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EFFECTS OF EXTREME pH ON THE PHYSIOLOGY OF THE AUSTRALIAN 'YABBY' *CHERAX DESTRUCTOR*: ACUTE AND CHRONIC CHANGES IN HAEMOLYMPH OXYGEN LEVELS, OXYGEN CONSUMPTION AND METABOLITE LEVELS

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Summary

Respiration and metabolism of the freshwater crayfish *Cherax destructor* were investigated with respect to the acidification and alkalization of its environment. Crayfish were exposed for up to 504 h (21 days) to pH 4.5, pH 7.1 (control) or pH 8.0 water and oxygen consumption rate, haemolymph oxygen transport and haemolymph glucose and lactate concentrations were determined. The effect of reducing environmental [Ca^{2+}] in acid water from 500 to 50 μmol l^{-1} was also examined.

In acid water (500 μmol l^{-1} Ca^{2+}), oxygen uptake by *Cherax* was reduced by 79 % after 504 h (21 days) compared with 'control' animals (pH 7.1, 500 μmol l^{-1} Ca^{2+}). Haemolymph lactate concentration (mean 0.6 mmol l^{-1}) remained constant, indicating that anaerobiosis was not important, while glucose concentrations were regulated within the range of control values (0.32±0.01 mmol l^{-1}). The arterial-venous C_{O_2} difference of *Cherax* haemolymph decreased after 288 h and Pa_{O_2} increased from 11.1±0.5 mmHg to 42.4±1.0 mmHg between 96 h and 288 h. Decreased oxygen uptake and delivery without compensatory increases in anaerobiosis or glucose levels describe a hypometabolic response to low pH.

The hypometabolic response of *Cherax* was greater in alkaline water as shown by a 53 % reduction in O_2 uptake rate compared with a 44 % reduction in acid-exposed (500 μmol l^{-1} Ca^{2+}) animals after 96 h. This decrease in $\dot{M}_{O_2}$ of alkaline-exposed animals was correlated with decreased haemolymph glucose levels (from 0.32±0.01 at 0 h to 0.06±0.01 mmol l^{-1} at 96 h).

Lowering the [Ca^{2+}] of the water both increased the magnitude of the effects of acid exposure and elicited further changes in haemolymph oxygen transport. The maintenance of high haemolymph P_{O_2} during pH stress appears to reduce the involvement of haemocyanin, since this promotes decreased a–v C_{O_2}. Hypometabolism probably permits *Cherax* to conserve resources that might otherwise be used, however, for growth and reproduction. The implications for the fitness of the animal are discussed.

Key words: acid, alkali, oxygen, metabolic rate, hypometabolism, crayfish, *Cherax destructor*.

Introduction

Variation in ambient pH is known to exert profound effects on ionoregulation and acid–base balance in decapod crustaceans, yet there is relatively little information on either metabolism or O_2 transport. Consideration of the respiratory as well as the ionic and acid–base responses to extreme pH environments is pertinent, since branchial ionoregulatory mechanisms also affect gas exchange *via* acid–base balance (Na^+/H^+, Cl^-/HCO_3^- or OH^-) (Wheatly and McMahon, 1981). The effect of extreme pH on the acid–base and ion physiology of the Australian crayfish *Cherax destructor* has been investigated (Ellis and Morris, 1995). The investigation reported here considers the effects of extreme pH on oxygen uptake and metabolic status.

It has been proposed that acid and alkaline environments may increase energy expenditure for maintenance of homeostasis, which could subsequently reduce energy available for growth and reproduction (e.g. Spaargaren, 1975; Neville, 1985; Mangum, 1986; Hargeby, 1990). In addition, low levels of calcium in the water may further increase the energy expenditure required for homeostasis (Wood and Rogano, 1986). Exposure of trout to pH 4.0 soft water, although impairing O_2 transport, does not limit resting O_2 consumption but reduces the scope for activity (Ye *et al.* 1991). More extreme acid conditions do impair resting O_2 uptake, suggesting that an acidosis may actually reduce O_2 consumption by direct action on metabolism at the tissue level.

The objectives of this study were, first, to determine the consequences of extreme environmental pH for the haemolymph O_2 transport and delivery to the tissues in *Cherax destructor* and the requirement for anaerobiosis, thereby assessing to what extent acid or alkaline toxicity represents an energetically 'expensive' environment for this species. The

second aim was to determine whether [Ca^{2+}] plays an important role in modulating any effects of pH exposure on metabolism.

Materials and methods

Experimental animals

Male and female *Cherax destructor* (45–60 g, N≈460) were donated by the commercial crayfish farm Crayhaven situated in Karuah, New South Wales. The animals were maintained in a recirculating aquarium system at the University of Sydney. Details on crayfish origin and holding have been described previously (see Ellis and Morris, 1995). The animals were not fed for the duration of the experiments. Experiments were carried out using five different combinations of pH and [Ca^{2+}] (Table 1).

Animals to be used in the high-Ca^{2+} experiments were acclimated for 1 week to water at pH 7.1 (treatment 1a) with the following salt composition (in μmol l^{-1}): NaCl, 250; KCl, 500; $CaCO_3$, 500; $MgCO_3$, 200; $MgSO_4$, 500. This served as the control condition for the high-Ca^{2+} treatments (500 μmol l^{-1}). Animals to be used in the low-Ca^{2+} experiments were acclimated for 1 week to Ca^{2+}-free water at pH 7.1 (treatment 2a) with a salt composition as follows (in μmol l^{-1}): NaCl, 250; KCl, 500; Na_2CO_3, 500; $MgCO_3$, 200; $MgSO_4$, 500.

Haemolymph oxygen

The sampling protocol was as outlined previously (Ellis and Morris, 1995). Six different animals were used at each time and each was sampled only once to ensure the collection of independent data. The control series served partly as a check on the possible disturbing effects of reduced food supply and the brief movement and air exposure involved in the transfers. The crayfish showed no obvious increase in activity during transfer. According to the protocol, both arterial and venous haemolymph samples were collected from crayfish over a maximum period of 504 h of exposure to the five different treatment combinations shown in Table 1.

