EFFECTS OF ACIDIFICATION ON THE CRAYFISH
CAMBARUS BARTONII BARTONII
IN SOUTHERN APPALACHIAN STREAMS

by

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(ABSTRACT)

Population biology and acid tolerance of the crayfish *Cambarus bartonii bartonii* (Fabricius) from southern Appalachian Mountain streams were investigated. Field studies were conducted primarily from May through October, 1985 at Coweeta Hydrologic Laboratory (U. S. Forest Service Southeastern Experimental Station) in Otto, North Carolina, to describe selected population parameters and life history events for this species in two first-order streams. Laboratory experiments were conducted using crayfish from Coweeta and from Craig Creek (Jefferson National Forest, Montgomery County, Virginia) to determine median lethal concentrations (LC_{50}) for sulfuric acid exposure, and to investigate physiological effects, influence of ambient water temperature, and effects of episodic acid events on molting.

Population estimates of crayfish ranged from 210 to 683 crayfish in 20 to 25m study sections ($\cong$9.2 to 29.8 per m^2) of Ball Creek and Pinnacle Branch. Mean catch per baited funnel trap and mean catch per hour of hand collecting were determined in three study sections of each stream in May and September to obtain relative abundance estimates and compare capture methods. Each method selected for different segments (size classes) of the population. Sex ratios were balanced (1:1) over the whole sampling season, but differed significantly from 1:1 in some months, probably due to reproductive activity. Sexually mature males and females (form I males, females with full cement glands) were observed in all months sampled.

Acute (96h) laboratory lethality tests yielded LC_{50} values for pH's of 2.43, 2.56, 2.85 and 2.43 for Coweeta intermolt adults (25.3 to 43.2mm carapace length, CL), late juveniles (11.5 to 19.9mm CL), early juveniles (3.9 to 12.8mm CL) and Craig Creek intermolt adults (23.0 to 41.3mm CL), respectively. Decreased water temperature resulted in increased acid tolerance of intermolt adults (LC_{50} = pH 2.33), and increased survival time during severe acid exposure. Acid exposure of intermolt adults in relatively soft water affected internal ion regulation, causing decreased hemolymph [Na^+] and increased [Ca^{++}]; no Ca^{++} mobilization (loss) from carapaces was observed. Preliminary work showed that extreme acidification had visible adverse effects on molting adults, causing failure of exoskeletons to become rigid.

Episodic acid events at Coweeta do not appear to be a direct mortality problem to *C. b. bartonii* at this time. However, this does not preclude the possibility of future problems, particularly sublethal effects to reproductive activity or early life history stages, if increasing acidification and gradual loss of soil buffering capacity of watersheds persists.

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I thank Dr. Richard J. Neves and Dr. Louis A. Helfrich for providing the opportunity for me to become acquainted with toxicological investigations and a fascinating group of animals, crayfish. They gave constant financial and professional support, friendly guidance, and freedom and encouragement to maintain and expand interests in other areas of aquatic science, while working on my assigned task. For that I am truly grateful. I would also like to thank the other members of my committee, Dr. Donald J. Orth and Dr. Alan G. Heath, for sharing timely insights and assistance whenever I asked, particularly in the analytical and physiological aspects of this study. Special thanks go to Dr. Robert J. Sheehan and Dr. Jerry L. Farris for generous giving of their expertise and friendship.

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Introduction

Acidification of aquatic ecosystems is one of the foremost environmental problems presently facing North America. Many scientists believe this problem can be primarily attributed to atmospheric transport and deposition of pollutants derived from anthropogenic activities (Oden 1976, Husain and Samson 1979, Hall et al. 1982, Ouellet and Jones 1983, Overrein 1983, LaZerte and Dillon 1984) and is influenced by chemical reactions in the atmosphere, forest canopy and soil (Dickson 1980, Howells 1983, Moore 1983). Trends over the past few decades indicate a general lowering of pH in rain and snow, and large regions of North America currently receive precipitation below pH 4.7 (Haines 1981, Likens and Butler 1981).

Reduced pH has affected aquatic life at all trophic levels, often causing irreparable damage (Hendrey et al. 1976, Overrein 1983). Alterations of species compositions, reduced diversity and abundance, and local extinctions of sensitive species and whole communities have been documented (Almer et al. 1974, Sprules 1975, Harvey 1980, Haines 1981, Singer 1982, Schindler 1985). These biological changes include direct effects of acid stress to organisms, which can lead to secondary effects such as restructuring of plant and animal communities caused by alterations in trophic relations (Eriksson et al. 1980, Eilers et al. 1984), changes in nutrient cycling rates (Sutcliffe and Carrick 1973, Burton et al. 1982, Burton et al. 1985) and increased incidence of disease (France and Graham 1985). Although detrimental effects of declining pH are most often reported, some individual species and whole communities appear to benefit from reduced pH, often through reduced predation and competition (Collins 1981, Schindler 1985, Mackay and Kersey 1985). Increased concentrations of metals and organic pollutants deposited from the atmosphere or leached from soil and sediments may react synergistically, additively or antagonistically with acid (Burton et al. 1982, Ouellet and Jones 1983, Andersson and Nyberg 1984, Schindler 1985). Aluminum in particular has been cited to have toxic and indirect effects on aquatic life (Muniz and Leivestad 1980, Hall et al. 1985, Malley and Chang 1985), while also serving as a buffer to acid in certain pH ranges (Henriksen 1980, Johannessen 1980).

In the last 30 years, precipitation in the southeastern United States has undergone the most rapid decline in pH of any region in the country (Likens and Butler 1981). This area is expected to exhibit the same pattern as regions previously affected, such as Scandinavia, United Kingdom, southeastern Canada and northeastern United States (Overrein 1983). Areas such as the southern Appalachian Mountains, where acid-neutralizing capacity of soils and water is poor, are considered at high risk for acidification (Harvey 1980, Burns et al. 1981, Hileman 1981), particularly in small, high-altitude headwater streams (Shaffer and Galloway 1982).

A real need exists to examine and document the influence of reduced pH on aquatic ecosystems, since many of the crucial aspects of this phenomenon are poorly understood (Malley 1980). Despite a recent dramatic increase in acidification research, information is still lacking on the susceptibility, resistance and recovery capacity of most aquatic organisms to acid pollution and its associated problems. Studies of this kind will facilitate prediction of the extent and magnitude of intensifying impacts (Haines 1981, Pratt and Hall 1981, Singer 1982), and help to identify specific groups of organisms which might serve as indicators of various stages of the problem. Insufficient knowledge of impacts of acid deposition in the aquatic environment demands immediate, innovative research. Extensive surveys and monitoring efforts in Scandinavia should serve as predictive models of possible consequences for other areas undergoing acidification (Overrein et al. 1980). Both field and laboratory manipulations to determine response mechanisms of aquatic organisms are needed (Pratt and Hall 1982, Singer 1982). Laboratory studies will provide precise dose-effect response information concerning predicted effects of a single pollutant on an organism, but these investigations never successfully duplicate all interacting variables of the natural environment (U.S. Environmental Protection Agency 1975). Specifically, laboratory bioassays and toxicity tests must distinguish limits of tolerance and identify often overlooked sublethal effects so that results can then be field tested (Singer 1982, France 1986).

Considerable field and laboratory research has been done on the lethal, physiological, and to a lesser extent, behavioral effects of acidification upon fishes (Fromm 1980, Spry et al. 1981, Schindler et al. 1985). Chronic reproductive failure due to acidification-induced effects during sensitive life cycle stages, interference with physiological mechanisms regulating active ion exchange across gill membranes, and complicating effects of metals and CO_2 are cited as probable causes of extirpated or declining fish populations in acid waters (Schofield 1976, Fromm 1980). Many factors including size, age, acclimation, genetics, and ionic content of ambient water interact in complex ways to determine acid tolerance of fish to natural and laboratory exposures (Schofield 1976).

Research on invertebrates, a prominent component of aquatic ecosystems, has involved mainly survey work (Sutcliffe and Carrick 1973, Økland 1980, Økland and Økland 1980, Raddum 1980, Collins et al. 1981, Eilers et al. 1984, Simpson et al.1985), with most experimentation being limited to the larval insect fauna (Bell 1970, Bell 1971, Hall et al. 1980, Zischke 1983, Burton et al. 1985, Hall et al. 1985, Lechleitner et al. 1985, Mackay and Kersey 1985, Schindler et al. 1985). Little is known of the basic toxicological and physiological responses of most invertebrates to acid pollution (Morgan and McMahon 1982). Crustaceans are considered highly sensitive indicators to acidification (Sprules 1975, Hendrey et al. 1976, Økland and Økland 1980, Schindler et al. 1985); however, crayfish (Decapoda: Astacidae), the largest and longest-lived of the freshwater crustaceans, have been largely neglected. Only recently have scientists attempted to assess the toxic effects of acid deposition on crayfish, and most studies have dealt with lake-dwelling species (France 1986).

Acidification and Crayfish

Crayfish are an important energy transfer link in aquatic trophic systems. They are unique in that they are polytrophic organisms, often functioning as the keystone predator (Momot et al. 1978). In high-elevation, southern Appalachian streams, *Cambarus bartonii bartonii* (the Appalachian brook crayfish) usually predominates over other crayfish species, and typically accounts for more biomass than most other species in these systems (Woodall and Wallace 1972, Huryn 1986). *C. b. bartonii* thrives on allochthonous organic matter common to these streams, as well as preying upon insects, salamanders and fish; cannibalism is also fairly common. This species serves as a staple diet item of trout and other fish predators (Tebo and Hassler 1961), as well as many wildlife species such as raccoon *Procyon lotor*, river otter *Lutrus canadensis*, mink *Mustella vison*, fisher *Martes pennanti*, opossum *Didelphus marsupialis* (Trippensee 1953), black bear *Ursus americanus* (Garner 1986), black-bellied salamander *Desmognathus quadramaculatus* (personal observation), queen snake *Regina septemvittata* (Behler and King 1979), hellbender *Cryptobranchus alleganiensis* and northern water snake *Nerodia sipedon* (Behler and King 1979). Loss of these crayfish populations would likely have an effect on the natural food web in and along these stream ecosystems.

Increased acidification of poorly buffered waters may pose a serious threat to long term survival of crayfish populations. Declining or extirpated crayfish populations have been reported in Scandinavia, the United Kingdom and Canada. *Astacus astacus* has been shown to be highly sensitive to acid pollution and is now rare in thousands of Swedish lakes with pH less than 6.0 (Almer et al. 1974, Hultberg 1976, Appelberg 1983). Repeated attempts to restock this species into acidified lakes have failed (Fürst 1977a).

Field experimentation indicates that crayfish may be quite susceptible to acidification. Placement of ovigerous *A. astacus* in a river of varying acidity resulted in hatchling mortality at pH below 5.2 (Fürst 1977b, cited in France 1985b). Schindler et al. (1985) experimentally acidified a small lake (L223) in northwestern Ontario from an original pH value of 6.8 down to 5.0 over an 8-year period. *Orconectes virilis* recruitment declined drastically at pH 5.4-5.6, as partial mortality of egg broods was experienced by 6.9-16.9 % of "berried" females (France in prep.). Prevalence of "porcelain disease" (*Thelohania contejeani*) and fungal plague increased in the population as pH decreased (France 1983, France and Graham 1985). France (1983) also found reduced carapace rigidity and Ca^{++} content in exoskeletons of these *O. virilis*, and noted an increased bioaccumulation of manganese and mercury. Growth of L223 crayfish was not affected by acidification to pH 5.35, but crayfish disappeared from this system at pH 5.13. *Orconectes rusticus* and *Orconectes propinquus* are rare in Killarney region (Ontario) lakes of less than pH 5.5 (Berrill et al. 1985). Experimental field transplants in conjunction with laboratory tests suggested that exposure to a pH range of 5.4-6.1 in soft water was toxic to stage I and II juveniles of these two *Orconectes* species, but not to adult females carrying broods (Berrill et al. 1985).

Alternatively, there are field reports of high tolerance by crayfish to acid pollution. Warner (1971) observed a *Cambarus* sp. in a stream known to have an average pH of 4.6. *Parastacoides tasmanicus* commonly occurs in waters of pH 4.5 in the button grass plains of Tasmania (Newcombe 1975). *Cambarus robustus* inhabits the lakes of the Killarney region of Canada at pH levels of 4.6-4.9. Collins et al. (1981) reported populations of *C. robustus*, and obtained evidence of *O. propinquus* reproducing in three recently acidified (pH less than 4.9) Ontario lakes devoid of fish life.

