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THE EFFECTS OF AERIAL EXPOSURE ON THE DISTRIBUTION OF BODY WATER AND IONS IN THE FRESHWATER CRAYFISH *AUSTROPOTAMOBIUS PALLIPES* (LEREBOULLET)

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SUMMARY

1. Submerged crayfish had a total body water content of 76% body mass. Haemolymph volume was around 30% and was dependent on mass, the relationship being described by the regression equation y (volume) = 3·98+0·177x (mass). The inulin space was greater than the copper space in abdominal muscle, suggesting the existence of an interstitial component of the extracellular fluid into which haemocyanin does not penetrate.

2. Crayfish exposed to water-saturated air (100% relative humidity, RH) did not lose mass and haemolymph levels of K^+ and Na^+ were unchanged from the submerged values.

3. When exposed to air (70–80% RH) crayfish progressively dehydrated at the rate of 0·38% body mass h^{-1} (0·5% body water h^{-1}) and died after 72 h when 27% of initial mass had been lost.

4. After a 10% reduction in mass, haemolymph volume was significantly reduced to about 75% of its submerged value in 10-g animals and to 65% in 60-g animals, the decrease in volume being approximately equivalent to the mass lost. After 48 h in air (19% reduction in mass) haemolymph volume was further reduced to 24% of the submerged volume.

5. The total water content of the abdominal muscles showed a small but significant reduction when animals were dehydrated which could be accounted for by a reduction in the extracellular fluid volume, including the interstitial space.

6. After 48 h dehydration in air the osmolarity of the haemolymph increased by 36%, $[K^+]$ by 47% and $[Cl^-]$ by 57% above the submerged levels whilst $[Na^+]$ showed an insignificant decrease of 10%. In the abdominal muscle $[K^+]$ increased by 15%, $[Na^+]$ increased by 23% and $[Cl^-]$ decreased by 27%.

7. $[Ca^{2+}]$ levels in the haemolymph doubled after 24 h in air, an increase which was independent of the degree of dehydration. The increase is thus a result of emersion and not dehydration and may be due to a mobilization of $CaCO_3$ from the exoskeleton to buffer a respiratory acidosis, as it corresponds to a doubling in $[HCO_3^- + CO_3^{2-}]$.

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Key words: crayfish, aerial exposure, dehydration, water and ion distribution.

8. A substantial loss of ions from the haemolymph during dehydration cannot be fully accounted for by uptake into the tissues or by urinary losses, but a 500 mmol l^{-1} increase in the Na^+ concentration of the proventricular contents suggests that the foregut may play an important role in ionoregulation.

INTRODUCTION

One of the greatest problems of terrestrial life is the threat of dehydration. Deprived of water most animals go into negative water balance since water output *via* the urine and faeces and by evaporation over the external membranes exceeds water uptake as preformed or oxidative water derived from food. Evaporative losses of water occur over the entire body surface and in particular the respiratory membranes. In terrestrial crustaceans these losses are reduced by low integumental permeabilities to water (Bliss, 1968; Herreid, 1969; Greenaway, 1980) and a progressive reduction in gill area per unit body mass with increasing 'terrestrialness' (Gray, 1957; Cameron, 1981*a*). Despite these adaptations, rates of integumental water loss in land crabs are generally higher than those in insects (Beament, 1959) and behavioural adaptations, such as burrowing and nocturnal activity, often help restrict water losses (Cameron, 1981*a*).

Water and ion balance during aerial exposure and dehydration has been studied in terrestrial, semi-terrestrial and amphibious crustaceans (Dandy & Ewer, 1961; Gross, 1963; Bliss, 1966; Lutz, 1969; Greenaway & MacMillen, 1978; Harris & Kormanik, 1981; Jones & Greenwood, 1982). These studies have revealed the ability of land and amphibious crabs to tolerate both substantial losses of body water and large ranges of sodium concentration in the haemolymph. These adaptations endow land crabs with an ability to tolerate dehydration when water availability is limited.

Most of the studies on dehydration in crustaceans have concentrated on species frequently exposed to air. Scant attention has been given to primarily aquatic crustaceans which only rarely encounter the aerial environment. The common British crayfish, *Austropotamobius pallipes* (Lereboullet), is an aquatic animal which voluntarily leaves hypoxic water to become a facultative air breather (Taylor & Wheatly, 1980). It is capable of correcting the acid–base disturbance which occurs immediately upon aerial exposure by elevating bicarbonate levels and lowering the circulating levels of lactic acid. This restores oxygen transport, and oxygen consumption returns to resting, submerged levels (Taylor & Wheatly, 1981).

Despite this ability to breathe in air, the survival of crayfish exposed in air over damp gravel [relative humidity (RH) 70–80 % at 15 °C] is limited to about 3 days. During these migrations into air the crayfish may experience severe problems with water and ion balance, due to loss of contact with water and subsequent evaporative loss of water and impairment of ionoregulation (Tyler-Jones & Taylor, 1986). The purpose of this investigation was to assess the effects of aerial exposure on water balance and the distribution of body water and ions during dehydration in the crayfish.

