

ACID TOLERANCE AND EFFECTS OF SUBLETHAL ACID EXPOSURE ON IONO-REGULATION AND ACID-BASE STATUS IN TWO CRAYFISH *PROCAMBARUS CLARKI* AND *ORCONECTES RUSTICUS*

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(Received 14 May 1981)

SUMMARY

Acid-toxicity tests were performed using two crayfish species, *Procambarus clarki* Girard and *Orconectes rusticus* Girard to determine the median lethal pH (LC_{50}) after 4 days exposure to acid. Four-day LC_{50} values of pH 2.5-2.8 were observed, indicating that these animals are more acid-tolerant than most fish species. Haemolymph acid-base variables and major ion concentrations were measured during 4 days exposure of *P. clarki* to sublethal acid H_2SO_4 , pH 3.8 levels. A major haemolymph acidosis was observed. While minimal changes were seen in haemolymph $[Cl^-]$, $[K^+]$, and $[Mg^{2+}]$, haemolymph $[Na^+]$ decreased. Haemolymph $[Ca^{2+}]$ increased significantly during acid exposure, suggesting that dissolution of exoskeletal carbonate buffers was being employed as a compensatory mechanism for the acidosis; this process would be expected to contribute to exoskeletal rigidity problems in the long term.

INTRODUCTION

Acid precipitation, resulting from the conversion of airborne sulphur and nitrogen dioxide pollutants to sulphuric and nitric acids, has been observed in a number of the industrialized regions of North America and Europe (Likens & Bormann, 1974; Jeffries, Cox & Dillon, 1979). The damaging effects are particularly apparent in those areas where rock and soil types are such that local freshwater possesses a limited buffering capacity; in these regions, acidification is a growing threat to freshwater ecosystems. Losses of freshwater fish populations in the affected areas have been well documented (Leivestad & Muniz, 1976; Beamish & Harvey, 1972), and considerable invertebrate population decreases have also been observed (Almer *et al.* 1974; Sprules, 1975).

A number of recent studies have dealt with the physiological effects of acid exposure in fish. These effects include disturbances in blood acid-base status (Packer, 1979; Neville, 1979a; McDonald, Hobe & Wood, 1980), ionic balance (Leivestad & Muniz, 1976; Packer & Dunson, 1970, 1972; McDonald *et al.* 1980; Hobe *et al.* 1980), and oxygen uptake and transport (Packer, 1979). Physiological responses to acid exposure

reported in the literature vary widely, but Neville (1979*b, c*) has shown that some of the confusion may be due to the unrecorded presence of hypercapnia in some experiments; McDonald *et al.* (1980) add that differences in environmental calcium levels can also create pronounced differences in responses to acid.

Although invertebrates constitute an important part of aquatic ecosystems affected by acid precipitation, and acid-related population decreases have been observed (Almer *et al.* 1974; Sprules, 1975), much less is known of basic physiological responses of any invertebrate to acid exposure. Several crayfish species inhabit waters potentially affected by acid precipitation and as basic respiratory physiology, acid base and osmotic and iono-regulatory processes of these animals are relatively well studied, two species were selected for a study of the physiological effects of acid exposure. A preliminary series of experiments was used to determine lethal levels of acidity in both *Procambarus clarki* and *Orconectes rusticus*; secondly, changes in haemolymph acid-base status and in ionic regulation have been investigated during sublethal acid exposure in *Procambarus clarki*.

MATERIALS AND METHODS

Experimental animals

Adult *Procambarus clarki* Girard and *Orconectes rusticus* Girard of both sexes were obtained from commercial suppliers in northern California and southern Ontario, respectively; animals were held at least 2 weeks prior to experimentation in running Calgary tap water at 15 °C. Analysis of this water using techniques described below yielded the following ionic composition: Na⁺, 0.08; K⁺, 0.02; Ca²⁺, 1.1; Mg²⁺, 0.6; Cl⁻, 0.5; SO₄²⁻, 0.03 (all values are mM). Animals were fed regularly before but not during the toxicity tests; in the physiological studies, which involved a 2-week acclimation period in decarbonated water (see below), animals were not fed during the second week of acclimation or during the subsequent acid exposure. All crayfish used were in the intermoult stage of the moulting cycle, as judged by exoskeleton rigidity (Vranckx & Durliat, 1978).

