

Growth and population size of crayfish in headwater streams: individual- and higher-level consequences of acidification

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SUMMARY

1. Environmental stress may have indirect positive effects on population size through modification of food-web interactions, despite having negative effects on individuals. Here we evaluate the individual- and population-level effects of acidification on crayfish (*Cambarus bartonii*) in headwater streams of the Allegheny Plateau (PA, U.S.A.) with field experiments and survey data. Median baseflow pH of 24 study reaches in nine streams varied from 4.4 to 7.4, with substantial variation found both among and within streams.
2. Two bioassays were conducted to evaluate the relationship between stream pH and crayfish growth rates. Growth rates were always higher in circumneutral reaches than in acidic reaches. Crayfish originating in acidic water grew less when transplanted into neutral water than did crayfish originating in neutral water, providing some evidence for a cost of acclimation to acidity.
3. Stream surveys showed that fish were less abundant and crayfish more abundant in acidified streams than in circumneutral streams. Crayfish density was sixfold higher in reaches with the lowest pH relative to circumneutral reaches. Large crayfish made up a higher proportion of crayfish populations at sites with high fish biomass, consistent with the hypothesis that fish predation on small individuals may be limiting crayfish population size at these sites.
4. Although individual crayfish suffered lower growth in acidified streams, increased acidity appeared to cause an increase in crayfish population size and shifts in size structure, possibly by relieving predation pressure by fish.

Keywords: acidification, crayfish, *Cambarus bartonii*, headwater streams, water quality

Introduction

Streams across a large portion of north-eastern North America continue to suffer from chronic acid deposition (Driscoll *et al.*, 2001). Acid deposition impairs streams in poorly buffered catchments by lowering pH, decreasing acid-neutralising capability, and increasing aluminium concentrations (Haines, 1981; Schindler, 1988). Studies of lakes show that acidification is usually associated with a reduction in species

diversity of lentic invertebrates (Eriksson *et al.*, 1980; Schindler, Kaison & Hesslein, 1989; Herrmann, 1990; Locke, 1992). Although some lentic invertebrates decline in abundance with acidification, other species may increase in abundance (e.g. Brezonik *et al.*, 1993; France & Collins, 1993; Klug *et al.*, 2000; Fischer *et al.*, 2001). In contrast to the large literature of lake acidification, the effects of acidification on stream food webs are not as well understood (but see Hildrew, Townsend & Francis, 1984; Rosemond *et al.*, 1992; Ledger & Hildrew, 2000).

The population-level effect of an environmental change like acidification may not be predictable from individual-level studies (Dunson & Travis, 1991).

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Although environmental stress can have direct negative effects on individual growth, survival, and reproduction, species typically differ in their tolerance. In such cases, environmental stress can have indirect positive effects on a population with relatively high tolerance by reducing predation or competition (e.g. Yan *et al.*, 1985; Menge & Sutherland, 1987; Wilbur, 1987; Menge & Olson, 1990; Webster *et al.*, 1992; Fischer & Frost, 1997). If the indirect positive effects are strong enough, they may offset the direct negative effect of the stressor, and the species can experience a net benefit (Eriksson *et al.*, 1980). Thus, when considered in the context of the stream food web (Power, 1990), the net effects of environmental stress on invertebrate populations can be complex and difficult to predict. Only empirical studies of the individual- and higher-level effects of abiotic stress will reveal the relative strengths of these direct and indirect effects, but few studies simultaneously evaluate the effects of environmental stress on individual and population level parameters (Dunson & Travis, 1991).

The crayfish *Cambarus bartonii* (Fabricius) is an important component of headwater stream ecosystems in the eastern United States. Studies of Appalachian headwater streams show that *C. bartonii* usually accounts for the majority of benthic macroinvertebrate biomass (Woodall & Wallace, 1972; Huryn & Wallace, 1987; Griffith, Perry & Perry, 1994; Griffith, Wolcott & Perry, 1996; Seiler, 2002). Crayfish in small streams can regulate periphyton standing crops (Creed, 1994), often constitute a large portion of fish diets (Rabeni, 1992; Roell & Orth, 1993), and play an important role in the processing of leaf litter (Huryn & Wallace, 1987; Usio, 2000). Both fish predation and interspecific competition may play a role in regulating crayfish population size (e.g. Momot, 1967; Momot & Gowing, 1977; Mather & Stein, 1993; Garvey, Stein & Thomas, 1994; Englund & Kruppa, 2000, reviews by Lodge & Hill, 1994; Dorn & Mittelbach, 1999; Hobbs, 2001). Because *C. bartonii* dominate benthic biomass and are key players in stream food webs, any perturbation to crayfish abundance will likely have consequences that extend through the ecosystem.