Laboratory investigations to determine lethal effects of acid are few, and results are conflicting. Park et al. (1940) found pond-dwelling *O. virilis* and *Cambarus diogenes* to be more tolerant to a pH of 1.1 than stream-dwelling *O. propinquus*. Newcombe (1975) reported that *P. tasmanicus* tolerated experimental pH's down to 2.75. Adult intermolt specimens of *Austropotamobius pallipes* survived 4.5 h at pH 1.5 (HCl), 7 d at pH 5.0 and indefinitely at pH 7.0-8.0 (Jay and Holdich 1977). Acute acid (H_2SO_4) exposures for 96 h produced median lethal pH's (LC_{50}, of pH 2.8 ($[H^+]=1.6 \times 10^{-3}$ M) for *Procambarus clarki* and pH 2.5 ($[H^+]=3.1 \times 10^{-3}$ M) for *O. rusticus* (Morgan and McMahon 1982). Appelberg (1984) determined that several stages of the reproductive cycle in *A. astacus* were acid sensitive. When egg-bearing females were exposed to pH 5.0, a drastic loss of attached eggs was recorded. Mortality increased at the moment of hatching, and remained high during early post-embryonic stages at low pH. France (1984) subjected three life stages of *O. virilis* to chronic lethality tests and found 2 week-old stage III hatchlings to be 14% as resistant as 2.5 month-old juveniles, and only 5% as resistant as 2 to 3 year-old adults. Acidification below pH 5.5 for hatchlings and below pH 5.0 for juveniles caused a progressive increase in mortality, while the intermolt adults exhibited survival for a month at a pH of 3.5. France (1984) cited similar results in Baker's experiments (unpublished data) with *O. rusticus*, in which hatchlings and juveniles exhibited acid tolerances within 95% confidence limits, and adults within 90% confidence limits of *O. virilis*. Stage III juveniles of *C. robustus* molted and survived in soft, acidic (pH 4.0) water, while juveniles of *O. rusticus* and *O. propinquus* experienced mortality between pH 5.4-5.9. In these experiments, adult female *O. rusticus* and *O. propinquus* died within 6 d exposure to pH 4.2-4.7 (Berrill et al. 1985). The high variability in acid-caused lethality of crayfish between studies is partially due to inconsistencies in laboratory methodology, but appears to indicate differences in acid tolerance between life history stages and some species.

Much of the recent literature concerning reduced pH effects on crayfish focuses on sublethal (physiological) aspects. Malley (1980) found *O. virilis* in the Experimental Lakes Area of Canada to be more sensitive to nominal pH's of 3.0 and 4.0 in early postmolt stages as opposed to later postmolt or intermolt periods. She noted that the uptake of Ca^{++} by postmolt individuals was inhibited by pH levels below 5.75 and ceased below pH 4.0. Dejours and Armand (1980) observed a decrease in hemolymph pH (acidosis) of *Astacus leptodactylus* when pH of the ambient water was reduced. Morgan and McMahon (1982) and McMahon and Morgan (1983) reported severe hemolymph acidosis in *O. rusticus* and *P. clarki* caused by acidification. Acute exposures to pH 3.8 resulted in mild disturbances in major hemolymph ion concentrations, including increased Ca^{++} and possible depressed Na^{++} levels. This work also showed that 1 d (vs. 14 d) acclimation to experimental conditions resulted in reduced ionic changes in *P. clarki*. Nikinmaa et al. (1983) recorded decreased hemolymph pH in *A. astacus* exposed to pH 4.0-4.5, and a pronounced decrease in hemolymph pH in hypoxic-acid stressed individuals. Intermolts of this species also showed difficulties in acid-base regulation and osmoregulation in hypoxic-acid water of pH 4.0 (Järvenpää et al. 1983). Net accumulation of Na^{+} and K^{+} was depressed in embryos and hatchlings, and Ca^{++} accumulation drastically increased after hatching, indicating an acid-linked ionoregulatory disturbance. Exposure to soft, acidified (pH 4.0) water decreased hemolymph concentrations of Na^{+}, K^{+} and Cl^{-}, while Ca^{++} fluctuated in *A. astacus*. Fourteen day exposure to Al^{+++} at pH 5.0 reduced hemolymph Na^{+} in *A. astacus* and *P. leniusculus* (Appelberg 1985). Most recently, Wood and Rogano (1986) observed severe metabolic acidosis, moderate depression of hemolymph $[Na^{+}]$ and $[Cl^{-}]$, and a substantial increase in $[Ca^{++}]$ in acid-exposed (soft water) *O. propinquus*. Responses of *O. rusticus* differed, showing lower reductions of $[Na^{+}]$ and $[Cl^{-}]$, and much greater elevation of $[Ca^{++}]$. Hollett et al. (1986) reported varying tolerance of low pH among species, manifested in a difference in ion regulation. Exposure to pH 3.8 soft water did not change hemolymph $[Na^{+}]$ or $[Ca^{++}]$of *C. robustus* adults or total body $[Na^{+}]$ of juveniles, whereas *O. rusticus* stage III juveniles showed increased total body $[Na^{+}]$. The increase in research on physiological effects of acid on crayfish has concentrated on internal ion regulation, and results implicate ionoregulatory effects as a probable toxic mechanism of reduced pH.

Effects of heavy metals in combination with low pH are also a concern to researchers, since acid-leached aluminum and iron have been shown to be a problem with some fish species (Schofield 1976, Muniz and Leivestad 1980, Andersson and Nyberg 1984). Chang et al.

(1983) demonstrated retarded bioaccumulation of ^{203}Hg in tissues of *O. virilis* when exposed to lowered pH. Reduced pH, alone or in combination with aluminum, was found to increase oxygen uptake rate in eggs of *A. astacus* under slightly acidic conditions, but severe acid stress (pH 3.0) reduced uptake rate (Appelberg 1983). Malley and Chang (1985) reported low pH to inhibit Ca^{++} uptake in postmolt *O. virilis* more than aluminum, which had no effect above pH 6.0, and only a slight influence below pH 5.0. Finally, elevated aluminum levels failed to increase mortality among juveniles of all 3 species at pH 4.5-5.0 (Berrill et al. 1985).

Information on the influence of acidification on crayfish behavior is limited. Adult and yearling *O. virilis* from a control population displayed strong avoidance of potentially lethal pH (4.5), while specimens from an acidified lake exhibited significantly reduced avoidance behavior at pH 4.0. Intensity of avoidance was inversely related to pH, and smaller individuals tended to be more sensitive in response to acid (France 1985a). France (1985b) also reported no apparent effect of low pH (down to 4.0) on pleopod vibration frequency or duration of vibration period in "berried" female *O. virilis*. However, percent of total time spent vibrating pleopods (thus aerating eggs) at pH 4.0 and 4.5 was significantly lower than treatments at pH 5.0, 5.5 and 7.0.

The impacts of reduced pH on lotic species of crayfish are not well documented, but the tolerance range to reduced pH has been reported to be narrower than lentic species (Park et al. 1940, Hobbs and Hall 1974). Stream dwellers may therefore be more likely to be affected by acid pollution. As acidification in the southeastern United States increases, research to determine acid tolerance levels of valuable indigenous crayfish species will allow managers to anticipate, predict and monitor long-term changes in crayfish populations in poorly buffered southern Appalachian streams. Such changes, should they occur, will not only affect the many fish and wildlife species dependent on these crayfish as an energy source, but they may be interpreted as possible early warning signals for overall stream degradation caused by acid pollution.

The present study was conducted to assess the current status of *C. b. bartonii* populations in two southern Appalachian streams, and integrate these data with results from laboratory experimentation on the effects of acute acid exposure on this crayfish species. Specific objectives of this study were as follows:

1. To determine the relative abundance, sex ratios and size class structure of *C. b. bartonii* populations in two first-order streams at Coweeta Hydrologic Laboratory, North Carolina.

2. To document the pH tolerance and acute lethal toxicity of episodic, low pH levels (sulfuric acid) on adult intermolt male and female, and juvenile *C. b. bartonii* from southern Appalachian streams.

3. To investigate the effect of ambient water temperature on pH tolerance of this species.

4. To document some specific sublethal (physiological) responses of this species to acute acid exposure.

Methods and Materials

Study Sites

In the fall of 1983, research was initiated at Coweeta Hydrologic Laboratory (U. S. Forest Service Southeastern Experiment Station) in Macon County, North Carolina, where a noted increase in acidity and sulfate levels in high elevation streams has occurred (Swank and Douglass 1975). Two high gradient, mountain streams, Ball Creek (Watershed 27) and Pinnacle Branch (Watershed 36), were selected for study. Mean annual pH values in 1984 were 6.51 (minimum pH = 5.60) for Watershed 27 and 6.70 (minimum pH = 4.90) for Watershed 36. Alkalinity ($HCO_3^{\ -}$) ranged from 0.50 to 2.61 mg l^{-1} in Watershed 27. Ball Creek (35°02′N, 83°28′W) originates at an elevation of roughly 1311 m in a relatively undisturbed, mixed hardwood forest (Swank et al. 1981). The stream flows approximately 4.8 km, dropping 640 m in elevation to its confluence with Shope Fork to become Coweeta Creek. The headwaters of Pinnacle Branch (35°03′N, 83°28′W) are at an elevation of approximately 1387 m. The stream drops a total of 717 m, joining Shope Fork at about 930 m. Sites on these streams were used to collect baseline data on *C. b. bartonii* populations, and to obtain specimens for acid toxicity tests. Specimens were also obtained from Shope Fork and Coweeta Creek (U. S. Forest Service Topographic Map 1972).

Craig Creek is a relatively pristine, high gradient tributary of the James River, originating in the Jefferson National Forest, Montgomery County, Virginia (Burkhead 1983). Collecting sites were established in the extreme headwaters (610 m) to obtain specimens of *C. b. bartonii* for toxicity tests. Acid tolerance of this population was compared to that of Coweeta crayfish.

Population Biology

Coweeta Sampling

Crayfish were collected in pool and riffle habitats from May to November, 1984, and May to October, 1985 at Coweeta (in conjunction with quantitative sampling), to obtain form I males for species identification (Hobbs 1976). Baited traps (overnight sets, baited with liver-flavored dog food), aquarium dip nets, and a known-area quadrat sampler (572.6 cm^2) were used to capture specimens. Adult females were also collected for reference. University of Georgia museum collections from the Coweeta watershed were examined to complete the survey of the crayfish fauna. All specimens were preserved in 6% formalin, transferred to 30% isopropanol (Hobbs 1976), and tentatively identified to species. Representative specimens were taxonomically verified by Dr. Horton H. Hobbs, Jr. at the Smithsonian Institution.

Six 20-25 m, first-order study sections with an approximate width of 1 m were established on Ball Creek and Pinnacle Branch to conduct mark-recapture studies in May and September, 1985 (Table 1). In each stream section, upper and lower boundaries were blocked with seines, and 12 baited traps were set overnight (12 h to 14 h). Captured crayfish were sexed, repro-

Table 1. Location of mark-recapture study sections in Ball Creek (WS27) and Pinnacle Branch (WS36) at Coweeta Hydrologic Laboratory, 1985.

Pinnacle Branch

Section	Elevation	Section Length	Location
Lower	1036 m	22.9 m	Between 12.2 m and 35.1 m upstream of weir 36
Middle	1044 m	23.8 m	Between 40.5 m and 64.3 m upstream of weir 36
Upper	1052 m	23.8 m	Between 74.4 m and 98.1 m upstream of weir 36

Ball Creek

Section	Elevation	Section Length	Location
Lower	1052 m	22.2 m	Between 22.2 m downstream and lower end of weir 27
Middle	1067 m	20.1 m	Between 3.0 m and 23.2 m upstream of weir 27
Upper	1082 m	22.9 m	Between 96.6 m and 119.5 m upstream of weir 27

ductive form of males (form I or II) and reproductive condition of females (immature, active cement glands, ovigerous, or with attached hatchlings) were recorded, specimens were wet-weighed to the nearest 0.1 g and marked with a coded combination of one uropod clip and green typewriter correction fluid (Stein 1976). Carapace length (CL) measurements were used in this and all other phases of this study. Crayfish were released in their respective capture locations. Twenty-four hours later, each section was hand-collected intensively for 2 man-hours, using aquarium nets. Captured specimens were sexed, measured (CL), wet-weighed, examined for marks and released. Data were used to estimate *C. b. bartonii* population size using Chapman's modification of the Lincoln-Petersen estimator (Ricker 1975). Captured specimens from these and adjacent streams were also used to examine relative abundance (catch per unit effort), estimate sex ratios and numbers of sexually mature versus immature, develop carapace length-wet weight relations for each sex, and develop length frequency histograms.

Life History Observations

In conjunction with qualitative and quantitative sampling, information was collected on life history aspects of *C. b. bartonii* at Coweeta, using sampling methods previously described. Notes were made on mating, occurrence of "berried" females, females bearing young, and other ecological observations.