MATERIALS AND METHODS

Measurements were taken from 192 specimens of the British freshwater crayfish *Austropotamobius pallipes* (Lereboullet) of either sex, collected and maintained as described by Taylor & Wheatly (1980). Each animal was sampled once only. The variables were measured on submerged crayfish and on crayfish following up to 72 h exposure to air of 70–80 % RH (unless otherwise stated) at 15 °C.

Mass during aerial exposure

The progressive reduction in mass during 72 h exposure to air was assessed by a standard weighing procedure (Taylor & Butler, 1978; Taylor & Wheatly, 1979). Total body water content was estimated using seven crayfish of mean mass 18·8 g (range 5·9–28·9 g). The animals were left in air for 1 h to allow the branchial chambers to drain of water, then surface-dried with tissue and weighed. They were then killed and dried to constant mass in an oven at 100 °C.

The distribution of body water

Haemolymph volume in submerged crayfish and following dehydration in air was measured by the dilution of an injected tracer. In one series of experiments, 0·2 ml of 5 % inulin in isosmotic saline was injected into animals *via* the infrabranchial sinus, subsequent to the removal of a similar volume of haemolymph for the preparation of blanks. Following injection, samples of haemolymph were removed at intervals, deproteinized by precipitation (Somogyi, 1930) and the concentration of inulin measured spectrophotometrically using the method of Roe, Epstein & Goldstein (1949). Possible interference by glucose could be discounted since the levels measured in the haemolymph of settled crayfish were relatively low ($0{\cdot}11 \pm 0{\cdot}01$ mmol l^{-1}; M. G. Wheatly, unpublished data).

In a second series of experiments, [^{3}H]inulin (Radiochemical Centre, Amersham) was used to determine the total inulin space (assumed to be equivalent to the total extracellular fluid volume, ECFV) and the tissue inulin space (tissue ECFV) of animals. The inulin was kept deep frozen until required, when it was dissolved in saline and kept at 0–5 °C. Animals were injected with 10–50 μl of crayfish saline containing 1 μCi 10 μl^{-1} of [^{3}H]inulin *via* a hole predrilled in the dorsal carapace over the heart and covered by a rubber septum to prevent leakage. Haemolymph samples were withdrawn at intervals into a hypodermic syringe *via* a needle inserted into the pericardial sinus behind the heart. Subsamples were immediately pipetted into 10 ml of scintillation fluid (9:1 mixture of toluene and Triton X-100). Immediately after the last haemolymph sample had been removed from each animal it was quickly killed and the abdominal extensor and flexor muscles were dissected out. The muscle was blotted on filter paper to remove excess haemolymph, placed into a preweighed glass vial and weighed. The tissue was then dried at 100 °C to a constant mass and the total water content of the muscle calculated. Dried tissue samples were digested overnight in 1 ml of 3 mol l^{-1} KOH at room temperature. An equal volume of 3 mol l^{-1} HCl was added to each vial to prevent chemiluminescence

(Wang & Willis, 1965). Finally, 0·5 ml of the digested tissue was added to 9 ml of scintillation fluid. Both blood and tissue samples were counted in a liquid scintillation counter (Philips PW4700) and corrected for quenching by the channels ratio method (Wang & Willis, 1965).

The concentration of injected inulin at time zero was obtained by extrapolation of the progressive dilution with time of haemolymph inulin concentration, and haemolymph volume was calculated from a knowledge of injection mass. Tissue ECFV was calculated from the activity of the tissue and haemolymph samples which had been taken simultaneously. The objection to the use of this technique in molluscs, that inulin could enter non-circulating fluid spaces (Potts, 1954), may not be critical in the present study as in the crayfish the only space to which inulin may gain access, which is not part of the circulating haemolymph volume, is the lumen and bladder of the excretory green gland (Riegel & Parker, 1960).

Haemolymph osmolarity and ion concentrations

Haemolymph samples were collected from submerged crayfish and from crayfish exposed to a desiccating atmosphere (70–80% RH) for 24 and 48 h. Prebranchial samples were taken from the infrabranchial sinus *via* an arthrodial membrane at the base of a walking leg. Samples, taken into melting-point tubes, were used to determine haemolymph osmolarity using a simple cryoscopic technique described by Welsh & Smith (1960) suitably adapted for this study (Taylor, Butler & Al-Wassia, 1977). The same sampling point was used to collect haemolymph which was diluted for measurement of $[K^+]$ and $[Na^+]$ using a flame photometer (Evans Electroselenium Ltd) and $[Cl^-]$ by titration as described by Schales & Schales (1941). Samples of postbranchial haemolymph, taken from a separate series of animals, were collected from the pericardial sinus *via* a septum glued over a hole in the carapace above the heart (Butler, Taylor & McMahon, 1978). Subsamples were diluted for measurement of $[Ca^{2+}]$ in an atomic absorption spectrophotometer (Pye Unicam SP2900) and for $[Cl^-]$ using an amperometric titrator (American Instrument Company).

In a later series of measurements, prebranchial haemolymph was sampled from submerged crayfish and from crayfish exposed for 48 h to non-desiccating (water-saturated) air. Subsamples were diluted for the measurement of $[K^+]$ and $[Na^+]$ in an atomic absorption spectrophotometer (Pye Unicam SP9).

