J. exp. Biol. 144, 417–436 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

OXYGEN TRANSPORT AND ACID–BASE BALANCE IN THE HAEMOLYMPH OF THE LOBSTER, *HOMARUS GAMMARUS*, DURING AERIAL EXPOSURE AND RESUBMERSION

BY E. W. TAYLOR AND N. M. WHITELEY

School of Biological Sciences, University of Birmingham, Birmingham B15 2TT, Institut für Zoologie IV, University of Düsseldorf, FRG and *Gatty Marine Laboratory, University of St Andrews, Scotland*

Accepted 20 February 1989

Summary

Submerged lobsters at 15°C were normoxaemic (Ca_{O_2} = 0·52 mmol l^{-1} at a Pa_{O_2} of 6·53 kPa) and normocapnic (Pa_{CO_2} = 0·44 kPa; $[HCO_3^-]$ = 9·3 mequiv l^{-1} and pHa = 7·78). After 3 h in air the haemolymph was markedly hypoxic and hypercapnic (Pa_{O_2} = 1·6 kPa; Ca_{O_2} = 0·2 mmol l^{-1}; Pa_{CO_2} = 0·7 kPa and pHa = 7·64). Disturbance after 3 h in air caused a greater increase in Pa_{CO_2} to 1·28 kPa and a fourfold increase in lactate levels to 3·6 mmol l^{-1}. The combined respiratory and metabolic acidosis reduced pHa to 7·39.

After 14 h in air, undisturbed lobsters remained hypoxic and hypercapnic (Pa_{O_2} = 1·2 kPa; Pa_{CO_2} = 1·2 kPa). Lactate levels had increased to 6·2 mmol l^{-1}. Despite this clear limit on respiratory gas exchange in air, oxygen transport by the haemolymph was restored. A rise in buffer base ($[HCO_3^-]$ = 15·8 mequiv l^{-1}) compensated for the potential respiratory and metabolic acidosis and pH was unchanged at 7·63. The combined effects of the increase in lactate ($\Delta \log P_{50}/\Delta \log[\text{lactate}] = -0{\cdot}175$) and calcium ($\Delta \log P_{50}/\Delta \log[Ca^{2+}] = -0{\cdot}20$ at pH 7·63) levels contributed to an increase in oxygen affinity of haemocyanin at constant pH. Consequently, mean Ca_{O_2} increased from 0·2 to 0·38 mmol l^{-1} between 3 h and 14 h in air.

Resubmergence after 14 h in air resulted in a transient alkalosis due to retention of bicarbonate; oxygen and CO_2 were rapidly restored to submerged levels. The lobster possesses the appropriate respiratory adaptations for survival during the relatively long periods of exposure in air encountered during commercial shipment.

Introduction

In the British Isles the lobster *Homarus gammarus* (*vulgaris*) is distributed along the coastal fringe from low-water mark down to 110 m (Anonymous, 1977) and therefore rarely comes into contact with air in its natural environment. Conse-

Key words: oxygen transport, acid–base regulation, aerial exposure, crustaceans, lobster, *Homarus*.

quently, lobsters can be regarded as fully aquatic, yet it is well known by the fishing industry that these animals can survive considerable periods (72–96 h) of aerial exposure, when they are removed from the sea and distributed to inland markets. It is possible that their present distribution below tidal rise and fall is due to intense fishing pressures (Cobb & Wang, 1985), so that their resistance to aerial exposure may be an adaptation for survival in the littoral zone.

Most of the current information relating to the response of primarily aquatic crustaceans to aerial exposure has been obtained from species that either voluntarily leave water to breathe air, when threatened by high temperatures or low oxygen levels in their aquatic environment, or are exposed to air by the tide leaving the littoral zone of the seashore. These include the intertidal crabs *Carcinus maenas* (Truchot, 1975*a*; Taylor & Butler, 1978; Wheatly & Taylor, 1979) and *Cancer productus* (deFur & McMahon, 1984*a*,*b*). An exception is the subtidal crab *Callinectes sapidus* (Batterton & Cameron, 1978). A detailed study of the respiratory consequences of relatively long-term aerial exposure (up to 48 h) is available for the freshwater crayfish *Austropotamobius pallipes*. The crayfish is amphibious, voluntarily leaving hypoxic water to become a facultative air-breather (Taylor & Wheatly, 1980). Exposure in air caused internal hypoxia and hypercapnia. An initial combined respiratory and metabolic acidosis was counteracted during long-term aerial exposure by an elevation of circulating bicarbonate buffer levels, a reduction in lactate concentrations and sequestration of protons in skeletal muscle (Tyler-Jones & Taylor, 1988). Oxygen transport to the tissues was restored by reversal of a Bohr shift on the haemocyanin, as the pH recovered towards the submerged level (Taylor & Wheatly, 1981), and the combined effects of increased levels of lactate and calcium (Taylor *et al.* 1987) which increase the oxygen affinity of haemocyanin (Morris *et al.* 1986*a*,*b*).

H. gammarus is closely related to *A. pallipes*. Both species belong to the suborder Reptantia, section Macrura, and have many anatomical similarities. For example, the lobster and the crayfish have trichobranchiate (filamentous) gills which may be less resistant to collapse in air than the phyllobranchiate (lamellar) gills found in brachyurans, such as *C. maenas*. The purpose of the present study was to determine the respiratory response of *H. gammarus* to exposure in air at 15 °C by describing the changes in haemolymph oxygen levels and acid–base status before, during and after 14 h of aerial exposure. This experimental regime was similar to that previously imposed on *A. pallipes*, enabling comparisons of the respiratory responses of a macruran species that rarely encounters the aerial environment (the lobster) with one that ventures voluntarily into air (the crayfish). This period of exposure in air also coincides with the average time that live lobsters are held in air during transport from suppliers in Scotland to the wholesale market in Birmingham (Whiteley, 1988). An increased understanding of the physiological changes imposed on lobsters during aerial exposure may lead to improvements in commercial handling, giving better survival, less wastage and eventually a reduction in the levels of exploitation of the natural population (Whiteley & Taylor, 1989).

Materials and methods

In vivo *blood gas, acid–base and ion determinations*

Adult lobsters, *Homarus gammarus* (*vulgaris*), of either sex and judged to be in intermoult, were used in this study. *In vivo* measurements of haemolymph oxygen levels and acid–base status were performed at the Gatty Marine Laboratory, University of St Andrews. These experiments required 31 lobsters (see Table 1 for details), ranging in mass from 0·14 to 0·35 kg. The animals were held in large tanks supplied with abundant recirculated and aerated sea water (salinity = 35 ‰) at approximately 15 °C, which was the experimental temperature, and were fed at regular intervals.

Prior to experiments, lobsters were prepared for the withdrawal of postbranchial (arterial) haemolymph samples from the pericardial sinus by drilling through the exoskeleton above the heart and covering the area with a rubber patch (Butler *et al.* 1978). Animals were then left for 1 week before experiments commenced. During experiments individual lobsters were transferred from the holding facilities into experimental tanks supplied with recirculated and well-aerated sea water at 15 °C. Each lobster was provided with a length of opaque plastic pipe in which they characteristically sought refuge. The tanks were covered with black polyethylene to minimize disturbance from external factors and were left undisturbed for 48 h, making it possible to collect data from settled animals (see Butler *et al.* 1978).

Careful sampling of postbranchial haemolymph did not disturb the animals. They were withdrawn gently backwards from their refuge in the pipe until the patch over the heart was exposed. A hypodermic needle was then inserted through the exoskeleton and a sample of haemolymph (approximately 0·5 ml) was withdrawn into a cooled glass syringe. Prebranchial (venous) haemolymph samples were withdrawn from the infrabranchial sinus *via* the base of a walking leg. This procedure was completed rapidly (< 20 s) to minimize handling and consequent disturbance of the animals. Post- and prebranchial samples were taken in quick succession, and each lobster was sampled once only. This introduced the problem of variations in haemolymph respiratory variables among individuals but avoided the changes resulting from repeated sampling (Truchot, 1975*a*).

The experimental regime included exposing animals for up to 14 h in air with a relative humidity of 70–80 %. This was imposed by gradually siphoning the sea water from the tank without handling the lobster, thus minimizing disturbance, prior to sampling. After 14 h of aerial exposure, water was reintroduced into the experimental tanks and recovery was followed for 3 h. Haemolymph samples were withdrawn from lobsters which were: settled and submerged in aerated sea water ($N = 7$); exposed in air for 3 or 14 h ($N = 10$); recovering in sea water for 30 min or 3 h, subsequent to 14 h in air ($N = 9$). In addition, five lobsters were handled for 1–2 min in air, after 3 h of aerial exposure, then subsequently sampled. These animals were termed 'disturbed'.

