Wayne – thanks for the opportunity and all the assistance. Bob D.

Response of the crayfish *Cambarus bartonii bartonii* to acid exposure in southern Appalachian streams

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Intermolt adult and juvenile *Cambarus bartonii bartonii* (Fabricius) from southern Appalachian Mountains streams tolerated considerable acidity when acutely exposed to greatly reduced pH levels in laboratory bioassays. Tolerance increased with increasing size or age of crayfish. Ninety-six-hour exposures yielded LC_{50} values of pH 2.43, 2.56, and 2.85 for adults, advanced juveniles, and early juveniles, respectively. Lowering the water temperature increased the acid tolerance and survival time of intermolt adults during severe acidification (temperatures ranged from 20.2 to 13.3°C). Acid exposure of intermolt adults in soft water up to 96 h caused a linear decrease in hemolymph [Na]. Hemolymph [Ca] increased through 48 h and then returned to near pre-exposure levels. An initial increase in [K] was followed by a decrease to slightly below pre-exposure levels. Hemolymph [Mg] remained unchanged. No Ca was lost from carapaces. These observations indicate that occasional episodes of higher than normal acidity in southern Appalachian streams are not necessarily a threat to intermolt adult and juvenile *C. b. bartonii*. Nevertheless, gradually increasing acidity and loss of watershed buffering capacity could produce sublethal effects such as altered reproductive activity, or changes in early life history stages and more sensitive molt cycle stages, that could damage these populations.

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Des individus adultes et juvéniles entre deux mues de *Cambarus bartonii bartonii* (Fabricius) du sud des Appalaches se sont avérés très tolérants à l'acidité lorsqu'ils ont été exposés de façon brusque à des pH très réduits au cours d'expériences en laboratoire. La tolérance augmentait en fonction de la taille ou de l'âge des écrevisses. Après 96 h d'exposition, la valeur du pH qui a entraîné 50% de mortalité (LC_{50}) a été estimée à 2,43 dans le cas des adultes, à 2,56 dans le cas des juvéniles avancés et à 2,85 dans le cas des très petits juvéniles. Le refroidissement du milieu au cours d'une forte acidification a augmenté la tolérance à l'acidité et la durée de la survie des adultes entre deux mues (températures entre 20,2 et 13,3°C). L'exposition à l'acide d'adultes entre deux mues dans de l'eau douce durant une période pouvant aller jusqu'à 96 h a entraîné une diminution linéaire du [Na] de l'hémolymphe. Le [Ca] de l'hémolymphe a augmenté au cours des 48 premières heures, puis est retourné à une valeur voisine de la valeur initiale. Le [K] a dabord augmenté, puis a diminué pour atteindre une valeur un peu inférieure à la valeur initiale. Le [Mg] de l'hémolymphe est resté à peu près inchangé. Il n'y a pas eu de perte du Ca des carapaces. Ces observations indiquent que l'augmentation occasionnelle de l'acidité dans les ruisseaux du sud des Appalaches ne constitue pas nécessairement une menace pour les adultes et juvéniles entre deux mues de *C. b. bartonii*. Néanmoins, une augmentation graduelle de l'acidité et une perte de la capacité tampon du bassin pourraient produire des effets sublétaux, notamment une modification de l'activité reproductrice, ou encore des modifications des tout premiers stades ou des stades plus sensibles du cycle de la mue, ce qui pourrait avoir des effets délétères sur la population.

[Traduit par la rédaction]

Introduction

High-altitude headwater streams in the southern Appalachian Mountains of the United States face a serious threat from acidification. If present trends continue, this area could soon have the same problems that have been described for Scandinavia, the United Kingdom, southeastern Canada, and the northeastern United States. In these areas, highly acidic precipitation and the poor acid-neutralizing capacity of soils and water have severely damaged the environment (Harvey 1980; Burns et al. 1981; Hileman 1981; Likens and Butler 1981; Overrein 1983). Aquatic systems have suffered alterations of species compositions, reduced diversity and abundance, and local extinctions of sensitive species and whole communities (Haines 1981; Singer 1982; Schindler 1985).

The crayfish *Cambarus bartonii bartonii* is an important trophic component of Appalachian headwater streams, usually predominating over other crayfish species, and often accounting for more biomass than all other invertebrates combined (Woodall and Wallace 1972; Huryn 1986). Increased acidification of poorly buffered high-elevation streams may pose a threat to long-term survival of these crayfish populations.