Animals in both the control condition (pH 7.1) and acid treatment (pH 4.5) containing 500 μmol l^{-1} Ca^{2+} were monitored over a 504 h period, whilst the exposure times for those in the alkaline (treatment 3) and low-Ca^{2+} treatment (treatment 2b) were 96 h and 288 h respectively.

Samples were immediately measured for oxygen partial pressure (P_{O_2}) and content (C_{O_2}). The haemolymph P_{O_2} was determined using a Radiometer electrode (type E5037/SI Radiometer, Copenhagen, Denmark) thermostatted at 20 °C in a BMS3 blood microsystem connected to a PHM73 blood gas analyzer. The O_2 content was measured using a Strathkelvin Instruments TC 500 Tucker cell (Tucker, 1967) as modified by Bridges *et al.* (1979) for haemocyanin.

Oxygen uptake

Animals were placed in separate jars (volume≈375 ml) which served as respirometers. Mesh 'lids' allowed gas exchange with the surrounding water and prevented animal escape until respirometry was to be initiated. Animals were exposed to the appropriate treatment water for the required period, after which the mesh covers were replaced with a gas-tight lid for 0.25 h. Water samples were taken immediately before and after sealing the jars and the rates of O_2 uptake were calculated from determinations of the change in P_{O_2} of each individual jar over 15 min. This method includes measurement of 'background' levels of $\dot{M}_{O_2}$ using jars containing only water. These background measurements were then subtracted from the experimental values. All water samples were introduced *via* capillary tubing into a thermostatted cell (D616, Radiometer, Copenhagen, Denmark) containing an oxygen electrode at the experimental temperature (20 °C). The electrode was connected to an acid–base analyzer (PHM71) containing an oxygen module (PHA930). The rates were then expressed as mass-specific values.

Table 1. *Details of experimental treatments*

Treatment	pH	[Ca^{2+}] (μmol l^{-1})	N
1a	7.1	500	120
2a	7.1	50	12
1b	4.5	500	120
2b	4.5	50	108
3	8.0	500	96

Haemolymph glucose and lactate concentrations

Haemolymph glucose concentration was determined by spectrophotometry (Varian techtron, model 635) using the enzymatic glucose oxidase assay method (Sigma, procedure no. 510). Haemolymph glucose concentrations were calculated from regressions of δAb/δ[glucose] for the standards over the range 0.03–0.28 mmol l^{-1}.

To determine L-lactate concentrations, 100 μl of 6% perchloric acid (PCA) was added to an equal volume of haemolymph to deproteinize the sample. After centrifugation, K_2CO_3 (2.5 mol l^{-1}) was added to neutralize the remaining PCA and then samples were frozen (−15 °C) until required for analysis. L-Lactate concentrations were determined, using 25 μl of the deproteinated haemolymph, by the ultraviolet test method (Boehringer Mannheim). Absorbances were determined using a Pharmacia LKB Ultraspec III spectrophotometer interfaced with a 80286 computer system running BIOCHROM kinetics software, allowing correction for any constant drift due to interfering substances.

Data analysis

Data have been reported as mean ± standard error unless otherwise stated. Two-way analyses of variance were used to detect differences between arterial and venous haemolymph and amongst treatments. The chosen limit of significance was P=0.05. *Post-hoc* testing was performed using Contrast testing and Tukey's HSD multiple means comparison test. All analyses were performed using the SYSTAT 5.01 statistical package.

Results

Haemolymph oxygen

Acid exposure: 500 μmol l^{-1} Ca^{2+}

The arterial and venous partial pressures of oxygen (Pa_{O_2} and Pv_{O_2} respectively) of *Cherax* held in neutral water both increased significantly at 0.5 h compared with the initial value ($F_{9,46}$=5.03; $F_{9,47}$=8.41, Tukey) but promptly decreased back towards the initial values (Fig. 1A,B). Acid-exposed crayfish exhibited more pronounced increases in haemolymph P_{O_2} since both Pa_{O_2} and Pv_{O_2} were significantly higher than the initial value after 0.25 h, 288 h and 504 h ($F_{9,47}$=3.48, arterial; $F_{9,47}$=4.70, venous) and higher compared with the control animals after 0.25 h, 1 h, 288 h and 504 h ($F_{8,90}$=2.02, Contrast) (compare Fig. 1C,D with Fig. 1A,B).

In *Cherax* exposed to either neutral or acid water (500 μmol l^{-1} Ca^{2+}), the initial increase in P_{O_2} was associated with a significantly decreased a–v difference ($F_{9,40}$=3.10, $F_{1,70}$=5.01 respectively, Tukey HSD) (Fig. 1A,C). The a–v P_{O_2} difference of the acid-exposed animals was significant only when the 288 h and 504 h values were excluded from the ANOVA, since the variance of these data and the apparently reversed a–v difference contributed substantially to the variance of the entire data set. The significance of the reversed a–v difference is difficult to determine since it occurs at a time of minimal oxygen demand (see below).

The arterial oxygen content (Ca_{O_2}) of crayfish held in neutral water increased significantly only at 0.5 h with respect to the initial value ($F_{9,47}$=3.89, Tukey), but also promptly returned to initial values (Table 2). Crayfish in acid water had significantly