The postbranchial and prebranchial haemolymph samples (approximately 0·5 ml each) were analysed immediately for oxygen partial pressure (Pa_{O_2} and Pv_{O_2}), total oxygen content (Ca_{O_2} and Cv_{O_2}), pH (pHa and pHv) and total carbon

dioxide (Ca_{CO_2} and Cv_{CO_2}). P_{O_2} was measured with an oxygen electrode (Radiometer E5047) housed in a cuvette maintained at 15°C and connected to an oxygen meter (Strathkelvin Instruments). Haemolymph pH was determined using a glass capillary electrode (Radiometer 5021a) maintained at the experimental temperature and connected to a Radiometer acid–base analyser (PHM 71). Subsamples of haemolymph (50 μl) were injected into small chambers held at 38°C for measurement of total CO_2 using the technique described by Cameron (1971), and total oxygen content following the technique devised by Tucker (1967) and modified by Bridges *et al.* (1979), for blood containing haemocyanin. The oxygen electrodes were calibrated between zero and air-saturated oxygen values with oxygen-free nitrogen gas and sea water equilibrated with air at 15°C. Calibration was checked immediately before each set of samples, and an accuracy of ±0·13 kPa was obtained from replicate samples. For measurement of C_{O_2} the electrode was calibrated with 50 μl samples of deionized water, equilibrated with air at 0°C. This contains 10 ml O_2 l^{-1} (Harvey, 1955), which is similar to the oxygen-carrying capacity of lobster haemolymph. The CO_2 electrode was calibrated with freshly prepared standard solutions of $NaHCO_3$.

Measured values of P_{O_2} and C_{O_2} were used to calculate dissolved oxygen ($[O_2]$) and combined oxygen (C_{Hcy,O_2}) in both post- and prebranchial haemolymph as described by Dejours (1981). The amount of oxygen delivered to the tissues was taken as the a–v oxygen content difference (C_{a-v,O_2}) and the proportion of oxygen delivered in solution or as oxyhaemocyanin was determined for each sample. Partial pressure of CO_2 (Pa_{CO_2} and Pv_{CO_2}) and haemolymph bicarbonate concentration ($[HCO_3^-]$) were derived from the measured levels of pH and total CO_2 *via* the Henderson–Hasselbalch equation using values for the constants, αCO_2 and pK_1' provided by Truchot (1976*b*).

Following the measurement of blood gas values, subsamples of haemolymph were diluted in deionized water and stored for eventual determination of calcium, magnesium, copper, potassium and sodium concentrations using an atomic absorption spectrophotometer (Pye Unicam SP9) and chloride concentrations using an amperometric titrator (Amino Cotlove). Additional subsamples were injected into 8% w/v perchloric acid and refrigerated. The supernatant was used for the estimation of haemolymph lactate levels using the standard technique presented in Sigma Technical Bulletin no. 826 UV. Samples plus lactic acid standards were incubated with enzyme for 1 h at 37°C and NAD absorbance was measured at 340 nm in a spectrophotometer (Beckman model 25). These measurements predated the recommendations of Graham *et al.* (1983), but avoided the problem of overestimating lactate levels by stopping the reaction after 1 h and including lactate standards.

In vitro *measurements*

The non-bicarbonate buffering capacity of lobster haemolymph was determined *in vitro* by equilibrating a pooled haemolymph sample (3 ml), taken from the experimental animals, with humidified gas mixtures containing a range of known

CO_2 levels, generated by a gas-mixing pump (Wöstoff, Bochum). At each P_{CO_2} an equilibrated haemolymph sample was withdrawn for the determination of pH and C_{CO_2}. $[HCO_3^-]$ was calculated using the Henderson–Hasselbalch equation and plotted against pH. The slope of this relationship (β) represents the *in vitro* non-bicarbonate buffer line (Truchot, 1976*b*; McDonald *et al.* 1979).

In vitro measurements of haemocyanin oxygen-affinity were performed at the Institut für Zoologie IV, University of Düsseldorf. Haemolymph collected from lobsters supplied by Sea Products International, Birmingham, was frozen and transported to Düsseldorf where it was dialysed at 4°C for 24 h against a series of Ringer's solutions containing concentrations of the major ions similar to those in lobster haemolymph. The Ringer which corresponded in composition to the haemolymph of submerged lobsters had the following composition (in $mmol\,l^{-1}$): NaCl, 480; KCl, 9; $CaCl_2$, 10; $MgCl_2$, 8; $NaHCO_3$, 6 (pH 8). The levels of calcium and L-lactate in the Ringer were adjusted during the experiment to mimic those measured *in vivo* in submerged lobsters and in lobsters during aerial exposure. L-lactate levels varied from 2 to 16 $mmol\,l^{-1}$ and calcium from 10 to 20 $mmol\,l^{-1}$. Oxygen equilibrium curves for dialysed haemocyanin at a range of pH, calcium and L-lactate levels were determined spectrophotometrically on 20 μl samples, using a modified version of the diffusion chamber apparatus described by Sick & Gersonde (1969). This was supplied with humidified gas mixtures containing varying proportions of nitrogen, oxygen and CO_2 from gas-mixing pumps (Wöstoff, Bochum), as described by Bridges *et al.* (1979) and summarized by Morris *et al.* (1986*b*). Problems associated with *in vitro* measurement of oxygen binding on previously frozen samples of lobster haemolymph were recently considered by Morris (1988). Despite changes in cooperativity, P_{50} was unaffected and it is this variable which is considered in the present study.

Measured values are given in the text as means ± S.E.M. (N). The significance of any apparent difference between mean values was tested using a two-tailed Student's t-test at the 95 % level of confidence ($P < 0.05$).

Results

Haemolymph oxygen levels

Changes in oxygen partial pressure (Pa_{O_2} and Pv_{O_2}) and oxygen content (Ca_{O_2} and Cv_{O_2}) in pre- and postbranchial haemolymph, prior to and during aerial exposure and following resubmergence are plotted in Fig. 1. Fig. 2 illustrates the relationships between these two variables at various sample intervals, plotted as speculative *in vivo* oxygen equilibrium curves. Lobsters settled in well-aerated sea water had relatively low values of Pa_{O_2} and Pv_{O_2}, 6·5 ± 1·5(7) and 2·4 ± 0·3(7) kPa, respectively, and a post- to prebranchial oxygen content difference (C_{a-v,O_2}) of 0·18 ± 0·03 $mmol\,l^{-1}$ (Table 1). Despite these low P_{O_2} values, 44 % of the available oxygen was carried in solution (Table 1).

After 3 h of aerial exposure, lobsters were internally hypoxic and hypoxaemic. Pa_{O_2} and Pv_{O_2} levels were 1·6 ± 0·3(4) and 1·2 ± 0·3(4) kPa, a drop of 76 % and

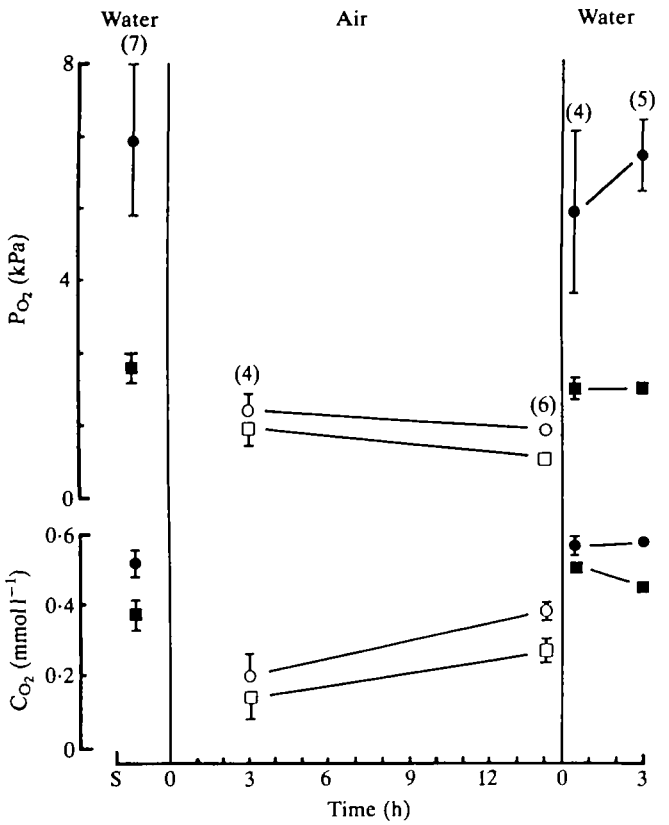

Fig. 1. Mean values (±S.E.M.) of oxygen partial pressure (P_{O_2}) and oxygen content (C_{O_2}) in prebranchial (■/□) and postbranchial (●/○) haemolymph of lobsters; submerged (S), exposed in air for 3 and 14 h (open symbols) and following 3 h resubmergence. The vertical lines demark the period in air. The numbers of observations for all variables are included in parenthesis above the Pa_{O_2} values.