Tissue water and ion content

The water content, $[Na^+]$, $[K^+]$ and $[Cl^-]$ of dissected abdominal extensor and flexor muscles was measured on tissue from submerged crayfish and from crayfish exposed for 24 and 48 h to air. Each animal was killed and the abdominal muscle dissected out. Samples weighing approximately 1 g were placed on clean aluminium foil, weighed, then dried to a constant mass at 105 °C to obtain tissue water content. Errors in the estimation of water content arising from the loss of lipids at these high

temperatures would be small as the lipid content of crayfish muscle is less than 1 % of fresh mass (O'Connor & Gilbert, 1969). The dried tissue was dissolved in 2 ml of concentrated nitric acid, heated in a boiling water bath, and subsequently diluted with deionized water for the measurement of $[Na^+]$, $[K^+]$ and $[Cl^-]$. Concentrations were related to tissue water content.

Haemolymph and abdominal muscle copper contents were measured in 12 animals (initial mass 13·5–36·3 g) of which six were submerged and six had been dehydrated in air to 90 % of initial mass. Haemolymph samples were withdrawn from the pericardial space and diluted with deionized water. The animals were then killed and samples of abdominal muscle weighing 0·1–0·4 g were placed on clean aluminium foil, weighed, and dried to a constant mass at 100 °C. The tissue was then dissolved in 300–500 μl of concentrated nitric acid, heated in a water bath to 70 °C and subsequently diluted with deionized water up to 10 ml. The copper concentration of the samples was determined on an atomic absorption spectrophotometer (Pye Unicam SP9).

Bladder volume

Bladder volume was determined in nine crayfish of mass 31·7 ± 3·2 g. The animals were killed and the antennal glands and bladders exposed by removing the dorsal carapace. The bladders were carefully removed intact and the contents emptied into preweighed glass vials. The vials were reweighed to allow the calculation of bladder volume assuming a specific density for urine of $1\,g\,ml^{-1}$. In most cases only one bladder from each animal was successfully removed. However, since there were no discernible differences in size between the two bladders in each animal, the volume for one bladder was doubled to give an approximation of total bladder volume.

Sodium concentration in the proventriculus

To investigate the possible redistribution of ions into the gut during dehydration, the sodium concentration in the contents of the proventriculus was measured in six submerged crayfish (mass, 19·4–42·6 g) and five crayfish following 24 h exposure to air (initial mass, 19·5–38·9 g). Animals were not fed for 1 week prior to experiments. The proventriculus of each animal was dissected out, blotted on filter paper to remove haemolymph and its contents were emptied into glass vials. Samples of the fluid were collected using 20 μl micropipettes, and transferred into 10 ml of deionized water. Sodium concentrations were determined in an atomic absorption spectrophotometer (Pye Unicam SP9).

Regression lines were calculated from the data for haemolymph volume for submerged and dehydrated animals and the two equations were compared by an analysis of covariance. The values for the other variables measured in submerged and dehydrated crayfish are expressed as mean ± S.E.M. and were compared using Student's t-test. Significant differences between variables were assigned at a confidence level of 95 %.

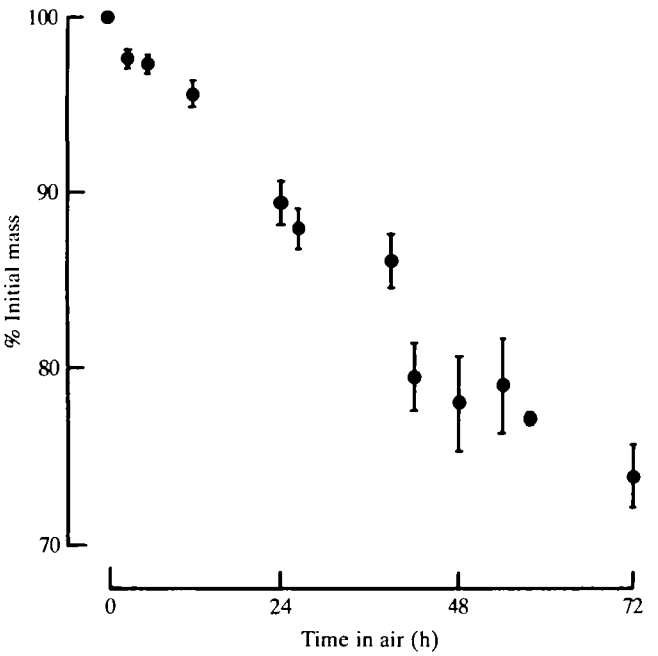

Fig. 1. The decrease in mass, expressed as percentage of initial mass, with time of crayfish exposed to air (at 15°C and 80% RH). Values expressed are means ± S.E.M. The decrease in mass was interpreted as water loss.

RESULTS

Rate of evaporative water loss

The total body water content of seven hydrated crayfish was 76 ± 1% of body mass. Evaporative water loss was measured for 25 animals of mean mass 31·2 g (range 12·6–49·3 g) over a 72 h period in air. The grouped data are expressed as percentage of initial mass against time in Fig. 1. The decrease in mass, interpreted as the rate of water loss, occurred at a relatively constant rate equivalent to 0·38% body mass h^{-1} which is 0·50% body water h^{-1} (BW h^{-1}). Thus, after 24 or 48 h of aerial exposure the body mass of an animal was approximately 90% or 81% of its submerged value, respectively. Death occurred after 27% of body mass (35·3% of body water) had been lost which corresponded to approximately 72 h of exposure to air under the present regime.

