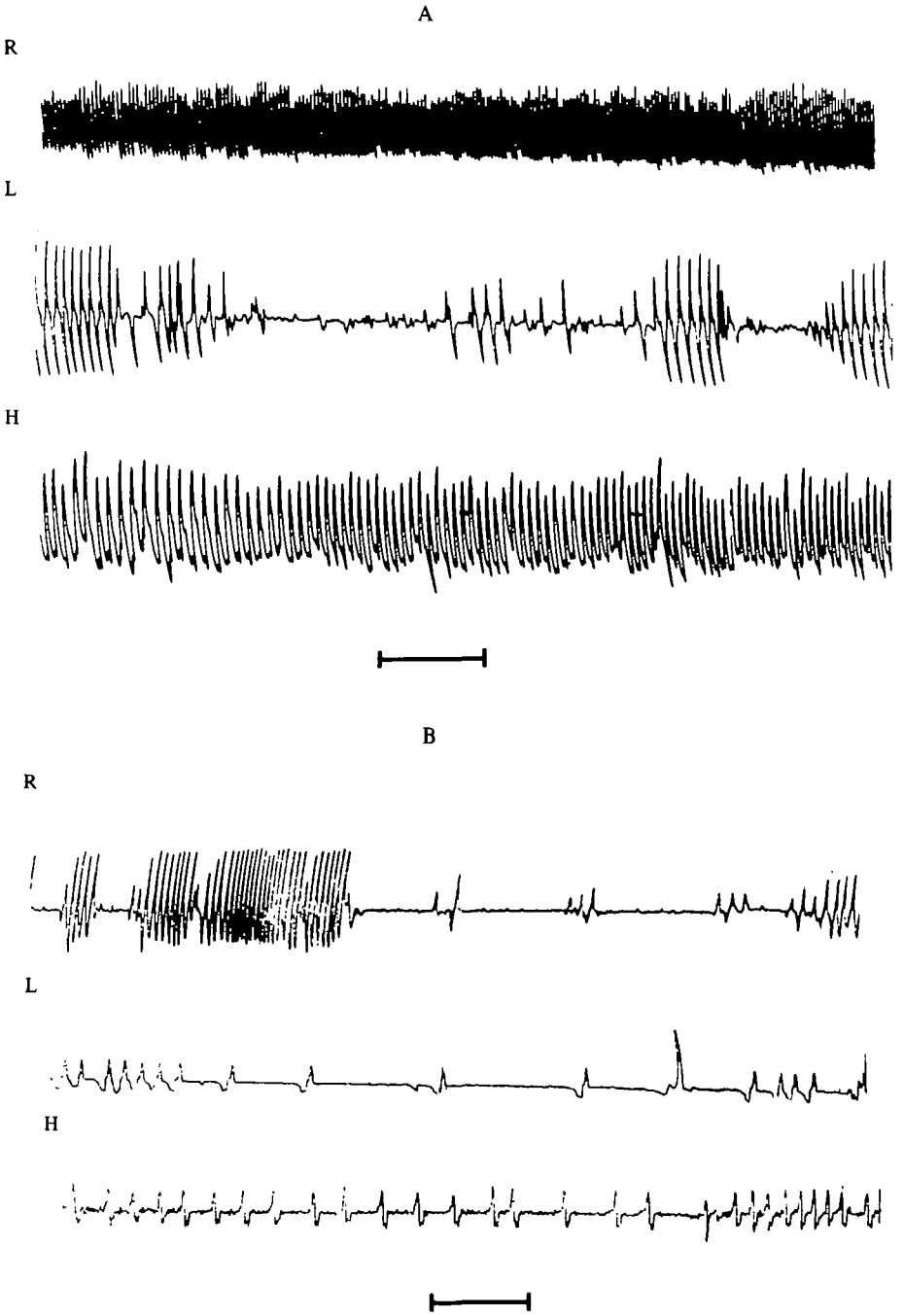

Fig. 1. Impedance recordings of heart (H), left (L), and right (R): scaphognathites showing (A) scaphognathite dominance during bilateral activity and transition to unilateral activity; and (B) bilateral pause with associated bradycardia. The bar represents 10 s.