50 %, respectively, from submerged values. Oxygen content values were 40 % of the values present in submerged animals, being $0{\cdot}20 \pm 0{\cdot}06(4)$ mmol l^{-1} in postbranchial and $0{\cdot}13 \pm 0{\cdot}06(4)$ mmol l^{-1} in prebranchial haemolymph. Oxygen in physical solution in the postbranchial haemolymph dropped from a submerged mean of $0{\cdot}07 \pm 0{\cdot}02(7)$ mmol l^{-1} to $0{\cdot}017 \pm 0{\cdot}006(4)$ mmol l^{-1} after 3 h in air, whereas oxygen bound to haemocyanin dropped from $0{\cdot}45 \pm 0{\cdot}01(7)$ to $0{\cdot}19 \pm 0{\cdot}06(4)$ mmol l^{-1}. The C_{a-v,O_2} difference decreased to $0{\cdot}07 \pm 0{\cdot}01(4)$ mmol l^{-1} owing to the reduction in both dissolved and haemocyanin-bound oxygen levels (Table 1). In lobsters disturbed after 3 h of aerial exposure Pa_{O_2} and Pv_{O_2} values were $2{\cdot}1 \pm 0{\cdot}3(5)$ and $1{\cdot}5 \pm 0{\cdot}1(5)$ kPa, respectively, whereas Ca_{O_2} and Cv_{O_2} were $0{\cdot}19 \pm 0{\cdot}03(5)$ and $0{\cdot}11 \pm 0{\cdot}01(5)$ mmol l^{-1}. The levels of P_{O_2} were slightly higher than those found in undisturbed lobsters after the same period in air; however, C_{O_2} values were the same due to an apparent rightward shift of the *in vivo* oxygen equilibrium curve (Fig. 2).

Following 14 h in air, undisturbed lobsters remained internally hypoxic with a further slight reduction in P_{O_2} levels to $1{\cdot}2 \pm 0{\cdot}1(6)$ and $0{\cdot}67 \pm 0{\cdot}1(6)$ kPa in post- and prebranchial haemolymph, respectively (Fig. 1). Oxygen content levels had, however, recovered back towards their resubmerged values. Haemocyanin-bound

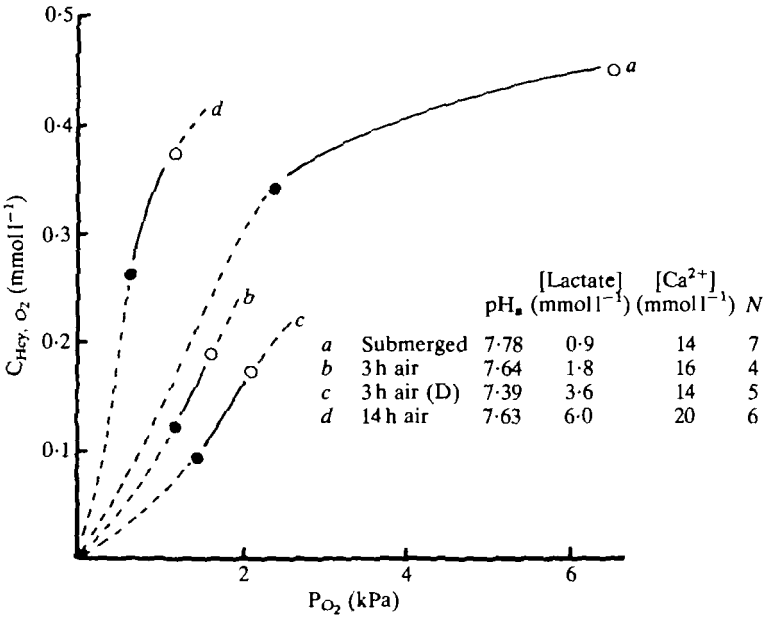

Fig. 2. The *in vivo* relationship between oxygen partial pressure (P_{O_2}) and the amount of oxygen bound to haemocyanin (C_{Hcy,O_2}) in post- (○) and prebranchial (●) haemolymph of undisturbed lobsters, submerged in sea water (*a*) and after 3 (*b*) and 14 h (*d*) of aerial exposure and of lobsters disturbed after 3 h in air (*c*). The broken and unbroken lines outline speculative *in vivo* oxygen equilibrium curves drawn through the mean values. Mean pHa, lactate and calcium values, and number of observations (N) for each curve are included on the figure. D, disturbed lobsters.

Table 1. *Haemolymph oxygen transport before, during and after 14 h of aerial exposure*

	C_{a-v,O_2} (mmol l^{-1})	O_2 delivered by oxyhaemocyanin (mmol l^{-1})	O_2 delivered from solution (mmol l^{-1})	No. of animals
Submerged	0·18 ± 0·03	0·12 ± 0·03	0·06 ± 0·01	7
3 h in air	0·07 ± 0·01	0·066 ± 0·01	0·004 ± 0·001	4
3 h in air (disturbed)	0·10 ± 0·02	0·093 ± 0·016	0·007 ± 0·002	5
14 h in air	0·122 ± 0·02	0·15 ± 0·02	0·007 ± 0·002	6
Resubmerged 0·5 h	0·08 ± 0·03	0·04 ± 0·01	0·04 ± 0·02	4
Resubmerged 3 h	0·12 ± 0·02	0·07 ± 0·02	0·05 ± 0·01	5

Derived variables include total oxygen delivered to the tissues (C_{a-v,O_2}) by haemocyanin or as dissolved oxygen.

oxygen levels in the postbranchial haemolymph increased significantly ($P < 0.01$) between 3 h and 14 h of aerial exposure from $0.19 \pm 0.06(4)$ to $0.37 \pm 0.01(6)$ mmol l^{-1}owing to an increase in haemocyanin oxygen-affinity, as shown by the apparent leftward shift of the *in vivo* oxygen equilibrium curve (Fig. 2). Levels of dissolved oxygen remained low and only contributed 6% of total oxygen supplied to the tissues. Oxygen delivery, therefore, was mainly enhanced by an increase in haemocyanin-bound oxygen levels (C_{Hcy,O_2}). C_{a-v,O_2} increased to $0.122 \pm 0.02(6)$ mmol l^{-1}, as shown in Table 1.

On resubmergence, Pa_{O_2} and Pv_{O_2} levels increased immediately to reach values of $5.2 \pm 1.5(4)$ and $2 \pm 0.1(4)$ kPa, respectively, after only 30 min. These values were similar to those obtained from settled submerged lobsters before aerial exposure (see Fig. 1). Ca_{O_2} and Cv_{O_2} levels increased to $0.57 \pm 0.02(4)$ and $0.50 \pm 0.01(4)$ mmol l^{-1}, respectively, after 30 min resubmergence (Fig. 1). Between 30 min and 3 h resubmergence there was little change in the mean values for P_{O_2} and C_{O_2} in both pre- and postbranchial haemolymph (Fig. 1). Following 3 h resubmergence, C_{a-v,O_2} was $0.12 \pm 0.02(5)$ mmol l^{-1}, which was not significantly different from that measured in settled submerged lobsters.

Haemolymph acid–base status

The effects of 14 h of aerial exposure and subsequent resubmersion on haemolymph pH, total CO_2 (C_{CO_2}), CO_2 partial pressure (P_{CO_2}) and bicarbonate concentration ($[HCO_3^-]$) are illustrated in Fig. 3. Aerial exposure for 3 h without disturbance was accompanied by a significant increase in Pa_{CO_2} and Pv_{CO_2} from $0.44 \pm 0.03(7)$ and $0.47 \pm 0.03(7)$ kPa to $0.7 \pm 0.08(6)$ and $0.73 \pm 0.05(6)$ kPa, respectively, and a significant increase in $[HCO_3^-]$ from $9.3 \pm 0.6(7)$ to $10.7 \pm 0.8(6)$ mequiv l^{-1} in postbranchial haemolymph and from $9.2 \pm 0.5(7)$ to $11.7 \pm 0.9(6)$ mequiv l^{-1} in prebranchial haemolymph. There was no significant change in mean lactate levels after 3 h in air (Fig. 4), but haemolymph pHa and pHv levels fell significantly ($P < 0.05$) from $7.78 \pm 0.02(7)$ and $7.76 \pm 0.01(7)$ to $7.64 \pm 0.05(6)$ and $7.65 \pm 0.05(6)$, respectively. In lobsters disturbed after 3 h of aerial exposure, the accumulation of CO_2 was greater, with mean Pa_{CO_2} reaching a value of $1.3 \pm 0.12(5)$ kPa which was 2.9 times the submerged value. This was accompanied by a fourfold increase in mean lactate concentration from a submerged value of $0.9 \pm 0.1(7)$ to $3.6 \pm 0.4(5)$ mmol l^{-1} (Fig. 4). This combined respiratory and metabolic acidosis resulted in a substantial drop in haemolymph pH of 0.39 units below the level in submerged lobsters. $[HCO_3^-]$ levels remained unchanged after 3 h in air.