For the physiological studies, large adult *Procambarus clarki* (40–60 g) were prepared for post-branchial haemolymph sampling by drilling a pair of small holes in the exoskeleton on opposite sides of the carapace midline above the pericardial sinus; holes were sealed with a rubber sheet glued to the carapace, and at least 4 days of recovery in normal tap water were allowed before experimentation.

Toxicity tests

Small adult *Procambarus clarki* and *Orconectes rusticus* (10–25 g) were exposed to a variety of acid levels for 4 days to estimate LC₅₀, the pH at which 50% mortality occurs. For these tests, and all other experiments in the present work, acidification was accomplished using sulphuric acid, the more common acid pollutant. Beamish (1972) for white sucker, Packer & Dunson (1972) for brook trout and Graham & Wood (1981) for rainbow trout all found H₂SO₄ generally slightly less toxic than HCl although the latter authors point out that the difference is variable depending on Ca²⁺ levels and probably other environmental factors.

For each toxicity test, nine or ten animals were placed in each of a series of tanks containing 12 l of static, well-aerated, charcoal-filtered tap water at 15 ± 0.5 °C. The pH levels in all tanks (except a single control) were lowered to the desired values 24 h prior to the experiment, to allow removal of CO₂ (aeration) resulting from titration of water bicarbonate.

P. clarki were exposed to pH 2.0, 2.5, 3.0, and 3.5, while *O. rusticus* were exposed to pH 2.0, 2.3, 2.6, and 3.0 (pH values were measured twice daily and maintained within 0.05 unit). Mortality, judged by a complete lack of response to stimulation of eyestalks, tail, or elsewhere, was recorded after 24, 36, 48 and 96 h of exposure. In both tests, no mortality was observed in groups of control animals maintained at pH 7.5–8.0. Using the mortality observed in these tests, LC₅₀ was estimated for each of the four exposure times using the Reed–Muench method as described by Woolf (1968).

Sublethal acid exposure experiments

Physiological studies were carried out in large non-recirculating water tanks at 15 ± 0.5 °C. Animals were first acclimated for 2 weeks in a shallow tank containing 95 l of tap water which had been decarbonated by lowering of pH to 3.0, 24 h of aeration to remove CO₂, and readjustment of pH to 7.5–8.0 using 10 M-NaOH. The acclimation tank was well aerated and charcoal-filtered, and the water was replaced with fresh decarbonated water every 3 days to prevent build-up of toxic wastes. After the 2-week acclimation period, half of the animals were placed in a second tank containing 120 l of tap water acidified to pH 3.8; this tank was also well aerated and charcoal-filtered, and acidification was carried out 24 h prior to placement of animals in the tank to allow CO₂ removal. pH 3.8 was selected using the results of the toxicity test above; it is 1 unit higher than the LC₅₀ at 4 days and is therefore a strong, but sublethal, level of acidity.

Acid exposure continued for 4 days, following which the animals were returned to the decarbonated water tank for a 4-day recovery period. Control animals remained in the decarbonated water throughout.

Haemolymph samples (80–100 µl) were withdrawn using gas-tight 100 µl Hamilton syringes. In order to obtain all desired haemolymph parameters and avoid the detrimental effects of excessive haemolymph removal, two groups of 11 animals (5 controls, 6 experimental animals) were used simultaneously. Samples from one group were analysed for pH and total CO₂, while samples from the second group were analysed for the concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻. Determination of pH, total CO₂, and [Cl⁻] were performed on freshly withdrawn haemolymph, but 50 µl aliquots of haemolymph were snap frozen in a dry-ice/acetone bath to allow cation determinations at a later date (see Analytical Procedures below).

In order to further minimise excessive haemolymph removal and sampling stress, samples were withdrawn only once in each 3- or 4-day period. Thus, the 'day zero' baseline measurements were actually made on samples withdrawn 2 days prior to acid exposure. Subsequent samples were taken after 1 and 4 days of acid exposure, followed by a final sampling after 4 days of recovery. Prior to all experiments, samples were withdrawn from crayfish in normal tap water for comparison with later samples from animals acclimated to decarbonated water.