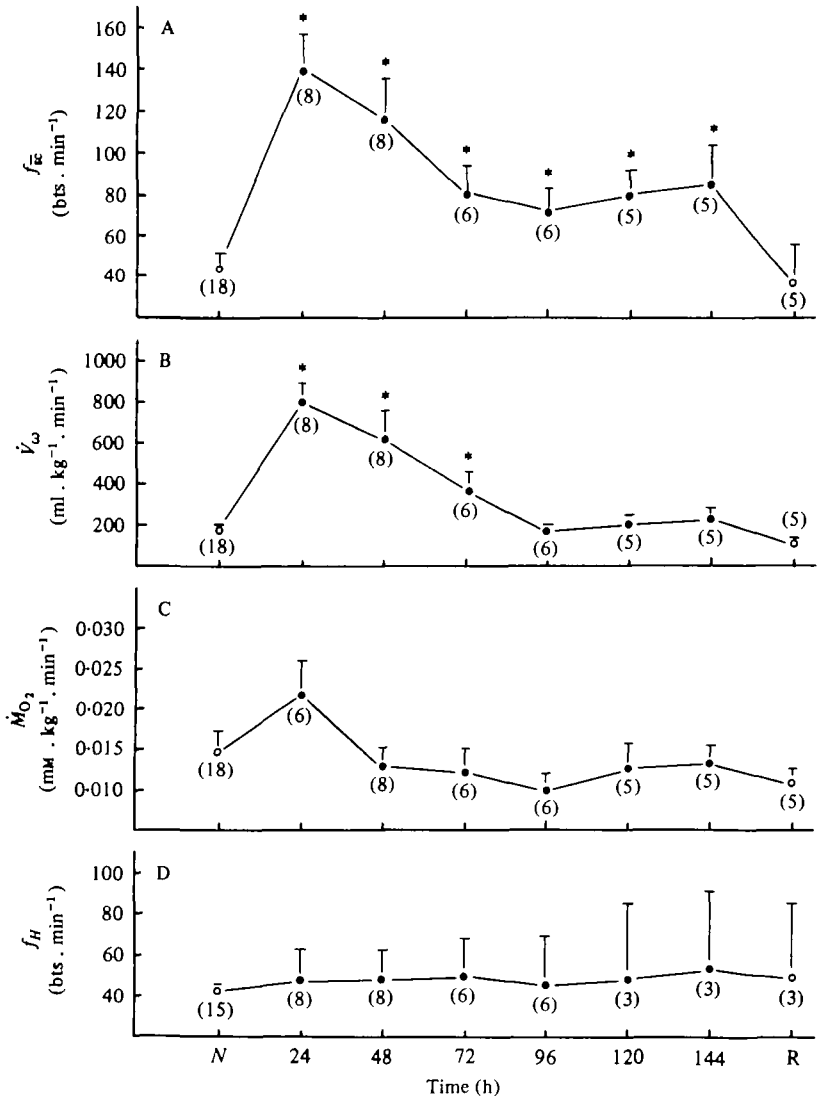

Fig. 2. Changes in (A) $f_{\dot{w}}$; (B) $\dot{V}_w$; (C) $\dot{M}_{O_2}$; and (D) f_H in *O. rusticus* from initial quiescent values in normoxia through 144 h hypoxia and 24 h recovery in normoxia. * Significance ($P < 0.05$) from initial values, numbers in parenthesis = n. ○, values measured during normoxic exposure.

RESULTS

Scaphognathite activity was recorded during normoxia for a total of 32 h from 6 quiescent crayfish. The most notable characteristic of routine scaphognathite patterns was an inequality of rates between the left and right sides, the dominant scaphognathite being $71{\cdot}4 \pm 6{\cdot}1\%$ faster. The slower scaphognathite would on occasion cease activity so that unilateral pumping occurred for 15% of the total recording time. The rate of both the dominant scaphognathite and the heart remained

Table 1. *Calculated expressions of oxygen exchange and transport in* O. rusticus *during normoxic and* 6 *days hypoxic exposure at* 15 °C: *values are presented as mean* ± S.E. (*n*) *where applicable*

	O_2 supply (C_{I,O_2}) ($\dot{V}_w$) (μmmol $O_2 . kg^{-1} . min^{-1}$)	Ext_b (%)	$\dot{V}_w/\dot{M}_{O_2}$ ($l . mmol^{-1}$ O_2)	O_2 extraction ($C_{I,O_2} . \%\, Ext_w$) (μM O_2)	*a–v* difference, $C_{a,O_2}-C_{v,O_2}$ (mmol O_2)	Ext_b	$\Delta P_{a,O_2}$ (torr)	T_{O_2} (mmol $O_2 . kg^{-1}$ min^{-1} $torr^{-1}$)	$\dot{V}_b/\dot{M}_{O_2}$ ($l . mmol\ O_2^{-1}$)	$\dot{V}_w/\dot{V}_b$
Normoxia	38·8 5·7 (18)	45·3 5·4 (18)	11·92	127·5	0·410	77·9	119·8	0·00028	2·4	5·0
24 h hypoxia	69·9 8·7 (8)	47·6 20·2 (6)	35·9	42·1	0·149	50·6	37·1	0·00142	6·25	5·2
48 h hypoxia	55·6 10·7 (8)	32·2 8·4 (8)	45·9	29·4	—	—	—	—	—	—
72 h hypoxia	34·3 5·2 (6)	45·4 12·8 (6)	29·6	42·1	—	—	—	—	—	—
96 h hypoxia	15·6 3·3 (6)	77·4 21·5 (6)	16·7	70·5	—	—	—	—	—	—
120 h hypoxia	19·8 4·0 (5)	72·1 12·7 (5)	16·4	69·4	0·224	67·0	35·1	0·00093	4·19	3·9
144 h hypoxia	21·2 5·7 (5)	72·9 12·5 (5)	16·9	67·1	—	—	—	—	—	—
24 h recovery	31·2 4·9 (5)	35·1 5·1 (5)	10·2	98·3	0·186	50·0	125·7	0·00020	4·93	2·1

unchanged over the transition to unilateral activity (Fig. 1 A). Complete simultaneous pausing of heart and scaphognathites was not observed but short periods of bilateral ventilatory pauses associated with bradycardia were observed occasionally (less than 1% of the recording time) (Fig. 1 B).