After 14 h of aerial exposure, undisturbed lobsters experienced an increase in haemolymph P_{CO_2} and lactate concentrations (Figs 3 and 4). Between 3 and 14 h of aerial exposure, Pa_{CO_2} and Pv_{CO_2} levels increased 1.8 times to give mean values of $1.2 \pm 0.3(6)$ and $1.1 \pm 0.24(6)$ kPa, respectively. Lactate concentrations increased from $1.8 \pm 0.2(6)$ to $6.2 \pm 1.3(6)$ mmol l^{-1} over the same period. After 14 h in air, $[HCO_3^-]$ levels in post- and prebranchial haemolymph were $15.8 \pm 0.5(6)$ an

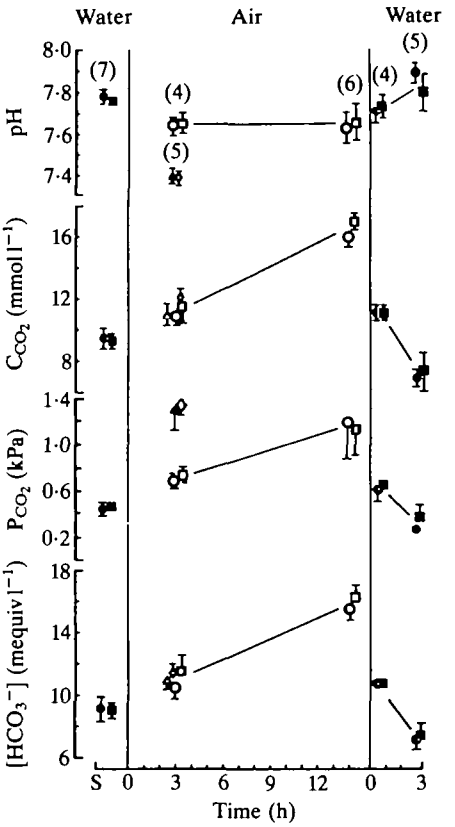

Fig. 3. Mean values (±S.E.M.) of acid–base variables in postbranchial (●/○) and prebranchial (■/□) haemolymph of lobsters during submergence (S), 14 h of aerial exposure (open symbols) and subsequent resubmersion. Values include: pH, total CO_2 (C_{CO_2}), CO_2 partial pressure (P_{CO_2}) and bicarbonate concentration ([HCO_3^-]). Values also include those obtained from lobsters subjected to a period of disturbance after 3 h in air (△ = postbranchial, ◇ = prebranchial). Number of observations are shown in parenthesis alongside appropriate mean pH values.

16·6 ± 0·6(6) mequiv l^{-1}, respectively, which marked an increase in concentration of 50 % from pre-exposure values.

The changes in related acid–base variables are illustrated by means of a pH–bicarbonate diagram (Davenport, 1974) in Fig. 5, which relates pH to [HCO_3^-] at various P_{CO_2} levels. The broken diagonal line represents the non-bicarbonate buffer line, which was determined *in vitro* and had a value of 15 mequiv l^{-1} pH unit^{-1} at 15 °C. Examination of Fig. 5 indicates that the initial acidosis in settled lobsters after 3 h in air was entirely respiratory in origin, due to the accumulation of CO_2, as the change in pH followed the gradient of the non-bicarbonate buffer line. This agrees with the lack of any significant increase in lactate levels after 3 h in air (Fig. 4). The pronounced acidosis in disturbed animals after 3 h in air appeared to be predominantly respiratory in nature, but the

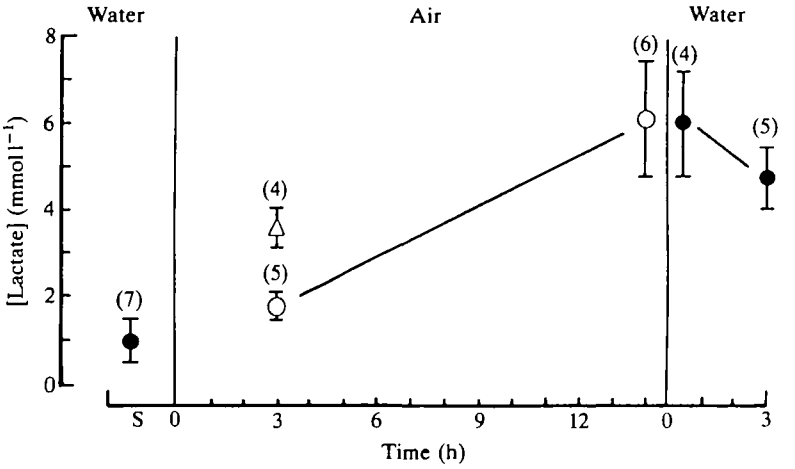

Fig. 4. Mean (±S.E.M.) lactate concentrations in the haemolymph of lobsters, submerged (S) in sea water, exposed in air for 14 h (open symbols) and following resubmergence for 3 h. Values at 3 h in air include those obtained from five lobsters after disturbance (△). Number of observations are included in parenthesis above their corresponding mean.

deviation from the buffer line indicated a metabolic component due to the accumulation of H^+ from lactic acid. After 14 h in air, haemolymph pH levels remained unchanged despite the accumulation of CO_2 and lactate (Figs 3 and 4). This was due to the marked elevation of $[HCO_3^-]$ in the haemolymph (Fig. 3) which served to compensate for the potential acidosis (Fig. 5).

When sea water was replaced in the experimental tanks after 14 h of aerial exposure, lobsters experienced a recovery in P_{CO_2} and $[HCO_3^-]$ back towards settled submerged values. After 30 min resubmergence, mean Pa_{CO_2} and Pv_{CO_2} levels had fallen to 0·61 ± 0·09(4) and 0·6 ± 0·1(4) kPa, respectively, and were not significantly different from submerged values, whereas mean $[HCO_3^-]$ remained significantly elevated at 10·9 ± 0·3(4) mequiv l^{-1} in the postbranchial haemolymph and 10·9 ± 0·4(4) mequiv l^{-1} in the prebranchial haemolymph (Fig. 3). As a result, a marked recovery alkalosis had developed after 3 h resubmersion (Fig. 3), as mean pHa was 7·89 ± 0·04(5), despite the fact that haemolymph lactate levels remained elevated at 4·7 ± 0·7(5) mmol l^{-1} (Fig. 4).

Haemolymph ion levels

Mean calcium, magnesium, copper, potassium, sodium and chloride concentrations were measured in the haemolymph of lobsters subjected to 14 h of aerial exposure and subsequent resubmersion for 3 h (Fig. 6). It can be seen that there was little change in the levels of $[Mg^{2+}]$, $[Cu^{2+}]$, $[K^+]$ and $[Na^+]$ in the haemolymph during aerial exposure, but there were noticeable and significant changes in $[Ca^{2+}]$ and $[Cl^-]$. Following 14 h in air, Ca^{2+} concentrations had increased gradually to a maximum level of 20·3 ± 0·6(6) mmol l^{-1}, which was 30 %

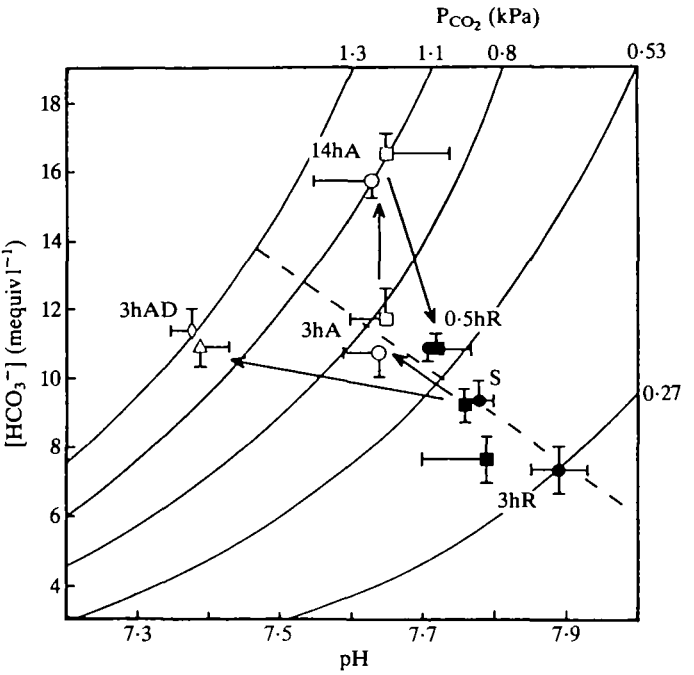

Fig. 5. A pH–bicarbonate diagram illustrating the changes in acid–base status in postbranchial (●/○) and prebranchial (■/□) haemolymph of lobsters; submerged (S), exposed in air for 3 and 14 h (3hA and 14hA) and resubmerged for 0·5 and 3 h (0·5hR and 3hR). The ◇/△ symbols represent lobsters disturbed after 3 h in air (3hA D). The broken diagonal line represents the non-bicarbonate buffer line. Closed symbols denote submerged animals and open symbols animals exposed in air. Number of observations are as given in Fig. 3.

above submerged values. On resubmergence, $[Ca^{2+}]$ was still significantly elevated from submerged values after 3 h, being $16{\cdot}8 \pm 0{\cdot}7(5)$ mmol l^{-1} ($P < 0{\cdot}01$). Haemolymph $[Cl^-]$ also increased during aerial exposure, but the difference was smaller and only significant after 3 h in air ($P < 0{\cdot}05$) and when $[Cl^-]$ was $453 \pm 3(5)$ mmol l^{-1}. On resubmergence, $[Cl^-]$ increased slightly, but insignificantly before returning to submerged levels (Fig. 6).