Changes in haemolymph volume

Haemolymph volume was determined in 31 submerged crayfish (mean mass 32·2 ± 2·8 g). Data from the two methods used did not differ significantly and were therefore combined. The volume of haemolymph increased with mass (Fig. 2A) but decreased as a proportion of mass with increasing mass (Fig. 2B). From the regression equation calculated for the data (see legend to Fig. 2A) 20- and 60-g animals typically have haemolymph volumes of 7·5 ml (38% body mass) and 14·6 ml (24% body mass), respectively.

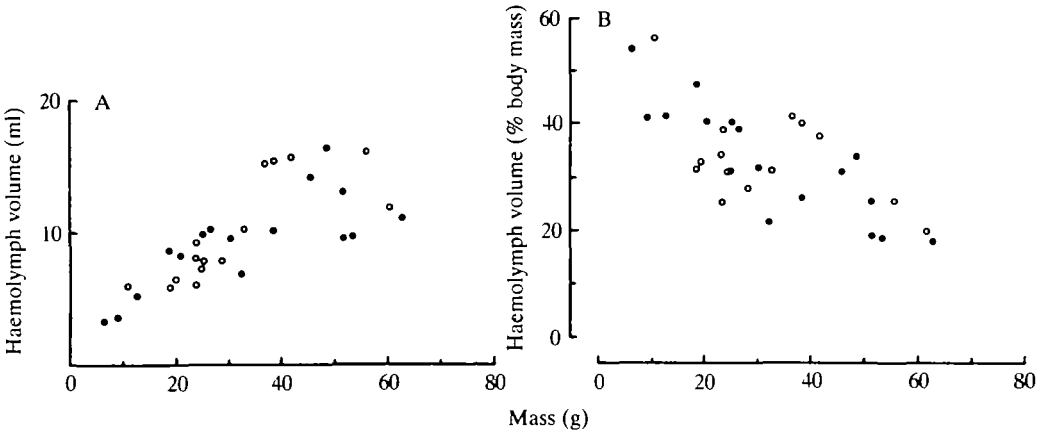

Fig. 2. (A) Haemolymph volume related to body mass in 31 animals. The equation for a regression line through these values is $y = 3{\cdot}98 + 0{\cdot}177x$ ($P < 0{\cdot}005$, $N = 31$). (B) Haemolymph volume, expressed as a percentage of body mass, related to body mass for the values shown in A. The filled symbols represent values obtained by the spectrophotometric determination of haemolymph inulin concentrations. The open symbols represent values obtained using tritiated inulin.

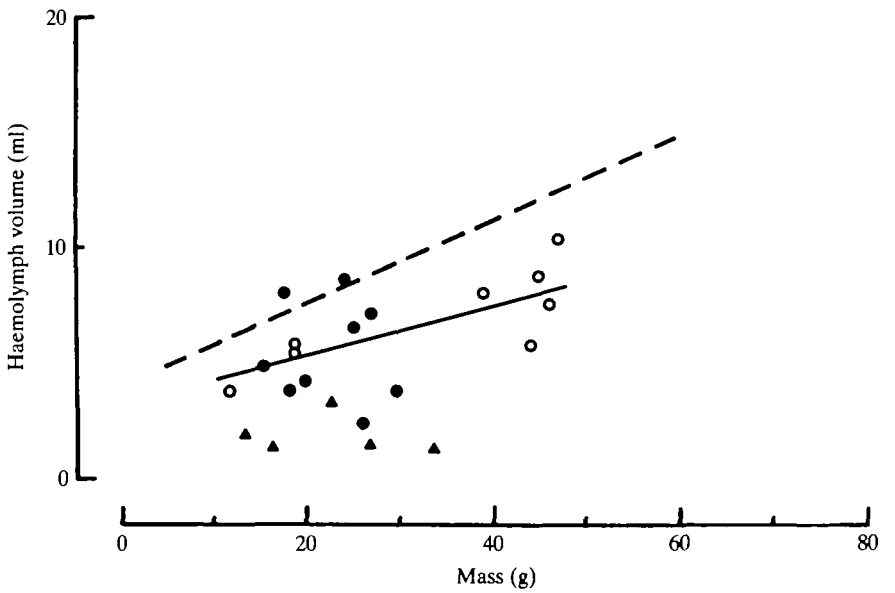

Fig. 3. Haemolymph volume related to initial body mass (mass prior to dehydration) in animals dehydrated in air to 90 % of initial mass. The equation for the regression line through these values is $y = 3{\cdot}27 + 0{\cdot}104x$ ($P = 0{\cdot}01$–$0{\cdot}05$, $N = 17$). The broken regression line is for submerged animals (taken from Fig. 2A) for comparison. Analysis of covariance shows that, although the gradients of the two regression lines do not differ significantly, the heights (adjusted means) are significantly different ($P < 0{\cdot}001$). Symbols are as described in the caption to (Fig. 2) with the addition that the filled triangles are values for crayfish dehydrated to 81 % of submerged mass.