Analytical procedures

Water pH was measured on samples at the experimental temperature, using a Fisher Accumet 140A pH meter and combination electrode, normally calibrated with Fisher buffers (pH 4.0 and 6.8); additional calibration with freshly mixed 1.00×10^{-2} M. HCl was employed during measurement of extremely acid samples.

Haemolymph pH was determined on 40 μ l aliquots using a liquid junction micro-electrode (Radiometer 299A, thermostatted to 15 °C), displayed on an acid base analyser (Radiometer PHM 73). This system was calibrated with precision buffers (Radiometer S1500 and S1510) corrected to 15°. Total CO₂ content of haemolymph was determined on 25 μ l aliquots using the micro-method of Cameron (1971), using bracketing of samples with sodium bicarbonate standards to improve accuracy. Radiometer CO₂ electrodes and a PHM 73 analyser were used.

Concentrations of Na⁺, K⁺, Ca²⁺, and Mg²⁺ were determined on a single 50 μ l sample, using a Jarrell-Ash Model 850 atomic absorption spectrophotometer as follows. Samples were diluted and divided such that each ion determination was made on the appropriate dilution: 1:4000 for Na⁺, 1:100 for K⁺, 1:400 for Ca²⁺, and 1:100 for Mg²⁺. Addition of excess CsCl to Na⁺ and K⁺ samples and LaCl₃ to Ca²⁺ and Mg²⁺ samples was employed to reduce chemical interference. Chloride determinations were made on 25 μ l samples using a digital chloridometer (Buchler 4-2500). Measurements of SO₄²⁻ were made using a technique based on that of Berglund & Sörbo (1960).

Calculations

Using measured values of pH and total CO₂ content (C_{CO_2}), P_{CO_2} was calculated using a method identical to that used for *Orconectes rusticus* by Wilkes, deFur & McMahon (1980); this technique provides a more accurate alternative to methods utilizing the Henderson-Hasselbalch equation (e.g. Truchot, 1976). Using these calculated P_{CO_2} values and a CO₂ solubility coefficient (α_{CO_2}) from the nomograms of Truchot (1976), $[HCO_3^- + CO_3^{2-}]$ could be calculated from the equation: $C_{CO_2} = \alpha_{CO_2} \cdot P_{CO_2} + HCO_3^- + CO_3^{2-}$. McDonald, McMahon & Wood (1979) point out that the dissociation of HCO₃ to CO₃²⁻ and H⁺ is not negligible in crustaceans at cool temperatures: thus, $[CO_3^{2-}]$ is included with $[HCO_3^-]$ in the display of data in the present study.

Statistical analysis

Differences in toxicity of acid between the two species were analysed using a 2-tailed Student's *t* test of the means. In the physiological studies, differences within the experimental or control groups were tested with a 2-tailed Student's *t* test (paired design). In those measurement sets where control animals exhibited a significant change between samples, an additional test was used. Here, the mean difference between experimental samples was compared with the mean difference between equivalent control samples. Again a Student's *t* test of the means was employed. Throughout the analysis, $P < 0.05$ was judged significant.

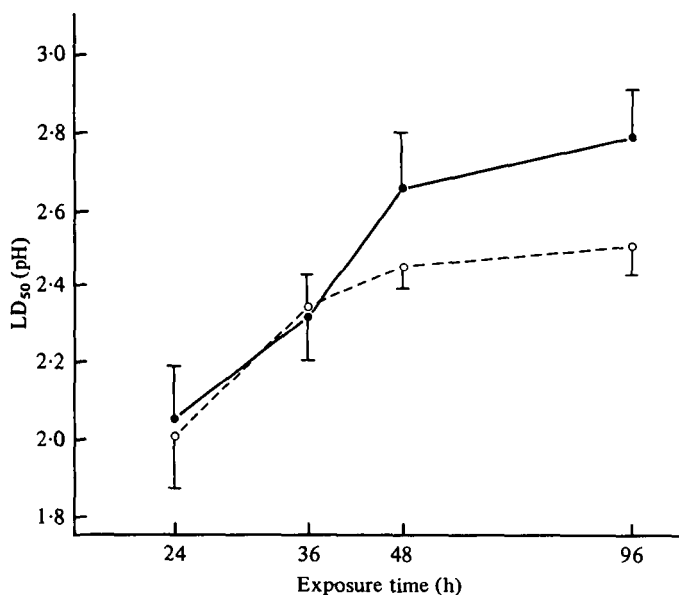


Fig. 1. Relationship between media lethal pH (LC_{50}) and exposure time for *Procambarus clarki* (●—●) and *Orconectes rusticus* (○---○). Values are means ± 1 S.E.M.