Differences in mean catch per trap among stream study sections were determined by a nonparametric Least Significant Differences Test on ranks (protected by a Kruskal-Wallis Test for Location Differences). A Wilcoxon Two-sample Test was used to detect differences between months (May vs. September) and streams (Ball Creek vs. Pinnacle Branch) (Hollander and Wolfe 1973). This test was also used to compare male and female carapace lengths from trap and hand collections. Chi-square analysis was used on sex ratios and ratios of reproductively active to non-active crayfish. Where mean values are followed by "±", 95% confidence limits are provided. In all statistical analyses, significance was set at $\alpha = 0.05$.

Effects of Acid Exposure

Acute Lethality Tests

Preliminary acute (96 h) lethality assays were conducted for several weeks prior to actual tests. These provided sufficient information to choose an appropriate range of test doses. All toxicity testing followed standard procedures for macroinvertebrates described by The Committee on Methods for Toxicity Tests With Aquatic Organisms (U.S. Environmental Protection Agency 1975) as closely as facilities would allow.

Intermolt adult (25.3-47.2 mm CL), late juvenile (11.5-19.9 mm CL) and early juvenile (3.9-12.8 mm CL) *Cambarus b. bartonii* were collected in conjunction with population sampling from May through October, 1985, in Ball Creek, Shope Fork and their first order tributaries in the Coweeta watershed. Collections were made by minnow traps (baited with liver-flavored canned dog food), hand-collecting with aquarium dip-nets, and seining. Crayfish were transported to the laboratory in Coleman coolers equipped with a 12-volt portable aerator. Specimens were acclimated to laboratory conditions for 10-14 days in aerated Frigid-Unit Min-O-Cool systems (Frigid Units, Inc., Toledo, Ohio). Transport and acclimation mortalities were reduced by addition of 127 mm PVC pipe sections for shelter (Capelli and Hamilton 1984).

All toxicity tests used a flow-through artificial stream system slightly modified from Farris (1986). Wooden, oval, paddle-driven streams had dimensions of 90 x 46 x 15 cm with a capacity of 30 liters, and a turnover time of approximately 90 min. (Figure 1). Dechlorinated, charcoal-filtered tap water entered the system via plexiglass headboxes, and continued by

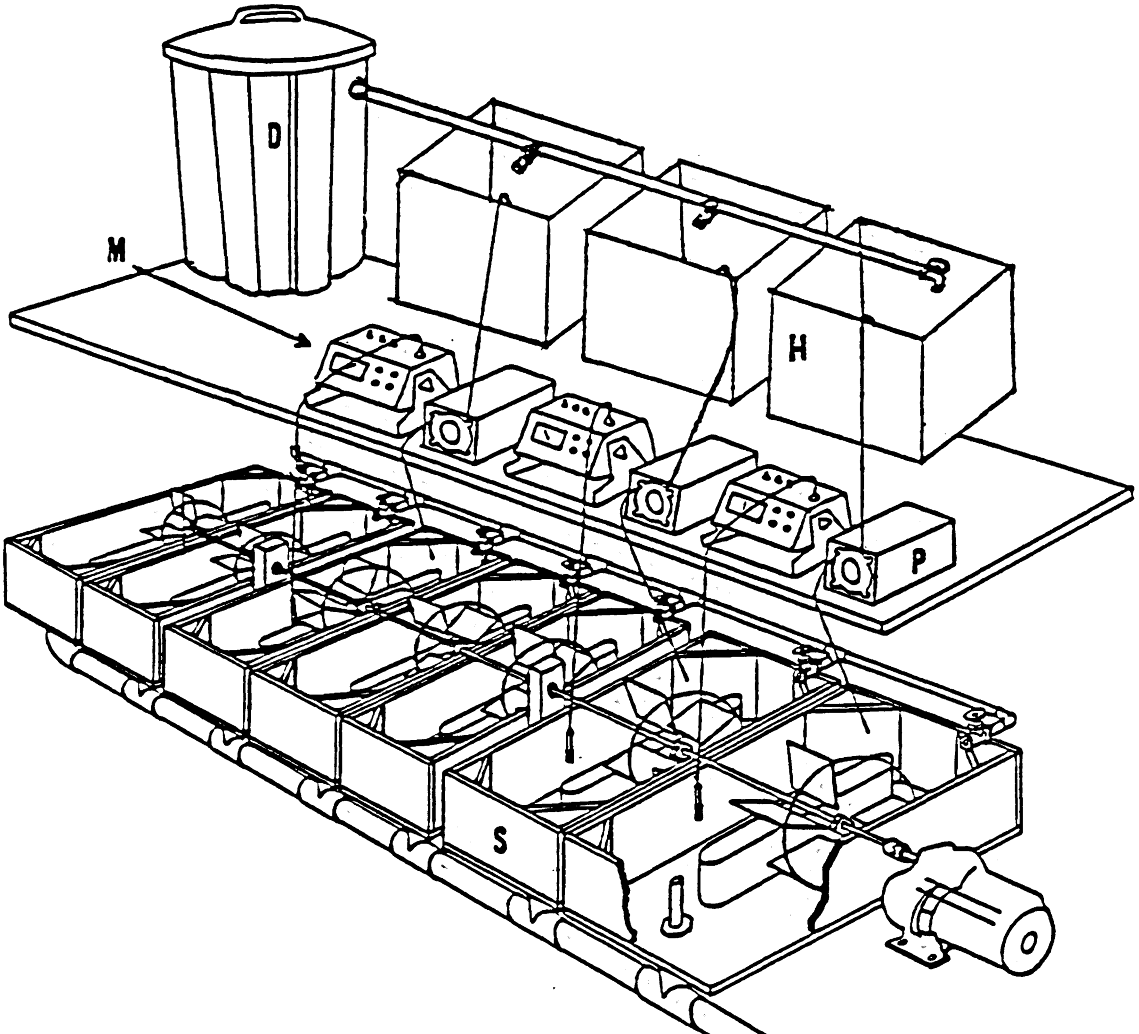

Figure 1. Artificial stream system used in laboratory experiments. The gravity-flow system includes (D) dechlorinator, (H) headboxes for acid dilution, (M) pH controller/meters, (P) peristaltic pumps for acid addition, and (S) paddle-driven, artificial streams.

gravity flow through 19 mm CPVC pipe to streams. Fisher Model 805MP pH meter/controllers linked to Cole-Parmer peristaltic pumps regulated pH by delivering a 10% solution of 12 M sulfuric acid (H_2SO_4) to headboxes. Mixing was facilitated by magnetic stirrers beneath headboxes. Probes in streams continously monitored pH. A 1/4 horsepower continuous-use motor propelled the plexiglass paddle wheels to provide constant aeration. Streams were covered with translucent screening to prevent escape of crayfish and allow light penetration.

Water chemistry parameters and major ion concentrations were measured periodically in experimental streams (Table 2); acidity levels and stream temperatures were also monitored. Stream and acclimation tank temperatures were stabilized around 20 to 22°C (U.S. Environmental Protection Agency 1975). A 16 h light: 8 h dark photoperiod was maintained throughout the period of acclimation and experiments. During acclimation, crayfish were fed canned dogfood every other day. No feeding occurred for 48h before and during experiments.

Ninety-six-hour exposures were used to simulate episodic acid events encountered by these crayfish under natural conditions. Acute median lethal concentrations (96 h LC_{50}'s) were determined for Coweeta intermolt adults (25.3-43.2 mm CL), Coweeta late juveniles (11.5-19.9 mm CL), Coweeta early juveniles (3.9-12.8 mm CL) and Craig Creek intermolt adults (23.0-41.3 mm CL).). Except for Coweeta early juveniles, all toxicity tests were conducted with five males and five females in each experimental chamber to test for possible tolerance differences between sexes. Determination of LC_{50} values and 95% fiducial intervals (FI) was made by Finney's Probit Analysis in the Statistical Analysis Systems (SAS) program (SAS Institute 1985).

Experimental animals were randomly removed from acclimation tanks and separated by sex. Following carapace length and wet-weight measurements, crayfish were randomly assigned to experimental chambers and held in individual cages (Capelli and Hamilton 1984). Mortality, pH (monitored continuously), and temperature were checked at 2 h intervals for the first 12 h, and every 12 h for the remaining 84 h of each experiment. During checks, all dead specimens were removed; and sex, wet-weight and carapace lengths were recorded. Crayfish were considered dead when thorough stimulation of antennae, eyestalks and walking ap-

Table 2. Water chemistry values in artificial stream systems during acidification experiments on crayfish, 1985.

Parameter	Measured Range
Conductivity	127 - 130 μ mhos
Dissolved Oxygen	86 - 98% saturation
Ammonia	< 0.01 mg/l (below detection)
Hardness	7 - 10 mg/l
Alkalinity	38 - 44 mg/l
Nitrites	below detection
Iron	95.1 - 119.7 μg/l
Aluminum	87.4 - 154.7 μg/l
Copper	below detection
Sodium	2.4 - 2.6 mg/l
Potassium	0.6 - 1.6 mg/l
Magnesium	0.9 - 2.6 mg/l
Calcium	4.6 - 9.6 mg/l
Phosphorus	.146 - .152 mg/l
Chlorine	< 0.01 mg/l (below detection)

pendages produced no observed response. Observations concerning behavior and condition of specimens were noted at this time. Measured pH values never fluctuated by more than ± 0.05 units from those reported, and temperature remained within ± 1.0°C in all streams. Following completion of experiments, dead and live crayfish were frozen for use in subsequent hemolymph ion analysis.

Effect of Temperature on pH Tolerance

The U. S. Environmental Protection Agency Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) suggests that toxicity testing with crayfish be done at a standardized water temperature of approximately 22°C. Because temperatures of Coweeta streams most commonly ranged between 10°C and 17°C throughout the field season, it was necessary to conduct additional experiments at more environmentally realistic ambient water temperatures.

A 96 h lethality test was conducted on Coweeta intermolt adults (27.5-47.2 mm CL) at a mean temperature of 15.1 ± 0.03°C. Aside from the difference in ambient water temperature, this experiment followed methods identical to those previously described for comparison to those tests conducted on adults tested at 20.2 ± 0.08°C. Water temperature was controlled by mixing valves and monitored by gauges in the laboratory.

In a follow-up experiment, two groups (12 each) of randomly chosen, adult intermolt crayfish were sexed, measured (CL), wet-weighed and placed in adjacent artificial streams with mean temperatures of 19.2 ± 0.3°C and 13.3 ± 0.6°C. These specimens were subjected to an extreme pH of 2.00 (continuously monitored) and examined hourly until all specimens died. Survival time of each individual was recorded. A Wilcoxon Two-sample Test compared differences in survival times. Statistical analysis of all laboratory studies incorporated a significance level of $\alpha = 0.05$.

Physiological Investigations

A series of experiments was conducted to examine possible modes of acute acid toxicity to *C. b. bartonii*. Effects on ionoregulatory mechanisms are thought to be a primary cause of acid toxicity in fish (Wood and Rogano 1986). Several researchers have attempted to assess physiological responses of acid stress in crayfish, including effects on ion regulation, specifically, Na^+, Ca^{++}, K^+, Mg^{++} and Cl^- (Shaw 1960, Malley 1980, Morgan and McMahon 1982, McMahon and Morgan 1983, Appelberg 1985, Wood and Rogano 1986). My experiments were designed to provide information on possible acid-induced changes in hemolymph ion balance, effects on exoskeleton Ca^{++} content, and influence on the molting process.

To determine the effects of low pH on ion regulation, 20 randomly selected, adult intermolt crayfish were used; 12 in acidified and 8 in reference streams for 4 d. Prior to experimentation, animals were sexed, measured and wet-weighed. A pH of 2.62 was selected, because it was previously determined to be the LC_{10}; a potentially lethal pH, but one that would not cause excessive loss of specimens.

Following treatments, 100 µl of hemolymph were withdrawn from the ventral (venous) sinus of each specimen using Jelco 26 gauge needles. Hemolymph was also taken from 5 crayfish remaining in acclimation tanks for the duration of the experiment. These were compared with reference animals to detect possible "stream effects" on ion balance. Concentrations of four major cations, Na^+, Ca^{++}, Mg^{++} and K^+, were analyzed using a Perkin-Elmer model 460 atomic absorption spectrophotometer following appropriate dilutions, and addition of $LaCl_3$ to Ca^{++} samples to reduce chemical interference. A nonparametric Least Significant Differences Test on ranks (protected by Kruskal-Wallis) was used to compare hemolymph cation concentrations among acclimation, reference and treatment groups.

In addition to 96 h treatments, crayfish were placed in streams that were treated to pH 2.62 for either 2 h (n=12), 24 h (n=12), 48 h (n=6) or 72 h (n=10). Hemolymph of these specimens was analyzed as above to provide information on possible trends over the 96 h period. No crayfish was sampled for hemolymph at more than one time period.