Field and laboratory observations of the effects of acidification on crayfish have focused primarily on lentic species. Results of those studies are somewhat variable, indicating differences in

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acid tolerance among life cycle stages and species (Furst 1977; Collins et al. 1981; France 1981; Morgan and McMahon 1982; Appelberg 1984; Berrill et al. 1985). Much of the recent literature has described sublethal effects, especially internal ion regulation (Malley 1980; Morgan and McMahon 1982; McMahon and Morgan 1983; Nikinmaa et al. 1983; Jarvenpaa et al. 1983; Appelberg 1985; Wood and Rogano 1986; Hollett et al. 1986). Many of these studies implicate ionoregulatory changes as a probable mechanism in the toxicity of reduced pH.

The effects of acidification on lotic crayfish are not well documented, but the tolerance range to reduce pH has been reported to be narrower than that of lentic species (Park et al. 1940; Hobbs and Hall 1974). The purpose of our experiments was to document the tolerance to acute acidification of three intermolt size classes of the primarily lotic *C. b. bartonii* from southern Appalachian Mountains headwater streams. We also investigated the influence of water temperature on acid tolerance, and the ionoregulatory response of adult intermolts to acute acid exposure.

Materials and methods

Three nominal sizes of intermolt *C. b. bartonii* were collected in baited traps during May to October, 1985: adults, 25.3–47.2 mm carapace length (CL); advanced juveniles, 11.5–19.9 mm CL; and early juveniles, 3.9–12.8 mm CL. They were taken from Ball Creek (annual mean pH 6.51, alkalinity 0.5–2.6 mg/L as $CaCO_3$) and Shope Fork (annual mean pH 6.70, alkalinity 0.5–2.6 as $CaCO_3$), two first-order mountain streams at the Coweeta Hydrologic Laboratory (U.S. Forest Service Southeastern Experiment Station) in Macon County, North Carolina (35°3′N, 83°25′W). For inter-regional comparison, we also obtained intermolt adults (23.0–41.3 mm CL) from the headwaters of Craig Creek (pH 7.1–7.2, annual mean alkalinity 67 mg/L as $CaCO_3$), a high-gradient tributary of the James River in the Jefferson National Forest, Montgomery County, Virginia.

In all experiments we followed standard procedures for macroinvertebrate testing described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (United States Environmental Protection Agency 1975), and used a flow-through artificial stream system slightly modified from Farris (1986). These oval wooden streams with paddle-driven flow measured 90 × 46 × 15 cm; their capacity was 30 L, and turnover time was about 90 min. Fisher model 805MP pH meter controllers linked to Cole–Parmer peristaltic pumps regulated pH by delivering dilutions of 12 M H_2SO_4 and dechlorinated, charcoal-filtered tap water originating from the New River (pH 7.75–8.00) to the artificial streams. Probes in the streams continuously monitored pH. Motor-driven Plexiglas paddle wheels provided constant aeration and flow. Streams were covered with translucent screening to prevent escape of crayfish and allow light to penetrate.

Selected water chemistry characteristics were measured periodically. Dissolved oxygen ranged from 86 to 98% saturation. Hardness and alkalinity (as $CaCO_3$) ranged from 50 to 70 mg/L and 38 to 44 mg/L, respectively. Major ion concentration ranges (mg/L) in artificial reference streams were as follows: Na, 2.4–2.6; Ca, 4.6–9.6; K, 0.6–1.6; Mg, 0.9–2.6. Stream and acclimation tank temperatures were stabilized at 20–22°C (United States Environmental Protection Agency 1975). A photoperiod of 16 h light : 8 h dark was maintained throughout the period of acclimation and experiments. During acclimation, crayfish were fed a commercially prepared canned dog food on alternate days. The crayfish were not fed for 48 h before (or during) experiments.

Acute lethality tests

Exposures of 96 h were used to simulate episodic acid events that might be encountered by crayfish under natural conditions. Water chemistry data from high-elevation Coweeta streams indicate that acid precipitation events tend to flush through the watershed in a matter of hours to a few days. Accordingly, we chose not to conduct chronic experiments. Acute median lethal concentrations for adults and advanced juveniles were determined with 10 crayfish in each experimental stream (5 males and 5 females, to test for tolerance differences between sexes). We based experimental pH levels (2.96–2.34) on preliminary range-finding tests. Determinations of LC_{50} values and 95% fiducial limits (FL) were made by Finney's probit analysis in the Statistical Analysis Systems program (SAS Institute Inc. 1985).