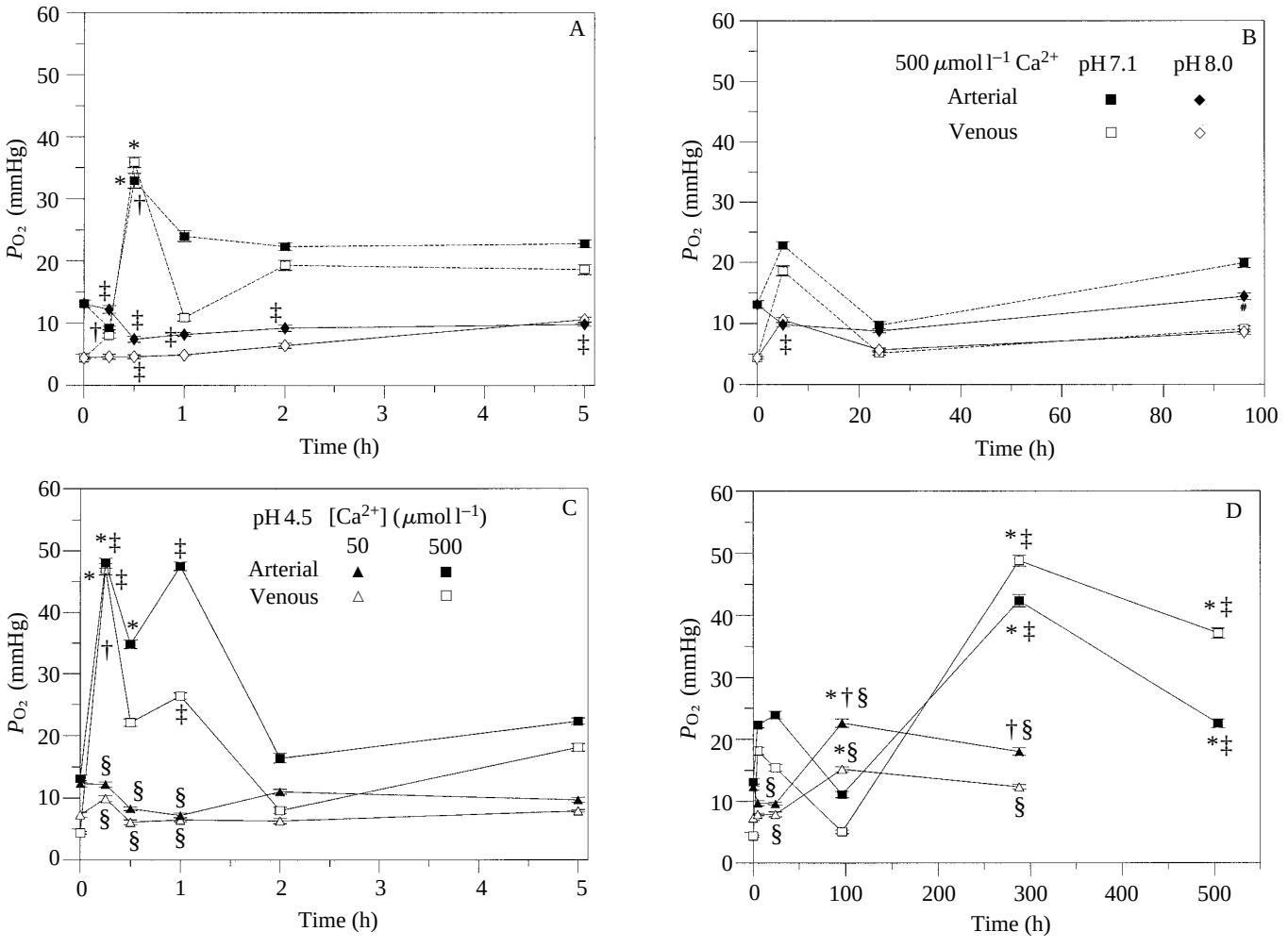

Fig. 1. Changes in the Pa_{O_2} and Pv_{O_2} of *Cherax destructor* placed in either neutral (pH 7.1, □, ■ broken line) or alkaline (pH 8.0, ◆, ◇) water ([Ca^{2+}] maintained at 500 μmol l^{-1}) are shown for (A) acute and (B) chronic exposures. The pre-exposure values are shown at 0 h. Results of exposure to acid water containing either 50 μmol l^{-1} (△, ▲) or 500 μmol l^{-1} Ca^{2+} (□, ■) are shown for (C) acute and (D) chronic exposures. Note that the low-Ca^{2+} acid-exposed crayfish were pre-acclimated to a low [Ca^{2+}] pH 7.1 condition (see text for details). *Significantly different from the initial value, shown at 0 h; †significant change in arterial-venous difference compared with that at 0 h; ‡significant difference between Pa_{O_2} and Pv_{O_2} of animals held in extreme pH water compared with those held in neutral water; §significant difference between the Pa_{O_2} or Pv_{O_2} of animals held in pH 4.5, 50 μmol l^{-1} Ca^{2+} water compared with those held in high-Ca^{2+} acid water. The difference between the arterial and venous values at each sample time was significant for all treatments (two-way ANOVA). Values are means ± S.E.M., N=228.

Table 2. C_{O_2} *(mmol* l^{-1}*) in the arterial and venous haemolymph of* Cherax destructor *during exposure to extreme pH in water containing 500* μ*mol* l^{-1} *[*Ca^{2+}*]*

Time (h)	High [Ca^{2+}]						Low [Ca^{2+}]	
	pH 7.1		pH 4.5		pH 8.0		pH 4.5	
	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
0.00	0.27±0.09	0.16±0.05	0.27±0.09	0.16±0.05	0.27±0.09	0.16±0.05	0.39±0.07§	0.29±0.07§
0.25	0.41±0.07	0.39±0.06	0.31±0.05	0.28±0.07	0.31±0.07	0.20±0.06	0.36±0.08	0.35±0.08
0.5	0.69±0.14*	0.31±0.07	0.23±0.05†	0.22±0.06	0.31±0.08†	0.28±0.08	0.32±0.06	0.22±0.04
1	0.33±0.06	0.23±0.05	0.40±0.09*,†	0.38±0.06*	0.31±0.05	0.26±0.08	0.33±0.04§	0.25±0.07
2	0.19±0.03	0.18±0.04	0.30±0.08	0.19±0.04	0.30±0.04	0.21±0.06	0.39±0.03	0.23±0.05
5	0.20±0.05	0.17±0.03	0.19±0.04	0.15±0.03	0.26±0.06	0.19±0.06	0.27±0.07	0.18±0.00
24	0.21±0.05	0.21±0.05	0.35±0.08	0.18±0.04	0.24±0.04	0.21±0.06	0.25±0.06	0.22±0.05
96	0.20±0.05	0.14±0.04	0.26±0.07	0.13±0.03	0.30±0.04	0.27±0.07	0.28±0.04	0.23±0.05§
288	0.19±0.04	0.30±0.09	0.34±0.06*,†,‡	0.34±0.06	–	–	0.30±0.05	0.23±0.04§
504	0.30±0.08	0.24±0.04	0.25±0.03‡	0.27±0.08	–	–	–	–

The response of *Cherax* to acid water containing only 50 μmol l^{-1} [Ca^{2+}] is also shown (S.E.M. shown for each time).