In vitro *oxygen equilibrium curves*

The effect of a progressive increase in L-lactate concentration on the oxygen affinity of lobster haemocyanin was measured *in vitro*, and is illustrated as changes in $\%S_{O_2}$ with P_{O_2} (Fig. 7) and as changes in $\log P_{50}$ with pH (Fig. 8). The oxygen affinity increased (i.e. $\log P_{50}$ decreased) with increasing L-lactate over the range of concentrations from 2 to 8 mmol l^{-1}, mimicking the measured *in vivo* changes (Fig. 4). The lactate effect between these two concentrations had a $\Delta\log P_{50}/\Delta\log[\text{lactate}]$ value of $-0{\cdot}175$. Above 8 mmol l^{-1}, the lactate effect saturated as an increase to 16 mmol l^{-1} had very little further effect on oxygen affinity. The

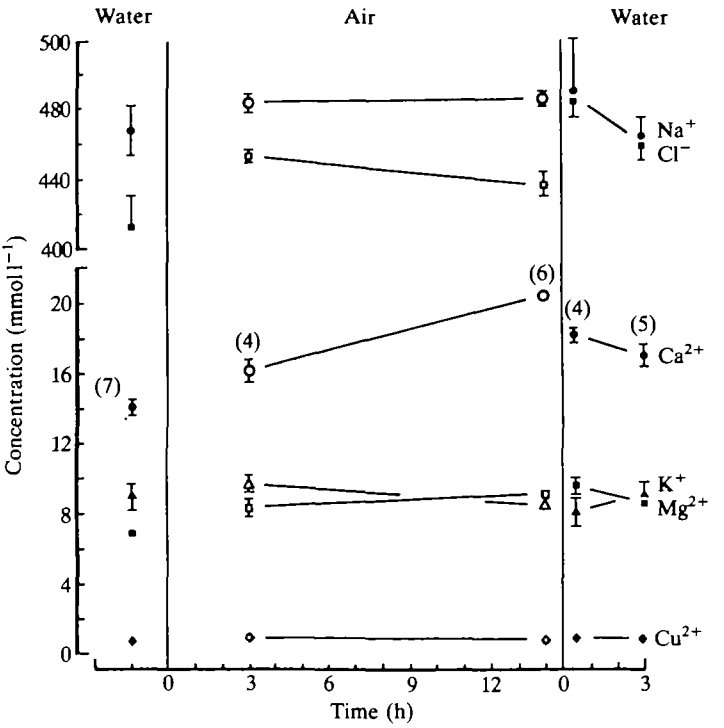

Fig. 6. Mean concentrations (±S.E.M.) of copper, magnesium, potassium, calcium, chloride and sodium ions in the haemolymph of lobsters during submergence, 14 h of aerial exposure (open symbols) and 3 h resubmergence. Number of observations are given above the mean values for Ca^{2+}.

gradient of the relationship between $\log P_{50}$ and pH did not differ significantly between whole haemolymph, dialysed haemolymph in the absence of L-lactate or haemolymph with added L-lactate as $\Delta\log P_{50}/\Delta pH$ ranged between −0·95 and −1·07. This infers that neither dialysing nor adding L-lactate influenced the Bohr effect, resulting from changes in pH. Whole haemolymph (undialysed) with a measured lactate level of 3 mmol l^{-1} had a higher oxygen affinity than dialysed haemolymph plus L-lactate at similar, or higher, concentrations of up to 16 mmol l^{-1}. The oxygen equilibrium curves presented in Fig. 7 illustrate this relationship. Lactate had no effect on the cooperativity of the oxygen equilibrium curves for lobster haemocyanin. The absence of effects of L-lactate on the Bohr factor and cooperativity of lobster haemocyanin was reported by Bouchet & Truchot (1985).

A separate *in vitro* experiment, in which the levels of calcium ions were increased from 10 to 20 mmol l^{-1}, revealed that this increase, which mimics the *in vivo* change, caused an increase in oxygen affinity. $\log P_{50}$ at a pH of 7·63 (the pH after 14 h in air) decreased from 1·34 to 1·28 ($\Delta\log P_{50}/\Delta\log[Ca^{2+}] = -0{\cdot}20$). In contrast to the lactate effect, calcium also increased the slope of the line relatin

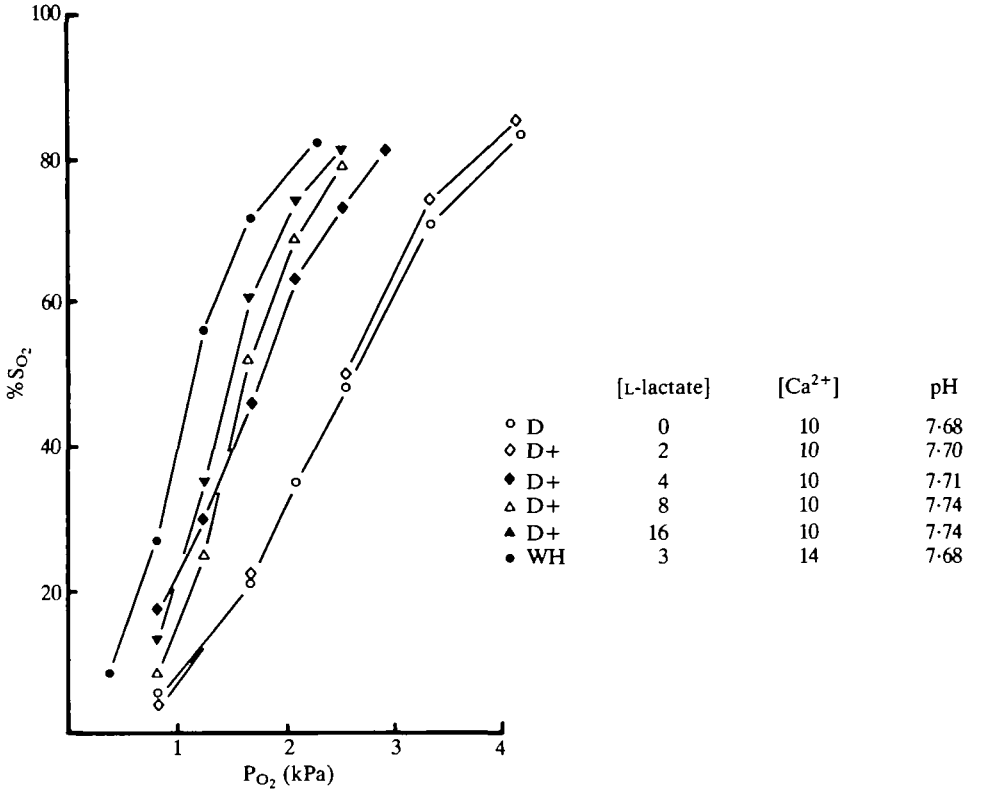

Fig. 7. *In vitro* oxygen equilibrium curves plotted as percentage saturation ($\%S_{O_2}$) against oxygen partial pressure (P_{O_2}) at 15°C for lobster haemocyanin, dialysed against a Ringer's solution containing 10 mmol l^{-1} [Ca^{2+}] but without L-lactate (D = dialysed) and Ringer containing levels of L-lactate varying between 2 and 16 mmol l^{-1}. An *in vitro* curve for whole haemolymph (WH) with an L-lactate concentration of 3 mmol l^{-1} and [Ca^{2+}] of 14 mmol l^{-1} is included for comparison. All concentrations are given in mmol l^{-1}.

$\log P_{50}$ to pH (Fig. 8) to give a $\Delta\log P_{50}/\Delta pH$ value of −1·25. This infers that changes in the concentration of calcium affected the pH-dependent Bohr shift in oxygen affinity of lobster haemocyanin.

Discussion

Lobsters, like other primarily aquatic crustaceans, experience chronic systemic hypoxia and hypercapnia when brought into air (Taylor, 1982). Their gills, normally supported by water, collapse in air (Taylor & Butler, 1978; Taylor & Wheatly, 1981; Cameron, 1981; deFur & McMahon, 1984*a*; Taylor & Greenaway, 1984). Clumping of the gill filaments leads to a reduction in the effective surface area for gas exchange, and could restrict adequate ventilation and perfusion of the gills (deFur & McMahon, 1984*a*). Lobster gills are filamentous (Phillips *et al.*