SCAPHOGNATHITE AND HEART RATES, VENTILATION VOLUME AND OXYGEN UPTAKE

Within 24 h of the commencement of hypoxic exposure f_{sc} had increased significantly by 3-fold (Fig. 2 A). Both scaphognathites now pumped at essentially the same rate. The marked increase in scaphognathite pumping, and hence ventilation volume (Fig. 2 B), was sufficient to enhance oxygen supply to the branchial chambers ($C_{I,O_2}.\dot{V}_w$, Table 1) despite the 70% reduction in the oxygen content of the ambient water. This allowed the rate of oxygen consumption to be sustained, or possibly increased, at this time (Fig. 2 C). The importance of the ventilatory response in maintaining oxygen consumption is reflected in a 4-fold increase in the convection requirement for water ($\dot{V}_w/\dot{M}_{O_2}$, Table 1). Nonetheless, the amount of oxygen extracted from the inhalent water ($C_{I,O_2}.\%\,\mathrm{Ext}_w$) was at a minimum at 24 h hypoxia (Table 1).

Peak scaphognathite rates were not maintained. After 72 h hypoxic exposure f_{sc} decreased significantly by 42% (Fig. 2 A). This level, still significantly elevated over the normoxic scaphognathite rate, was maintained for the duration of hypoxic exposure. Although the rate of oxygen delivery to the branchial chambers was reduced after 72 h hypoxia, $\%\,\mathrm{Ext}_w$ tended to increase throughout hypoxic exposure resulting in a concomitant increase in the volume of oxygen extracted (Table 1). Thus oxygen consumption was maintained at normoxic levels (Fig. 2 C) but a 1·5-fold elevation in the convection requirement for water persisted throughout the hypoxic period (Table 1). No significant change in heart rate occurred during hypoxic exposure (Fig. 2 D).

HAEMOLYMPH ACID-BASE STATUS

During normoxia, postbranchial pH and C_{CO_2} were 7·782 ± 0·027 and 5·84 ± 0·27 mM respectively, while P_{CO_2}, calculated from these data, was 3·4 ± 0·1 torr.

Following 24 h hypoxic exposure postbranchial haemolymph pH had risen significantly by 0·2 units (Fig. 3). This alkalosis was associated with a significant (almost 3-fold) decline in C_{CO_2} and P_{CO_2}. No further significant change in postbranchial acid-base status had occurred at 72 h hypoxia. By 144 h, however, partial compensation was apparent; haemolymph pH decreased significantly from its 24 h hypoxic value, while both C_{CO_2} and P_{CO_2} had increased significantly. These values were still however significantly lower than prehypoxic levels (Figs. 3 A–C).

HAEMOCYANIN OXYGEN STATUS AND HAEMOLYMPH OXYGEN TRANSPORT

After 24 h in hypoxic water mean P_{a,O_2} had fallen to 20%, but mean C_{a,O_2} only to 60% of normoxic values. Both decreases were significant. Neither mean P_{v,O_2} nor [illegible] decreased significantly (Fig. 4), thus the a–v difference in oxygen content

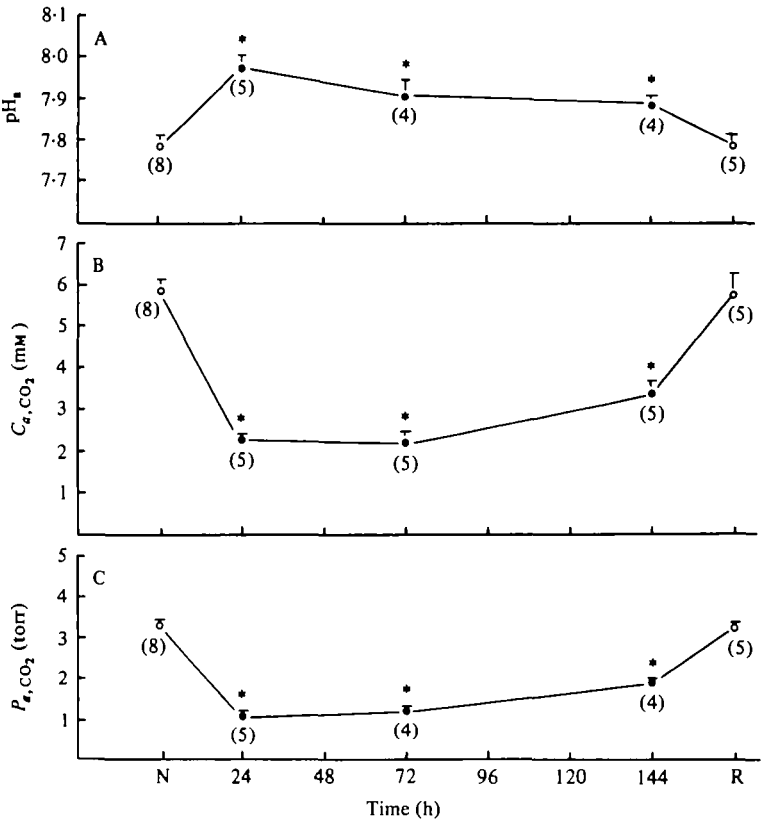

Fig. 3. Changes in postbranchial haemolymph (A) pH, (B) C_{CO_2} and (C) P_{CO_2} in *O. rusticus* from initial quiescent values in normoxia, 24, 72 and 144 h hypoxia, and 24 h recovery. * Significance ($P < 0{\cdot}05$) from initial values, number in parenthesis = n. ○, Values measured during normoxic exposure.