Statistical analyses were carried out to test for significant changes over time within each treatment (shown by *), and for significant differences between treatments (†significantly different from pH 7.1, 500 μmol l^{-1} [Ca^{2+}] treatment value; §significantly different from pH 4.5, 500 μmol l^{-1} [Ca^{2+}] treatment value).

Two-way ANOVA showed that the difference between the arterial and venous values at each sample time was significant. Thus, ‡ indicates a significant change in the a–v difference compared with the initial value.

lower Ca_{O_2} compared with control animals (pH 7.1, [Ca^{2+}]=500 μmol l^{-1}) after 0.5 h, but higher Ca_{O_2} compared with the initial value and that measured in control animals after 288 h ($F_{9,47}$=3.05; $F_{8,87}$=2.27) (Table 2). The chronic increase in Ca_{O_2} after 288 h of exposure to pH 4.5 water (500 μmol l^{-1} Ca^{2+}) was associated with a significantly smaller arterial–venous difference (effectively none) compared with the initial value ($F_{9,100}$=3.17) (a–v difference 0.01 and 0.11 mmol l^{-1} respectively).

Acid exposure: 50 μ*mol* l^{-1} Ca^{2+}

Lowering the [Ca^{2+}] of the acid water to 50 μmol l^{-1} significantly decreased the Pa_{O_2} and Pv_{O_2} below that of animals placed in high-Ca^{2+} water between 0.25–1 h and 288 h ($F_{8,90}$=4.59; $F_{8,87}$=13.85) (Fig. 1C,D). It is interesting that lowering the [Ca^{2+}] of the acid water also accelerated the chronic increase in haemolymph P_{O_2} seen in acid-exposed animals after 288 h, to 96 h (cf. 50 and 500 μmol l^{-1} Ca^{2+} acid treatments) ($F_{8,44}$=4.36, venous; $F_{8,44}$=4.28, arterial; Tukey) (Fig. 1D). The significant a–v P_{O_2} difference of *Cherax* in low-Ca^{2+} acid water ($F_{1,88}$=10.52) at this time also increased significantly ($F_{8,36}$=3.84, Tukey HSD).

Unlike animals held in high-Ca^{2+} pH 4.5 water, animals exposed to low-Ca^{2+} acid water exhibited an a–v C_{O_2} difference that was maintained throughout the 12 day exposure period ($F_{1,86}$=28.94) and there were no significant changes in either venous oxygen content (Cv_{O_2}) or Ca_{O_2} over time ($F_{8,42}$=1.05; $F_{8,45}$=1.80). Lowering the [Ca^{2+}] of the water at pH 7.1 prior to acid exposure initially increased Ca_{O_2} and Cv_{O_2} significantly compared with those of animals held in high-Ca^{2+} pH 7.1 water ($F_{8,85}$=3.02; $F_{8,87}$=2.27, Contrast) (Table 2 at 0 h). Both Ca_{O_2} and Cv_{O_2} decreased significantly below those in animals held in high-Ca^{2+} water at various times over the 288 h exposure period ($F_{8,85}$=3.02 arterial; $F_{8,87}$=2.27 venous, Contrast) (Table 2).

Alkaline exposure

Unlike those crayfish in neutral and acid 500 μmol l^{-1} Ca^{2+} water, an a–v P_{O_2} difference ($F_{7,79}$=18.40) of crayfish exposed to pH 8.0 was maintained even after 96 h (Fig. 1A,B). Whilst the Pv_{O_2} of alkaline-exposed crayfish was only significantly lower than that of the control animals after 0.5 h ($F_{6,67}$=11.07, Contrast), the Pa_{O_2} values of animals in alkaline water were comparatively lower at almost all times ($F_{1,70}$=10.99) (Fig. 1A,B).

There was a significant difference between the Ca_{O_2} and Cv_{O_2} ($F_{1,78}$=8.81) which was maintained throughout the 96 h exposure period ($F_{7,39}$=0.37) (Table 2). Animals held in alkaline water had significantly lower Ca_{O_2} values than animals in neutral water after 0.5 h ($F_{6,68}$=3.04, Contrast) (Table 2).

Oxygen uptake and anaerobiosis

Acid exposure

Animals held in acid water containing either 500 μmol l^{-1} or 50 μmol l^{-1} Ca^{2+} had significantly lower O_2 uptake rates ($\dot{M}_{O_2}$) than animals placed in neutral water ($F_{2,117}$=9.61, Tukey) (Fig. 2A,B). Whilst there were no significant changes over time in the $\dot{M}_{O_2}$ of 'control' crayfish placed in neutral pH water (500 μmol l^{-1} Ca^{2+}) ($F_{9,48}$=3.64, Tukey), crayfish had significantly decreased $\dot{M}_{O_2}$ after 2 h, 24 h, 96 h and 504 h of exposure to acid high-Ca^{2+} water compared with the initial value ($F_{9,50}$=5.83, Tukey). In fact, after 504 h (21 days), the rates for acid-exposed animals were only 25 % of those before the start of the experiment and were significantly reduced