Table 1. *Haemolymph parameters of Procambarus clarki in tap water and after 2 weeks acclimation to decarbonated water*

(Means \pm one S.E.M. (n). Ions are in mM.)

	Tap water	Decarbonated water
pH	7.930 \pm 0.22 (15)	8.167 \pm 0.033 (13)*
[HCO ₃ ⁻ + CO ₃ ²⁻]	9.94 \pm 0.56 (15)	17.78 \pm 1.67 (12)*
P _{CO₂} (torr)	3.64 \pm 0.18 (15)	3.28 \pm 0.15 (12)
[Na ⁺]	166.4 \pm 5.9 (14)	169.5 \pm 9.6 (13)
[Cl ⁻]	196.5 \pm 4.3 (15)	128.9 \pm 10.3 (13)*
[K ⁺]	4.19 \pm 0.15 (14)	3.70 \pm 0.21 (12)
[Ca ²⁺]	9.15 \pm 0.54 (14)	6.65 \pm 0.64 (13)*
[Mg ²⁺]	2.69 \pm 0.15 (14)	2.26 \pm 0.93 (12)
SO ₄ ²⁻	2.43 \pm 0.30 (15)	2.26 \pm 0.31 (6)†

* Significantly different from tap water value ($P < 0.05$).

† Decarbonated water for only 120 h, separate experimental series.

RESULTS

Toxicity tests

The purpose of these tests was not to provide a detailed analysis of acid toxicity in crayfish, but rather to provide an indication of sublethal levels of acid for use in subsequent physiological studies. LC_{50} values at 4 days of exposure were pH 2.8 and 2.5 for *Procambarus clarki* and *Orconectes rusticus*, respectively (Fig. 1); there were no significant differences between the two species at any exposure time.