Table 1. *Haemolymph [Na^+], [K^+], [Cl^-] and osmolarity in submerged crayfish and following 24 or 48 h of exposure to air (15 °C, 70–80 % RH)*

	Ionic concentrations in the haemolymph (mequiv l^{-1})			Osmolarity (mosmol l^{-1})
	Na^+	K^+	Cl^-	
Submerged animals	188 ± 3·0 (8)	4·7 ± 0·2 (6)	203 ± 4·2 (6)	422 ± 8 (7)
24 h in air	188 ± 3·8 (6)	5·4 ± 0·3 (6)	256 ± 4·7 (6)*	434 ± 8 (6)
48 h in air	170 ± 9·2 (7)	6·9 ± 1·0 (7)*	318 ± 5·8 (6)*	578 ± 5 (6)*

Values are means ± S.E.M. with the number of observations in parentheses.
* Indicates a significant change from the submerged level.

Haemolymph volume for animals dehydrated in air to 90 % of initial mass [mean 27·7 ± 2·9 (17) g] was significantly reduced over the whole size range (Fig. 3). After a 19 % reduction in mass (48 h in air) a third group of animals [mean initial mass 22·2 ± 3·6 (5) g] showed a further highly significant ($P < 0·001$) reduction in haemolymph volume to 24 % of the submerged level (Fig. 3).

Changes in haemolymph osmolarity and ion concentrations

The osmolarity, [Na^+], [K^+] and [Cl^-] of haemolymph samples taken from 48 animals with mean mass 29·6 g (range 12·6–49·3 g) submerged in normoxic water and after 24 or 48 h exposure to air (70–80 % RH) are listed in Table 1. [Na^+] underwent a statistically insignificant decrease of 10 % over a 48-h period in air. Both [K^+] and [Cl^-] increased progressively, to 47 % and 57 %, respectively, above the submerged level after 48 h in air. Accompanying these changes in ionic concentration, the total osmolarity of the haemolymph increased progressively during aerial exposure to 37 % above the submerged level at 48 h.

In a separate experiment, [Ca^{2+}] in the haemolymph of crayfish (mean mass 31 ± 4 g) exposed for 24 h to either 'dry' (60 % RH) or 'damp' (90 % RH) air was measured and compared to the mean value in submerged animals. After 24 h in dry air, mean mass was reduced significantly by 12·5 ± 0·3 % and [Ca^{2+}] increased from the submerged level of 17·0 ± 2·8 (6) mequiv l^{-1} to 49·0 ± 7·2 (7) mequiv l^{-1}. In damp air, mass decreased by only 1·8 ± 0·2 % and yet [Ca^{2+}] was again significantly elevated to 44·6 ± 6·6 (6) mequiv l^{-1}. The proportional changes in the concentration of the major ions in the haemolymph, together with its volume and osmolarity, are related to the reduction in mass during aerial exposure in Fig. 4.

Haemolymph [K^+] and [Na^+] were measured in a control group of seven crayfish (mean mass 18·9 ± 3·9 g) submerged in normoxic water and on seven crayfish (mean mass 23·7 ± 2·5 g) exposed to water-saturated air for 48 h. The mass of the air-exposed animals had not changed significantly by the end of the 48-h period of exposure. Haemolymph [K^+] and [Na^+] in submerged crayfish were 3·89 ± 0·23 (7) mmol l^{-1} and 221·1 ± 5·0 (7) mmol l^{-1}, respectively. After 48 h exposure the levels were 3·92 ± 0·23 (7) mmol l^{-1} for [K^+] and 231·2 ± 2·8 (7) mmol l^{-1} for [Na^+], neither of which was a significant change from the control values.

Tissue water content

The total water content and ECFV of abdominal extensor and flexor muscles was measured in nine submerged animals (mean mass 34·5 g) and in nine dehydrated animals which had lost 11·2 ± 0·5 % of their initial mass (mean 37·3 g). The muscle water content of submerged crayfish was 81·84 ± 0·45 % of fresh mass and there was a small but significant reduction to 80·05 ± 0·43 % when animals were dehydrated in air. The ECFV of the muscles in submerged animals was 23·6 ± 1·3 ml $100\,g^{-1}$ fresh tissue and in dehydrated animals it was significantly reduced to 17·1 ± 2·6 ml $100\,g^{-1}$ fresh tissue, a reduction of 27 %.

Haemolymph copper concentration measured in four submerged crayfish was 0·97 ± 0·16 mmol l^{-1} and in six crayfish dehydrated in air to 90 % of initial mass it was not significantly different, being 1·33 ± 0·18 mmol l^{-1}. There was an increase in the oxygen content of air-equilibrated haemolymph ($C_{max}O_2$) from 615 to