DiStefano *et al.* (1991) studied the acute tolerance of *C. bartonii* to acid stress and found that although increased acidity resulted in lower survivorship, *C. bartonii* are quite tolerant of acidification relative

to other freshwater organisms (see also Berrill *et al.*, 1985). Survivorship of adult *C. bartonii* over a 96-h trial was 50% at a pH of 2.43 (DiStefano *et al.*, 1991). The fish fauna of small streams is considerably less tolerant to acidification. Daye & Garside (1975) report that brook trout (*Salvelinus fontinalis* Mitchell), the most acid-tolerant fish of eastern coldwater streams, have a 50% survival rate at pH 3.5 for 167-h trials. Several studies show that fish of headwater streams prey on crayfish and may limit crayfish population size (Momot, 1967; Englund, 1999; Englund & Kruppa, 2000; Flinders & Magoulick, 2003; Townsend, 2003). Thus, it is possible that food-web level processes could reverse the direct effects of acidification, and acidification could have a net positive effect on crayfish populations (Eriksson *et al.*, 1980).

Here we examine the individual and population-level effects of acidification on crayfish in headwater streams. We document the relationship between acidity and crayfish growth with enclosure experiments, and we measure the relationship between stream acidity and population size of fish and crayfish through electrofishing surveys. Results of the experiments and surveys suggest that acidification has contrasting effects on individuals and populations.

Methods

Study system

We surveyed 24 reaches in nine first-order streams within the Tionesta Creek catchment of north-western Pennsylvania, U.S.A. (Fig. 1). Our study streams lie within the boundaries of the Allegheny National Forest (ANF), which encompasses more than 250 000 ha of forested lands on the Allegheny Plateau. Each of our study streams drain catchments that are entirely forested and have experienced minimal landuse disturbance in recent years. Eighteen of our 24 study sites are bordered by mature, second growth forest, dominated by Eastern hemlock (*Tsuga canadensis* Carriere), Yellow birch (*Betula alleghaniensis* Britton), American beech (*Fagus grandifolia* Erhart), and Black birch (*Betula lenta* Linnaeus). The remaining six study sites lie within the old-growth forest of the Tionesta Scenic and Research Natural Area, a 2000 ha tract also dominated by the hemlock-birch-beech association. Wetted channel width of our study reaches at summer baseflow ranged from 2.2 to

5.9 m (Table 1). Our study streams are characterised by high gradients, low colour, low fertility, and low embeddedness. We specifically avoided sampling any sites with highly embedded substrata, as crayfish are often markedly less abundant in such reaches (S. Seiler, unpublished data).

The Allegheny plateau of northern Pennsylvania experiences sulphate (SO_4^{2-}) and nitrate (NO_3^-) deposition rates that are among the highest in North America [National Atmospheric Deposition Program (NADP), 2002]. Sulphates and nitrates form strong acids, which reduce the pH of rainfall. The NADP/National Trends Network monitors a location in the Kane Experimental Forest (NADP/NTN PA29), which is within 40 km of all our study sites. The mean hydrogen ion concentration of precipitation at this site, measured as pH, was 4.35 in 2001 (NADP, 2002), which was among the most acidic of the more than 200 sites monitored nationwide. As this region is sparsely populated and has few local point-source emissions of sulphates and nitrates, most of the acid deposition is probably because of long-range atmospheric transport from the mid-western region of the United States.

The soils of the unglaciated Allegheny Plateau are predominately Ultisols, derived from sandstones and shale, and are typically quite nutrient poor and acidic (Drohan & Sharpe, 1997). Headwater streams of the plateau are often poorly buffered, with acid neutralising capability typically $<50 \mu\text{eq L}^{-1}$ (Seiler, 2002), and thus are vulnerable to acidification. However, local variation in bedrock geology and soil properties causes adjacent catchments to differ in their ability to buffer acid deposition. We took advantage of variation in the acidity of otherwise similar headwater streams to assess the effects of stream acidification on crayfish growth and population size.

Water chemistry

We measured the pH of each study site on at least five occasions in the summers of 2000 and 2001. pH was measured *in situ* with field meters that were calibrated daily. Under normal flow conditions in summer, pH was quite stable, and most of our replicate readings were within 0.1 pH unit. Therefore, we selected the median of these readings, and use this value