Mortality, pH, and temperature were recorded at 2-h intervals for the first 12 h, and every 12 h for the remainder of the 96-h experiments. During these observation periods, all dead crayfish were removed. Death was recorded when stimulation of antennae, eyestalks, and walking appendages produced no observable response. Measured pH values (+0.05 units) and temperatures (20.2 ± 1.0°C) varied little among replicate streams. After completion of the experiments, dead and live crayfish were frozen for use in carapace Ca analysis.

Temperature-dependent pH tolerance

In addition to acute lethality tests conducted at a standardized water temperature of about 22°C (United States Enviromental Protection Agency 1975), we also performed tests in the 10–17°C range typical of Coweeta streams from May to October. A 96-h lethality test was conducted on Coweeta crayfish (27.5–47.2 mm CL) at a mean temperature of 15.1 ± 0.03°C, following procedures identical with those previously described.

In a related experiment, two groups (12 each) of randomly chosen adult intermolt crayfish were placed in adjacent artificial streams with mean temperatures of 19.2 ± 0.3 and 13.3 ± 0.6°C. They were subjected to an extreme pH of 2.00 and examined hourly until all specimens had died. A Wilcoxon two-sample test was used to compare differences in survival times. All hypothesis tests were performed at the 0.05 significance level.

Hemolymph cation concentrations

To determine the effects of reduced pH on ion regulation of adult *C. b. bartonii*, we exposed randomly selected crayfish to pH 2.62 (96-h LC_{10}), for 2 h (n = 12), 24 h (n = 12), 48 h (n = 6), 72 h (n = 10), and 96 h (n = 20), at 20.2 ± 0.1°C. Five crayfish from acclimation (stock) tanks were used as controls to test for the effects of artificial streams on ion balance.

We sampled hemolymph with a 100-μL automatic pipettor (Gilson) fitted with a Jelco 26 gauge needle. The needle was inserted into the ventral (venous) sinus via the first joint of the third pereiopod, and 100 μL of hemolymph were withdrawn and immediately dispensed into 1-mL polyethylene test tubes (each crayfish being sampled only once). We analyzed concentrations of four major cations, Na, Ca, K, and Mg, using a Perkin–Elmer model 460 atomic absorption spectrophotometer, following appropriate dilutions and the addition of $LaCl_3$ to Ca samples to reduce chemical interference.

Linear and quadratic regression models were used to describe changes in ion concentrations over time. We used pooled t-tests to test normally distributed ion data for differences between reference and treatment (acidified) groups. Possible differences between pre-exposure (acclimation) and reference crayfish due to the use of artificial streams were also determined with t-tests.

We also investigated the effects of acute acid exposure on exoskeleton (carapace) Ca content. Crayfish were freeze-dried after 96-h exposure to pH levels of 7.85 (reference, n = 20), 2.96 (n = 20), 2.74 (n = 15), 2.53 (n = 20), and 2.47 (n = 28). Carapaces were removed and cleaned of debris. Length (CL) and dry weight were determined, and carapaces were ground in test tubes and digested with concentrated nitric acid. Atomic absorption spectrophotometry was used to determine [Ca] (and percent Ca) of carapaces. Carapace Ca concentrations are expressed in millimoles of Ca per kilogram of dry weight (mmol/kg). Percent Ca refers to the percentage of dry weight of each carapace that was made up of Ca. Treatment-dependent changes in carapace mass were assessed by comparing carapace dry weight per unit CL. We applied Jonckheere's test for ordered alternatives to test the a priori hypothesis that a decrease in environmental pH would cause a mobilization of internal Ca stores, thus decreasing the Ca level in the carapace.

TABLE 1. Ninety-six-hour median lethal (LC_{50}) values, with 95% fiducial limits (FL), for life stages of *Cambarus bartonii bartonii* in flow-through acid toxicity tests

	Carapace length (mm)		LC_{50}			
	Mean	Range	$[H^+]$ (mmol)	FL	pH	FL
Coweeta						
Adults	33.5	25.3–43.2	3.69	3.02–4.77	2.43	2.52–2.32
Craig Creek						
Adults	32.2	23.0–41.3	3.70	3.45–4.01	2.43	2.46–2.40
Coweeta						
Advanced juveniles	16.4	11.5–19.9	2.78*	2.59–3.00	2.56	2.59–2.52
Early juveniles	9.1	3.9–12.8	1.42†	1.22–1.62	2.85	2.92–2.79

*Twenty-five percent less than 3.69.
†Forty-nine percent and 62% less than 2.78 and 3.69, respectively.