decreased substantially to 36% of the normoxic value (Table 1). $\%Ext_b$ declined from its normoxic value of 77·9% to 50·6% at 24 h hypoxia (Table 1). After 120 h P_{a,O_2} had increased significantly from the 24 h value. Although there was no significant change in either C_{a,O_2} or C_{v,O_2} the a–v difference was nonetheless elevated to 55% of the normoxic value and $\%Ext_b$ partially recovered to 67·0% (Table 1).

Mean oxygen gradient across the gills ($\Delta P_{G,O_2}$) fell from 119·8 to 37·1 torr within the first 24 h and remained low for the duration of hypoxic exposure (Table 1). Despite the reduction in $\Delta P_{G,O_2}$ the transfer factor or oxygen conductance (T_{O_2}), which provides an indication of the effectiveness of oxygen diffusion across the gill epithelium, had increased 5-fold over normoxic values by 24 h hypoxia and remained 3-fold greater at 120 h hypoxia (Table 1).

The Bohr value for *O. rusticus* at 15 °C as established from *in vitro* oxygen equilibrium curves (Wilkes & McMahon, 1982) was used in conjunction with *in vivo* haemolymph pH values in the present study to assess changes in haemocyanin affinity occurring during the hypoxic period. Based on the *in vivo* pH changes haemocyanin oxygen affinity had increased (i.e. P_{50} decreased) by 24 h hypoxic exposure and remained significantly elevated throughout the hypoxic period (Fig. 5). The *in*

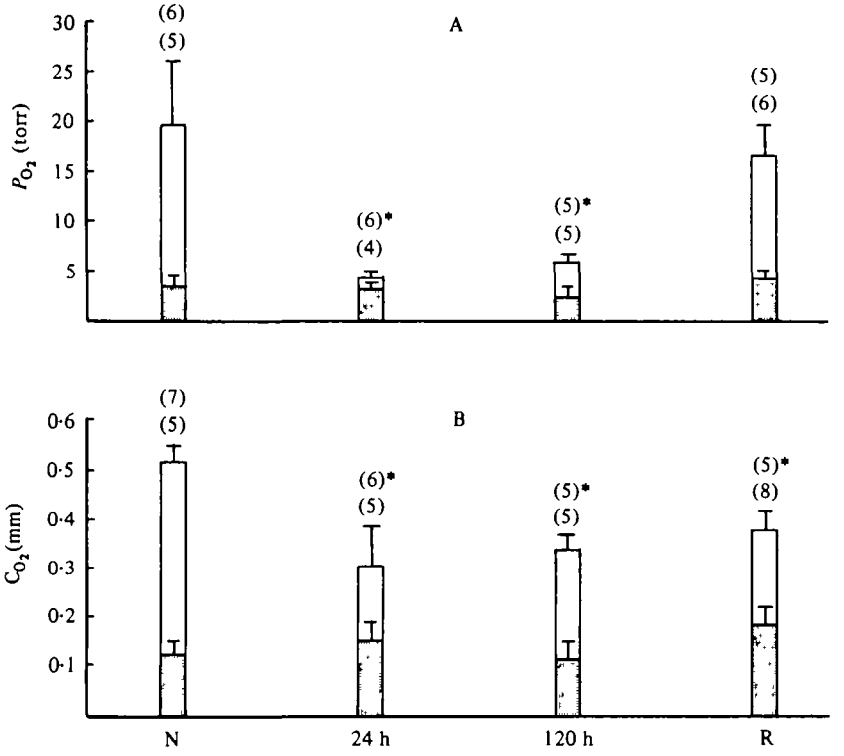

Fig. 4. P_{O_2} and C_{O_2} for postbranchial (□) and prebranchial (□) haemolymph during normoxia, 24, 120 h hypoxia and 24 h recovery. * Significance ($P < 0{\cdot}05$) from initial values, number in parenthesis = n.

oxygen dissociation curves provide qualitative confirmation of these calculated results. The *in vivo* data suggest a greater increase in oxygen affinity during hypoxia, however these are necessarily less precise than the calculated data, being influenced by individual variability in P_{a,O_2}, pH and oxygen content. The importance of such an increase in haemocyanin oxygen affinity in oxygen transport is best demonstrated by reference to Fig. 6; should P_{50} have remained at normoxic values, maximum saturation of postbranchial haemolymph would have been less than 20%.