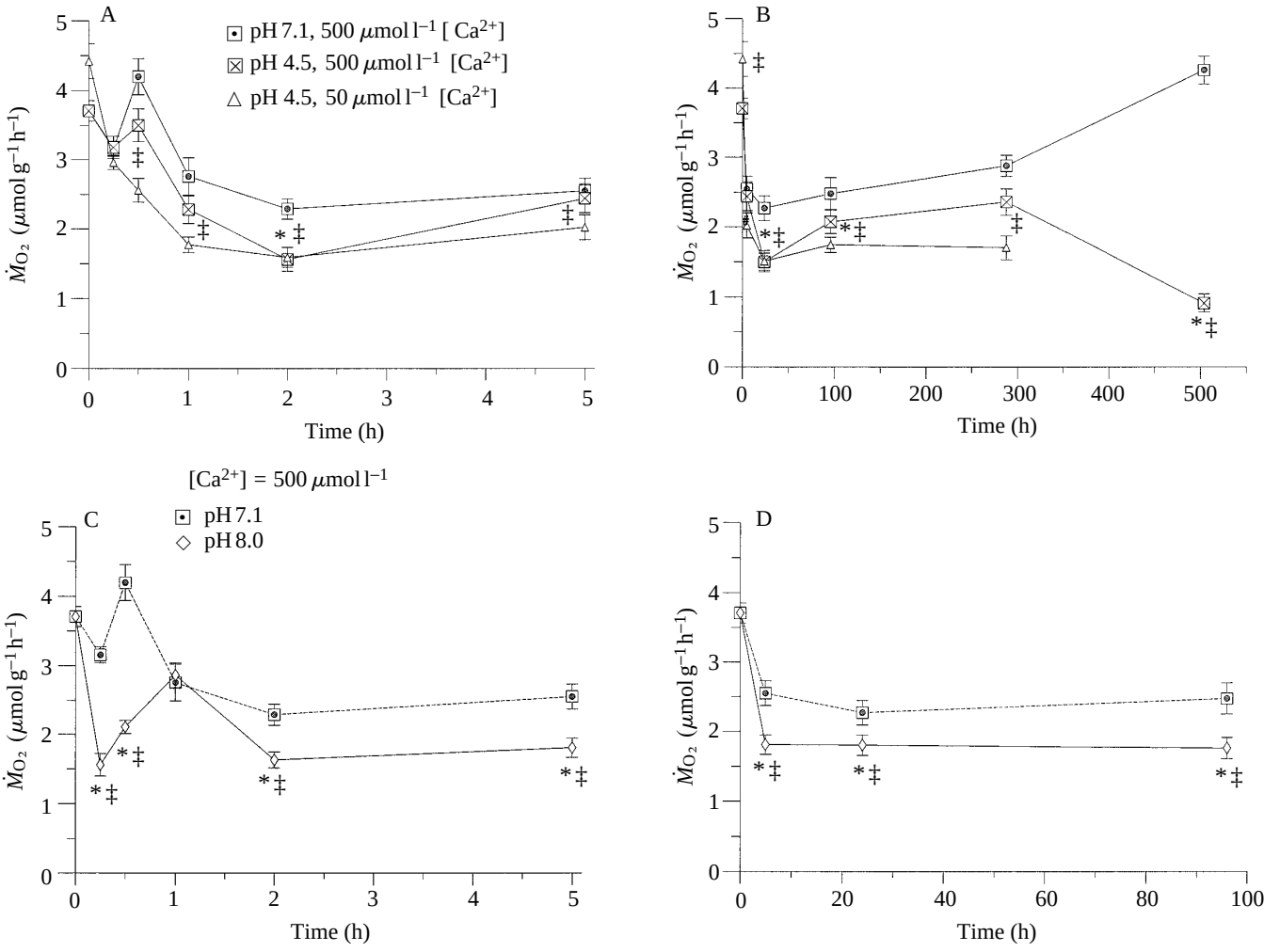

Fig. 2. Effects of pH and environmental [Ca^{2+}] concentrations on the respiratory gas exchange of *Cherax destructor*. The acute and chronic changes in $\dot{M}_{O_2}$ of *Cherax* held in either neutral water (pH 7.1 control, ⊡) or pH 4.5 water (50 μmol l^{-1} Ca^{2+}, △ compared with 500 μmol l^{-1} Ca^{2+}, ⊠) are shown in A and B respectively. The initial value for the pH 7.1 condition is obscured under the ⊠ symbol. * denotes points significantly different from the initial value; ‡ denotes a significant decrease in the $\dot{M}_{O_2}$ of animals held in the acid-exposure treatment containing 500 μmol l^{-1} Ca^{2+} compared with those held in pH 7.1, 500 μmol l^{-1} Ca^{2+} water. The $\dot{M}_{O_2}$ of animals held in the 50 μmol l^{-1} Ca^{2+} acid water decreased significantly at all times below the initial value (0 h). Acid exposure in 50 μmol l^{-1} Ca^{2+} water significantly reduced the $\dot{M}_{O_2}$ below that of animals in pH 4.5 high-Ca^{2+} water at all times over the 288 h treatment period, except at 0 h. The oxygen uptake rates ($\dot{M}_{O_2}$) of *Cherax destructor* held in pH 8.0 water containing a high [Ca^{2+}] (◇) are compared with the control condition (⊡, broken line in A and B). (C) Acute exposure; (D) chronic exposure. * represents a significant change from the initial value; ‡ denotes a significant decrease in $\dot{M}_{O_2}$ of animals exposed to the alkaline treatment compared with those in control conditions. Values are means ± S.E.M., N=228.

compared with those of animals in the control condition ($F_{8,88}$=4.74, Contrast) (Fig. 2B).

Interestingly, lowering the [Ca^{2+}] of the acid water further significantly reduced $\dot{M}_{O_2}$ below the initial value after 0.25 h; it then remained low and significantly less than that of control animals up to 288 h ($F_{8,44}$=11.76, Tukey) (Fig. 2A,B).

The haemolymph lactate concentrations of acid-exposed crayfish (500 μmol l^{-1} Ca^{2+} water) were significantly depressed below those in the control animals in both the acute (0.5 h, 1 h, 5 h; $F_{14,116}$=1.94, Contrast) and chronic responses (504 h; $F_{8,87}$=2.04) (Table 3).

Similarly, exposure of the crayfish to acid, low-Ca^{2+} water (pH 4.5) significantly depressed anaerobic metabolism below the lowest rate observed in animals from both the control condition after 0.5 h and 1 h ($F_{14,116}$=1.94, Contrast) and the high-Ca^{2+} acid water (treatment 1b) during a 288 h exposure ($F_{1,78}$=5.76) (Table 3), despite the already depressed haemolymph lactate concentrations recorded.

Alkaline exposure

Cherax held in alkaline water at pH 8.0 showed both significantly reduced $\dot{M}_{O_2}$ compared with the pre-exposure condition ($F_{9,48}$=3.64) and considerably depressed $\dot{M}_{O_2}$ compared with those in control animals for almost the entire 96 h exposure period ($F_{1,68}$=21.86) (Fig. 2C,D).