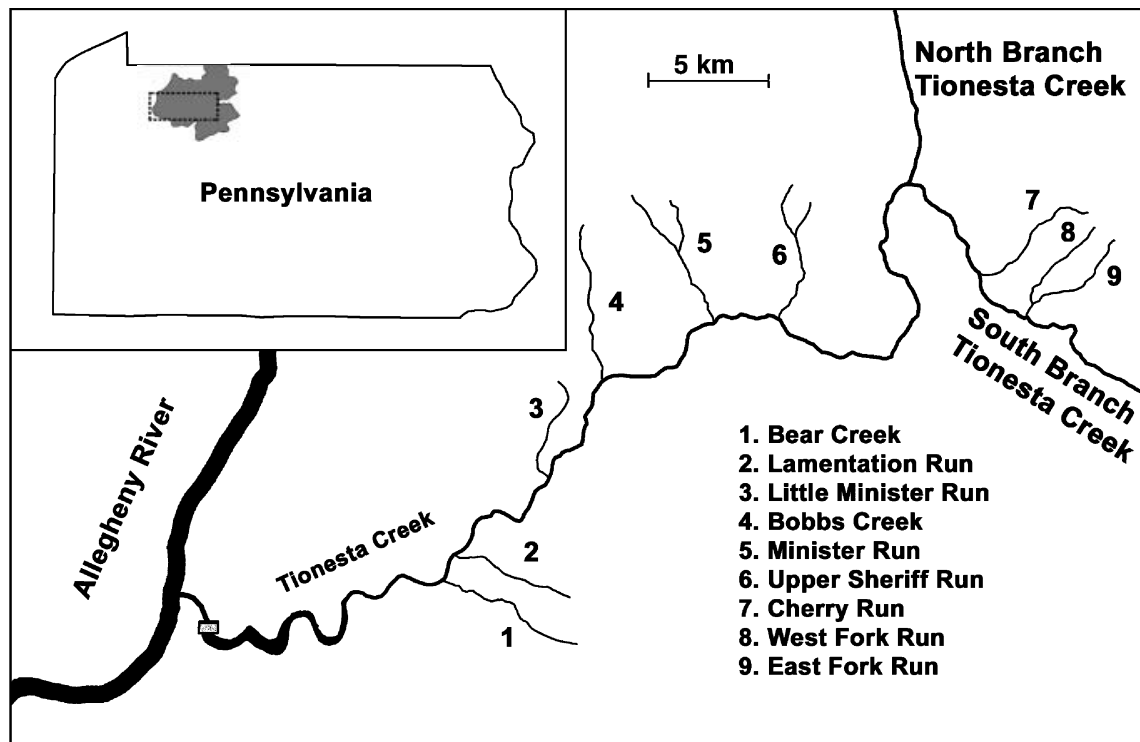


Fig. 1 Location of study streams within the Allegheny National Forest, PA, U.S.A. Inset: Location of the Allegheny National Forest within north-west Pennsylvania. Dashed box indicates the area shown in the main figure.

Table 1 Description of study sites. Distance from mouth is measured from confluence with Tionesta Creek. Summer baseflow pH is the median of several measurements; spring pH and aluminium concentrations are based on a single sampling event, 1 March 2001

Site	UTM Coordinates Zone 17 T		Distance from mouth (km)	Width (m)	pH-summer baseflow	pH-spring snowmelt	Aluminium ($\mu\text{g L}^{-1}$)
Bear Creek	643725 E	4610567 N	4.06	2.7	5.5	—	—
Bobbs Creek	652553 E	4618174 N	0.79	5.4	7.1	—	—
Cherry Run 1	651941 E	4611723 N	0.27	3.4	6.9	5.8	11
Cherry Run 2	656123 E	4612091 N	0.60	3.6	6.9	5.8	12
Cherry Run 3	648505 E	4620044 N	1.58	3.8	6.9	5.7	7
Cherry Run 4	652289 E	4623672 N	1.98	4.4	6.9	5.4	8
Cherry Run 5	662294 E	4634877 N	2.90	4.3	6.9	5.5	22
East Fork Run 1	667742 E	4619018 N	0.85	2.8	6.0	5.5	13
East Fork Run 2	655587 E	4625008 N	1.55	2.2	5.6	5.3	50
East Fork Run 3	664727 E	4627404 N	2.18	3.6	5.7	5.0	137
East Fork Run 4	659543 E	4626854 N	3.17	3.9	5.2	5.2	143
Lamentation Run 1	622977 E	4625977 N	0.20	5.0	6.4	—	—
Lamentation Run 2	640351 E	4598406 N	1.24	3.0	6.0	—	—
Lamentation Run 3	625912 E	4608782 N	3.37	1.7	4.9	—	—
Little Minister Run	643500 E	4605595 N	0.34	2.5	6.9	—	—
Minister Creek	640045 E	4634189 N	1.77	5.2	6.7	—	—
West Fork Run 1	668210 E	4619770 N	0.85	2.9	6.2	5.2	128
West Fork Run 2	655375 E	4637901 N	2.20	2.3	5.0	4.3	331
West Fork Run 3	662860 E	4618134 N	2.90	2.7	4.5	5.0	459
West Fork Run 4	667718 E	4622999 N	3.23	2.5	4.6	4.1	413
West Fork Run 5	653835 E	4628055 N	3.95	2.5	4.5	4.1	459
Upper Sheriff Run 1	642000 E	4613556 N	0.33	5.9	7.4	5.7	97
Upper Sheriff Run 2	655617 E	4636364 N	3.75	3.5	5.2	4.4	319
Upper Sheriff Run 3	645592 E	4630289 N	5.37	2.3	4.4	3.8	228

(hereafter baseflow pH) in all further analyses. These pH values should reflect the degree of chronic stress associated with acidification. Acid stress in poorly buffered catchments can be particularly acute during spring snowmelt and rain showers (Wigginton *et al.*, 1996). We measured pH and collected water samples for analysis of aluminium concentrations at 17 of our 24 sites during spring thaw on 1 March 2001. Concentrations of total aluminium were analysed using the Eriochrome Cyanine R method (American Public Health Association, American Water Works Association & Water Environment Federation, 1998).