CARDIAC PERFORMANCE

Cardiac output ($\dot{V}_b$), as calculated by the Fick principle, increased within 24 h hypoxia to 137·4 ml.kg^{-1}.min^{-1}, 3-fold above normoxic values. By 120 h, however, $\dot{V}_b$ decreased to 53·6 ml.kg^{-1}.min^{-1} (Fig. 7). Since no significant change in f_H occurred during hypoxic exposure (Fig. 7) the changes in $\dot{V}_b$ must have resulted from an increase in cardiac stroke volume (Sv). Following the changes in circulatory performance the convection requirement for haemolymph ($\dot{V}_b/\dot{M}_{O_2}$) increased 2·5-fold during the initial 24 h hypoxic period but subsequently declined to a level 1·7 times the normoxic values by 120 h hypoxia (Table 1). Changes in calculated ventilation volume were mirrored by changes in cardiac output, thus maintaining a constant ventilation/perfusion ($\dot{V}_w/\dot{V}_b$) ratio throughout the hypoxic period (Table 1).

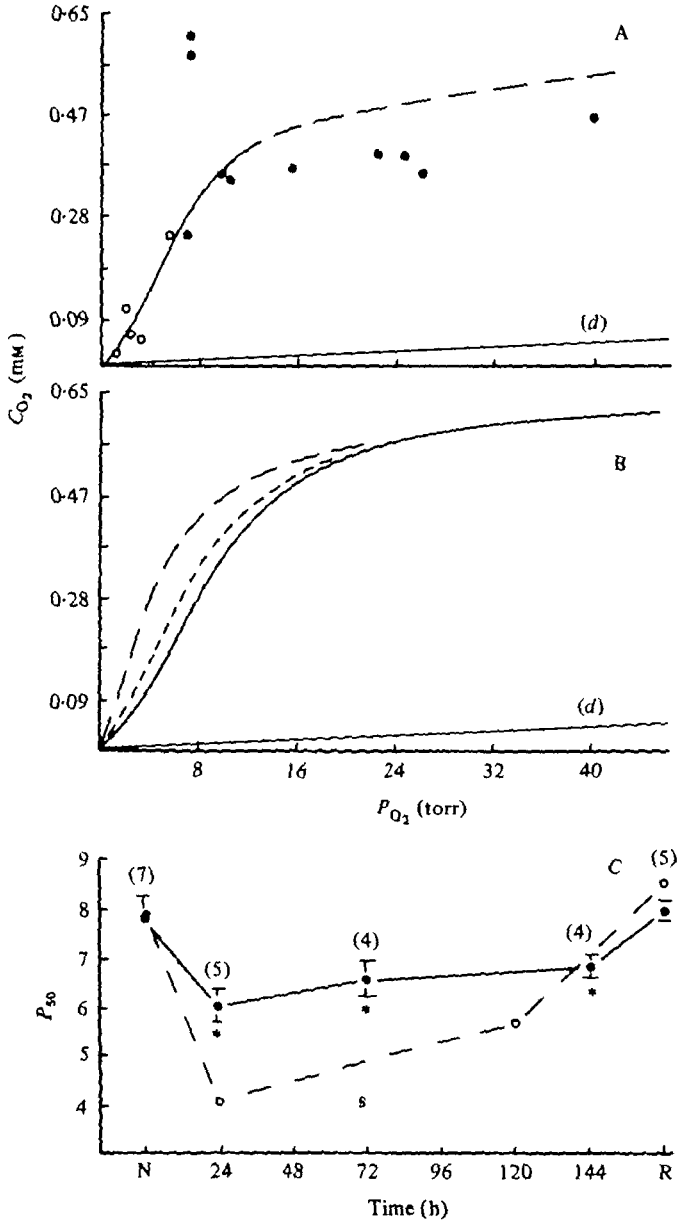

Fig. 5. (A) Representative *in vivo oxygen equilibrium curve* for *O. rusticus* based on mean C_{a,O_2} of 0·507 mM during normoxia; ●, post-branchial samples; ○, prebranchial samples. (B) Calculated oxygen equilibrium curves based on *in vivo* pH and a Bohr value of −0·628 (Wilkes & McMahon, 1982); ——, normoxia and 24 h recovery; – – –, 24 hr hypoxia; ----, 120 hr hypoxia. (*d*) Amount of oxygen carried in physical solution. (C) Changes in P_{50} from calculated equilibrium curves (–●–) and from *in vivo* oxygen equilibrium curves (----○----). * Significant ($P < 0·05$) from initial values, number in parentheses = *n*.

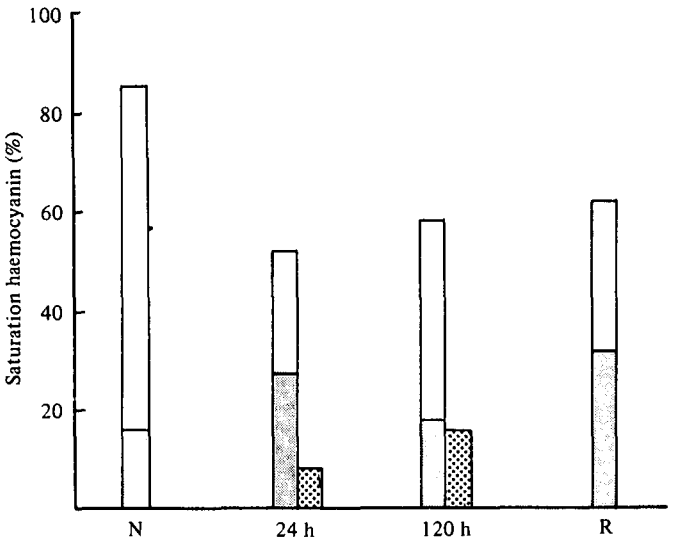

Fig. 6. Changes in percentage saturation of post- (□) and pre- (▨) branchial haemocyanin during normoxia, 24 and 120 h hypoxia, and 24 h recovery. (▦) Calculated percentage saturation of postbranchial haemocyanin if the increase in oxygen affinity had not occurred.