Results

Acute lethality tests

All three size classes of *C. b. bartonii* from Coweeta streams and Craig Creek were highly tolerant of acidic conditions during 96-h exposures (Table 1). Exposures yielded LC_{50} values of pH 2.43 for both Coweeta and Craig Creek adults, 2.56 for advanced juveniles, and 2.85 for early juveniles. As judged by these values, early juveniles were only 51% as tolerant as advanced juveniles, and 38% as tolerant as Coweeta adults. Advanced juveniles were 75% as tolerant as Coweeta adults. There was no overlap in 95% FLs among any of these size classes. Acid tolerance did not depend on sex. Adult crayfish from two geographically isolated populations, Coweeta and Craig Creek, had the same acid tolerance, based on LC_{50} values; however, the 95% FLs were much wider for the Coweeta adults.

Temperature-dependent pH tolerance

Acid tolerance of adult crayfish was increased at lower water temperatures (<17°C) in artificial streams. A 96-h lethality test conducted at a mean temperature of 15.1°C (±0.3) resulted in a median lethal pH of 2.33 (95% FL = 2.37–2.30) for adult crayfish, whereas an LC_{50} of pH 2.43 (95% FL = 2.52–2.32) was observed at 20.2°C (±0.1). In terms of hydrogen ion concentration, this difference is considerable and indicates a 25% difference in acid tolerance for similar-sized crayfish, although the FLs exhibited some overlap.

When subjected to severe acidification (pH 2.00) in replicate streams, adult crayfish survived significantly longer at 13.3°C (±0.6) than at 19.2°C (±0.3). A 6°C decrease in temperature nearly doubled survival time (24.6 ± 4.8 to 44.3 ± 5.9 h).

Hemolymph cation concentrations

Acidification to pH 2.62 in moderately soft water for 96 h did not cause mortality in adult *C. b. bartonii*, but affected hemolymph ionic status (Fig. 1). Among animals from acclimation tanks and reference animals (from streams), no significant differences were found in the concentrations of four cations: [Na], $P = 0.3524$; [Ca], $P = 0.4905$; [K], $P = 0.7088$; and [Mg], $P = 0.6439$; this suggests that there were no laboratory "stream effects" on ion regulation. However, [Na] changed significantly ($P = 0.0095$), and [Ca] seemed to change somewhat (not significantly, $P = 0.0675$) in acid-treated crayfish (Table 2).

Stepwise regression produced a linear model to describe the significant ($P = 0.0001$) decrease in hemolymph [Na] for acid-treated crayfish throughout the 96-h exposure (Fig. 1). The significant ($P = 0.0003$) regression relation between duration of acid exposure and hemolymph [Ca] contained both linear and quadratic terms. Calcium concentrations first increased, and then decreased to pre-exposure levels (Fig. 1). A significant ($P = 0.0040$) relation, containing both linear and quadratic terms, was also observed for hemolymph [K] over 96 h. These concentrations appeared to decrease to slightly below pre-exposure levels after an initial increase (Fig. 1). Hemolymph [Mg] showed no change ($P > 0.7518$) in animals exposed for 96 h. Acute exposure to five pH levels did not decrease carapace [Ca], as indicated by Jonckheere's test for ordered alternative ($P = 0.70442$; Table 3). In fact, comparison of acid-exposed crayfish ($n = 83$) with reference specimens ($n = 20$) showed Ca levels to be significantly higher (Wilcoxon two-sample test, $P = 0.0344$) in treated animals (Table 3). Analysis of covariance using CL as a covariate showed that the carapace weights of the four groups of acid-treated crayfish were not significantly different from each other ($P > 0.10$), but the mean carapace weight of the reference crayfish group was significantly lower ($P < 0.05$) than that of all acid-treated groups (Table 3).