Crayfish growth

We conducted two experiments with in-stream crayfish enclosures to assess how crayfish growth rates might differ between acidified and circumneutral sites. Enclosures allowed growth rates to be measured on individual crayfish and prevented size-selective mortality as a result of predation. Each enclosure was cylindrical, measuring 60 cm long by 20 cm in diameter, and was constructed of a coarse plastic mesh

(5 mm square mesh). Before crayfish were added, each enclosure was partially filled with cobbles from the study site to provide habitat and to anchor the enclosures in the stream. Each enclosure received two crayfish (one male, one female), with a maximum carapace length difference of 2 mm between crayfish sharing an enclosure. The cages were built with relatively coarse mesh so that invertebrate prey and other resources (e.g. detritus) could pass into the cage. Therefore, the observed growth within each cage is a metric of the growth potential of that site relative to other sites. Crayfish density within enclosures (16.7 m^{-2}) was higher than we observed in these streams, but within the range of natural variation in *C. bartonii* densities (e.g. Surber, 1936; Huryn & Wallace, 1987).

In the first experiment, the effect of acidity on crayfish growth was tested by rearing crayfish in Cherry Run (circumneutral) and in West Fork Run (acidic). *Cambarus bartonii* were collected from Cherry Run by seining. Eleven enclosures on Cherry Run and eleven enclosures on West Fork Run were stocked with crayfish on 25 May 2000. An additional eleven

enclosures on each stream were stocked on 6 June 2000. At the time of stocking, pH for Cherry Run sites was 6.5 and pH for West Fork Run sites was 4.2. Initial carapace lengths, averaged across enclosures, was 21.0 mm for Cherry Run and 20.3 mm for West Fork Run (*t*-test for initial size differences: $P > 0.05$). At the end of the summer (122 days after stocking for each set of enclosures) we recovered surviving crayfish, measured their carapace lengths, and calculated the average change in carapace length for each cage. Repeated observations in both streams and throughout the summer found crayfish molting, suggesting that crayfish in both streams underwent at least one, and likely two molts. Two cages (one from each stream) were left dry by receding water and were eliminated from the study. Using cages as our unit of observation, we used ANOVA to test whether growth rates differed between Cherry Run and West Fork Run. Location/loading date was incorporated as a blocking factor in the ANOVA in order to control for the time between stocking each set of enclosures. There was some mortality during the study, and we also included final density of each cage (one or two crayfish) as a blocking factor in the ANOVA to account for any effect of density on growth.

If crayfish originating from a circumneutral stream grow more slowly in an acidified stream, the growth effect could be because of low pH *per se*, or it could reflect acclimation to local conditions, regardless of pH. Therefore, we performed a reciprocal transplant experiment in the summer of 2001 in order to test whether crayfish exhibited any local acclimation that could account for the growth patterns observed in summer 2000. Upper Sheriff Run was chosen for the reciprocal transplant study because pH increased from 4.4 in the headwaters to 7.4 at a site just 5.05 km downstream. *Cambarus bartonii* are abundant throughout this stream. Twenty enclosures were placed at a downstream location near Tionesta Creek and twenty enclosures were placed in the headwaters of Upper Sheriff Run. At the downstream site, 10 enclosures were stocked with crayfish originating locally and 10 enclosures received crayfish collected from the headwater site. The upstream enclosures were stocked in a similar manner with 10 receiving local crayfish and 10 receiving crayfish from the downstream site. Averaged across enclosures within a treatment, initial carapace lengths ranged from 18.9 to 20.9 mm, and there were no significant differences

among treatments in initial size ($P > 0.05$). The experiment was initiated on 10 June 2001. After 114 days, crayfish were recovered and carapace lengths measured, and the average change in carapace length was calculated for each enclosure. To test for site effects and acclimation effects on growth, a 2×2 ANOVA was performed with site (upstream or downstream) and crayfish source (upstream or downstream) as treatments and final density (one or two) as a blocking term.

Field surveys of fish and crayfish

We surveyed fish and crayfish populations of 24 reaches across nine streams in the summers of 2000 and 2001. A 50 m transect was established at each site, blocked off with nets to contain fish and crayfish, and surveyed by three-pass removal sampling with a backpack electrofishing unit (pulsed DC current, 500–700 V). Electrofishing can be an effective method of censusing crayfish populations (Taylor, McIntosh & Peckarsky, 2001). Crayfish abundance varies across small scales in headwater streams of the ANF (Seiler, 2002), and we consider multiple transects on a stream to be independent samples. Crayfish carapace length was measured to the nearest 0.1 mm. The fish fauna of these streams, ranked by biomass, is dominated by creek chub (*Semotilus atromaculatus* Mitchell), brook trout (*S. fontinalis*), mottled sculpin (*Cottus bairdi* Girard), blacknose dace (*Rhinichthys atratulus* Hermann) and white suckers (*Catostomus commersoni* Lecepede). Of these, only the brook trout, creek chub, and mottled sculpin were large enough to prey upon crayfish, so we calculated the biomass of these three taxa (hereafter 'fish biomass'). Fish were identified and total length measured, and their wet mass was calculated using length–weight regressions generated from specimens sampled from headwater streams of north-western Pennsylvania (Seiler, 2002). Estimates of crayfish density (No. m^{-2}) and fish biomass (g m^{-2}) were calculated from the total catch of the three passes divided by transect area.