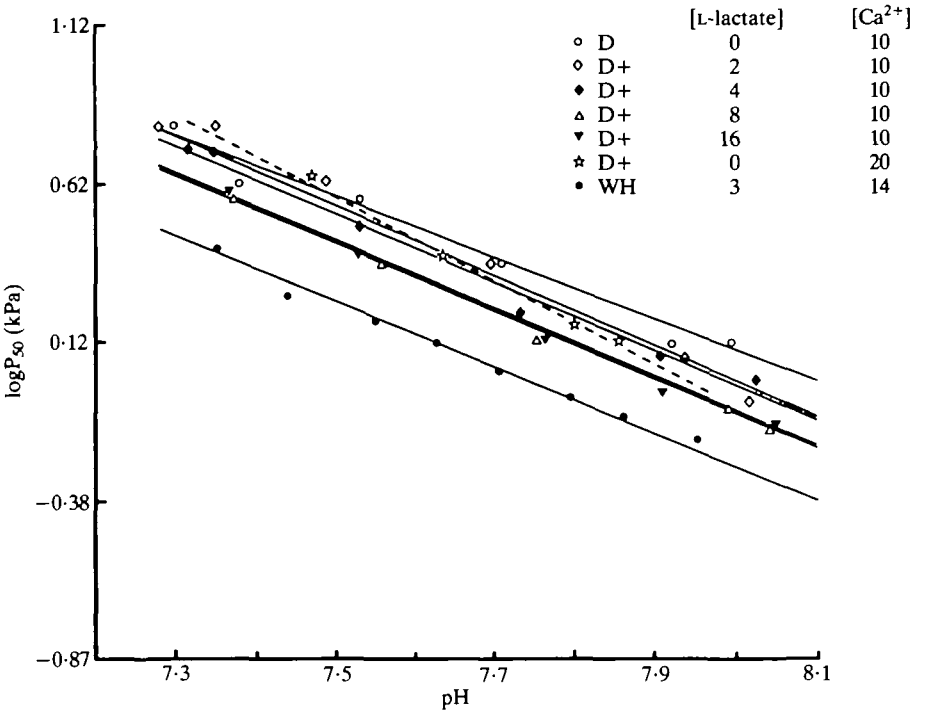

Fig. 8. The relationship between pH and $\log P_{50}$ at 15°C for lobster haemocyanin dialysed against Ringer's solutions containing different concentrations of L-lactate and Ca^{2+}. Concentrations are expressed in mmol l^{-1}. The dependence of $\log P_{50}$ on pH is also shown. Dialysed haemolymph (D) contained no L-lactate and Ca^{2+} at a concentration of 10 mmol l^{-1}. Different concentrations of L-lactate were added as indicated and for one series of equilibrium curves [Ca^{2+}] was increased to 20 mmol l^{-1} in the absence of L-lactate (broken line). Whole haemolymph (WH) contained 3 mmol l^{-1} L-lactate and 14 mmol l^{-1} [Ca^{2+}].

1980), and may be less resistant to collapse than the lamellate gills typical of *C. maenas* which are fairly well spaced and supported by their covering of chitin (Taylor & Butler, 1978). The gills of terrestrial crabs, such as *Cardisoma carnifex* and *Birgus latro*, are reduced in area with small lamellae that are stiffened at the margins and well spaced (Cameron, 1981; Innes & Taylor, 1986). The gills in these species are adapted for ion exchange with water held in the branchial chambers, respiratory gas exchange taking place over the well-perfused walls of the branchial chambers which act as lungs (Taylor & Innes, 1988; McMahon & Burggren, 1988).

After 3 h in air, lobsters were hypoxaemic and had developed a respiratory acidosis. Accumulation of CO_2 caused haemolymph pH to titrate along the non-bicarbonate buffer line (Davenport, 1974; Truchot, 1975*a*; Taylor & Wheatly, 1981). The non-bicarbonate buffer value obtained in this study was typical for marine crustaceans in intermoult (Truchot, 1976*a*; McMahon *et al.* 1978; McDonald *et al.* 1979). The decline of circulating P_{O_2} values to very low levels (< 2 kPa) substantially decreased the amount of oxygen delivered to the tissues

either as dissolved oxygen or as oxygen combined with haemocyanin. Disturbed lobsters experienced greater reductions in haemolymph oxygen content after 3 h in air, as the marked acidosis (both respiratory and metabolic in origin) caused a decrease in oxygen affinity *via* the Bohr shift. The magnitude of the *in vitro* Bohr effect ($\Delta\log P_{50}/\Delta pH$) for whole haemolymph was $-1{\cdot}0$, which agrees with the *in vivo* value obtained by Butler *et al.* (1978) for lobster haemocyanin.

Throughout the remaining period in air, from 3 to 14 h, CO_2 continued to accumulate while anaerobic metabolism began to contribute lactic acid to the increase in H^+ concentration. Initially, some CO_2 production resulted from aerobic respiration, but after prolonged aerial exposure CO_2 may have been generated by the buffering action of bicarbonate ions on lactic acid (Taylor, 1982). After 14 h in air, lobsters displayed P_{CO_2} levels similar to those found in air-breathing land crabs such as *C. carnifex* (Wood & Randall, 1981; Burggren & McMahon, 1981) and *Gecarcinus lateralis* (Taylor & Davies, 1981). These elevated P_{CO_2} and lactate values corresponded with the values recorded in the crayfish after 14 h of aerial exposure at 15°C (Taylor & Wheatly, 1981; Taylor, 1982). Despite this incipient respiratory and metabolic acidosis, mean haemolymph pH remained unchanged at the 3 h value after 14 h in air, owing to the progressive elevation of bicarbonate ions which increased the buffering capacity of the haemolymph by a process of metabolic compensation (Davenport, 1974). This elevation of buffer base must come from an internal source, as the branchial route for bicarbonate ion exchange was unavailable. Accumulation of bicarbonate by branchial ion exchange predominates in primarily aquatic decapods (Truchot, 1983; Cameron, 1985), though metabolic compensation by the exchange of HCO_3^- between internal compartments is utilized where the gills loose contact with water e.g. in *C. maenas* (Truchot, 1975*a*) and *A. pallipes* (Taylor & Wheatly, 1981) during long-term aerial exposure. The increases in $[Ca^{2+}]$ and $[Mg^{2+}]$ observed during aerial exposure infer that buffering bicarbonates were released into the haemolymph from the mineralized exoskeleton, as described in the crayfish during prolonged emersion (Taylor & Wheatly, 1981; Morris *et al.* 1986*a*). Similar compensatory mechanisms are employed during hypercapnic acidosis in both aquatic and terrestrial decapodan crustaceans (McMahon *et al.* 1978; deFur *et al.* 1980; Henry *et al.* 1981; Cameron, 1985; Cameron & Wood, 1985).

After 14 h of aerial exposure, oxygen content values in the haemolymph gradually returned towards submerged levels, despite a persistent systemic hypoxia. This was attributable to a marked increase in the relative affinity of haemocyanin for oxygen, at constant pH. Oxygen delivery to the tissues was enhanced, as the high oxygen affinity of the respiratory pigment allowed relatively large changes in C_{Hcy,O_2} (e.g. $0{\cdot}11\,mmol\,l^{-1}$) for small changes in P_{O_2} (e.g. $0{\cdot}5\,kPa$). During aerial exposure haemocyanin played the dominant role in oxygen transport, delivering 94 % of the oxygen transported by the haemolymph to the tissues (Table 1). The *in vitro* measurements suggest that the increase in haemocyanin oxygen-affinity at constant pH was in part due to the combined allosteric effects of lactate and calcium ions on the haemocyanin molecule. Both

ions gradually accumulated in the haemolymph during the time in air. The effect of Ca^{2+} on haemocyanin oxygen-affinity is well documented (e.g. Pickett *et al.* 1966; Larimer & Riggs, 1964; Hwang & Fung, 1970; Miller & Van Holde, 1974; Truchot, 1975*b*; Mason *et al.* 1983; Morris *et al.* 1986*a*). The effect of an increase in calcium concentration on oxygen affinity of *Homarus* haemocyanin measured *in vitro* ($\Delta\log P_{50}/\Delta\log[Ca^{2+}] = -0{\cdot}20$ at pH 7·63) is similar to the value of −0·28 calculated for *C. maenas* by Truchot (1975*b*), but lower than the value of between −0·70 and −0·80 described for *Callinectes sapidus* (Mason *et al.* 1983) and *A. pallipes* (Morris *et al.* 1986*b*). The increase in oxygen affinity of crustacean haemocyanin, due to accumulation of lactate ions, is also well documented (Truchot, 1980; Booth *et al.* 1982; Graham *et al.* 1983; Mangum, 1983; Morris *et al.* 1986*a*). Recently, Bouchet & Truchot (1985) described the lactate effect in *H. vulgaris* (*gammarus*), induced by exposure to hypoxic water, and obtained a value of $\Delta\log P_{50}/\Delta\log[\text{lactate}]$ of −0·158. This is similar to the *in vitro* value of −0·175 obtained in the present study between L-lactate concentrations of 2 and 8 mmol l^{-1}, which approximate the *in vivo* changes encountered during aerial exposure (Fig. 4).

The potentiating effects of calcium and L-lactate ions on haemocyanin oxygen-affinity *in vitro* were found to be interdependent in crayfish haemolymph (Morris *et al.* 1986*b*). This possibility was not tested in the present study. If the two ions are assumed to act independently on *Homarus* haemocyanin, and the increase in oxygen affinity resulting from an increase in $[Ca^{2+}]$ from 10 to 20 mmol l^{-1} in dialysed haemocyanin (a change in P_{50} of 0·37 kPa at pH 7·63) is added to the effect of an *in vitro* increase in L-lactate from 2 to 6 mmol l^{-1}, obtained by interpolation on Fig. 8 (a change in P_{50} of 0·31 kPa at pH 7·63), a combined reduction in P_{50} of 0·68 kPa is obtained. This compares with an apparent *in vivo* change of about 0·93 kPa estimated from Fig. 2 after 14 h in air when $[Ca^{2+}]$ and [L-lactate] have changed over similar ranges. The shortfall between the *in vivo* and *in vitro* enhancement of P_{50} may be attributed to the effects of other metabolites, such as urate, accumulating in the haemolymph during prolonged aerial exposure (Bridges & Morris, 1986; Lallier *et al.* 1987). A similar role for a dialysable factor, other than lactate, was described for lobster haemocyanin by Bouchet & Truchot (1985).