Discussion

Acute lethality tests

Adult *C. b. bartonii* were more acid tolerant than most other adult crayfish and freshwater organisms. Previously reported 96-h LC_{50} values were pH 2.5 for *Procambarus clarki* and 2.8 for *Orconectes rusticus* (Morgan and McMahon 1982). *Orconectes virilis* adults were more tolerant (LC_{50} = pH 2.35) than adult *C. b. bartonii*, but juveniles were not (LC_{50} = pH 2.95) (France 1984). The 96-h LC_{50} values obtained for *C. b. bartonii* showed their acid tolerance to be considerably higher than that of many adult fishes and insects. Previously reported LC_{50} values include pH 3.5 for 167 h for brook trout (*Salvelinus fontinalis*) (Daye and Garside 1975), 4.0–4.2 for 96 h (McDonald et al. 1980) and 4.1–4.5 for 167 h (Graham and Wood 1981) for rainbow trout (*Oncorhynchus mykiss*), and 4.0–4.2 for 96 h for yearling Arctic char (*Salvelinus alpinus*) (Jagoe et al. 1984). White suckers (*Catostomus commersoni*) exhibited an LT_{50} of 100 h at pH 3.9 (Beamish 1972). Values for aquatic insects include 96-h LC_{50} values ranging from pH 3.15 to 4.65 for immatures of nine species of caddisflies, stoneflies, dragonflies, and mayflies (and pH 1.5 for one caddisfly, *Brachycentrus americanus* (Bell and Nebeker 1969)). Lechleitner et al. (1985) recorded 96-h LC_{50} values of pH 2.8–3.3 for three species of