Since crayfish may have the ability to buffer internal acidosis caused by an influx of H^+, and this buffering is thought to be the result of mobilization (dissolution) of $CaCO_3$ stores in the hepatopancreas, gastrolith, intracellular muscle compartments and exoskeleton (Morgan and McMahon 1982, Wood and Rogano 1986), an experiment was conducted to study potential effects of acute acid exposure on exoskeleton (carapace) Ca^{++}. Following 96 h exposure of adult intermolt crayfish 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), they were freeze-dried. Carapaces were removed, cleaned of debris with toothbrushes, measured and weighed (dry weight). They were ground-up in test tubes and digested in reagent grade (concentrated) HNO_3. Following appropriate dilutions and addition of $LaCl_3$, atomic absorption spectrophotometry was used to determine Ca^{++} concentrations (and percent Ca^{++}) in carapaces. Jonckheere's Test for Ordered Alternatives was used to test the hypothesis that a decrease in environmental pH would result in a decrease in carapace $[Ca^{++}]$.

Effects of Acute Acid Exposure on Molting

General effects of severe acute acid exposure on six molting *C. b. bartonii* were studied in animals found molting in laboratory acclimation tanks. Specimens were removed, sexed, measured and wet-weighed. Each specimen was paired with an intermolt crayfish of similar size to serve as a reference, and each pair was placed together in an artificial stream and acidified to a pH of approximately 2.00. Animals were monitored every 15 minutes until death, and survival times were recorded.

Results

Population Biology

Abundance

From May to November, 1984, and May to October, 1985, approximately 2,235 crayfish, including 392 form I males, were collected in the Coweeta drainage by quantitative sampling and collecting for laboratory specimens. In addition, 50 crayfish from Ball Creek and 32 from Coweeta Creek (at University of Georgia museum; collected on 21 October, 1982) were examined to complete the qualitative survey of the crayfish fauna. All crayfish were identified as *Cambarus bartonii bartonii* (Fabricius), and these findings were verified by Dr. Horton H. Hobbs, Jr., Smithsonian Institution.

Abundance estimates of crayfish, from 6.0 mm to 44.5 mm CL, in 20-25 m study sections of Ball Creek (WS27) and Pinnacle Branch (WS36) were variable. Three population estimates calculated for crayfish in Ball Creek in May 1985, ranged from 210 to 525 ($\cong$ 9.2 to 23.6 per m^2), with 95% confidence intervals (C.I.) of 77 to 1313. Pinnacle Branch estimates ranged from

397 to 564 (≅ 16.7 to 24.6 per m^2), with 95% C.I. of 145 to 1409. Estimates for Ball Creek increased in September, ranging from 408 to 683 (≅ 18.4 to 29.8 per m^2) with C.I. of 124 to 1,707. Failure to recapture marked individuals negated calculation of two of the Pinnacle Branch estimates for September (Table 3).

Mean catch per trap night was calculated for each study section of Ball Creek and Pinnacle Branch in May and September, 1985 (Table 4). Values for upper, middle, and lower study sections of Ball Creek did not differ significantly (Kruskal-Wallis Test, $n = 24$, $P = 0.3316$). A significant difference was detected in mean catch per trap between study sections of Pinnacle Branch; specifically, between the middle and lower sections.

Overall catch per trap data for Ball Creek and Pinnacle Branch ranged from 0 to 8 in May and 0 to 11 in September. A significant difference was detected in mean catch per trap between creeks in September, but not in May ($n = 36$, $P = 0.0859$). Significant differences between months in Ball Creek were also detected, but no significant difference was found between months for Pinnacle Branch ($n = 36$, $P = 0.5187$).

Catch per hour by hand-collecting during recapture runs was calculated for stream study sections, although not analyzed statistically due to small sample size (Table 5). Mean catch per hour for Ball Creek (17.8, based on 3 sampling sites) and Pinnacle Branch (17.3) was similar in May. In September, mean values for Ball Creek (29.5) and Pinnacle Branch (30.2) were substantially higher, but again similar (Table 5).

Mean carapace lengths (mm) of male (31.5 ± 1.0) and female (31.3 ± 0.7) crayfish trapped in Ball Creek and Pinnacle Branch were similar in May (Wilcoxon Two-sample Test, $n = 36$,$P = 0.6867$), but differed significantly between sexes in September (Table 6). Ranges for CL showed that there were some females larger than males in May; however, that was reversed in September. Hand collections resulted in similar mean carapace lengths (Wilcoxon Two-sample Test, $n = 36$, $P = 0.4469$) of males and females in May and in September (Wilcoxon Two-sample Test, $n = 36$, $P = 0.1831$). Carapace length ranges showed the same trend as trap data; some females attained a larger size than males in May, but the situation was apparently reversed in September (Table 6).

Table 3. Population estimates and 95% confidence intervals (C.I.) for crayfish in 20-25 m study sections of Ball Creek (WS27) and Pinnacle Branch (WS36) in May and September, 1985.[1]

		May		September	
Stream	Section	Population Estimate	95% C.I.	Population Estimate	95% C.I.
WS27	Lower	525	192 - 1313	408	124 - 742
WS27	Middle	343	153 - 858	476	174 - 1190
WS27	Upper	210	77 - 525	683	250 - 1707
WS36	Lower	564	230 - 1409	—	————
WS36	Middle	496	150 - 902	589	216 - 1473
WS36	Upper	397	145 - 992	—	————

[1] No recaptures in WS36 Upper and WS36 Lower.

Table 4. Mean catch (95% C.I.) per trap of crayfish in three study sections[1] each of Ball Creek (WS27) and Pinnacle Branch (WS36), May 20-23 and September 8-11, 1985.[2]

	May		September	
Stream	Mean Catch Per Trap	Range	Mean Catch Per Trap	Range
Ball Creek	2.83 ± 0.59	0 - 8	1.86 ± 0.37	0 - 5
Pinnacle Branch	3.28 ± 0.80	0 - 8	2.86 ± 0.71	0 - 11
Combined	3.07 ± 0.53	0 - 8	2.35 ± 0.36	0 - 11

[1] A Kruskal-Wallis Test for location differences detected a significant difference ($P = 0.0213$, $n = 24$) in mean catch per trap between the upper, middle and lower sections of Pinnacle Branch. A Protected Rank L.S.D. Test showed a significant difference ($P = 0.0087$, $n = 24$) between the middle and lower sections.

[2] Significant differences were detected in mean catch per trap between creeks in September (Wilcoxon 2-Sample Test, $n = 36$, $P = 0.0251$), and between months in Ball Creek (Wilcoxon 2-Sample Test, $n = 36$, $P = 0.0131$).

Table 5. Mean catch (and range) per hour of crayfish in three study sections each of Ball Creek (WS27) and Pinnacle Branch (WS36), May 20-23 and September 8-11, 1985.

	May		September	
Stream	Catch/hour	Range	Catch/hour	Range
Ball Creek	17.8	14.5 - 22.0	29.5	23.5 - 33.5
Pinnacle Branch	17.3	15.5 - 20.0	30.2	27.5 - 33.5
Combined	17.6	14.5 - 22.0	29.8	23.5 - 33.5

Table 6. Mean carapace length (CL) and 95% C.I. of male and female crayfish trapped and hand-collected in study sections of Ball Creek (WS27) and Pinnacle Branch (WS36), May 20-23 and September 8-11, 1985.[1]

		May			September		
Sampling Method	Sex	Mean CL (mm)	Range	Sample Size	Mean CL (mm)	Range	Sample Size
Trap	Male	31.5 ± 1.0	11.9 - 39.2	82	26.9 ± 1.5	10.2 - 44.5	104
Trap	Female	31.3 ± 0.7	12.4 - 41.1	137	22.6 ± 1.4	13.3 - 37.1	66
Hand	Male	16.0 ± 1.3	6.0 - 37.1	106	13.6 ± 0.8	6.4 - 37.4	163
Hand	Female	17.6 ± 1.7	6.4 - 41.0	90	13.9 ± 0.7	6.6 - 31.7	195

[1] Carapace lengths from September trapping were significantly different between males and females (Wilcoxon 2-Sample Test, P = 0.0002).

Length-Frequency Distributions

Carapace length-frequency distributions were analyzed for 958 crayfish trapped and hand-collected in study sections of Ball Creek and Pinnacle Branch in May and September, 1985 (Figures 2 and 3). Trapping was selective for larger crayfish, while hand-collecting primarily yielded smaller individuals. Therefore, catch totals in each carapace length increment for both methods of capture were expressed as percent frequency of overall catch, and results were plotted together on histograms. Histograms of twenty, 2 mm CL size classes exhibited polymodal distributions in May and September, indicating several age classes. A distinct shift in population structure from May to September was apparent (Figures 2 and 3). In May, a large portion (≅34%) of sampled crayfish ranged from 26.0 to 37.9 mm CL, with about 13% of all captures between 32.0 and 33.9 mm CL. A pronounced peak was observed in size classes from approximately 8.0 to 17.9 mm CL (≅31%). It appeared that another peak was present in the 4.0 to 7.9 mm CL range, possibly representing young-of-the-year recruited to the population. The largest specimens taken in May were in the 40.0 - 41.9 mm CL increment, while the smallest measured 4.0 - 5.9 mm CL. Plots of crayfish collected in September samples showed a substantial recruitment (young-of-year) effect. Approximately 49% of the catch fell within the 8.0 - 17.9 mm CL range. This peak appeared to overlap with another that occurred between 18.0 and 25.9 mm CL (≅26%). Approximately 14% of the captured crayfish occurred between 28.0 and 31.9 mm CL. A possible fourth peak (age III+) was evident from 36.0 mm up to the largest specimens in the 44.0 to 45.9 mm CL increment. The smallest individuals captured in September were between 6.0 and 7.9 mm CL.

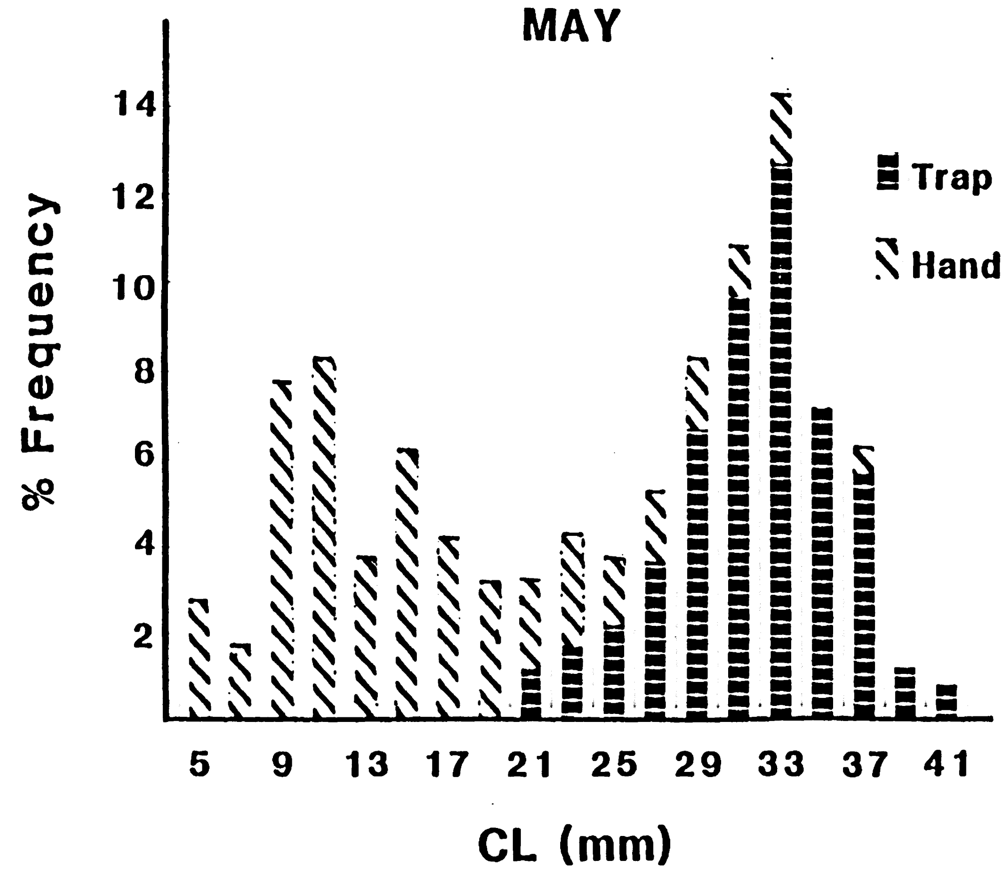

Figure 2. Histogram depicting carapace length-frequency distribution of 429 crayfish trapped and hand-collected in Ball Creek and Pinnacle Branch during May 1985.