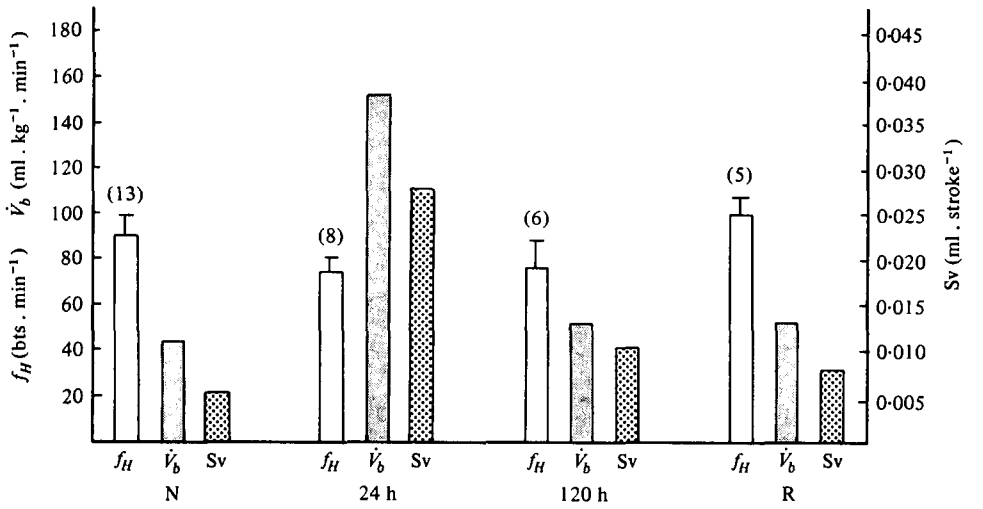

Fig. 7. Changes in f_H (□), $\dot{V}_b$ (▨), and Sv (▦) during normoxia, 24 and 120 h hypoxia and 24 h recovery.

RECOVERY FROM HYPOXIC EXPOSURE

After 24 h return to normoxic water scaphognathite patterns, f_{sc} and haemolymph acid-base components had all returned to levels not significantly different from their prehypoxic values. Although mean P_{a,O_2} returned to prehypoxic levels mean C_{a,O_2} increased only slightly from the 120 h hypoxic values and remained significantly below pre-hypoxic levels. Neither mean P_{v,O_2} nor C_{v,O_2} were significantly different from normoxic or hypoxic levels 24 h after return to normoxia. The *a–v* difference

thus remained at one half the prehypoxic level, while $\%Ext_b$ fell from 67% at 120 h hypoxia to 50%. Mean $\Delta P_{a,O_2}$, T_{O_2} and P_{50} were all re-established at prehypoxic levels by 24 h return to normoxia.

There was no significant change in heart rate 24 h following return to normoxic water. Similarly cardiac output, stroke volume and the convection requirement for haemolymph did not change during recovery. Since ventilation volume decreased, the ventilation/perfusion ratio fell below prehypoxic values.

DISCUSSION

Ventilatory and cardiac pumping patterns characteristic of quiescent *O. rusticus* during normoxia consist of bilateral activity associated with scaphognathite dominance, unilateral pumping and occasional bilateral pausing accompanied by bradycardia. These characteristics are similar to those recorded under quiescent conditions for another crayfish, *P. clarki* (Hassall, 1979; Hassall & McMahon, 1980). Similarly scaphognathite and heart rate, oxygen consumption, post- and prebranchial oxygen content and pressure, and postbranchial acid-base status measured in quiescent *O. rusticus* during normoxic exposure agree well with values reported for the crayfish species *O. virilis* (McMahon *et al.* 1974), *Astacus leptodactylus* (Angersbach & Decker, 1978; Dejours & Beekenkamp, 1977), *Austropotamobius pallipes* (Taylor & Wheatly, 1980); (Wheatly & Taylor, 1981), *Pacifasticus leniusculus* (Rutledge, 1981) and *P. clarki* (Hassall, 1979).