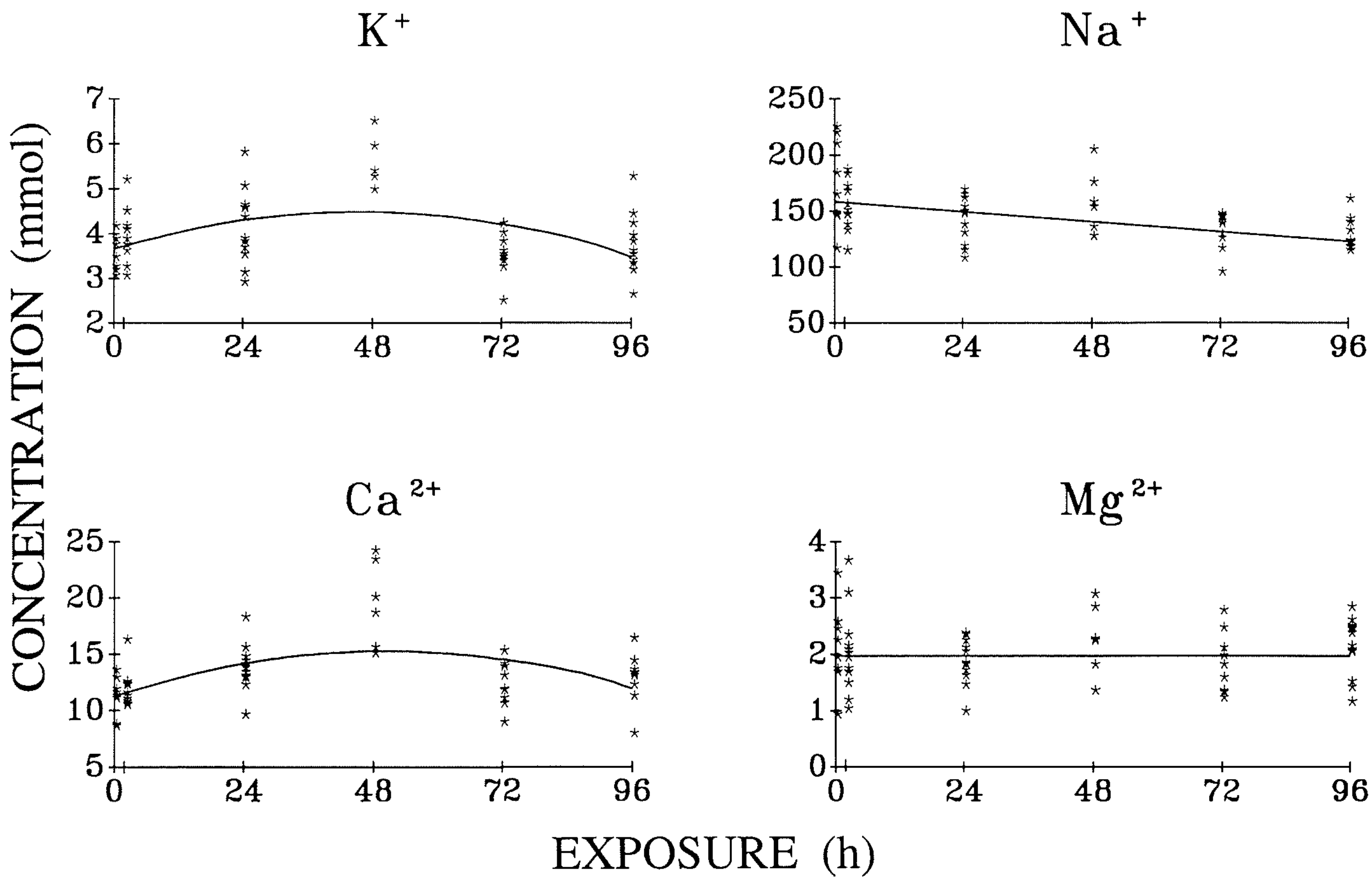

FIG. 1. Cation concentrations in hemolymph of adult intermolt *Cambarus bartonii bartonii* following laboratory exposure to reduced pH (2.62). Reference specimens are shown at 0 h. Regression models for concentrations are described by the following equations: Na^+: 158.434 − 0.369 × exposure; Ca^{2+}: 11.237 + 0.161 × exposure − 0.0016 × exposure^2; K^+: 3.658 + 0.036 × exposure − 0.0004 × exposure^2.

TABLE 2. Concentration and 95% confidence limits of major cations in hemolymph of Coweeta *Cambarus bartonii bartonii* from an acclimation tank, a reference stream (96-h exposure), and an acidified treatment stream (96-h exposure, pH 2.62)

	Mean concentration (mmol)		
	Acclimation (n = 5)	Reference (n = 8)	Treatment (n = 12)
[Na]	191.91±13.79	173.83±27.19	125.13±7.82*
[Ca]	11.40±0.44	10.94±1.23	12.63±1.14
[Mg]	1.94±0.17	2.07±0.51	2.08±0.30
[K]	3.58±0.35	3.50±0.26	3.68±0.38

*P = 0.0095 for [Na] treatment versus reference.

TABLE 3. Concentration and percent composition (±95% confidence interval) of calcium in carapaces of Coweeta crayfish acutely exposed to five pH levels

Group	n	Mean pH	Mean carapace $[Ca^{2+}]$ (mmol/kg)	Mean carapace Ca^{2+} content * (%)
1	28	2.47	3.75±0.46	15.0±0.02
2	20	2.53	4.11±0.29	16.5±0.02
3	15	2.74	4.16±0.60	16.7±0.02
4	20	2.96	4.08±0.44	16.3±0.02
5†	20	7.85	3.04±0.63	12.2±0.03

*Percentage of dry weight of each carapace that was composed of Ca.
†Reference specimens.

stonefly nymphs. Our results identify *C. b. bartonii* as being among the most acid-tolerant freshwater organisms tested, and substantiate previous claims of variation in tolerance to pH stress among crayfish species (Hollett et al. 1986).

The susceptibility of *C. b. bartonii* from Coweeta streams to acutely toxic acid exposures is related to size or perhaps life cycle stage. A similar relation has been demonstrated in *Orconectes virilis* (France 1984). In this species, 96-h LC_{50} values were pH 2.35 for adults, 2.95 for juveniles, and 3.70 for stage III independent hatchlings. Similar size-dependent relations have been reported for freshwater fishes (Schofield 1976). The mechanism of acid toxicity is believed to be similar in fish and crayfish: failure to maintain internal ion regulation (Shaw 1960; Muniz and Leivestad 1980; Morgan and McMahon 1982; Wood and Rogano 1986). Size-specific (age-specific) response to acid exposure has been attributed to the larger surface area to volume ratio of the smaller animals (Schofield 1976), and an increased resistance with physiological maturation, possibly due to a decreased metabolic rate (France 1984). These findings stress the importance of including several size classes or life history stages in acid toxicity investigations. Appelberg (1984) suggested that reproductive failure may be a serious threat to acid-exposed *Astacus astacus*. It is possible that additional experiments would show specific periods of *C. b. bartonii*'s life cycle, particularly early life stages such as hatchlings or eggs, to be more susceptible to reduced pH than the three size groups we studied.

The observed similarity in LD_{50} values between Coweeta and Craig Creek adult crayfish indicates no apparent influence of the lower ambient Coweeta stream pH on acid tolerance. Annual mean pH values are about 6.51 (5.60 recorded minimum) in Ball Creek and 6.70 (4.90 recorded minimum) in Pinnacle Branch (W. Swank, personal communication), whereas pH in Craig Creek ranged between 7.1 and 7.2 during our study. In spite of geographical segregation, the toxicological response to reduced pH was similar in crayfish from these two areas. However, these results do not preclude the possibility of genetic adaptation to a continuing reduction in stream pH by Coweeta crayfish or other populations.