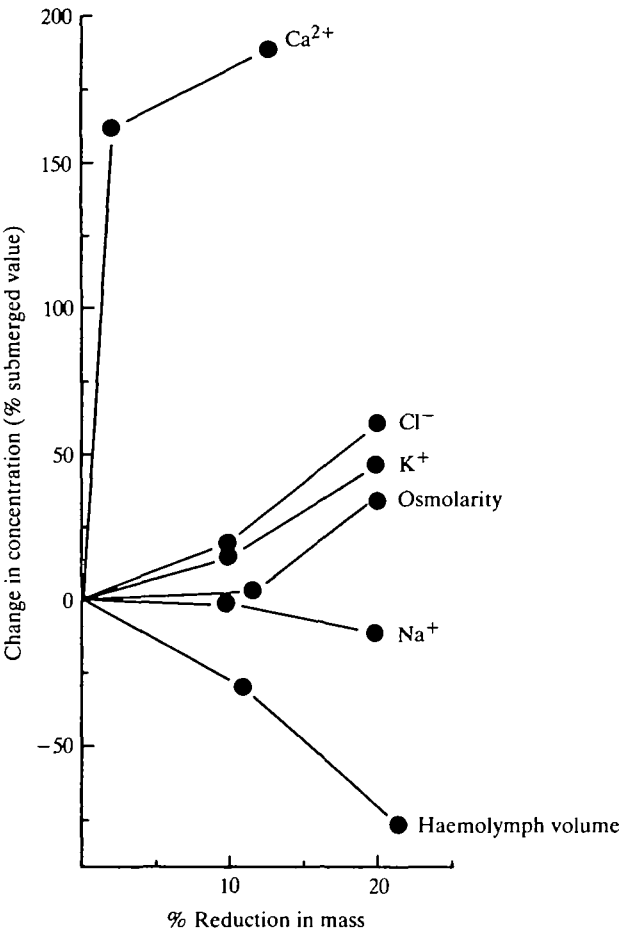

Fig. 4. Proportional changes in volume, osmolarity, [Na^+], [K^+], [Cl^-] and [Ca^{2+}] of the haemolymph in crayfish during progressive dehydration, expressed as a percentage of the submerged level and plotted against percentage reduction in body mass.

Table 2. *[K^+], [Na^+] and [Cl^-] in the abdominal muscle of crayfish submerged in normoxic water and following periods of desiccation in air at 15°C (70–80% RH) for 24 and 48 h*

	[K^+] (mequiv kg^{-1})	[Na^+] (mequiv kg^{-1})	[Cl^-] (mequiv kg^{-1})
Submerged animals	126·3 ± 4·0 (13)	50·6 ± 2·4 (13)	33·8 ± 2·1 (13)
24 h in air	127·9 ± 5·2 (6)	51·4 ± 1·8 (6)	26·7 ± 3·4 (6)
48 h in air	144·9 ± 3·5 (7)*	62·9 ± 1·8 (7)*	24·7 ± 1·5 (7)*

Values are means ± S.E.M. with the number of observations in parentheses.
* Indicates a significant change from the submerged level.

725 μmol l^{-1} (E. W. Taylor, unpublished results). The copper content of the abdominal muscles of submerged crayfish was 24·9 ± 7·9 (6) μmol 100 g^{-1} fresh tissue, not significantly different from the value of 15·5 ± 4·9 (6) μmol 100 g^{-1} fresh tissue in dehydrated crayfish. The water content of abdominal muscle from these submerged animals was 81·0 ± 0·4 (6) % and again showed a small but significant reduction after dehydration. Assuming all the copper in the tissues is contained in haemocyanin suspended in the circulating haemolymph, the volume of the copper space in each tissue sample was calculated. The copper space in abdominal muscles of submerged animals was 9·85 ± 1·43 (6) ml 100 g^{-1} tissue, which is 42% of the tissue ECFV. After dehydration the copper space [7·3 ± 0·98 (6) ml 100 g^{-1} tissue] had not significantly changed.

Changes in tissue ion concentrations

Changes in [Na^+], [K^+] and [Cl^-] in abdominal muscle taken from crayfish submerged in normoxic water and following 24 and 48 h exposure to air are listed in Table 2. The mean levels of Na^+ and K^+ increased progressively during aerial exposure so that after 48 h, when the animals had lost approximately 20% of their initial mass, [Na^+] in the tissues had increased by 24% and [K^+] had increased by 15%. The mean level of Cl^- in the tissues decreased by 27% during 48 h exposure to air.

Bladder volume

The total bladder volume of nine crayfish submerged in water was estimated as 0·29 ± 0·06 ml. The proportion of the inulin space occupied by the bladders was calculated as 3·4 ± 0·5 (9) %. Although no intact bladders were removed from crayfish which had been dehydrated in air, the bladders of dehydrated animals did not appear on inspection to be any different in size from those of submerged animals.

Sodium concentration in the proventriculus

The sodium concentration of the fluid in the proventriculus of submerged animals was 803 ± 127 (6) mmol l^{-1} and after 24 h dehydration in air it was 1302 ± 263 (5) mmol l^{-1}, which was not a significant change from the submerged value.

DISCUSSION

This investigation demonstrates that when the freshwater crayfish *Austropotamobius pallipes* is exposed to air (15 °C, 70–80 % RH) it progressively dehydrates at a rate of 0·50 % body water h^{-1} (0·38 % body mass h^{-1}) and dies after 72 h, when 35·3 % of body water has been lost. Thus, although it is primarily an aquatic species, *Austropotamobius* compares favourably with terrestrial and amphibious species of crabs in both the degree of dehydration it can tolerate and in the rate of water loss in air (see Jones & Greenwood, 1982).