Lactate concentrations in animals exposed to pH 8.0 did not

Table 3. *Lactate concentrations (mmol l^{-1}) in the haemolymph of* Cherax destructor *during exposure to extreme pH in water containing 500 μmol l^{-1} [Ca^{2+}]*

Time (h)	High [Ca^{2+}]			Low [Ca^{2+}]
	pH 7.1	pH 4.5	pH 8.0	pH 4.5
0.00	0.722±0.166	0.722±0.166[a]	0.722±0.166	0.280±0.139[a]
0.25	1.032±0.186	1.082±0.191	0.518±0.094	0.317±0.076
0.50	1.294±0.153	0.523±0.110†	0.331±0.095†	0.752±0.200§
1	0.910±0.133	0.280±0.119†	0.835±0.161	0.608±0.106*,§
2	0.963±0.127	0.545±0.117	1.023±0.164	0.575±0.144
5	1.697±0.231	0.503±0.123†	0.362±0.102†	0.213±0.089§
24	0.560±0.109	0.597±0.113	0.380±0.111	0.272±0.084
96	0.633±0.111	0.760±0.168	1.283±0.186	0.187±0.059
288	1.483±0.197	0.523±0.133		0.460±0.102
504	0.989±0.147	0.475±0.141†		

The response of *Cherax* to acid water containing only 50 μmol l^{-1} [Ca^{2+}] is also shown (S.E.M. shown for each time).

Statistical analyses were carried out to test for significant changes over time within each treatment, and for significant differences between treatments.

* indicates a significant increased compared with t_0; † indicates a significantly reduced [lactate] compared with animals held in the pH 7.1, 500 μmol l^{-1} [Ca^{2+}] water at the specified sample time; §significantly different from pH 4.5, 500 μmol l^{-1} [Ca^{2+}] treatment value.

[a] indicates the effect of low-Ca^{2+} acclimation at neutral pH (t_0) on the [lactate] of *Cherax*.

change over time with respect to the initial value, but were significantly below those in control animals after 0.5 h (mean 0.33 and 1.29 mmol l^{-1} respectively) and 5 h ($F_{6,67}$=2.90) (Table 3).

Haemolymph glucose concentrations

Acid exposure

There was no significant change in the haemolymph glucose concentration of 'control' crayfish held in pH 7.1, 500 μmol l^{-1} Ca^{2+} water ($F_{9,49}$=0.21). Exposure to acid water (treatment 1b) produced a significant, but only transient, increase in haemolymph glucose concentration of *Cherax* between 1 h and 2 h compared with the initial value ($F_{9,49}$=4.67) and with values for animals held in the control condition ($F_{8,88}$=2.69, Contrast) (Fig. 3A, inset). At all other times, even over the long term, glucose concentrations in animals exposed to acid and neutral water were essentially equivalent (Fig. 3A).

However, acclimation of *Cherax* to low-Ca^{2+} neutral water (treatment 2b) significantly decreased the haemolymph glucose concentration compared with values for animals kept in the 500 μmol l^{-1} Ca^{2+} pH 7.1 water (0 h mean 0.15 mmol l^{-1} and 0.32 mmol l^{-1} respectively). This trend to decrease continued up to 5 h ($F_{8,86}$=3.46, Contrast). Interestingly, at 24 h, the low-Ca^{2+} acid water transiently but significantly increased the haemolymph glucose concentration of *Cherax* ($F_{8,43}$=3.75), which then returned to within the range of initial values (Fig. 3A).

Alkaline exposure

Animals exposed to pH 8.0 water exhibited variable haemolymph glucose concentration, but the glucose concentration declined progressively and after 5 h was significantly below control levels (Fig. 3B). Consequently, after 96 h, the haemolymph glucose concentration was significantly lower (mean 0.06 mmol l^{-1}) than the initial value (0 h) (mean 0.32 mmol l^{-1}) ($F_{7,38}$=2.32).

Discussion

Environmental pH

Oxygen uptake and transport

Crustaceans completely at rest are occasionally referred to as 'sleeping' since they exhibit very low P_{aO_2} values, within the range 7–22 mmHg (Forgue *et al.* 1992). The transient increases in P_{aO_2} of *Cherax destructor* held in either acid or neutral 500 μmol l^{-1} Ca^{2+} water during the first 0.25–1 h were, however, relatively small (35 and 20 mmHg respectively) and indicative of a classic 'startle' response. This conclusion was supported by a decreased P_{CO_2} (Ellis and Morris, 1995) and an apparent decrease in a–v O_2 content difference. However, the absence of any decrease in $\dot{M}_{O_2}$ or increase in haemolymph lactate suggests that any hypoxia and/or anaerobiosis is minor.

A similar increase in P_{aO_2} with low a–v content difference, after 288 h of acid exposure (500 μmol l^{-1} Ca^{2+}) of *Cherax*, occurred without changes in haemolymph CO_2 content or pH (Ellis and Morris, 1995). Furthermore, there were no increases in $\dot{M}_{O_2}$ or circulating L-lactate concentration and thus there is no evidence for increased metabolic rate. Chronic exposure of *Cherax* to pH 4.5 water (500 μmol l^{-1} Ca^{2+}) reduced extraction of O_2 by the tissues from the haemolymph to effectively zero. Since $\dot{M}_{O_2}$ decreased significantly both with exposure time and with respect to 'control' animals, concomitant with a real reduction in circulating lactate concentration, the animals were clearly hypometabolic with respect to crayfish in pH 7.1 water (cf. sleeping crayfish, Forgue *et al.* 1992).