The ventilatory responses of *O. rusticus* to hypoxic exposure are similar to those recorded previously for three other species subjected to protracted hypoxic exposure; i.e. the crayfish *O. virilis* (McMahon *et al.* 1974), the lobster *H. vulgaris* (Butler *et al.* 1978) and the shore crab *Carcinus maenas* (Burnett & Johansen, 1981). An initial phase was characterized by a marked elevation in $f_{\bar{w}}$ and hence $\dot{V}_w$. The present study did not measure the early time course of the elevation in $f_{\bar{w}}$ but, in this species (McMahon & Wilkes, in preparation), *O. virilis* (McMahon *et al.* 1974), *H. vulgaris* (Butler *et al.* 1978) and *A. pallipes* (Wheatly & Taylor, 1981) $f_{\bar{w}}$ increases within minutes of detection of hypoxia and peaks within 6–24 h. In the former three cases, which involved long-term studies, $f_{\bar{w}}$ declined by 72 h to a new steady level significantly above normoxic values. In the present study these new rates were maintained for the remainder of the hypoxic period and McMahon *et al.* (1974) have shown maintenance for up to 12 days in *O. virilis*.

During the initial period the increased scaphognathite pumping was sufficient to elevate oxygen delivery to the gills above normoxic levels in both *O. rusticus* and *O. virilis* (McMahon *et al.* 1974). Subsequent diminution of ventilation reset oxygen delivery close to prehypoxic levels. The importance of the ventilatory response to the maintenance of $\dot{M}_{O_2}$ is reflected in the convection requirement for water which mirrored changes in scaphognathite pumping. Nevertheless, the amount of oxygen extracted during hypoxia is well below normoxic values, a fact not reflected by $\%Ext_w$ (Herreid, O'Mahoney & Shah, 1979). Thus the maintenance of oxygen consumption during hypoxic exposure cannot result solely from the increase in oxygen supply either in *O. rusticus*, *O. virilis* (McMahon *et al.* 1974) or the lobster *H. vulgaris* (Butler *et al.* 1978) and further internal compensatory adjustments must occur.

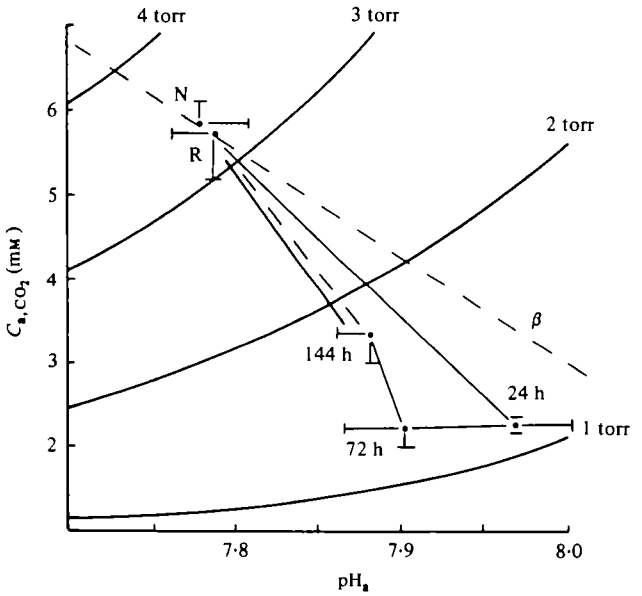

Fig. 8. Changes in postbranchial haemolymph acid-base status presented on a 'Davenport' diagram in which C_{CO_2} is plotted against pH. The curved lines represent P_{CO_2} isopleths. The dashed line labelled β is a mean buffer curve obtained for *O. rusticus* under normoxic conditions at 15 °C (Wilkes & McMahon, 1982). ○, Values measured during normoxic exposure.

Increase in the participation of the respiratory pigment haemocyanin, i.e. utilization of venous reserve oxygen, plays an important role in oxygen transport, and also serves to lessen the decrease in $\Delta P_{a,O_2}$ which results from hypoxia in two marine crustaceans (A. C. Taylor, 1976; Butler *et al.* 1978). This mechanism however, is not available in *O. rusticus* where, even in normoxia, venous oxygen tensions are sufficiently low that little reserve occurs either in oxygen capacity or in the gradient for oxygen delivery to the tissues (Fig. 6). Instead increase in oxygen affinity as previously reported for both *A. pallipes* (Wheatly & Taylor, 1981) during short-term oxygen depletion and *H. vulgaris* during chronic hypoxic exposure (McMahon *et al.* 1978*a*) plays an essential role in *O. rusticus*. As is apparent from the oxygen equilibrium curves (Fig. 5) and as shown in Fig. 6, the marked increase in haemocyanin oxygen affinity allows 60% oxygen saturation of postbranchial haemocyanin at 6 torr, a tension which would have allowed only 20% saturation under normoxic conditions.

In *O. rusticus*, *A. pallipes* (Wheatly & Taylor, 1981) and *H. vulgaris* (McMahon *et al.* 1978*a*) the increased oxygen affinity which occurs during hypoxia results from a respiratory alkalosis in which CO_2 washout is usually attributed to hyperventilation (McMahon *et al.* 1978*a*; Sinha & Dejours, 1980; Wheatly & Taylor, 1981). However, recent results from Burnett & Johansen (1981) on *C. maenas* and Wood & Jackson (1980) on *Salmo gairdneri* suggest that ventilation alone may not be as important in controlling blood P_{CO_2} levels as changes in CO_2 production and/or gill perfusion. In both *O. rusticus* (present study) and *C. maenas* (Burnett & Johansen, 1981) the respiratory alkalosis is associated with a base deficit, i.e. the haemolymph acid-base status deviates below the normal buffer line (Fig. 8). This latter aspect was not, how-