We used regression analysis to test whether there was a relationship between pH and crayfish density, or pH and fish standing crop. Sampling sites (reaches) constituted the unit of observation for all analyses of survey data. Crayfish densities were \log_{10} transformed, and fish standing crops were $\log_{10}(x+1)$ transformed (five sites lacked fish), in order to

produce residuals with variances that were homogenous with respect to the independent variable (pH). We also tested whether there was a relationship between fish standing crop and crayfish density.

Fish are highly selective when feeding on crayfish, preferring small individuals (Momot, 1967; Stein & Magnuson, 1976). If fish predation is playing an important role in regulating crayfish populations there should be a predictable shift towards larger crayfish at the sites with the most fish. We used regression analysis to evaluate the relationship between fish biomass and the proportion of large crayfish (carapace length > 25 mm) and small crayfish (carapace length < 15 mm).

Results

Water chemistry

Median baseflow pH of the 24 study sites ranged from 4.4 to 7.4 (Table 1). The site with the lowest pH was on the same stream as the site with the highest pH (Upper Sheriff Run), and upstream just 5.05 km, illustrating the large within-stream variation in pH that is typical of some catchments. Of the five streams with three or more sample sites, four showed large within-stream changes in pH (0.8–3.0 pH units), with the lowest pH values always recorded in the headwaters and pH generally increasing with each successive downstream measurement (Table 1). Cherry Run showed no within stream shifts in baseflow pH, with a uniform value of 6.9 along its length.

Conditions were harsher during spring snowmelt (Table 1). Considering the 17 sites for which we have spring runoff data, the median decline in pH from summer baseflow values was 0.7 pH units. Concentrations of total dissolved aluminium ranged from 7 to 460 $\mu\text{g L}^{-1}$. Summer baseflow pH was correlated with both aluminium concentrations in spring (Pearson's $r = 0.84$) and spring pH (Pearson's $r = 0.87$). Thus, although pH was lower during spring melt, the relative ranking of sites based on spring pH measurements or aluminium concentrations was quite similar to rankings based on summer pH measurements.

Crayfish growth

In both summers of study crayfish growth was lower at acidified sites than at circumneutral sites (Fig. 2).

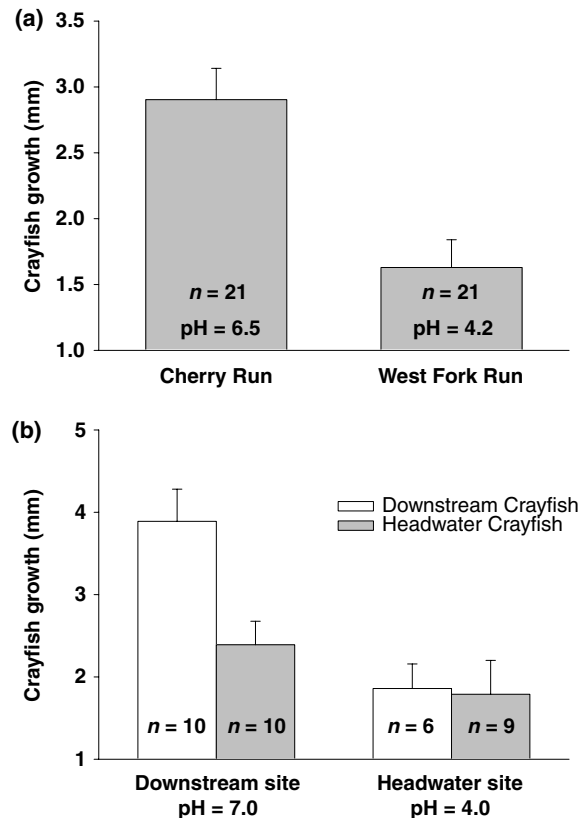


Fig. 2 (a) Crayfish growth in enclosures placed in a circumneutral and acidic stream. Growth was measured as average change in carapace length in each enclosure averaged across all enclosures in each stream (± 1 SE). Stream pH at beginning of experiment and number of enclosures in each stream is shown. (b) Crayfish growth in reciprocal transplant study conducted in Upper Sheriff Run. Number of enclosures in each treatment and pH at end of study is shown.