Most of the mass lost during aerial exposure can be attributed to the evaporative loss of water, since urine flow does not contribute substantially to the loss of mass of air-exposed animals (Taylor & Tyler-Jones, 1985; Tyler-Jones & Taylor, 1986). Taylor & Wheatly (1980) emphasized that the rate of branchial ventilation with air is low in *Austropotamobius* (5 % of the ventilation rate in water) and this may, together with the relatively low permeability to water of the freshwater crayfish (Rudy, 1967; Subramanian, 1975), minimize evaporative water loss.

The body fluids of crayfish are not as concentrated as in aquatic marine crustaceans and a greater degree of dehydration may be tolerated before internal concentrations reach a lethal level. The prolonged survival of crayfish in water osmotically and ionically equivalent to the levels found in the haemolymph at the point of death (M. G. Wheatly, unpublished data) and with haemolymph osmolarity similar to those levels (Bryan, 1960*b*) indicates that internal ion concentrations are not the ultimate cause of death.

The values for haemolymph volume and the relationship between haemolymph volume and mass for hydrated *Austropotamobius* are similar to the results obtained by Riegel & Parker (1960) for two crayfish species. Mild dehydration of *Austropotamobius* results in a reduction in haemolymph volume, which is approximately equivalent to the mass lost, and a slight decrease in muscle water content attributable to a decrease in tissue ECFV. During severe dehydration, haemolymph volume is further reduced to levels which are likely severely to limit the efficacy of the circulatory system. It appears that during dehydration *Austropotamobius* protects the intracellular fluid volume whilst preferentially losing water from the ECFV.

One of the main functions of crustacean haemolymph is the transport of respiratory gases. Crayfish, which are primarily aquatic, experience substantial reductions in haemolymph volume during dehydration which ultimately may present problems with circulation and oxygen delivery. These problems may be compounded by increases in viscosity resulting from concentration of the haemolymph. Crustaceans may be able to tolerate substantial reductions in haemolymph volume as the relatively large volume and low pressures of the open circulation (Taylor, 1982) render it less likely to failure following reductions in volume. Nevertheless, in most of the terrestrial decapodan crustaceans studied, the intracellular fluid compartment is not conserved during dehydration and water losses occur from both the extra- and intracellular compartments (Gross, 1963; Lutz, 1969; Greenaway & MacMillen, 1978; Harris & Kormanik, 1981). The terrestrial anomuran, *Birgus latro*, avoids the

problem of reduced haemolymph volume since, although the haemolymph acts as a store of water, the abdomen decreases in volume during dehyration (Harris & Kormanik, 1981) thus maintaining the functional integrity of the circulatory system.

The haemolymph of insects acts as a reserve of water for the maintenance of intracellular fluid volume during dehydration (Edney, 1977). Large decreases in haemolymph volume can occur in the wide range of terrestrial habitats exploited by insects, without the concomitant respiratory problems faced by other animals, as the tracheal system is responsible for the transfer of respiratory gases, thereby effectively bypassing the haemolymph used by the closely related crustaceans.

Vertebrates not adapted to xeric habitats maintain the intracellular fluid volume at the expense of the circulating body fluid during dehydration (MacFarlane, Morris & Howard, 1956; Shoemaker, 1964; Kutscher, 1968) whereas vertebrates which inhabit dry environments maintain blood volume by dehydrating the tissues (MacFarlane *et al.* 1963; Schmidt-Nielsen, 1964; Kutscher, 1968; Denny & Dawson, 1975). This ability to maintain blood volume during dehydration may be essential to these animals as the closed circulation, with its relatively low volume and high operating pressures, is particularly susceptible to failure following loss of volume.

The difference between the inulin and copper spaces of crayfish muscles suggests the existence of an interstitial fluid space which lacks haemocyanin. Similar discrepancies between marker spaces have previously been reported for other crustacean species and it has been proposed that the extracellular fluid space of crustaceans may consist of functionally separate compartments: a circulating compartment containing haemocyanin and an interstitial fluid lacking haemocyanin (Flemister, 1958; Robertson, 1960, 1961; Zuckerkandl, 1960; Smith & Dall, 1982). Despite the lack of any statistically significant change in tissue copper space following dehydration, the post-dehydration value was only 74% of the submerged value. This may represent a physiologically significant change and evince a decrease in interstitial fluid space concomitant with the decrease in tissue ECFV. Thus, although the extracellular space may be divided into functionally separate compartments, possibly with a thin membrane separating the blood and interstitial spaces (Smith & Dall, 1982), these results would indicate that, in the crayfish, the ECFV acts as a continuous fluid space with no differential partitioning of water losses between the extracellular compartments.

The levels of ionic constituents and osmolarity in the haemolymph of submerged animals agree closely with previous measurements on freshwater crayfish (Bryan, 1960*a*; Kerley & Pritchard, 1967). The sum of the major cations is similar to the sum of the major anions and the two together almost total the measured osmolarity (see Table 1).

Crayfish exposed to water-saturated air did not dehydrate (see also Tyler-Jones & Taylor, 1986) and haemolymph $[K^+]$ and $[Na^+]$ remained unchanged. Thus the changes in haemolymph osmolarity, $[K^+]$, $[Na^+]$ and $[Cl^-]$ observed in crayfish exposed to air of 70–80% RH resulted from dehydration and were not consequences of emersion alone.