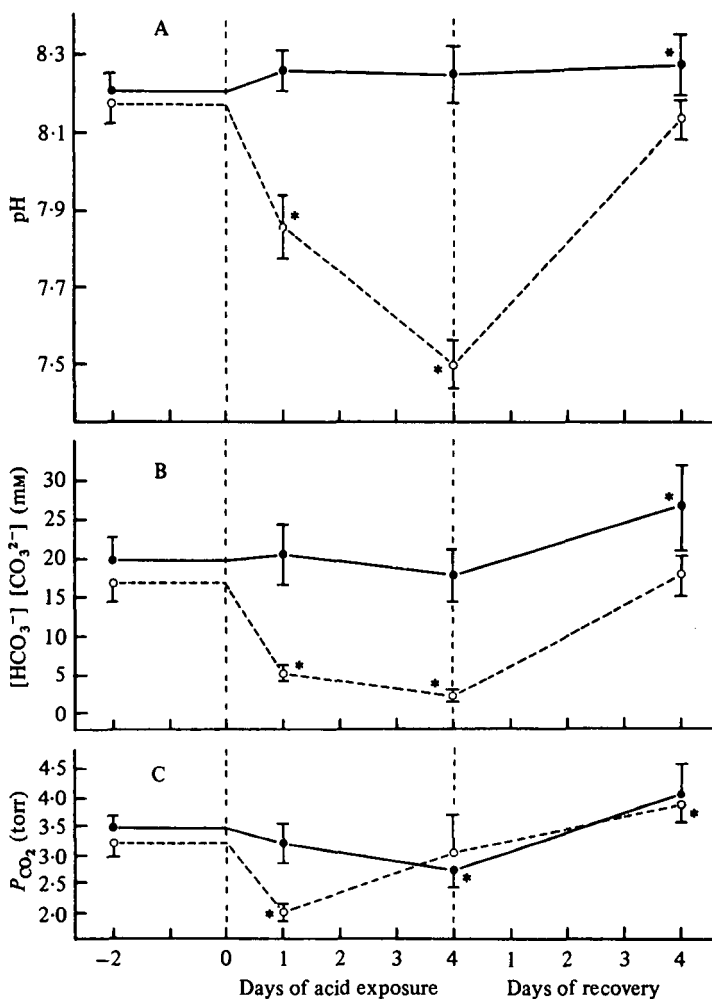


Fig. 2. Haemolymph pH (A), bicarbonate concentration (B), and CO_2 tension (C) (means ± 1 S.E.M.) in *Procambarus clarki* acclimated to decarbonated water. Two days after the initial sample in decarbonated water, experimental animals (\bigcirc --- \bigcirc , $n = 6$) were transferred to a pH 3.8 environment for 4 days, followed by a 4-day recovery in decarbonated water. Control animals (\bullet — \bullet , $n = 5$) remained in decarbonated water throughout. Asterisks indicate a significant difference ($P < 0.05$, paired t test) from initial (day -2) values.

Physiological studies

(i) *Acclimation to decarbonated water.* Prior to carrying out the acid exposure experiment, a comparison was made of haemolymph parameters of animals in running tap water and after the 2-week acclimation period in decarbonated water (Table 1). An increase in pH and $[HCO_3^- + CO_3^{2-}]$, together with unchanged P_{CO_2} , indicates that the animals underwent a significant metabolic alkalosis during the acclimation period. In addition, chloride and calcium decreased significantly. As a result of the readjustment of pH following decarbonation (see Methods), Na^+ levels of decarbonated water

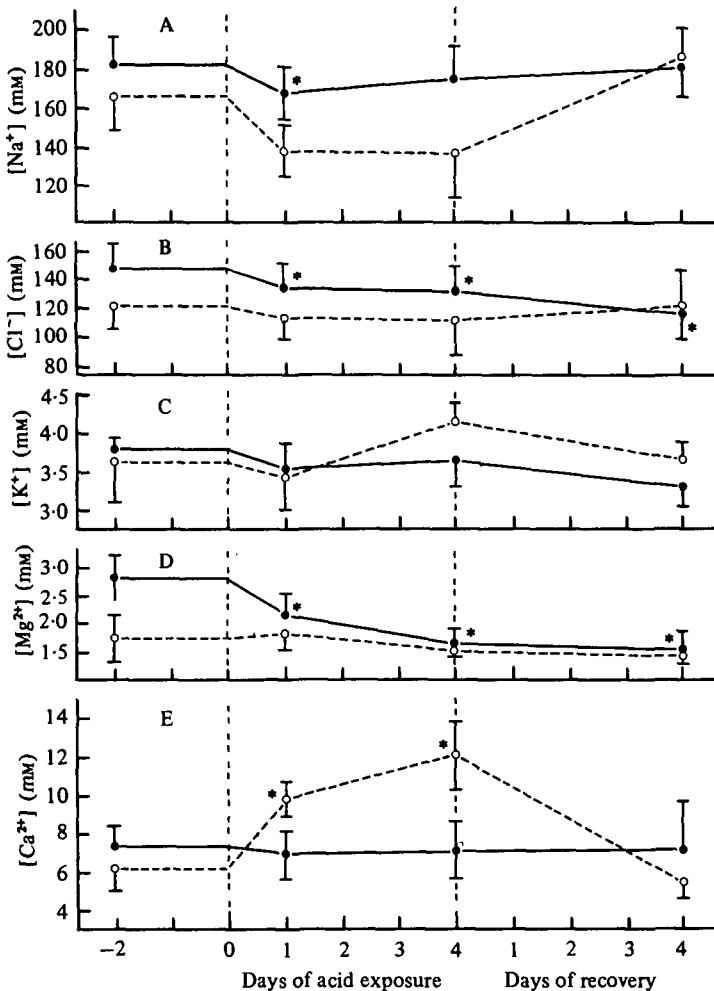


Fig. 3. Haemolymph concentrations of Na^+ , Cl^- , K^+ , Mg^{2+} , Ca^{2+} , (means \pm one S.E.M.) in *Procambarus clarkii* acclimated to decarbonated water. Two days after the initial sample in decarbonated water, experimental animals (\bigcirc --- \bigcirc , $n = 6$) were transferred to a pH 3.8 environment for 4 days, followed by a 4 day recovery in decarbonated water. Control animals (\bullet — \bullet , $n = 5$) remained in decarbonated water throughout. Asterisks indicate a significant difference ($P < 0.05$, paired 't' test) from initial (day -2) values.