Temperature-dependent pH tolerance

Coweeta crayfish showed temperature-dependent differences in acute acid tolerance. Our results indicate that acid tolerance decreased as stream temperatures approached maximum thermal tolerance levels for the organisms. Servos et al. (1987) reported that specimens of *Elliptio complanata* were unusually inefficient accumulators of metals in streams during spring melt of acidic snow, possibly due to reduced metabolic activity at low temperatures (0–4°C). There are few other relevant data on aquatic invertebrates; however, experiments with fish, although somewhat contradictory (Cairns et al. 1975), generally indicate that most other pollutants are more toxic as temperatures approach maximum thermal tolerance levels (Sprague 1970). Acid tolerance of fingerling rainbow trout (*Oncorhynchus mykiss*) and survival time of brook trout (*Salvelinus fontinalis*) at low pH were inversely related to water temperature (Kwain 1975; Robinson et al. 1976), but Daye and Garside (1975) reported no difference in acid resistance between 10 and 20°C for fingerling brook trout. Cairns et al. (1975) also concluded that temperature is more of a factor in acute versus long-term exposure. Direct comparisons of acute LC_{50} values for *C. b. bartonii* with values for other crayfish species should include experimental temperature differences, because temperature seems to affect acid toxicity (Morgan and McMahon 1982; France 1984).

Assuming that the influences of temperature on the acid tolerance of *C. b. bartonii* in the wild is similar to that observed in the laboratory, any discussion of the effects of acidification on wild crayfish populations must take seasonality into account. Northern Appalachian Mountains streams typically receive the highest doses of acid during spring snowmelt, when temperatures are relatively low and crayfish may be more tolerant (Jeffries et al. 1979; DeWalle et al. 1983). Coweeta streams usually do not receive acidic meltwater from a large snowpack, and mean annual precipitation is distributed uniformly over the year (Swank and Douglass 1977). However, stream chemistry data for Ball Creek and Pinnacle Branch show that periods of depressed pH occur mostly during winter months (February, March) when stream temperatures are below 10°C. Populations of *C. b. bartonii* should then be relatively tolerant because of low temperatures and the absence of more sensitive early life stages. Conversely, occasional episodes of significantly reduced pH (often caused by summer storms) may occur during periods of elevated stream temperatures. The relation between temperature and acid tolerance may also be important in warmer, lower elevation stream reaches, or regions such as the southeastern United States, as acidification intensifies. Higher temperatures than those recorded in first-order Coweeta streams may pose greater acidification related problems for populations of crayfish and other aquatic organisms.

Hemolymph cation concentrations

Acute exposure of adult *C. b. bartonii* to extremely acidic conditions affected hemolymph cation balance. However, crayfish were able to partly compensate for these changes and apparently reestablish ion balance within a few days (except for [Na]). Similar compensatory trends have been reported for *Procambarus clarki* after longer periods of sublethal acid exposure (McMahon and Stuart 1989).

The decrease of about 35% in hemolymph [Na] after acid exposure is consistent with reported Na decreases in aquatic insects (Lechleitner et al. 1985), fish (Fromm 1980; McWilliams 1980), and most other crayfish species (Shaw 1960; Morgan and McMahon 1982; McMahon and Morgan 1983; Appelberg 1985; Wood and Rogano 1986). However, Hollett et al. (1986) found no change in [Na] of acid-tolerant *C. robustus*. Our use of lower pH values and higher temperatures may account for this discrepancy.

Reduction of hemolymph [Na] may result from a decrease in Na uptake across the gills, possibly due to disturbance of the Na^+–H^+ exchange mechanism (Shaw 1960; Appleberg 1985). Passive efflux of Na to the environment would also reduce [Na] (Morgan and McMahon 1982; Wood and Rogano 1986), and may be increased by acid exposure. Acid exposure may cause ionoregulatory failure in chinook salmon (*Oncorhynchus tshawytscha*) and freshwater mussels (*Anodonta californiensis*) through loss of primary amines, which destroys the integrity of membranes and membrane processes (Swinehart and Cheney 1984). Whether caused by inhibition of uptake, increased efflux, or both the decrease in [Na] in *C. b. bartonii* hemolymph suggests a disturbance in ion regulation as one cause of sublethal acid toxicity.