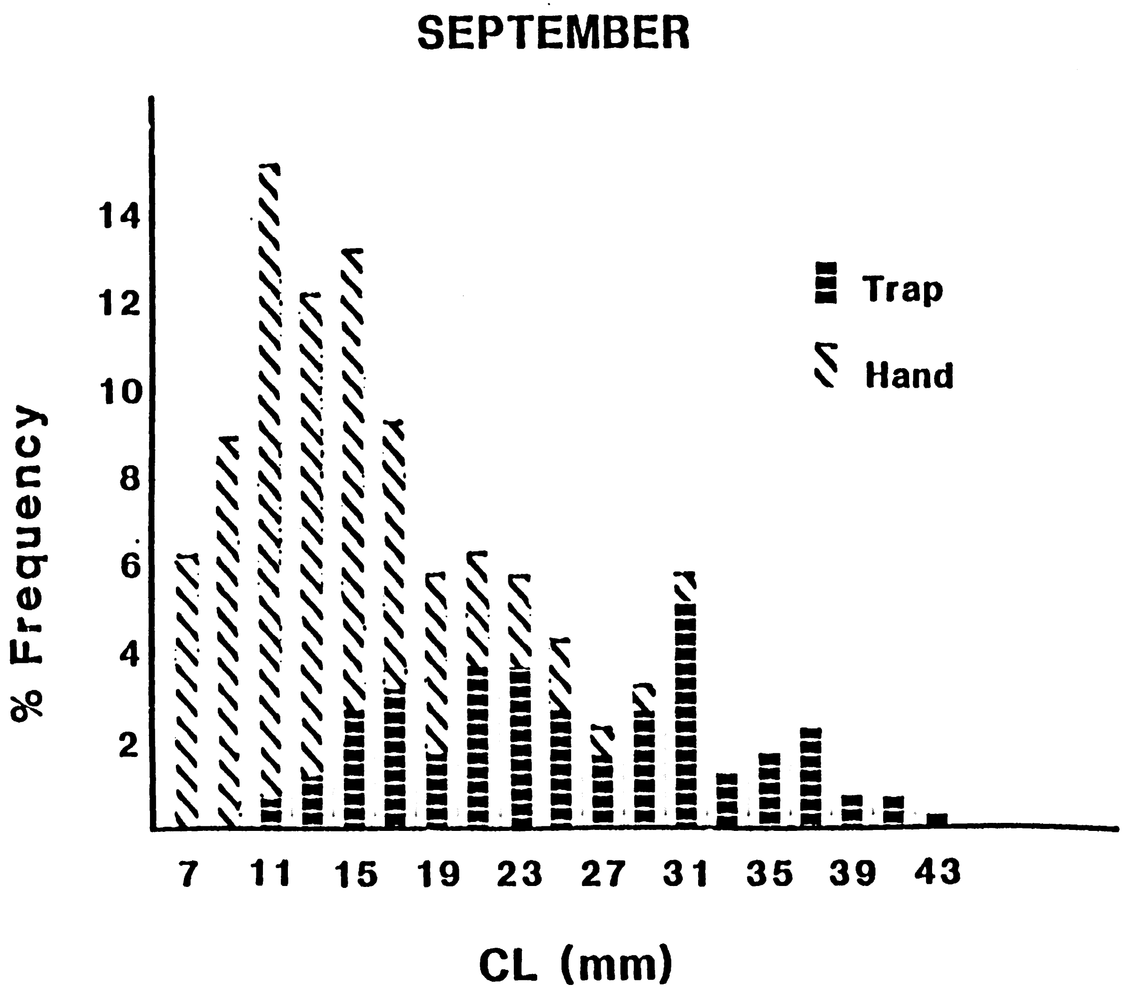

Figure 3. Histogram depicting carapace length-frequency distribution of 528 crayfish trapped and hand-collected in Ball Creek and Pinnacle Branch during September 1985.

Carapace Length-Weight Relation

Analysis of carapace length (mm) and wet weight (g) data for *C. b. bartonii* from Coweeta streams yielded the following equations:

males: $\text{Log } W\ (g) = -3.6169 + 3.1150 \text{ Log } CL\ (mm)$ $r = 0.986$ $N = 476$

females: $\text{Log } W\ (g) = -3.5372 + 3.0532 \text{ Log } CL\ (mm)$ $r = 0.987$ $N = 536$

Plots of wet weight versus carapace length regressions exhibited nearly identical regressions, indicating little difference between sexes (Figures 4 and 5).

Life History Observations

Of 1,497 crayfish trapped between May and October, 1985 in Ball Creek, Pinnacle Branch and adjacent woodland streams, 734 (49.0%) were males and 763 (51.0%) were females (Table 7). Hand-collections yielded 269 (48.6%) males and 285 (51.4%) females. These data indicate an overall balanced sex ratio in the populations. However, analysis of monthly trap samples showed sex ratios significantly different from 1:1 ($X^2 = 60.353$, $P = 0.0001$). Females dominated trap catches in May; sex ratios were equal in June; and males dominated trap captures from August through October. Hand-collections showed no significant variation from a 1:1 sex ratio in May or September; males represented 54.1% of May samples, and 45.5% of September captures.

Sexually mature males (form I) were collected from May through October, 1985 (also in November, 1983). Monthly abundance of form I males in traps was significantly different from form II (Table 8). In June, form I males reached their lowest frequency, comprising only 23.1% of the males captured. They increased in frequency and peaked at 79.5% in October. Trap collections were a better indicator of the form I to form II (adult) ratio, as hand collections

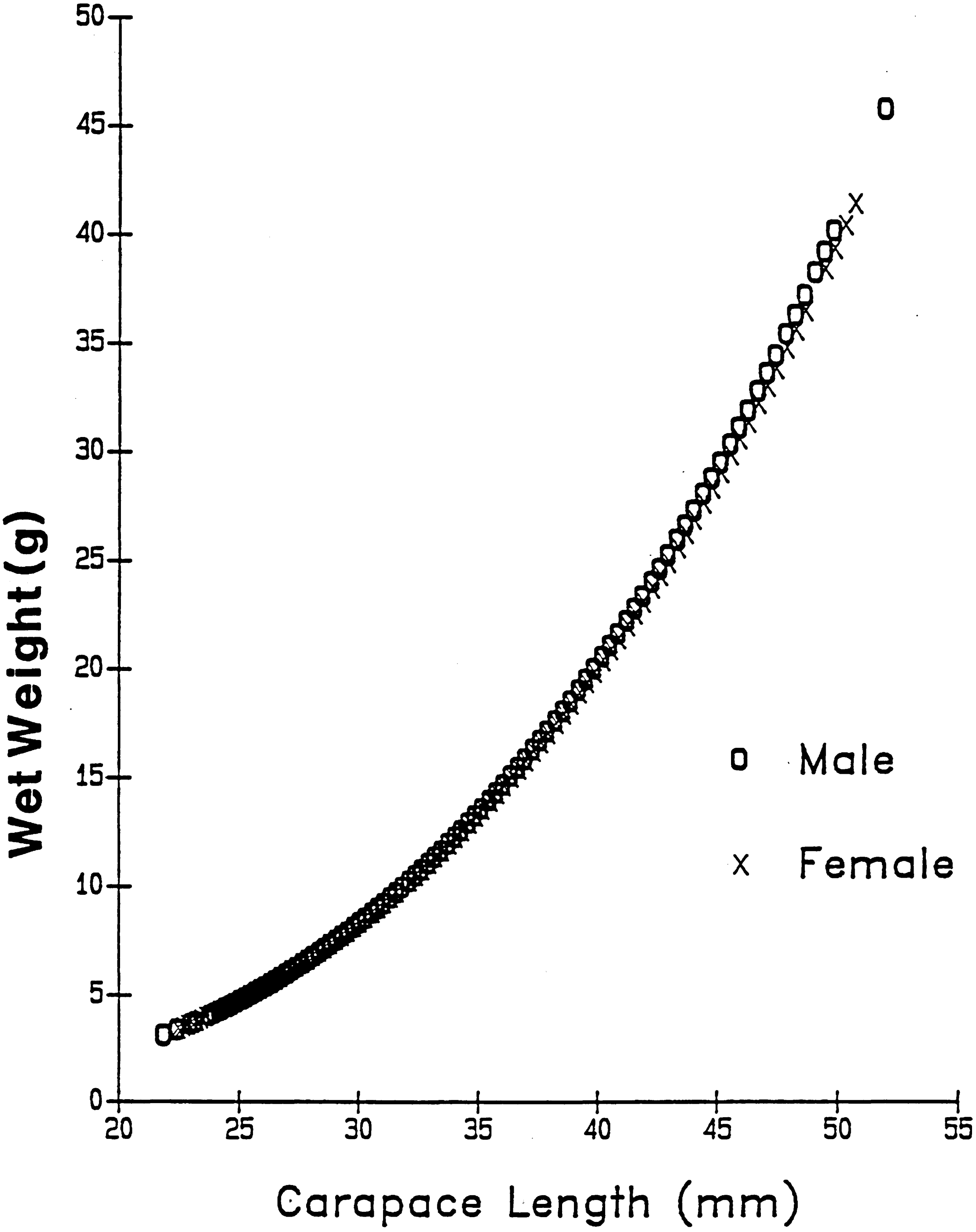

Figure 4. Comparison of carapace length (mm) - wet weight (g) relations for male and female crayfish from Coweeta streams.

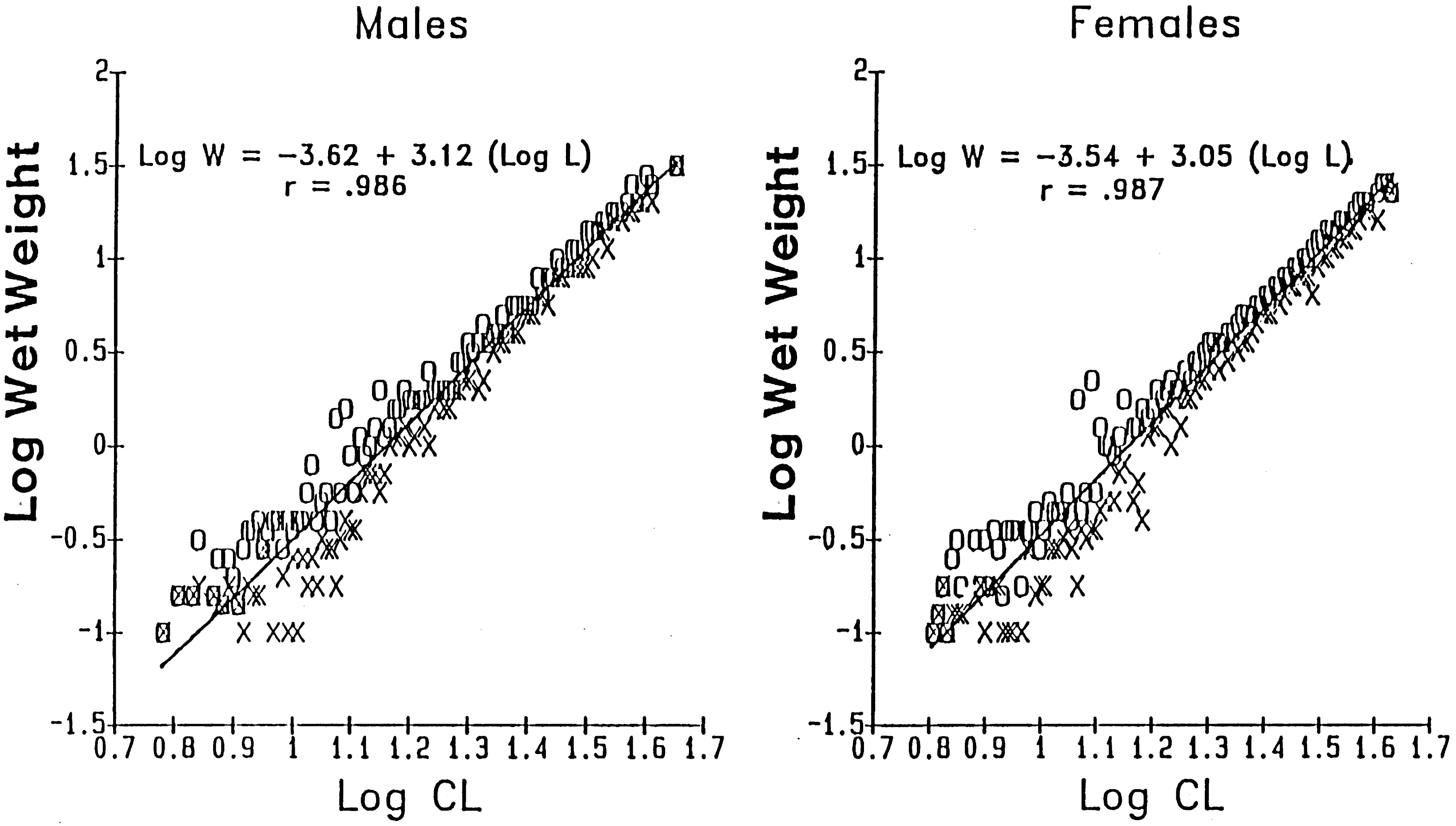

Figure 5. Carapace length-wet weight relations (log transformation) for male and female crayfish from Coweeta streams. Minimum (X) and maximum (O) wet weights for recorded carapace lengths are displayed.