(3 mm) were substantially elevated over tap water (0.1 mm) levels. This increase in Na^+ may have contributed in some way to the alkalosis observed.

(ii) *Acid exposure.* Exposure of these acclimated animals to pH 3.8 resulted in significant decreases in post-branchial haemolymph pH and $[\text{HCO}_3^- + \text{CO}_3^{2-}]$ (Fig. 2A, B). No increase in P_{CO_2} was observed during acid exposure (Fig. 2C); thus, the acidosis was entirely metabolic. The 4 days of recovery resulted in a return of pH and $[\text{HCO}_3^- + \text{CO}_3^{2-}]$ to pre-exposure levels, although the control animals also underwent a small but significant metabolic alkalosis during this time. P_{CO_2} of acid stressed animals was significantly reduced at 24 h, but throughout the experiment

P_{CO_2} levels were extremely variable in both experimental and control animals and, no clear trends were apparent in the data (Fig. 2C).

In general, acid exposure caused only mild disturbance in major haemolymph ion concentrations (Fig. 3). There were significant increases in calcium levels during acid exposure; no significant changes occurred in any other ion level, although, sodium, chloride, and magnesium all decreased significantly in the control animals. It is possible that these decreases in ion levels in the control animals obscured concomitant increases in ion levels in the experimental animals; in order to test this possibility, a second statistical test was performed, in which mean differences between control and experimental samples were compared (see Methods). Using this test, there were no significant differences between controls and experimental animals in any ion level in which controls underwent a significant decrease.

During recovery, the mean rise in sodium levels in the experimental animals was significantly different from the corresponding change in the controls. This apparent rise in sodium levels during recovery suggests that these were depressed (but not significantly) during acid exposure. It should be realized that ion levels in crustaceans are extremely variable in all situations, and it is quite possible that a slight decrease in sodium level occurred during acid exposure but was obscured by this variability.

Chloride levels decreased significantly in the controls during recovery, but this decrease was not significantly different from the mean change in the experimental animals over the same period of time. Acidification of water with H_2SO_4 resulted in a 5-fold increase in $[SO_3^{2-}]$ over tap-water levels. This increase was not reflected in haemolymph where no significant increase in $[SO_4^{2-}]$ could be demonstrated.

Following return to 'neutral' waters pre-exposure acid-base status and haemolymph ionic levels are accurately re-established within 4 days (Fig. 2, 3). No permanent acid-base or ionic imbalance thus resulted from short-term exposure to pH 3.8.

DISCUSSION

Crayfish acid tolerance

The toxicity tests performed in the present work, together with evidence from previous studies (Beamish, 1974; Newcombe, 1975), suggest that adult intermoult crayfish are remarkably acid tolerant. The 96 h LC_{50} values of 2.8 and 2.5 for the two species studied (Fig. 1) are considerably lower than those commonly found in fish; for example, Beamish (1972) reports an LC_{50} of pH 3.9 at 100 h for white sucker, Daye & Garside (1975) report an LC_{50} of pH 3.5 at 167 h for brook trout, while McDonald *et al.* (1980) report pH 4.0–4.2 as the 96 h LC_{50} and Graham & Wood (1981) report pH 4.1–4.5 as 167 h LC_{50} for rainbow trout. Compared with other fish, cardinal tetras appear to be relatively acid-tolerant, since Dunson, Swartz & Silvestri (1977) report that they survive indefinitely at pH 3.5, but, in general, the crayfish studied in the present work are among the most tolerant animals investigated.

The high tolerance of adult intermoult crayfish may be offset by the greater sensitivity of postmoult (Malley, 1980) and juvenile (R. France, pers. comm.) animals. France (pers. comm.) also notes that female reproductive success may be hindered in acid environments. Thus, a proper evaluation of acid sensitivity in these animals would require a broad range of studies involving all aspects of the crayfish life-cycle.