Water loss in the lobster was minimal during 14 h of aerial exposure as haemolymph ion concentrations, with the exception of $[Ca^{2+}]$ and $[Cl^-]$, remained unchanged. The increase in $[Ca^{2+}]$ was in stoichiometric proportion to the increase in $[HCO_3^-]$, and probably arises as a result of the mobilization of $CaCO_3$ from internal sources such as the calcified exoskeleton (Cameron, 1985). The increase in $[Cl^-]$ suggests that this ion was withdrawn from the tissues during aerial exposure, as reported in the crayfish under similar conditions (Taylor *et al.* 1987). This may represent an exchange of chloride for bicarbonate ions resulting in the regulation of $[HCO_3^-]$ levels in the haemolymph or passage of buffer base into the intracellular compartment.

On resubmergence after 14 h in air, oxygen levels in the haemolymph were

replenished within 30 min. Pa_{O_2} levels were high enough to allow 100 % saturation of the haemolymph with oxygen. At the same time, a venous reserve was re-established (i.e. Cv_{O_2} increased) which accounted for 86 % of the total oxygen combined to haemocyanin. Oxygen delivery to the tissues actually decreased after 30 min resubmergence due to the increase in Cv_{O_2} levels, which increased the contribution of dissolved oxygen to overall oxygen transport. Elimination of accumulated CO_2 was rapid, presumably due to the high initial concentration gradient across the gills, the relatively high capacity coefficient for CO_2 in water (Dejours, 1981) and the occurrence of hyperventilation (N. M. Whiteley, A. H. Al-Wassia & E. W. Taylor, in preparation). The loss of bicarbonate and calcium ions from the haemolymph was slower, resulting in the short-term retention of buffer base and development of an internal alkalosis. Lactate levels also remained elevated on resubmergence, as crustaceans cannot excrete or rapidly mobilize this metabolic end product (McDonald *et al.* 1979; Bridges & Brand, 1980; Booth *et al.* 1982; Ellington, 1983). Consequently, haemocyanin oxygen-affinity will have remained high during initial resubmersion, which may have increased the effectiveness of oxygen removal into the haemolymph at the gills. Haemolymph $[Cl^-]$ levels increased on initial resubmersion, indicating that $[HCO_3^-]$ may be excreted across the gills in exchange for $[Cl^-]$ from the environment (Cameron, 1979, 1985). A similar increase in $[Cl^-]$ following resubmersion was recorded in the crayfish (M. G. Wheatly & E. W. Taylor, unpublished observations). It is also possible that HCO_3^- was enzymatically dehydrated to CO_2 for diffusion across the gill surface by carbonic anhydrase located in the gill epithelium (Burnett *et al.* 1985). After the initial rapid drop in haemolymph P_{CO_2} levels, further acid–base adjustments between 0·5 and 3 h of resubmersion were due to a progressive reduction in CO_2 levels and followed the non-bicarbonate buffer line.

Despite the impairment of respiratory gas exchange during aerial exposure, resulting in a persistent systemic hypoxia and hypercapnia and progressive accumulation of lactic acid, the gill-breathing lobster manages to survive relatively long periods out of water. This is achieved by partial compensation for an incipient respiratory and metabolic acidosis with an increase in haemolymph bicarbonate levels. This mitigates the development of a Bohr shift on the haemocyanin. The modulating effects of calcium and lactate ions, and possibly other metabolites, on haemocyanin oxygen-affinity at constant pH then combine to improve oxygen transport to the tissues. Lobsters subjected to a period of disturbance during aerial exposure experience a rapidly developed, and uncompensated, respiratory and metabolic acidosis which proves fatal within 8 h (E. W. Taylor, unpublished observations). Important generalizations about the proper treatment of live lobsters in air during commercial handling and transport have arisen from these observations (Whiteley & Taylor, 1989).

The authors wish to thank Professor M. S. Laverack and his staff for their hospitality and help at the Gatty Marine Laboratory during the *in vivo* study in 1981. The *in vitro* work was performed at the Institut für Zoologie IV, University

of Düsseldorf in 1983 and we are grateful to Professor M. K. Grieshaber and Dr C. R. Bridges for their help and advice during this study, and to Ms A. Lundkowski for her technical expertise. We would also like to thank Mrs J. Hill for processing the manuscript. This work was funded by a Royal Society European Exchange award to EWT. NMW received a SERC research studentship.

References

ANONYMOUS (1977). The lobster: its biology and fishery. *Lab. leafl. MAFF Fish. Lab., Burnham-on-Crouch, U.K.*

BATTERTON, C. V. & CAMERON, J. N. (1978). Characteristics of resting ventilation and response to hypoxia, hypercapnia and emersion in the blue crab *Callinectes sapidus* (Rathburn). *J. exp. Zool.* **203**, 403–418.

BOOTH, C. E., MCMAHON, B. R. & PINDER, A. W. (1982). Oxygen uptake and the potentiating effects of increased haemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *J. comp. Physiol.* **148**B, 111–121.

BOUCHET, T. Y. & TRUCHOT, J. P. (1985). Effects of hypoxia and L-lactate on the haemocyanin oxygen affinity of the lobster, *Homarus vulgaris*. *Comp. Biochem. Physiol.* **88**A(1), 69–73.

BRIDGES, C. R., BICUDO, J. E. P. W. & LYKKEBOE, G. (1979). Oxygen content measurement in blood containing haemocyanin. *Comp. Biochem. Physiol.* **62**A, 457–462.

BRIDGES, C. R. & BRAND, A. R. (1980). The effect of hypoxia on oxygen consumption and blood lactate levels of some marine crustacea. *Comp. Biochem. Physiol.* **65**A, 399–409.

BRIDGES, C. R. & MORRIS, S. (1986). Modulation of haemocyanin oxygen affinity by L-lactate. A role for other cofactors. In *Invertebrate Oxygen Carriers* (ed. B. Linzen), pp. 341–352. Berlin: Springer-Verlag.

BURGGREN, W. W. & MCMAHON, B. R. (1981). Haemolymph oxygen transport, acid–base status, and hydromineral regulation during dehydration in three terrestrial crabs, *Cardisoma*, *Birgus* and *Coenobita*. *J. exp. Zool.* **218**, 53–64.

BURNETT, L. E., DUNN, T. N. & INFANTINO, R. L. (1985). The function of carbonic anhydrase in crustacean gills. In *Transport Processes, Iono- and Osmoregulation* (ed. R. Gilles & M. Gilles-Baillien), pp. 159–168. Berlin: Springer-Verlag.

BUTLER, P. J., TAYLOR, E. W. & MCMAHON, B. R. (1978). Respiratory and circulatory changes in the lobster (*Homarus vulgaris*) during long-term exposure to moderate hypoxia. *J. exp. Biol.* **73**, 131–146.

CAMERON, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**(4), 632–634.

CAMERON, J. N. (1979). Excretion of CO_2 in water-breathing animals. *Mar. Biol. Letts* **1**, 3–13.

CAMERON, J. N. (1981). Brief introduction to the land crabs of the Palau Islands: stages in the transition to air breathing. *J. exp. Zool.* **218**, 1–5.

CAMERON, J. N. (1985). Compensation of hypercapnic acidosis in the aquatic crab, *Callinectes sapidus*: the predominance of external sea water over carapace carbonate as the proton sink. *J. exp. Biol.* **114**, 196–206.

CAMERON, J. N. & WOOD, C. M. (1985). Apparent H^+ excretion and CO_2 dynamics accompanying carapace mineralization in the blue crab (*Callinectes sapidus*) following moulting. *J. exp. Biol.* **114**, 181–196.

COBB, J. S. & WANG, D. (1985). Fisheries biology of lobsters and crayfishes. In *Biology of Crustacea, Economic Aspects: Fisheries and Culture*, vol. 10 (ed. A. J. Provenzano, Jr), pp. 168–247. New York: Academic Press.

DAVENPORT, H. W. (1974). *The ABC of Acid–Base Chemistry*. Chicago: University of Chicago Press.

DEFUR, P. L. & MCMAHON, B. R. (1984*a*). Physiological compensation to short-term air exposure in red rock crabs, *Cancer productus* Randall, from littoral and sublittoral habitats. I. Oxygen uptake and transport. *Physiol. Zool.* **57**, 137–150.

DEFUR, P. L. & MCMAHON, B. R. (1984*b*). Physiological compensation to short-term a

exposure in red rock crabs, *Cancer productus* Randall, from littoral and sublittoral habitats. II. Acid–base balance. *Physiol. Zool.* **57**, 151–160.