Mild stress or sudden disturbance is often met by complete

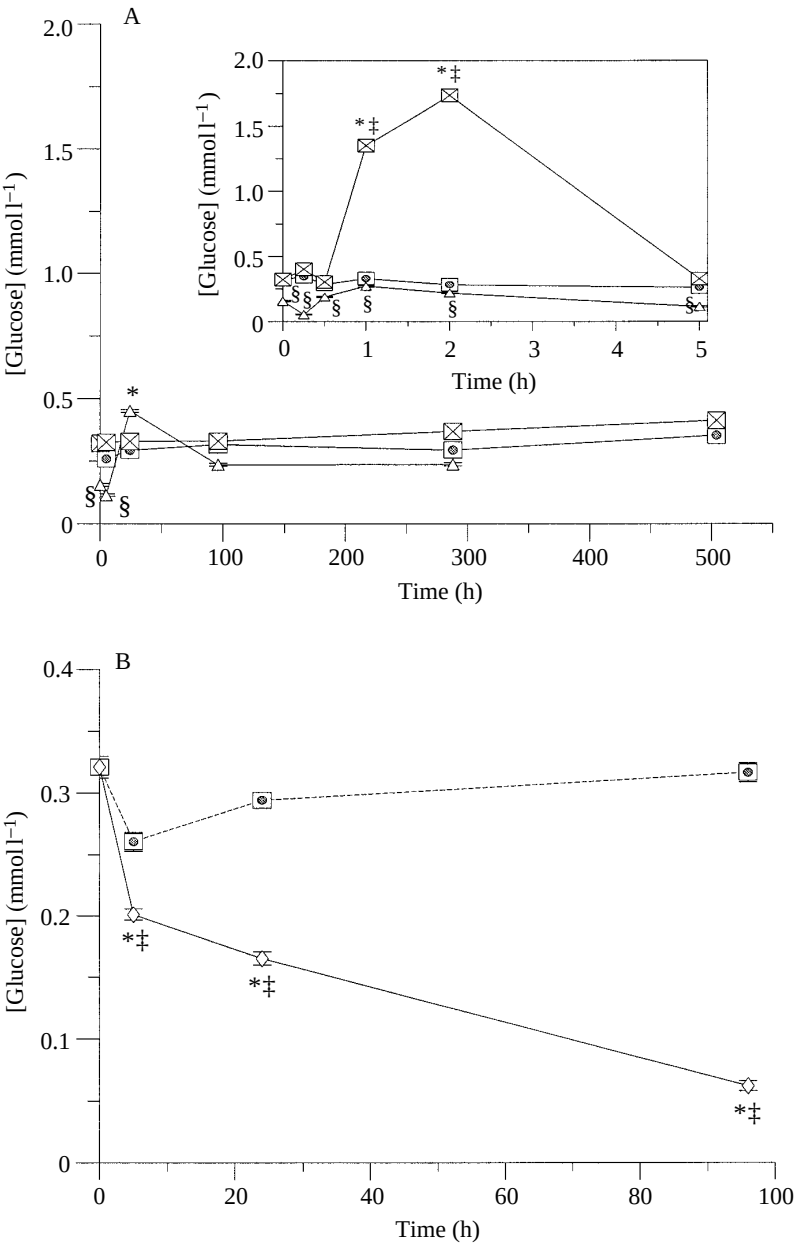

Fig. 3. Haemolymph glucose concentrations of *Cherax destructor* during a 504 h exposure to (A) high-Ca^{2+} water at pH 7.1 (control, ⊡) and pH 4.5 (⊠). Inset: the acute (5 h) response. The figures also demonstrate the effects of water hardness ($[Ca^{2+}]$) on the haemolymph glucose concentrations during 5 h and 288 h exposures to low-Ca^{2+} pH 4.5 water (△). Chronic effects of high-Ca^{2+} alkaline water (◇, pH 8.0) on the haemolymph glucose concentrations of *Cherax destructor* compared with the control condition (as in A) are shown in B. Note the different *y*-axis scales used for A and B. * represents a significant change from the initial value; ‡ represents a significant difference between the haemolymph glucose concentrations of animals held in extreme pH water (500 μmol l^{-1} Ca^{2+}) compared with crayfish held in neutral water; § indicates significantly lower haemolymph glucose concentrations of animals held in high-Ca^{2+} water compared to low-Ca^{2+} water. Error bars are sometimes obscured by the symbols. Values are means ± S.E.M., *N*=228.

O_2 saturation of the respiratory pigment (Piiper, 1986). Circulating Ca^{2+} and L-lactate potentiate the O_2 affinity of *Cherax* haemocyanin ($\Delta\log P_{50}/\Delta\log[\text{lactate}] = -0.12$, $\Delta\log P_{50}/\Delta\log[Ca^{2+}] = -0.63$; S. Morris and J. Callaghan, in preparation) such that, over the pH range and the Ca^{2+} and L-lactate concentration ranges in the haemolymph of pH-challenged animals, the P_{50} varied from 2.6 to 3.5 mmHg and the P_{95} from 6.2 to 8.1. Therefore, the increase in Pa_{O_2} from 11.1 mmHg after 96 h to 42.4 mmHg after 288 h of acid exposure would encourage 100 % haemocyanin O_2-saturation (compare with acid-exposed *Astacus astacus*, which exhibit greater than 95 % saturation, Jensen and Malte, 1990). Pigment saturation reduces the role of haemocyanin in O_2 transport (McMahon and Wilkens, 1983). Similarly, increased haemolymph pH and $[Ca^{2+}]$ in alkaline-exposed animals would increase oxygen affinity. Thus, in both acid- and alkaline-exposed *Cherax*, relatively high O_2 affinity and elevated P_{O_2} of the haemolymph lead to reduced a–v O_2 content differences, which is quite different from the true 'quiescent' state (see Forgue *et al.* 1992). Unlike some crayfish species (e.g. *Procambarus clarkii*; Patterson and Defur, 1988), haemolymph pH may, *via* haemocyanin O_2-affinity, be an important component of the hypometabolic response in *Cherax destructor*.

Effects of acid water on energy metabolism

At rest, respiratory gas exchange and transport are matched to the metabolic rate (Piiper, 1986). Acid soft water environments depressed the major aerobic and anaerobic metabolic pathways in *Cherax destructor* compared with those in similarly treated animals at pH 7.1 (i.e. not a starvation effect). The extent of this depression also depended on the

environmental calcium concentration. Facultative hypometabolism is often a mechanism for eluding harsh environmental conditions (e.g. Brooks and Storey, 1989; Storey, 1987). The 75 % decrease in oxygen uptake rate (compared with 0 h) of *Cherax* in high-Ca^{2+} pH 4.5 water is comparable, for example, to 50–85 % decreases in aestivating lungfish (Swan *et al.* 1968) and 85 % decreases in diving turtles (Jackson, 1968). Metabolic depression may be maladaptive in part, since it seems that ion balance requiring ATP may be disrupted at a time when salt regulation is also challenged (Spaargaren, 1975; Mangum, 1986; Ellis and Morris, 1995).

Assuming that 1 mol of O_2 taken up by the tissues produces 6.33 mol of ATP during aerobic metabolism (Herreid and Full, 1984), the mass-specific energy expenditure of *Cherax* over a 504 h (21 day) exposure is only 7.5×10^3 mol of ATP in acid water compared with 9.8×10^3 mol of ATP in neutral water, conserving 2.3×10^3 mol of ATP. The hypometabolic state can therefore be viewed as a basic survival strategy (Storey, 1987), economizing on fuel reserves.