ever, observed during acute hypoxia in *A. leptodactylus* (Sinha & Dejours, 1980) Although some recovery occurs within 120 h in *O. rusticus* as scaphognathite pumping decreases it is not completed within 3½ weeks maintained hypoxia (Wilkes & McMahon, 1982). In contrast, both Truchot (1975) and McMahon *et al.* (1978*a*) observed a gradual increase in haemolymph bicarbonate levels during hypoxic exposure, i.e. a base excess. This difference may be related to varying degrees of metabolic acid production occurring during hypoxic exposure (Burke, 1979; Bridges & Brand, 1980; Burnett & Johansen, 1982). Few studies have examined metabolic acid production (anaerobic metabolism) in chronic hypoxia. Butler *et al.* (1978) were able to report no significant change in lactate in *H. vulgaris*. Haemolymph lactate levels were not measured in this study but increases are unlikely since $\dot{M}_{O_2}$ was maintained throughout. The physiological significance of these species differences are not fully understood. With the exception of the study by Burnett & Johansen (1982) on *C. maenas* and Sinha & Dejours (1980) on *A. leptodactylus* partial compensation for the respiratory alkalosis is effected by elevation of haemolymph bicarbonate. These, exceptions may reflect differences in experimental methods. For instance, in the former study the severe level of hypoxia used may not have allowed significant reduction in scaphognathite pumping; while in the latter study the short duration of hypoxic exposure may not have permitted sufficient time for compensation to occur.

No published study has analysed the role of the cardiovascular system in compensation to chronically maintained hypoxic exposure in decapods. As oxygen levels gradually decline, heart rate generally remains stable until a critical ambient P_{O_2} is reached, beyond which rates decline (Herreid, 1980; McMahon & Wilkes, in prep.). The critical level varies with species (deFur & Mangum, 1979), temperature (Taylor, Butler & Sherlock, 1973) and, to a certain extent, the oxygen-carrying capacity of the haemolymph (Spoek, 1974; A. C. Taylor, 1976). Less variability is seen in cardiac output, which often increases with or without a concomitant increase in heart rate (McMahon & Wilkens, 1975; Burnett, 1979). Decrease in cardiac output in response to moderate hypoxia has been reported (A. C. Taylor, 1976), but less commonly. No significant change in heart rate occurs in *O. rusticus* during long-term exposure to this level of hypoxia but estimates of cardiac output increase 3- to 4-fold, presumably by increased stroke volume.

Increase in cardiac output and hence gill perfusion could further improve on the benefits derived from increased haemocyanin oxygen affinity (above) by rapidly removing oxygenated haemolymph from the gills and thus maintaining an effective gradient for oxygen transfer, and secondly, by increasing the effective gill surface area. A possible mechanism for the latter action has been described for crayfish by Burggren, McMahon & Costerton (1974). Briefly, when perfusion is low small lacunae in the gill filaments may allow diversion of haemolymph from afferent to efferent channels, thus reducing the effective length of the gill filament. Elevated cardiac output and hence gill perfusion could reduce the relative magnitude of this shunt and increase the effective gas exchange area. The observed increase in oxygen conductance (T_{O_2}) may be accounted for by such a mechanism.

It would appear that during hypoxic exposure each species demonstrates its own particular pattern of adaptive mechanisms, the blend not being constant but varyin

with the extent and duration of exposure. For instance in all cases there is an increase in ventilation which contributes to a respiratory alkalosis. Although the respiratory alkalosis is never fully compensated, in most species partial recovery of haemolymph pH occurs by elevation of bicarbonate levels. Exceptions may simply reflect differences in experimental methods, a case in point being *C. maenas* (Truchot, 1975; Burnett & Johansen, 1981). Exploitation of a venous reserve, which serves to lessen the decrease in $\Delta P_{G,O_2}$ and increase oxygen transport, occurs where it does not encroach on the haemolymph to tissue oxygen gradient. In *O. rusticus* there was no venous reserve and the increased oxygen affinity, effected by the respiratory alkalosis, facilitates oxygen binding to haemocyanin at the gills, while elevation in cardiac output provides adequate tissue oxygenation.

Clearly, in the case of *O. rusticus* the initial 24 h hypoxic period is characterized by large increases in both scaphognathite pumping and cardiac output which decline by 72 h. It is not known whether the transitory nature of the initial responses results from a short lived 'avoidance' response (McMahon & Wilkes, in prep.) mediated by increased levels of circulating hormones such as 5-HT or dopamine (see Wilkens, 1981) or, if it simply takes time to achieve a balance between the energetic cost of (Jones, 1971; Jones, Randall & Jarman, 1970; Herreid, 1980) and the benefits derived from elevated ventilation and perfusion. In any case these initial responses decrease so that the respective convection requirements for water and haemolymph are maintained only slightly above normoxic levels. Using the mechanisms described above *Orconectes rusticus* is well able to maintain resting oxygen uptake at the hypoxic level used, i.e. 45–55 torr but the maximum recorded mean P_{a,O_2} of 6 torr allowing 60% oxygenation of the postbranchial haemolymph indicates that this level may be approaching the lower long-term tolerance levels of this species.

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