DEFUR, P. L., WILKES, P. R. H. & MCMAHON, B. R. (1980). Non-equilibrium acid–base status in *Cancer productus*: role of exoskeletal carbonate buffers. *Respir. Physiol.* **42**, 247–261.

DEJOURS, P. (1981). *Principles of Comparative Physiology* (2nd edn). Amsterdam: North Holland Publishing Co.

ELLINGTON, W. R. (1983). The recovery from anaerobic metabolism in invertebrates. *J. exp. Zool.* **228**, 431–444.

GRAHAM, R. A., MANGUM, C. P., TERWILLIGER, R. C. & TERWILLIGER, N. B. (1983). The effect of organic acids on oxygen binding of haemocyanin from the crab *Cancer magister*. *Comp. Biochem. Physiol.* **74**A, 45–50.

HARVEY, H. W. (1955). *The Chemistry and Fertility of Sea Waters*. Cambridge: Cambridge University Press.

HENRY, R. P., KORMANIK, G. A., SMATRESK, N. J. & CAMERON, J. N. (1981). The role of $CaCO_3$ dissolution as a source of HCO_3^- for buffering hypercapnic acidosis in aquatic terrestrial decapod crustaceans. *J. exp. Biol.* **94**, 269–274.

HWANG, J. C. & FUNG, C. P. (1970). Effect of calcium ions on oxygen equilibrium of haemocyanin of Asiatic horseshoe crab *Tachypleus tridentatus*. *Comp. Biochem. Physiol.* **37**, 573–579.

INNES, A. J. & TAYLOR, E. W. (1986). The evolution of air-breathing in crustaceans: a functional analysis of branchial, cutaneous and pulmonary gas exchange. *Comp. Biochem. Physiol.* **85**A, 621–637.

LALLIER, F., BOITEL, F. & TRUCHOT, J. P. (1987). The effect of ambient oxygen and temperature on haemolymph L-lactate and urate concentrations in the shore crab *Carcinus maenas*. *Comp. Biochem. Physiol.* **86**A(2), 255–260.

LARIMER, J. L. & RIGGS, A. F. (1964). Properties of hemocyanins. I. The effect of calcium ions on the oxygen equilibrium of crayfish hemocyanin. *Comp. Biochem. Physiol.* **13**, 35–46.

MCDONALD, D. G., MCMAHON, B. R. & WOOD, C. M. (1979). An analysis of acid–base disturbances in the haemolymph following strenuous activity in the Dungeness crab, *Cancer magister*. *J. exp. Biol.* **79**, 47–58.

MCMAHON, B. R. & BURGGREN, W. W. (1988). Respiration. In *Biology of the Land Crabs* (ed. W. W. Burggren & B. R. McMahon), pp. 249–297. Cambridge: Cambridge University Press.

MCMAHON, B. R., BUTLER, P. J. & TAYLOR, E. W. (1978). Acid–base changes during recovery from disturbance and during long-term hypoxic exposure in the lobster, *Homarus vulgaris*. *J. exp. Zool.* **205**, 361–370.

MANGUM, C. P. (1983). Oxygen transport in the blood. In *The Biology of Crustacea*, vol. 5 (ed. L. H. Mantel), pp. 373–429. New York: Academic Press.

MASON, R. P., MANGUM, C. P. & GODETTE, G. (1983). The influence of inorganic ions and acclimation salinity on hemocyanin oxygen affinity in the blue crab *Callinectes sapidus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **164**, 104–163.

MILLER, K. & VAN HOLDE, K. E. (1974). Oxygen binding by *Callianassa californiensis* hemocyanin. *Biochemistry, N.Y.* **13**(8), 1668–1674.

MORRIS, S. (1988). Effects of freezing on the function and association state of crustacean haemocyanins. *J. exp. Biol.* **138**, 535–539.

MORRIS, S., TYLER-JONES, R., BRIDGES, C. R. & TAYLOR, E. W. (1986*a*). The regulation of haemocyanin oxygen affinity during emersion of the crayfish *Austropotamobius pallipes*. II. An investigation of *in vivo* changes in oxygen affinity. *J. exp. Biol.* **121**, 327–337.

MORRIS, S., TYLER-JONES, R. & TAYLOR, E. W. (1986*b*). The regulation of haemocyanin oxygen affinity during emersion of the crayfish *Austropotamobius pallipes*. I. An *in vitro* investigation of the interactive effects of calcium and L-lactate on oxygen affinity. *J. exp. Biol.* **121**, 315–326.

PHILLIPS, B. F., COBB, J. S. & GEORGE, R. W. (1980). General biology. In *The Biology and Management of Lobsters*, vol. 1 (ed. J. S. Cobb & B. F. Phillips), pp. 1–82. New York: Academic Press.

PICKETT, S. M., RIGGS, A. F. & LARIMER, F. L. (1966). Lobster haemocyanin: properties of the minimum functional subunit and of aggregates. *Science* **151**, 1005–1007.

SICK, H. & GERSONDE, K. (1969). Method for continuous registration of O_2-binding curves of hemoproteins by means of a diffusion chamber. *Analyt. Biochem.* **32**, 362–376.

TAYLOR, A. C. & DAVIES, P. S. (1981). Respiration in the land crab, *Gecarcinus lateralis*. *J. exp. Biol.* **93**, 315–323.

TAYLOR, E. W. (1982). Control and co-ordination of ventilation and circulation in crustaceans: responses to hypoxia and exercise. *J. exp. Biol.* **100**, 289–319.

TAYLOR, E. W. & BUTLER, P. J. (1978). Aquatic and aerial respiration in the shore crab *Carcinus maenas* (L.) acclimated to 15 °C. *J. comp. Physiol.* **127**, 315–323.

TAYLOR, E. W. & INNES, A. J. (1988). A functional analysis of the shift from gill to lung breathing during the evolution of land crabs (Crustacea, Decapoda). *Biol. J. Linn. Soc.* **34**, 229–247.

TAYLOR, E. W., TYLER-JONES, R. & WHEATLY, M. G. (1987). The effects of aerial exposure on the distribution of body water and ions in the freshwater crayfish, *Austropotamobius pallipes* (Lereboullet). *J. exp. Biol.* **128**, 307–332.

TAYLOR, E. W. & WHEATLY, M. G. (1980). Ventilation, heart rate and respiratory gas exchange in the crayfish *Austropotamobius pallipes* (Lereboullet) submerged in normoxic water and following 3 h exposure in air at 15 °C. *J. comp. Physiol.* **138**, 67–78.

TAYLOR, E. W. & WHEATLY, M. G. (1981). The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. *J. exp. Biol.* **29**, 109–124.

TAYLOR, H. H. & GREENAWAY, P. (1984). The role of the gills and branchiostegites in gas exchange in a bimodally breathing crab, *Holthuisana transversa*: evidence for a facultative change in the distribution of the respiratory circulation. *J. exp. Biol.* **111**, 103–121.

TRUCHOT, J. P. (1975*a*). Blood acid–base changes during experimental emersion and reimmersion of the intertidal crab, *Carcinus maenas* (L.). *Respir. Physiol.* **23**, 351–360.

TRUCHOT, J. P. (1975*b*). Factors controlling *in vitro* and *in vivo* oxygen affinity of the haemocyanin in the crab, *Carcinus maenas* (L.). *Respir. Physiol.* **24**, 173–189.

TRUCHOT, J. P. (1976*a*). Carbon dioxide combining properties of the blood of the shore crab *Carcinus maenas* (L.): carbon dioxide dissociation curves and Haldane effect. *J. comp. Physiol.* **112B**, 283–293.

TRUCHOT, J. P. (1976*b*). Carbon dioxide combining properties of the blood of the shore crab, *Carcinus maenas* (L.): carbon dioxide solubility coefficient and carbonic acid dissociation constants. *J. exp. Biol.* **64**, 45–57.

TRUCHOT, J. P. (1980). Lactate increases the oxygen affinity of crab haemocyanin. *J. exp. Zool.* **214**, 205–208.

TRUCHOT, J. P. (1983). Regulation of acid–base balance. In *The Biology of Crustacea*, vol. 5 (ed. L. H. Mantel), pp. 431–457. New York: Academic Press.

TUCKER, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. appl. Physiol.* **23**(3), 410–414.

TYLER-JONES, R. & TAYLOR, E. W. (1988). Analysis of haemolymph and muscle acid–base status during aerial exposure in the crayfish, *Austropotamobius pallipes*. *J. exp. Biol.* **134**, 409–422.

WHEATLY, M. G. & TAYLOR, E. W. (1979). Oxygen levels, acid–base status and heart rate during emersion of the shore crab *Carcinus maenas* (L.) into air. *J. comp. Physiol.* **132**, 305–311.

WHITELEY, N. M. (1988). The physiological basis of the ability of the lobster, *Homarus gammarus* (L.), to survive out of water and its commercial exploitation. PhD thesis. University of Birmingham, 303pp.

WHITELEY, N. M. & TAYLOR, E. W. (1989). *Handling, Transport and Storage of Live Crabs and Lobsters*. An open learning module for the Sea Fish Industry Authority. 104pp.

WOOD, C. M. & RANDALL, D. J. (1981). Oxygen and carbon dioxide exchange during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 7–22.