Regulation of Ca in crayfish tissues is an active and essential process which is highly dependent on environmental sources and susceptible to environmental disturbances such as acidification (McWhinnie 1962; Adegboye 1983; Greenaway 1985; Wood and Rogano 1986). The trend towards increased hemolymph [Ca] in acid-exposed *C. b. bartonii* is consistent with results from studies involving exposure of other crayfish species to reduced pH (Morgan and McMahon 1982; Wood and Rogano 1986). Increased hemolymph [Ca] may be due to mobilization of $CaCO_3$ from internal stress such as those in the gastrolith (McWhinnie 1962), hepatopancreas (Huner et al. 1976), muscle (Wood and Rogano 1986), or exoskeleton (Morgan and McMahon 1982). Acid exposure had unexpected effects on carapace Ca content in *C. b. bartonii*. The concomitant increase in carapace dry weight and Ca content suggests Ca deposition rather than decrease. However, 4 days of acid exposure may be insufficient to allow mobilization of exoskeleton (carapace) $CaCO_3$ stores, since more readily mobilized stores are available in the gastrolith and hepatopancreas (McWhinnie 1962; Huner et al. 1976). Permeability to and excretion of H^+ affect Ca flux between the crayfish and its environment (Appleberg 1985). Therefore, a more detailed examination of Ca metabolism in acid-exposed *C. b. bartonii* is required to explain Ca fluxes between tissues.

The availability of Ca from the environment is also important. Hardness of natural waters and experimental media may influence Ca levels in crayfish tissues (Morgan and McMahon 1982; Huner and Lindqvist 1985). Because our experiments were conducted in water of moderate hardness (50–70 mg/L) and alkalinity ($CaCO_3$ 38–44 mg/L), sources of Ca were not abundant. However, alkalinities ($CaCO_3$) in Coweeta streams are even lower (0.5–2.6 mg/L, W. Swank, personal communication), possibly forcing crayfish to rely heavily on internal stores of $CaCO_3$ for hemolymph buffering. Therefore, it is possible that *C. b. bartonii* in our laboratory studies exhibited tolerance equal to or greater than that which might be expected of crayfish in Coweeta streams.

Hemolymph [K] followed a pattern similar to [Ca] over 4-d exposures; however, after initial increases in [K] eventually subsided, final concentrations were no different from those of acclimation or reference animals. In contrast, hemolymph [K] of acid-exposed (pH 4.0, extremely soft water) *O. rusticus* showed a continuous increase in [K] to nearly double that of controls after 5 d (Wood and Rogano 1986).

Differences in acid tolerance of crayfish species have been reported (McMahon and Stuart 1989) and sometimes correlated with geographical differences in environmental pH (Berrill et al. 1985; Hollett et al. 1986). Our results lend some support to earlier suggestions (Berrill et al. 1985; Wood and Rogano 1986; Hollett et al. 1986) that *Cambarus* species are more acid tolerant than *Orconectes* species. There are also differences in acid tolerance among *Cambarus* species. The 96-h LC_{50} for *C. robustus* was pH 3.8 at 15°C (Hollett et al. 1986), a $[H^+]$ more than 10-fold that of the LC_{50} for *C. b. bartonii* (20°C). Since differences in experimental protocol could affect estimates of LC_{50}, a study comparing the acid tolerance of *Cambarus* species from different habitats would be required to confirm this difference.

We conclude that because of their relatively high tolerance to reduced pH, Coweeta populations of *C. b. bartonii* are not immediately threatened by increasing acidification in the southern Appalachian Mountains, especially during colder months, when metabolic rates are low and postmolt and younger juvenile stages are not present. Although the usefulness of acute experimental acid exposures (as opposed to chronic studies) in predicting long-term population responses to acidification can be limited, crayfish in high-altitude southern Appalachian streams are extremely unlikely to encounter chronic acidification, or the low pH ranges we used. However, if present trends continue, there are reasons for concern. Our results indicate that disturbance of internal ion regulation is a mechanism of acid toxicity in this species. This type of physiological stress may weaken crayfish in Coweeta streams and increase their susceptibility to disease, parasites, or predators; or it may induce behavioral changes, affecting critical activities such as feeding or reproduction. Preliminary observations suggest that molting crayfish may be less resistant to severe acid stress than intermolt individuals (DiStefano 1987). In addition, acute toxicity results indicate that early life history stages of crayfish will be affected more than adults by certain environmental conditions (such as elevated stream temperatures) in poorly buffered headwater streams. It seems likely that if reduced pH begins to affect *C. b. bartonii* in the southern Appalachians, the physiological damage will be apparent first in these more sensitive life stages or molt cycle stages.

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