Acclimation to decarbonated water

The marked metabolic alkalosis which developed during acclimation to decarbonated water (Table 1) is puzzling. Since decarbonation resulted in a rise in water $[Na^+]$ from 0.08 to about 3 mM, increased influx of Na^+ via an Na^+/H^+ exchange may have been involved. In crayfish, Short & Haswell (1979) have reported that Na^+ -free environments lead to acidosis, while in rainbow trout, Perry *et al.* (1981) report that an increase of water $[Na^+]$ from 0.04 to 3 mM led to metabolic alkalosis but also to a 10% rise in plasma $[Na^+]$. No change in haemolymph $[Na^+]$ was seen in the present study (Table 1), and it is not clear to what extent increased Na^+ influx contributed to the observed alkalosis. Further work with ion flux measurements will be necessary before definite conclusions can be made.

Sublethal acid exposure

The magnitude of the haemolymph acidosis resulting from sublethal acid (H_2SO_4) exposure of crayfish (Fig. 2) is similar to that reported for the rainbow trout (Neville, 1979*a*) but more severe than that reported for white sucker (H_2SO_4 ; Hobe *et al.* 1980). McDonald *et al.* (1980) observed acidosis in rainbow trout, but noted that pronounced acidosis occurred only in high $[Ca^{2+}]$ waters (1.6–2.7 m-equiv/l), similar to those used in the present study (see Methods).

The additional H^+ ions may have come from two sources: internal (i.e. lactate) or external (i.e. H^+ influx from the environment). Packer (1979) found decreased gill oxygen transfer and blood oxygen capacity in brook trout acutely exposed to severe acid levels, and he suggested that lactate arising from anaerobic metabolism may have produced a portion of the acidosis. However, no increase in blood lactate levels were observed in rainbow trout exposed to pH 4.0 (Neville, 1979*a*; McDonald *et al.* 1980) leading the latter authors to suggest that increased lactate levels probably resulted only from severe acid exposure. In the present study, animals were exposed to sublethal acidity, and remained quiescent throughout the experiment, so increased lactate levels probably contributed little to the acidosis. McWilliams & Potts (1978) found an increased H^+ influx across brown trout gills during exposure to acid; thus, it seems likely that branchial influx, rather than metabolic production, is the cause of the acidosis observed in crayfish.

The fluctuation in P_{CO_2} observed during acid exposure in the present study (Fig. 2C) are not consistent with those reported for fish. While McDonald *et al.* (1980) found no significant change in P_{CO_2} during sublethal acid exposure in rainbow trout, Hobe *et al.* (1980) report an elevation in white sucker. In crayfish, the decrease in P_{CO_2} observed after 24 h of acid exposure and subsequent increase, may reflect an initial hyperventilation. Although no studies of ventilation during acid exposure have been carried out in crayfish, Neville (1979*c*) reported that no change in ventilation occurred in rainbow trout exposed to normocapnic sublethal acid levels. The issue is further complicated by the recent work of deFur, Wilkes & McMahon (1980), who found that large discrepancies between calculated and measured P_{CO_2} may occur in crustacean haemolymph during non-equilibrium conditions, i.e. in situations where the concentration of one or more of the components of the haemolymph acid-base status is undergoing rapid change. In the present study, calculated P_{CO_2} values may be considered

inaccurate for this reason, since there is evidence that continuing metabolic compensation for acidosis is occurring. A significant rise in haemolymph $[Ca^{2+}]$ occurred during acid exposure (Fig. 3E), suggesting that $CaCO_3$ stores in the exoskeleton were being mobilized to compensate for the haemolymph acidosis. deFur *et al.* (1980) have postulated a similar mechanism for *Cancer productus* during respiratory acidosis induced by emersion. Skeletal dissolution may result from acid exposure in fish, as indicated by the spinal deformities resulting from long-term acid exposure of white sucker (Beamish, 1972) and the increased Ca^{2+} and phosphate excretion measured for acid exposed rainbow trout by McDonald & Wood (1981).