Table 7. Numbers and percentages of male and female crayfish trapped and hand-collected at Coweeta, 1985.

		Sampling Period					
Sampling Method	Sex	May 20-23	June 21-22	August 3-4	September 8-11	October 9-10	Totals
Trap	Male	188 (36.6%)	139 (47.1%)	76 (60.3%)	182 (57.4%)	149 (60.6%)	734 (49.0%)
Trap	Female	325 (63.4%)	156 (52.9%)	50 (39.7%)	135 (42.6%)	97 (39.4%)	763 (51.0%)
Contribution to X^2		31.4*	0.4	6.4*	8.9*	13.1*	60.353[1]
Hand	Male	106 (54.1)	------------	------------	163 (45.5%)	------------	269 (48.6%)
Hand	Female	90 (45.9%)	------------	------------	195 (54.5%)	------------	285 (51.4%)
Contribution to X^2		2.4			1.3		3.707[2]

[1] X^2 value is significant (P = 0.0001).

[2] X^2 value is not significant (P = 0.0541).

* Asterisk indicates significance ($\alpha = 0.05$).

Table 8. Numbers and percentages of reproductively active (form I) and inactive (form II) male crayfish trapped and hand-collected at Coweeta, 1985.

		Sampling Period					
Sampling Method	Form	May 20-23	June 21-22	August 3-4	September 8-11	October 9-10	Totals
Trap	I	83 (44.2%)	31 (23.1%)	43 (59.7%)	110 (71.4%)	116 (79.5%)	383 (55.2%)
Trap	II	105 (55.9%)	103 (76.9%)	29 (40.3%)	44 (28.6%)	30 (20.6%)	311 (44.8%)
Contribution to X^2		9.3	55.6*	0.6	16.5*	34.8*	116.713[1]
Hand	I	6 (5.7%)	————	————	3 (14.3%)	————	9 (7.1%)
Hand	II	100 (94.3%)	————	————	18 (85.7%)	————	118 (92.9%)
Contribution to X^2		0.3			1.6		1.980[2]

[1] X^2 value is significant ($P = 0.0001$).

[2] X^2 value is not significant ($P = 0.1593$).

* Asterisk indicates significance ($\alpha = 0.05$).

yielded only 7.1% form I males. One incidence of crayfish mating was observed on 20 May 1985 at 10.6°C (38.9 mm CL male, 39.8 mm CL female).

Sexually mature (reproductively active) females, identified by their full, white cement glands located on the ventral surface of the uropods (Stephens 1952), were collected in Coweeta streams from May through October, 1985. Mature females typically were outnumbered by immatures or nonreproductive females, but frequency of occurrence in traps followed a pattern similar to that of form I males (Table 9). Chi-square analysis suggested that numbers of sexually developed females were lowest in June and August (8.5% and 2.1%, respectively), and peaked in October (51.6%). As with males, trapping was a better indicator of sexually mature and immature females than hand-collecting.

Coweeta crayfish collections suggested that both sexes reached a carapace length of approximately 26.0 mm before becoming reproductively mature. Form I males averaged 34.1 ± 0.4 mm CL (n = 351), but ranged from 26.4 to 44.5 mm. Sexually mature females (with full cement glands) averaged 33.1 ± 0.5 mm CL (n = 161) and ranged from 26.6 to 41.8 mm; however, one female with eggs internally measured only 24.8 mm CL.

Only three "berried" females were trapped at Coweeta; one in late June and two in early August, when water temperatures were 14.2°C and 16.5°C, respectively. These crayfish ranged from 33.8 to 38.2 mm CL and 14.2 to 17.3g (wet weight). Four young-bearing females (27.1 to 35.2 mm CL) were collected between early September and mid-November, 1985. Water temperatures at these times ranged from approximately 11.5 to 16.2°C. Carapace lengths for young were between 5.0 and 5.4 mm.

Incidence of recently molted *C. b. bartonii*, based on field observations of trapped specimens, appeared to increase in September, and remained at high levels through October, 1985. No crayfish were observed directly in the process of molting. September sampling yielded 8 form I males, 2 form II males and 1 female specimen which had retained the marks (typewriter correction fluid) from marking studies done in May. These crayfish ranged in size from 26.2 to 39.0 mm CL. It appears that some Coweeta form I males may not molt to the form II (non-reproductive) stage during summer.

Table 9. Numbers and percentages of reproductively active and inactive female crayfish trapped and hand-collected at Coweeta, 1985.

		Sampling Period					
Sampling Method	State	May 20-23	June 21-22	August 3-4	September 8-11	October 9-10	Totals
Trap	Active	117 (36.2%)	13 (8.5%)	1 (2.1%)	26 (25.5%)	49 (51.6%)	206 (28.6%)
Trap	Inactive	206 (63.8%)	140 (91.5%)	47 (97.9%)	76 (74.5%)	46 (48.4%)	515 (71.4%)
Contribution to X^2		9.2	30.2*	16.5*	0.4	24.6*	81.096[1]
Hand	Active	8 (8.9%)	------------	------------	1 (4.8%)	------------	9 (8.1%)
Hand	Inactive	82 (91.1%)	------------	------------	20 (95.2%)	------------	102 (91.9%)
Contribution to X^2		0.1			0.3		0.389[2]

[1] X^2 value is significant ($P = 0.0001$).

[2] X^2 value is not significant ($P = 0.5327$).

* Asterisk indicates significance ($\alpha = 0.05$).

C. b. bartonii was most active when water temperatures exceeded 10.0°C. In November, 1983, when water temperatures were approximately 10.0°C, very few active crayfish were observed in Coweeta streams. During their peak activity from May to October, water temperatures ranged from 11.5 to 17.0°C in Coweeta streams (Figure 6).

Acute Lethality Tests

All three life stages of *C. b. bartonii* from Coweeta streams and Craig Creek were highly resistant to extreme acid pH conditions under acute 96 h exposures (Tables 10, 11, 12, 13 and 14). The 96 h LC_{50} values and 95% fiducial intervals (FI) for pH were 2.43 (2.52 to 2.32) for Coweeta adults, 2.56 (2.59 to 2.52) for Coweeta late juveniles, 2.85 (2.92 to 2.79) for Coweeta early juveniles and 2.43 (2.46 to 2.40) for Craig Creek adults. There was an apparent positive relationship between increasing crayfish size (or age) and tolerance to acid; the median lethal concentration value for late juveniles was 96% lower than that of early juveniles, and Coweeta adults were 33% and 160% more tolerant than late juveniles and early juveniles, respectively. There was no overlap in 95% FI's for $[H^+]$ among any of these size classes. Crayfish intermolt adults from two geographically isolated populations (Coweeta versus Craig Creek) exhibited similar acid tolerance with nearly identical LC_{50}'s; however, 95% FI's were much wider for the adult group from Coweeta.

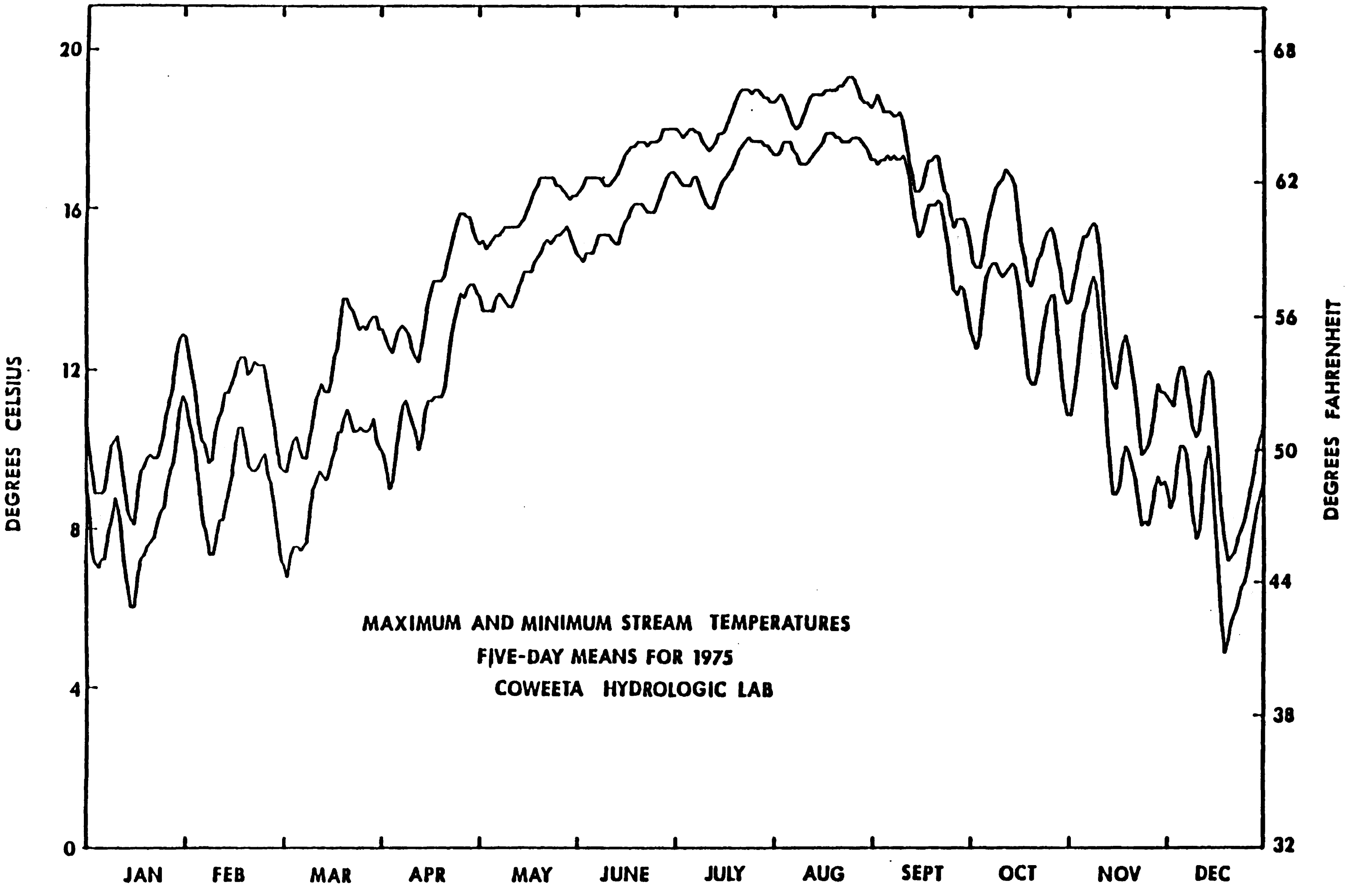

Figure 6. Ambient temperature ranges in a Coweeta drainage stream (WS7), Otto, North Carolina.

Table 10. Mean acidity level, ambient water temperature, and mortalities in individual experimental chambers (streams) from 96 h toxicity test with adult intermolt crayfish from Coweeta, 1985.[1] Each chamber initially contained 10 specimens.

Mean $[H^+]$ M	Mean pH	Mean Temp.(°C)	Mortalities
4.90×10^{-3}	2.31	19.6	10
4.90×10^{-3}	2.31	19.9	10
4.02×10^{-3}	2.40	20.9	9
3.94×10^{-3}	2.40	21.0	6
3.47×10^{-3}	2.46	21.1	3
3.30×10^{-3}	2.48	21.2	0
3.01×10^{-3}	2.52	20.1	0
2.92×10^{-3}	2.54	19.6	0
1.92×10^{-3}	2.72	20.1	3
1.75×10^{-3}	2.76	19.7	0
1.09×10^{-3}	2.96	20.0	0
1.09×10^{-3}	2.96	20.0	0
1.62×10^{-8}	7.79	20.0	0
1.55×10^{-8}	7.81	20.6	0
1.00×10^{-8}	8.00	19.9	0
0.98×10^{-8}	8.01	20.6	0

[1] Test yielded an LC50 value of 3.69×10^{-3} M $[H^+]$ (pH=2.43).

Table 11. Mean acidity level, ambient water temperature, and mortalities in individual experimental chambers (streams) from 96 h toxicity test with late juvenile crayfish from Coweeta, 1985.[1] Each chamber initially contained 10 specimens.