The measured 'resting' haemolymph glucose concentrations of *Cherax destructor* were within the 'normal' range (<0.56 mmol l^{-1}; Telford, 1975) and comparable with previously observed levels for *Cherax destructor* (0.22 mmol l^{-1}) (B. A. Ellis and S. Morris, unpublished data) and for other crayfish species (Telford, 1974). Elevation of haemolymph glucose concentration is a common response in decapods during stress (Telford, 1974) and up to tenfold increases have been recorded (Sedlmeier and Keller, 1981). The transient, yet considerable, hyperglycaemia in *Cherax* after 2 h in acid 500 μmol l^{-1} Ca^{2+} water (1.73 mmol l^{-1}) was higher than those observed in *Nephrops norvegicus* caused by handling and asphyxiation stresses (1.38 mmol l^{-1}) (Spicer *et al.* 1990) and in *Orconectes propinquus* during exposure to 2,4-dichlorophenol (0.68 mmol l^{-1}) (Telford, 1974).

An increased glucose concentration implies decreased glycolysis relative to glycogenolysis and would explain the almost immediate depression of aerobic metabolism in *Cherax* (within 2 h). The recovery of haemolymph glucose concentrations within 5 h (see Keller and Andrew, 1973) depends on achieving a new balance between glucose mobilisation, glucose utilisation and possibly glycogen synthesis. This biphasic pattern, characterized by an initial 'startle' and then a protracted phase, is common during environmental fluctuations (McMahon and Wilkens, 1983). Animals were noticeably 'lethargic' after 504 h (21 days) of exposure to acid water but not after a similar period in 'control' conditions (B. A. Ellis and S. Morris, personal observation). Thus, glucose mobilisation must ultimately slow to match reduced aerobic glycolysis, representing slowed flux, and therefore reduced scope for activity and perhaps even growth. Hypoactivity and cessation of feeding are common behaviour traits in fish during environmental acidification, especially in the pH range 4.5–4.75 (Peterson and Martin-Robichaud, 1983; Jones *et al.* 1985). Slower and less complete moult cycles of the crayfish *Orconectes rusticus* and *Astacus astacus* (France, 1981) in naturally acid waters support this suggestion.

Anaerobiosis requires significantly increased glycolysis, while lactate concentrations can potentially increase 40- to 60-fold in decapods during environmental stress (Tyler-Jones and Taylor, 1988; Van Aardt and Wolmarans, 1987; Lowery and Tate, 1986). In most crayfish species, acidification does not promote accumulation of lactate (e.g. Morgan and McMahon, 1982; *Astacus astacus*, Jensen and Malte, 1990). In *Cherax*, L-lactate concentrations did not increase (compared with the control condition) ($F_{2,116}$=11.333, Contrast), showing the animals to be aerobically competent but, together with decreased $\dot{M}_{O_2}$, suggesting metabolic 'shut-down' (Storey, 1987).

Effects of alkaline water on energy metabolism

Alkaline water produced the most rapid and extensive depression of metabolism, as shown by the immediate and sustained depression of $\dot{M}_{O_2}$ and circulating [lactate]. Also, while *Cherax* exposed to pH 4.5 (500 μmol l^{-1} Ca^{2+}) showed a 'normal' stress-induced hyperglycaemia, alkaline exposure induced a progressive severe hypoglycaemia such that after 96 h haemolymph glucose concentration had fallen by 80 %. Sustained imbalances in the rates of metabolic processes signify irreversible damage (Storey, 1988) and long-term survival may be unlikely.

The effect of environmental calcium

Low environmental calcium concentrations have been proposed to increase the energy expenditure required for homeostasis (McDonald and Milligan, 1988). Indeed, acclimation of *Cherax* to low-Ca^{2+} water at neutral pH significantly increased $\dot{M}_{O_2}$, consistent with decreased haemolymph glucose concentrations. However, haemolymph oxygen levels, $\dot{M}_{O_2}$ and haemolymph lactate levels provide strong evidence that lowering the [Ca^{2+}] of acid water from 500 to 50 μmol l^{-1} both accelerates and amplifies the effects of acid exposure. This is supported by the 28 % decrease in $\dot{M}_{O_2}$ of *Cherax* held in 50 μmol l^{-1} water relative to that of animals in high-Ca^{2+} acid water and the 62 % decrease in $\dot{M}_{O_2}$ compared with the initial value at pH 7.1. The mechanism for such an exacerbation of metabolic depression during low-Ca^{2+} acid exposure is unknown. This Ca^{2+} effect is important, since the [Ca^{2+}] of many natural water bodies and rivers is extremely low due to the binding of Ca^{2+} by humic acids produced in the soil (Hargeby, 1990).

Cherax held in acid (50 μmol l^{-1} Ca^{2+}) water had a surprisingly higher haemolymph pH and haemolymph Ca^{2+} concentration than animals held at neutral pH in water with ten times the amount of Ca^{2+} (see Ellis and Morris, 1995). These changes in haemolymph conditions during exposure to acid low-Ca^{2+} water would cause a leftward shift of the O_2 equilibrium curve, thereby explaining the higher C_{vO_2} but quite similar P_{O_2} values compared with those of crayfish in pH 7.1, high-Ca^{2+} water (see McMahon and Wilkens, 1983). The mean a–v C_{O_2} difference of crayfish during the 288 h of acid exposure was only 0.07 mmol l^{-1}. High Ca^{2+} concentration in acid water therefore probably acts by some mechanism to increase haemolymph P_{O_2} and thus oxygenation.

To conclude, acid and alkaline waters are indeed challenging environments for *Cherax destructor* and probably reduce growth and fecundity, but do not promote increased energy expenditure for homeostasis (see Neville, 1985; Hargeby, 1990). It seems unlikely that the energy-conserving responses to extreme pH could form the basis of any long-term survival strategy, although clearly they would enable the animals to survive transient fluctuations in environmental pH. Given the increasing frequency with which such abnormal disturbances of the environment are occurring, considerable further studies of both the effect of extreme pH and the hypometabolic response are required.

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