In general, disturbance in the concentrations of Cl^- , K^+ , Mg^{2+} in acid-exposed crayfish is minimal (Fig. 3B–D). Hobe *et al.* (1980) for white sucker and McDonald *et al.* (1980) for rainbow trout (Mg^{2+} not measured) report similar findings at high environmental Ca^{2+} levels but the latter authors report that low Ca^{2+} acid waters lead to a more pronounced ionic disturbance.

The use of an Na^+/H^+ exchange as a compensatory mechanism during acid/base disturbances in fish has been suggested by Cameron (1976), while McDonald *et al.* (1980) further suggest that this exchange may be involved in elevated proton excretion during acid exposure of rainbow trout in low $[Ca^{2+}]$ water. In contrast, McWilliams & Potts (1978) and McWilliams (1980) report inhibition of Na^+ uptake in acid-exposed fish. This inhibition together with increased Na^+ efflux (McWilliams & Potts, 1978) leads to decreased plasma Na^+ levels in fish (Packer & Dunson, 1970, 1972; Leivestad & Muniz, 1976; Neville, 1979b; McDonald *et al.* 1980) and may contribute to the decreasing trend in haemolymph $[Na^+]$ in the present study (Fig. 3A). The actual extent to which sodium exchange is involved in acid-base regulation during acid exposure, however, remains unclear in either case.

The significant increase in haemolymph calcium levels during acid exposure (Fig. 3E) contrasts sharply with the small decrease seen in acid-exposed rainbow trout (McDonald *et al.* 1980). The increase probably results from exoskeletal dissolution (see above) and this has a number of implications. Although the removal of $CaCO_3$ from the exoskeleton may provide beneficial compensation for acidosis, it could become detrimental in the long term if exoskeletal breakdown becomes excessive. Malley (1980) has found that Ca^{2+} uptake in the crayfish *Orconectes virilis* is completely inhibited in postmoult animals exposed to pH 4.0, and this apparently leads to reduced calcification of the exoskeleton. Clearly, the combination of Ca^{2+} uptake inhibition and acidosis-induced exoskeletal dissolution could create severe problems with exoskeleton rigidity, particularly in postmoult animals; this may be the reason for their higher acid sensitivity (Malley, 1980). R. France (pers. comm.) has observed decreasing exoskeletal rigidity in crayfish in Lake 223 (pH 5.2) of the Experimental Lakes Area of north-western Ontario.

Preliminary results with *Orconectes rusticus* show that this crayfish species also undergoes a large metabolic acidosis during acid exposure, but in this species P_{CO_2} remains stable throughout. Ionic changes in *O. rusticus* are essentially similar to those seen in *P. clarki*.

In conclusion, crayfish are apparently considerably more tolerant of increased external acidity than are fish species, at least in the short term. Although in the present study crayfish were exposed to a somewhat greater acid-load, with the exception of

Increased haemolymph Ca^{2+} levels, their physiological responses were essentially similar to those of fishes when exposed to acid waters of similar Ca^{2+} content (McDonald *et al.* 1980; Hobe *et al.* 1980). Perhaps the major difference between the two groups stems from the presence, in the crustacean, of the chitinous and calcified exoskeleton. A chitin layer overlays the gills and thus the exoskeleton could ameliorate the effects of acid stress both by acting as a barrier to the diffusion of hydrogen ions across the gills as well as a source of carbonate used to buffer some of the remaining hydrogen ion flux. McDonald *et al.* (1980) suggest that failure of ionoregulation is a major contributor to death from acid stress in fish. In the present study, little disruption of ionic levels was apparent after 4 days sublethal exposure but haemolymph $[\text{Na}^+]$ did exhibit a decreasing trend and failure of ionoregulation may prove to be important in longer term exposures. Following 4 days exposure to pH 3.8, both acid-base and ionic levels of haemolymph were accurately re-established within 4 days indicating that no sustained damage to gill or other regulatory tissues resulted from acute acid exposure.

The authors wish to express their gratitude to H. Hobe, P. R. H. Wilkes and A. Pinder for advice and assistance. The work was supported by N.S.E.R.C. grant A5762 to B. R. McMahon and an N.S.E.R.C. Summer Fellowship to D. O. Morgan.

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