Crayfish growth in summer 2000, measured as change in carapace length, was 1.57-fold higher in Cherry Run than West Fork Run (Fig. 2a; stream effect: $F_{1,38} = 12.7$, $P < 0.001$). Crayfish density (one or two survivors) had no effect on growth rates ($F_{1,38} = 2.94$, $P = 0.36$), suggesting a lack of density dependence within enclosures.

The reciprocal transplant study also showed that crayfish, regardless of their origin, grow more at a circumneutral site than an acidified site (Fig. 2b, site effect: $F_{1,30} = 13.5$, $P < 0.001$). Growth rates also depended on site of origin. When transplanted into the downstream site, crayfish from the headwaters site grew substantially slower than did crayfish of local origin (origin effect: $F_{1,30} = 5.1$, $P = 0.04$; site \times origin interaction: $F_{1,30} = 4.2$, $P = 0.05$). There

were no appreciable origin effects at the upstream site (Fig. 2b). As with the first growth experiment, final density had no significant effect on growth rates ($F_{1,30} = 3.2$, $P = 0.10$).

Overall survivorship was 72% in 2000 and 76% in 2001. Because mortality was due in part to receding water leaving a few enclosures dry, we did not test for location effects on survivorship. During mid-summer monitoring and upon final collection detritus, invertebrates (plecopteran nymphs and dipteran larvae), and young of year crayfish were observed within enclosures, suggesting that they were permeable to food resources.

Fish and crayfish survey

Both crayfish density and fish biomass were related to variation across sampling sites in median baseflow pH. Across the gradient of increasing pH, crayfish density declined more than sixfold (Fig. 3a; $F_{1,22} = 29.4$, $P < 0.0001$) from a mean of 1.38–0.22 individuals m^{-2} . Across this same pH gradient, fish biomass increased from 0 at pH 4.4 to a mean of 2.21 $g m^{-2}$ at pH 7.4 (Fig. 3b; $F_{1,22} = 33.1$, $P < 0.0001$). Because crayfish and fish responded in different ways to the pH gradient, crayfish density declined more than eightfold with increasing fish standing crop ($F_{1,22} = 8.79$, $P = 0.007$).

Shifts in crayfish size structure were consistent with those expected if fish predation were driving patterns of crayfish abundance. There was a threefold increase in the proportion of crayfish with carapace lengths >25 mm as fish standing crop increased from 0 to 3.1 $g m^{-2}$ (Fig. 4a, $P = 0.001$). Conversely, the proportion of crayfish with carapace lengths <15 mm declined more than threefold across the same gradient (Fig. 4b, $P < 0.001$).

Discussion

Ecologists appreciate that abiotic factors can affect the outcome of food web processes (e.g. Menge & Sutherland, 1987; Wilbur, 1987; Dunson & Travis, 1991). Unfortunately, relatively few studies actually attempt to simultaneously evaluate the individual- and population-level effects of abiotic factors along environmental gradients, and thus the extent to which abiotic factors alter the outcome of species interactions is still an open question for most types of ecosystems

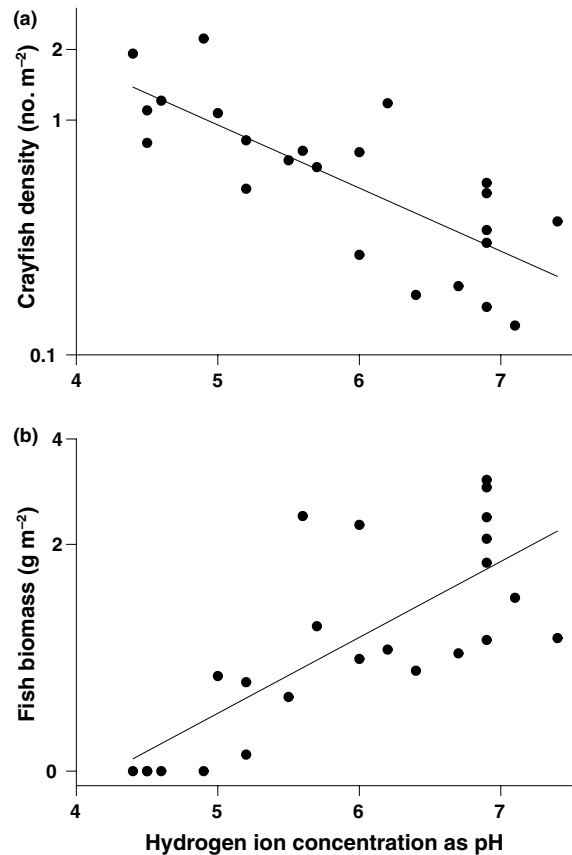


Fig. 3 (a) Relationship between hydrogen ion concentration, measured as pH, and crayfish mean density for 24 sites in nine streams ($P < 0.0001$, $R^2 = 0.60$). Crayfish density is \log_{10} transformed. $\log_{10}C = 1.312 - 0.267 \times \text{pH}$ where C is crayfish density (No. m^{-2}). (b) Relationship between pH and fish biomass for the 24 study sites ($P < 0.0001$, $R^2 = 0.57$). Fish biomass is $\log_{10}(x+1)$ transformed, and includes creek chub, brook trout, and mottled sculpin. $\log_{10}F = -0.677 + 0.160 \times \text{pH}$ where F is [fish standing crop ($g m^{-2}$) + 1].