Dehydration resulted in an increase in $[K^+]$ in both the haemolymph and tissues which seems straightforward as it conformed to the progressive dehydration experienced in air, with the larger change occurring in the haemolymph from which water was lost. The changes in $[Na^+]$ and $[Cl^-]$ were, however, more complex. $[Na^+]$ did not change in the haemolymph but was significantly raised in the tissues. This implies that Na^+ passes from the haemolymph as its volume decreases, and may be taken up intracellularly by the tissues. In contrast to these changes in $[Na^+]$ there was a relatively large proportional increase in $[Cl^-]$ in the haemolymph, accompanied by a significant withdrawal of Cl^- from the tissues. These fluxes may constitute components of the mechanisms of intracellular acid–base regulation (Thomas, 1984; Galler & Moser, 1986), although preliminary investigations have indicated that an acidosis in the abdominal muscles incurred on exposure to air remains uncompensated throughout the period of exposure (R. Tyler-Jones, E. W. Taylor & R. C. Thomas, unpublished observations).

The value for total body water in hydrated crayfish (76·4 %) is the same as that reported by Scudamore (1947) and, with the data for haemolymph volume, allows the total volume of intracellular water to be estimated. Assuming that the intracellular water content does not change during dehydration and that the changes in intracellular $[Na^+]$ occur in all tissues, the increase in total intracellular sodium was estimated as approximately 270 μmol. The substantial decrease in haemolymph volume during dehydration results in an overall loss of ions from the haemolymph. For sodium this loss is equivalent to about 540 μmol in a 30-g animal. The increase in intracellular sodium therefore accounts for 50 % of the loss from the haemolymph. Urinary sodium losses over 24 h in air total 73 μmol in a 30-g crayfish (estimated from data in Tyler-Jones & Taylor, 1986) and account for about 14 % of the loss from the haemolymph, leaving a deficit of approximately 197 μmol.

Movements of ions and water may occur between fluid compartments other than the haemolymph and muscles. A possible involvement of the foregut in ion balance has been assessed in the present investigation. Although the sodium concentration of the proventricular contents did not show any statistically significant change, there was a substantial increase ($500\,mmol\,l^{-1}$) which may account for the deficit of haemolymph sodium. Over 48 h exposure to air haemolymph volume is reduced to 24 % of its submerged level although osmolarity increased by only 36 % (Table 2). If the haemolymph constituents are not redistributed during dehydration an increase in osmolarity of 4·2 times might be expected. The deficit may be accounted for by the accumulation of ions in the gut with the proventriculus acting as a temporary storage site, preventing the haemolymph concentration increasing beyond tolerable limits during dehydration. In many terrestrial crabs the gut has been implicated as an organ important in the regulation of ions (see Bliss, 1968, for a review) and may therefore replace the gills when branchial exchanges are limited by aerial exposure.

The changes in the levels of $[Ca^{2+}]$ in the haemolymph of crayfish after 24 h in air were independent of the degree of dehydration (Fig. 4) and therefore result from emersion and the change to aerial respiration. The relatively large increase in $[Ca^{2+}]$ to 2·6 times the submerged level may relate to the similar proportional increase, to

2·1 times the submerged level, in $[HCO_3^- + CO_3^{2-}]$ in the haemolymph of crayfish after 24 h in air (Taylor & Wheatly, 1981). These authors postulated that an internal source of $CaCO_3$, possibly the exoskeleton, was mobilized to provide HCO_3^- and the present results would tend to support that hypothesis. A similar mobilization of an internal source of fixed base has been postulated in crustaceans by DeFur, Wilkes & McMahon (1980), Cameron (1981*b*), Henry, Kormanik, Smatresk & Cameron (1981), and Wood & Randall (1981) and in molluscs by Burton (1975, 1976). The urine concentration of calcium increased by only 24 % during exposure to air (compared with 51 % for sodium and 76 % for chloride) suggesting that it is retained (Tyler-Jones & Taylor, 1986), a mechanism which may aid the restoration of oxygen transport in air, noted by Taylor & Wheatly (1981), by increasing the oxygen affinity of the haemocyanin (Morris, Tyler-Jones, Bridges & Taylor, 1986).

The relationship between the major cations, anions and the measured osmolarity is complicated by the differential changes in ionic concentration after 48 h in air. The total concentration of the major cations Na^+ and K^+ is considerably less than the concentration of the major anion Cl^- and the overall total is less than the measured osmolarity. This imbalance arises from the unchanging level of Na^+ which has subsequently been confirmed (E. W. Taylor, unpublished results) and the disproportionate increase in $[Cl^-]$, also confirmed by M. G. Wheatly (unpublished results) who noted an even larger proportional increase. The imbalance is not satisfied by the inclusion of the total of $[Ca^{2+}] + [Mg^{2+}]$, which is approximately 70 mequiv l^{-1} (E. W. Taylor, unpublished results). The change in the equivalent charge balance (total cations minus total anions) from 4 to -88 mequiv l^{-1} implies that the imbalance is due to a cation(s) as yet unidentified. Further investigation is required before these problems are resolved.

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