Mean [H$^+$] M	Mean pH	Mean Temp. (°C)	Mortalities
3.33×10^{-3}	2.48	20.3	10
3.33×10^{-3}	2.48	20.4	8
2.82×10^{-3}	2.55	20.7	4
2.81×10^{-3}	2.55	20.7	4
2.16×10^{-3}	2.67	21.6	3
2.15×10^{-3}	2.67	21.6	1
1.71×10^{-3}	2.77	21.4	0
1.70×10^{-3}	2.77	21.4	0
1.82×10^{-8}	7.74	22.0	0
1.56×10^{-8}	7.81	21.7	0
1.17×10^{-8}	7.93	20.8	0
1.07×10^{-8}	7.97	20.7	0

[1] Test yielded an LC50 value of 2.78×10^{-3} M [H$^+$] (pH=2.56).

Table 12. Mean acidity level, ambient water temperature, and mortalities in individual experimental chambers (streams) from 96 h toxicity test with early juvenile crayfish from Coweeta, 1985.[1] Each chamber initially contained 10 specimens.

Mean $[H^+]$ M	Mean pH	Mean Temp. (°C)	Mortalities
2.12×10^{-3}	2.67	21.5	10
2.10×10^{-3}	2.67	21.4	8
1.75×10^{-3}	2.78	21.4	5
1.74×10^{-3}	2.78	21.2	5
1.41×10^{-3}	2.87	20.2	4
1.39×10^{-3}	2.87	20.2	6
1.11×10^{-3}	2.97	20.3	4
1.11×10^{-3}	2.97	20.2	6
1.10×10^{-8}	7.96	21.3	0
1.00×10^{-8}	8.00	20.1	0

[1] Test yielded an LC50 value of 1.42×10^{-3} M $[H^+]$ (pH=2.85).

Table 13. Mean acidity level, ambient water temperature, and mortalities in individual experimental chambers (streams) from 96 h toxicity test with adult intermolt crayfish from Craig Creek, 1985.[1] Each chamber initially contained 10 specimens.

Mean $[H^+]$ M	Mean pH	Mean Temp. (°C)	Mortalities
4.59×10^{-3}	2.34	21.2	9
4.59×10^{-3}	2.34	21.0	9
3.49×10^{-3}	2.46	21.2	6
3.48×10^{-3}	2.46	21.0	3
2.86×10^{-3}	2.54	21.6	0
2.84×10^{-3}	2.55	21.3	0
2.29×10^{-3}	2.64	21.9	0
2.26×10^{-3}	2.65	21.6	1
2.45×10^{-8}	7.61	21.9	0
1.82×10^{-8}	7.74	21.5	0

[1] Test yielded an LC50 value of 3.70×10^{-3} M $[H^+]$ (pH=2.43).

Table 14. Size classes (carapace lengths, CL) of crayfish from Coweeta and Craig Creek (CA=Coweeta Adults, CLJ=Coweeta Late Juveniles, CEJ=Coweeta Early Juveniles, CCA=Craig Creek Adults) and 96 h median lethal (LC50) values (with 95% fiducial intervals) for flow-through acid toxicity tests, 1985.

Life Stage	Mean CL (mm)	Range CL (mm)	Median Lethal $[H^+]$ M×10^{-3}	95% Fiducial Interval	Median Lethal pH	95% Fiducial Interval
CA	33.5	25.3 - 43.2	3.69**	3.02 - 4.77	2.43	2.52 - 2.32
CLJ	16.4	11.5 - 19.9	2.78*	2.59 - 3.00	2.56	2.59 - 2.52
CEJ	9.1	3.9 - 12.8	1.42	1.22 - 1.62	2.85	2.92 - 2.79
CCA	32.2	23.0 - 41.3	3.70	3.45 - 4.01	2.43	2.46 - 2.40

** 3.69 is 96% greater than 2.78. * 2.78 is 33% greater than 1.42.

Effect of Temperature on pH Tolerance

Lowered water temperatures in artificial streams, to more environmentally realistic levels (< 17.0 °C), increased acid tolerance of adult crayfish (Tables 15 and 16). A 96 h lethality test conducted at a mean temperature of 15.1°C resulted in a median lethal concentration of pH 2.33. Tolerance (based on LC_{50}) at this temperature appeared to be 25% greater than at 20.2°C for similar-sized *C. b. bartonii*, although 95% FI's exhibited some overlap.

When exposed to extremely acidic (pH 2.00) conditions in adjacent artificial streams at a lower ambient water temperature (13.3 ± 0.6 °C), adult intermolt crayfish survived significantly longer than those at 19.2°C (Table 17). A 6 °C decrease in temperature resulted in crayfish surviving nearly twice as long (44.3 ± 5.9 h) as at the higher temperature (24.6 ± 4.8 h).

Physiological Investigations

Exposure of intermolt adult *C. b. bartonii* to pH 2.62 in relatively soft water ($[Ca^{2+}] = 4.6 - 9.6$ mequiv) for four days failed to cause any mortalities, but did affect hemolymph ionic status (Table 18). No significant differences for any of the tested cations ($[Na^+]$ $P = 0.27909$, $[Ca^{2+}]$ $P = 0.33006$, $[Mg^{2+}]$ $P = 0.33027$, $[K^+]$ $P = 0.35682$) were found between acclimation animals (day 0) and reference animals (from stream chambers), suggesting no laboratory "stream effects" on ion balance. However, 4 d of acid exposure resulted in significantly less hemolymph $[Na^+]$ with a difference of approximately 67 mM, than on day 0 (acclimation), and approximately 49 mM difference from those of the reference animals. Hemolymph $[Ca^{2+}]$ in treatment animals (12.63 ± 1.14mM) was significantly higher than that in acclimation animals

Table 15. Mean acidity level, ambient water temperature, and mortalities in individual experimental chambers (streams) from 96 h toxicity test with adult intermolt crayfish from Coweeta, 1985.[1] Each chamber initially contained 10 specimens.

Mean $[H^+]$ M	Mean pH	Mean Temp. (°C)	Mortalities
5.92×10^{-3}	2.23	15.1	9
5.87×10^{-3}	2.23	15.1	9
4.46×10^{-3}	2.35	15.0	6
4.45×10^{-3}	2.35	15.0	4
3.61×10^{-3}	2.44	15.1	1
3.56×10^{-3}	2.45	15.1	1
2.94×10^{-3}	2.53	15.1	0
2.91×10^{-3}	2.54	14.8	0
2.19×10^{-8}	7.66	14.9	0
1.86×10^{-8}	7.73	15.5	0

[1] Test yielded an LC50 value of 4.63×10^{-3} M $[H^+]$ (pH=2.33).

Table 16. Results of 96 h lethality tests (LC50's with 95% fiducial intervals) for *C. b. bartonii* adults from Coweeta in flow-through artificial streams at two ambient water temperatures, 1985.

Group	Mean CL (mm)	Ambient Water Temp. (°C)	Median Lethal $[H^+]$ $Mx10^{-3}$	95% Fiducial Interval	Median Lethal pH	95% Fiducial Interval
1	33.5	20.2 ± 0.1	3.69*	3.02 - 4.77	2.43	2.52 - 2.32
2	33.9	15.1 ± 0.0	4.63**	4.31 - 5.01	2.33	2.37 - 2.30

**This value is 25% greater than value marked with *.

Table 17. Effects of ambient water temperature on survival times of Coweeta crayfish acutely exposed to an extreme pH (2.00) in artificial streams, 1985. Groups were significantly different from each other (Wilcoxon 2-Sample Test, P=0.0007).

Group	Sample Size	Mean CL (mm)	Mean Temp.(°C.)	Survival (h) (X ± 95% C.I.)
1	12	34.0 ± 2.1	19.2 ± 0.3	24.6 ± 4.8
2	12	33.7 ± 2.0	13.3 ± 0.6	44.3 ± 5.9

Table 18. Mean concentrations (mM) and 95% confidence limits of major cations in hemolymph of Coweeta *C. b. bartonii* from acclimation tank, reference stream (96 h exposure) and an acidified treatment stream (96 h exposure, pH =2.62), 1985.

	Mean Concentration		
Cation	Acclimation n=5	Reference n=8	Treatment n=12
[Na^+]	191.91 ± 13.79	173.83 ± 27.19	125.13 ± 7.82*
[Ca^{2+}]	11.40 ± 0.44	10.94 ± 1.23	12.63 ± 1.14 *
[Mg^{2+}]	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

* Indicates a significant difference between treatment and reference and between treatment and acclimation group. There were no significant differences detected between acclimation and reference groups. Differences were tested using a nonparametric Rank L.S.D. Test (protected by a Kruskal-Wallis Test for location differences, $P = 0.0013$ for Na^+, $P = 0.0353$ for Ca^{++}); Na^+ treatment vs. reference ($P = 0.00273$) and treatment vs. acclimation ($P = 0.00078$), Ca^{++} treatment vs. reference ($P = 0.02019$) and treatment vs. acclimation ($P = 0.01324$).

(11.40 ± 0.44 mM) and reference animals (10.94 ± 1.23 mM), although much variation was present in these concentration values. There was no significant difference in [Mg^{2+}] among treatment (2.08 ± 0.30 mM), acclimation (1.94 ± 0.17) and reference animals (2.07 ± 0.51) (P = 0.67956), or in [K^+] between treatment (3.68 ± 0.38), acclimation (3.58 ± 0.35) and reference specimens (3.50 ± 0.26) (P = 0.80802).

Additional exposure of crayfish to pH 2.62 for 2 h, 24 h, 48 h, and 72 h to observe changes in hemolymph ions over time produced variable results. Failure to obtain adequate hemolymph samples from some specimens caused uneven sample sizes of treatment groups. Ion concentrations were not statistically analyzed, but were plotted graphically so as to observe trends in the data. Only [Na^+] and [Ca^{2+}] exhibited any apparent patterns over time (Figures 7 and 8). A large increase in all hemolymph cation concentrations measured after 48 h disrupted any consistent trend in these data.

Anticipated effects of acute exposure (96 h) on crayfish carapace calcium to a range of 5 pH values were not apparent (Table 19). No decrease in carapace [Ca^{2+}] consistent with a decrease in pH level was observed.

Due to inconclusive results of Jonckheere's Test, further statistical analysis was conducted on carapace [Ca^{2+}] of all acid exposed crayfish (pooled, n = 83) versus reference animals (n = 20). Analysis indicated that [Ca^{2+}] was unexpectedly higher in the treatment group (Table 20).

Effects of Acid Exposure on Molting Crayfish

In a preliminary experiment, acute exposure to extreme acid (pH 2.00) had pronounced effects on molting crayfish in the laboratory (Table 21). In 5 of 6 cases, intermolt adult crayfish survived longer (X = 836 min) than molting specimens (X = 590 min); and only one molting

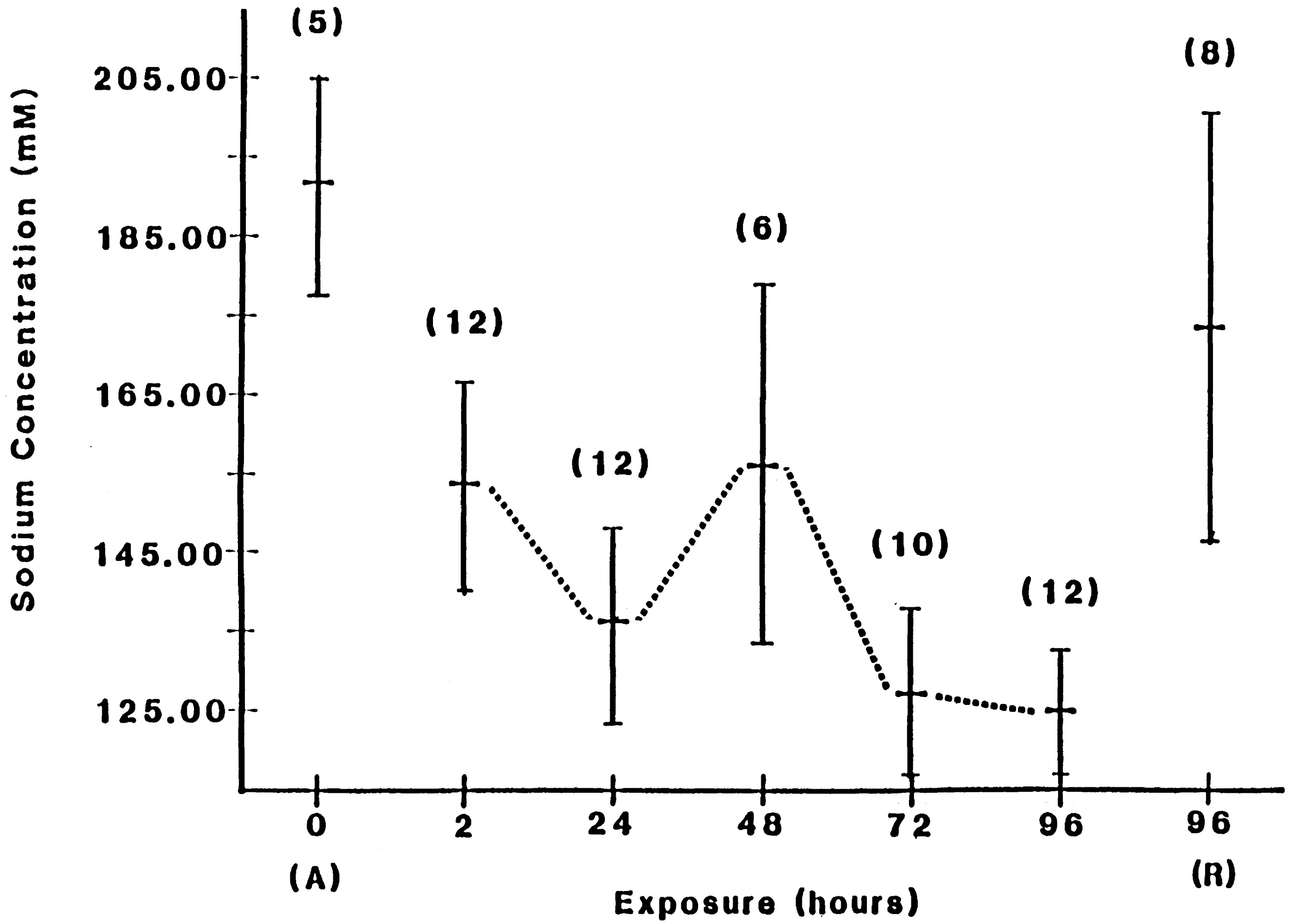

Figure 7. Sodium concentrations (means and standard error) in hemolymph extracted from Coweeta crayfish following laboratory exposure to reduced pH (2.62) for 2h, 24h, 48h, 72h, and 96h; including specimens from acclimation water (A) and reference streams (R). Numbers in parentheses refer to sample size.