(Warner, Travis & Dunson, 1993). Our results suggest that acidification of headwater streams has contrasting effects on individuals and populations. Acidification had a negative effect on the growth of individual crayfish, and these effects persisted even when crayfish were transplanted into circumneutral reaches. Despite the negative effects of acidification on individuals, crayfish population sizes were more than sixfold larger in acid streams relative to neutral streams, perhaps due to modification of food web interactions.

Most of the work concerning the effects of acidification on stream invertebrates, and crayfish in particular, have been laboratory studies examining

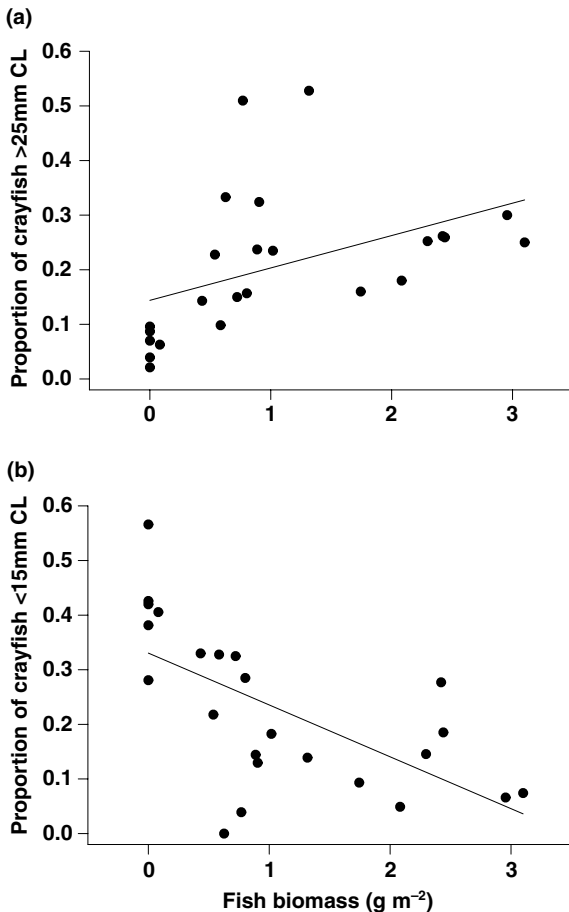


Fig. 4 (a) Relationship between fish biomass and density of large crayfish ($P = 0.029$, $R^2 = 0.20$). (b) Relationship between fish biomass and density of small crayfish ($P = 0.001$, $R^2 = 0.40$).

the direct effects of low pH on survival (e.g. Berrill *et al.*, 1985; DiStefano *et al.*, 1991). These studies confirm that acidification is an environmental stressor, and that extreme acidification will ultimately prove lethal to crayfish. However, the results of these individual-level studies are not easily extrapolated to a field setting, where both direct and indirect effects play out (Peckarsky, 1983; Menge & Sutherland, 1987; Dunson & Travis, 1991). Our field surveys show, in fact, a positive relationship between acidity and crayfish population size. Field surveys, by themselves, suffer from the weakness that underlying mechanisms are often unclear, and a combination of field surveys, manipulative experiments, and laboratory studies will ultimately prove to be the most powerful approach (Sadinski & Dunson, 1992; Gonzalez & Frost, 1994).

Our growth experiments showed that crayfish placed in acidified sites experienced substantially lower growth rates than did crayfish in circumneutral reaches. Lower growth in acidified streams could reflect the energetic cost of tolerating high levels of acidity or dissolved metals, or it could be because of lower resource availability. The negative effects of low pH on growth shown in this study are consistent with the results of other studies (Rodgers, 1984; Freda & Dunson, 1985). In other taxa, acidification is thought to impair growth rates by reducing conversion efficiency of food to growth (Lee, Gerking & Jezierska, 1983; Hargeby & Petersen, 1988). Acidification increases the energetic costs associated with osmoregulation, ion retention, and respiration (Økland & Økland, 1986). Inhibition of crayfish growth in acidified water may have important ecological consequences, as it is likely that foraging ability, vulnerability to predators, and reproductive output are size-dependent (e.g. Moore & Folt, 1993).

In the first growth study, all crayfish originated from Cherry Run and experienced near-neutral pH prior to the experiment. Thus, the observed growth depression in an acidified stream reflects how crayfish might respond in a system undergoing episodic acidification. However, this experiment by itself does not parse the potential effects of local acclimation from the effects of acidification. The reciprocal transplant experiment demonstrates that reducing pH results in lower growth, but increasing pH results in higher growth rates. In addition, at the downstream site, crayfish transplanted from acidified upstream sites grew more slowly than local crayfish, suggesting that there may be physiological costs associated with acclimation to acidity (see also Daveikis & Alikhan, 1996).

The apparent success of crayfish populations in acidified streams is a conspicuous exception to the general pattern of few calcareous shelled organisms in soft waters (Williams, 1984). The crayfish cuticle is composed of a matrix of protein and calcite, but the calcium and especially carbonate ions necessary for calcification of the cuticle are virtually absent in these acidified streams. The supply of calcium ions to the mineralisation tissue comes from the hypodermal cells, but the production and delivery of carbonate ions to the site of mineralisation is not well understood (Roer & Dillaman, 1984). Regardless of the mechanism, crayfish are clearly adept at inducing

carbonate deposition in an acidic environment, but the process of mineralising the exoskeleton may be energetically expensive (Horne, Tarsitano & Lavalli, 2003) and may contribute to lower growth rates in acidified streams.

Several other field studies found, like we did, that crayfish are surprisingly successful in stressful environments (e.g. Kullberg, 1992; Griffith *et al.*, 1996). For example, Flinders & Magoulick (2003) found that crayfish were more abundant in intermittent streams than in permanent streams, and conclude that fish predation played a large role in shaping this pattern. Although these studies did not include measurements of predator abundance, the predator release hypothesis is reasonable explanation for positive effect of abiotic stress on crayfish population size, and provides motivation for further experimental work in these systems.

The negative relationship between fish biomass and crayfish abundance provides only indirect evidence for the predator release hypothesis and must be interpreted with caution. However, several lines of evidence support the predation hypothesis. (i) Shifts in crayfish size structure are consistent with that expected if size-selective predation were regulating population size. (ii) In a pilot study of creek chub, sculpin, and brook trout diets in our study streams, we found that that crayfish were a common dietary component of fish diets (Seiler, 2002). Crayfish were found in 17% of the creek chub guts, 33% of brook trout guts, and 75% of sculpin guts (Seiler, 2002). (iii) Other experimental and observational studies have concluded that fish in headwater streams have a strong effect on crayfish population size and size structure (Englund, 1999; Flinders & Magoulick, 2003). Other mechanisms could also account for these patterns. Increased crayfish abundance at low pH could reflect a release from competition if competitors are more sensitive to acidity than are crayfish (Bell & Nebeker, 1969; Lechleitner *et al.*, 1985), or crayfish populations could be responding to some other factor correlated with pH.

The relationship between pH and fish biomass observed in these Appalachian Plateau streams is similar to the relationship documented in other systems (e.g. Baker *et al.*, 1996; Van Sickle *et al.*, 1996; Baldigo & Lawrence, 2001). For example, Baldigo & Lawrence (2001) found that Catskill Mountain streams that experienced a mean pH < 5.0

were generally without fish, whereas streams at pH = 5.0 and above contained fish, with fish biomass related to pH. This result mirrors those presented here, suggesting that although factors such as episodic acidification and metal toxicity may ultimately limit fish populations in these streams, the baseflow pH of a stream is still a good predictor of its ability to support fish. In contrast to the fish studies, we are not aware of any other landscape-scale studies of stream acidification and crayfish dynamics with which to compare our results. Toxicological studies of crayfish tolerance to low pH show that results vary a great deal among species tested, with the cambarids generally being more tolerant than the orconectids (Hobbs & Hall, 1974; Morgan & McMahon, 1982; Berrill *et al.*, 1985). Thus, the results presented here may not be easily generalised to other streams lacking highly tolerant taxa.

The relationships among pH, fish biomass, and crayfish abundance are striking when considered in light of all the other biotic and abiotic factors known to affect fish and crayfish abundance in streams. Factors such as stream productivity, substrate size, gradient, and riparian vegetation undoubtedly play an important role in determining fish and crayfish abundance. Yet pH, by itself, explained more than half of the variation among sites in both fish biomass and crayfish density ($R^2 = 0.60$ for crayfish, 0.57 for fish; Fig. 3). Our study streams were similar to each other with respect to many biotic and abiotic factors, and a study encompassing a wider variety of stream types would likely find that other factors also play an important role in regulating fish and crayfish population sizes. Nevertheless, our data show that at the landscape scale variation in pH is a good predictor of the variation in fish and crayfish population size.

The direct and indirect effects of acidity on lake communities are well documented, and many of these studies highlight the sometimes contrasting effects of acidification on individuals and populations, and thus the importance of considering population level effects of environmental stress in the context of food-web interactions (Eriksson *et al.*, 1980; Yan *et al.*, 1985; Locke, 1992; Gonzalez & Frost, 1994; Fischer *et al.*, 2001). In contrast, there are relatively few studies examining how acid stress may reorganise stream food webs. This study suggests that the net effect of moderate acidity on *C. bartonii* populations is positive, illustrating how indirect effects, possibly mediated

through shifts in food web structure, can outweigh the direct, negative effects of acidity. Thus, the response of populations to changes in the environment may be contingent on the structure of the food web in which they are embedded.

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