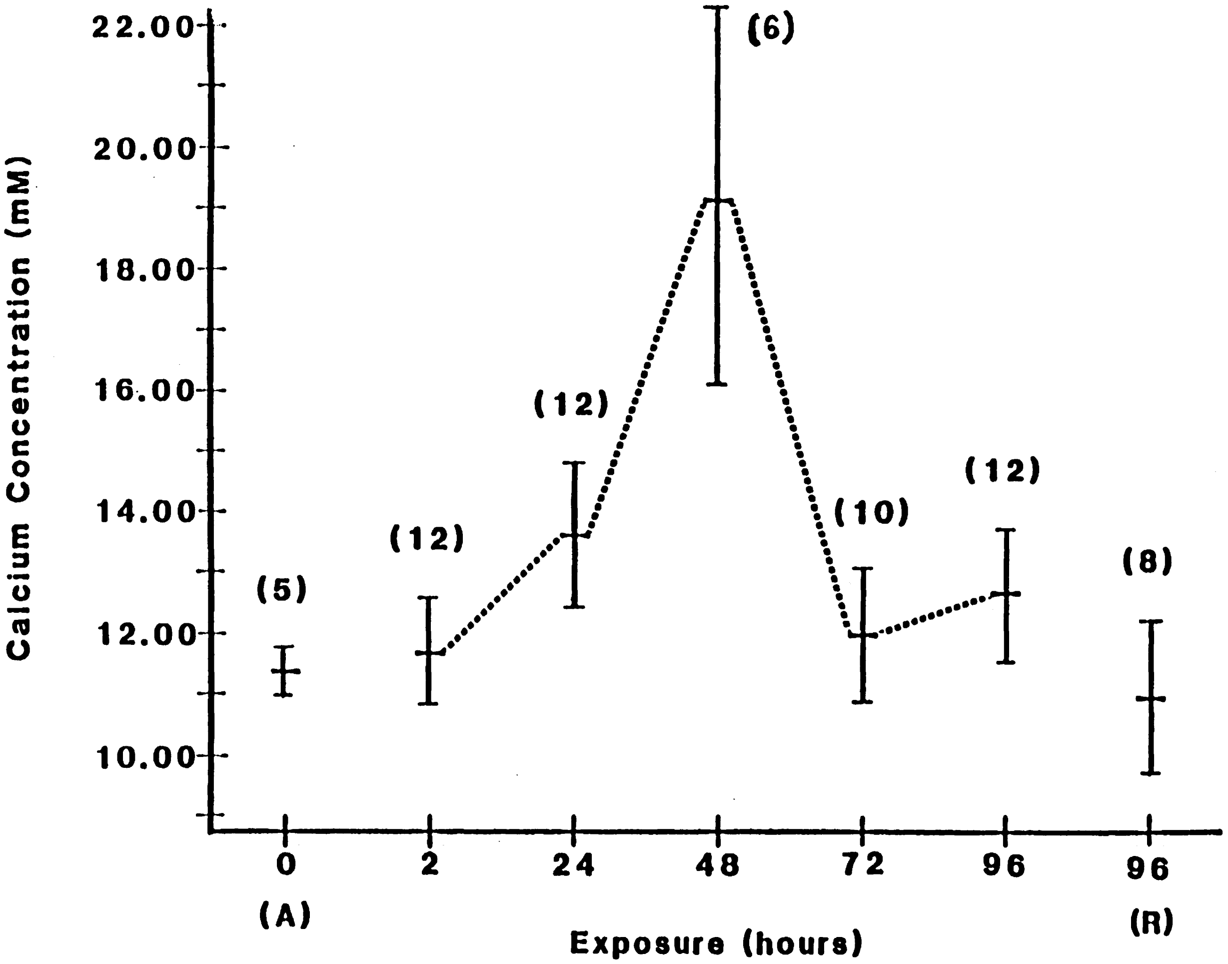

Figure 8. Calcium concentrations (means and standard error) in hemolymph extracted from Coweeta crayfish following laboratory exposure to reduced pH (2.62) for 2h, 24h, 48h, 72h, and 96h; including specimens from acclimation water (A) and reference streams (R). Numbers in parentheses refer to sample size.

Table 19. Millimolar (mM) concentrations and percent composition (± 95% C.I.) of Ca^{++} in carapaces of Coweeta crayfish acutely exposed to 5 pH levels in artificial streams, 1985. Group 5 is a reference.

Group	Mean pH	Sample Size	Mean CL (mm)	Mean Carapace Ca^{++}] mM[1]	Mean % Ca^{++} in Carapace
1	2.47	28	33.0	3.75 ± 0.46	15.0 ± 0.02
2	2.53	20	32.7	4.11 ± 0.29	16.5 ± 0.02
3	2.74	15	34.4	4.16 ± 0.60	16.7 ± 0.02
4	2.96	20	33.5	4.08 ± 0.44	16.3 ± 0.02
5	7.85	20	32.5	3.04 ± 0.63	12.2 ± 0.03

[1] Mean carapace [Ca^{++}] mM values tested with Jonckheere's Test For Ordered Alternatives under a priori hypothesis that groups 1 (τ_1), 2 (τ_2), 3 (τ_3), 4 (τ_4),and 5 (τ_5) were not different (Alternative hypothesis: $\tau_1 < \tau_2 < \tau_3 < \tau_4 < \tau_5$). P = 0.70442, fail to reject.

Table 20. Comparison of millimolar (mM) concentrations and percent composition (± 95% C.I.) of Ca^{++} in carapaces of Coweeta crayfish acutely exposed (treatment group) to various pH levels (2.47 to 2.96) and a reference group (pH=7.85), 1985.

Group	Number Of Carapaces	Mean CL (mm)	Mean Carapace $Ca^{++}]$ mM	Mean Percent Ca^{++} in Carapace
Reference	20	32.6 ± 1.7	3.04 ± 0.63	12.2 ± 0.03
Treatment	83	33.3 ± 0.8	4.00 ± 0.24*	16.0 ± 0.01

* Indicates significant difference from references (Wilcoxon 2-Sample Test, P = 0.0344).

Table 21. Results of preliminary experiment to determine survival time of pairs of molting and intermolt crayfish in artificial streams (pH = 2.00), 1985.[1]

Test Pair	Molt Status	Sex	CL (mm)	Survival (minutes)
1	Molting	Female	24.4	660
1	Intermolt	Female	25.4	1065
2	Molting	Female	31.1	Indefinite[2]
2	Intermolt	Female	34.2	1020
3	Molting	Female	34.6	375
3	Intermolt	Female	35.6	930
4	Molting	Female	24.4	780
4	Intermolt	Female	29.6	810
5	Molting	Female	27.3	665
5	Intermolt	Female	27.2	710
6	Molting	Male	34.6	470
6	Intermolt	Female	28.1	665

[1] All exoskeletons of molting crayfish failed to become rigid in acid.

[2] This specimen survived (with softened exoskeleton) until termination of experiment (48 h).

specimen survived the full 48 h experiment. No significant difference was detected between survival times of molting and intermolt animals (nonparametric paired Sign Test, n = 6, P = 0.109). Exoskeletons of all 6 molting crayfish failed to become rigid.

Crayfish Behavior During Acid Exposure

Although observations of crayfish behavior during acid exposure were not regimented or quantified, certain responses were apparent. Reference animals remained relatively inactive throughout exposure periods, but responded to gentle prodding with typically aggressive defensive posturing. Upon initial placement in acidified artificial streams, treatment animals tended to be quite active. In preliminary experiments prior to use of individual crayfish cages, several of these animals escaped from stream chambers within the first few hours. Aggressive behavior, including cannibalism, was common. Following an initial period of activity, treatment crayfish became very quiescent. Gentle prodding failed to produce reactions typical of this species, such as defensive posturing, strikes with chelae or flight. When close to death, most crayfish were observed lying ventral side up, and would respond to prodding only by slow movements of appendages, particularly pleopods.

Discussion

Population Biology

Petersen-Lincoln population estimates (Chapman's modification) for Ball Creek and Pinnacle Branch appeared to be fairly consistent among study sections, and probably are representative of crayfish numbers in 20 to 25m reaches of study streams which have abundant cover. The high degree of variability in estimates was probably due to low values for m_2 (marked recaptures) in the estimator. Low sample sizes for n_1 and n_2 may have caused a downward bias in most population estimates (Robson and Regier 1964). It is possible that seines placed at upper and lower boundaries of study sections did not adequately impede crayfish movements, thus violating the assumption of a closed population. It is also conceivable that the odor from baited traps in such small streams would be sufficient to attract downstream crayfish able to negotiate the block nets.

Higher Petersen-Lincoln estimates in September (as compared to May) were due chiefly to an overall increase in hand-collected (n_2) crayfish, and reflected a real increase in the population, primarily of young-of- the-year individuals collected by the hand-capture method.

It is also possible that experienced field personnel and low flows in September contributed to a greater collecting efficiency.

The combination of baited traps and hand-capture used in this study has been shown to yield unbiased population estimates, and alleviates potential problems associated with single sampling methods (Brown and Brewis 1978). Although baited traps are the most common means of crayfish capture because of their applicability to aquatic systems, they are generally known to violate the assumption of equal probability of capture of all population members by selecting for larger individuals (Mason 1975, Brown and Bowler 1977, Reid 1977, Brown and Brewis 1978, Westman et al. 1978). Mason (1975) successfully estimated numbers of *O. virilis* in a population of known size with traps, but determined that when gear was fished on consecutive days without replacement to the population, average size of captured crayfish decreased. Brown and Brewis (1978) found that trapped samples of *A. pallipes* contained 96.1% adults, and underestimated a known population by three-fold. Reid's (1977) traps yielded 97.1% adult *C. b. bartonii.*

My length-frequency histograms confirm trap bias toward larger crayfish in Coweeta streams, to include September samples, when the population structure shifted and many more immature individuals were present. Smaller crayfish were rarely trapped, but frequently observed around the traps. Possibly, smaller individuals were able to escape through the mesh or were inhibited from entering traps by the presence of larger, more aggressive individuals (Brown and Bowler 1977, Westman et al 1978). Partially eaten remains of smaller crayfish were frequently encountered in traps, indicating that adult cannibalism on smaller individuals was common. Generally, adult crayfish are more active than juveniles, particularly at night, and therefore more available to capture by traps (Berrill and Arsenault 1982). Other suspected biases inherent in trapping are chiefly behavior-related in association with reproductive events (Momot and Gowing 1972, Mason 1975, Brown and Bowler 1977, Momot and Gowing 1977, Reid 1977, Brown and Brewis 1978, Westman et al. 1978, Taylor 1983). Molting crayfish were never captured, and are thought to be less apt to enter traps (Momot 1967, Reid 1977). Predator densities and water levels will affect trapability of crayfish (Stein and

Magnuson 1976, Collins et al. 1983). Taylor (1983) demonstrated that activity of *C. latimanus* is synchronous with rising water levels, thereby affecting availability to capture. Ambient water temperatures also influence activity and thus trapping success for *O. virilis* (Momot 1967, Momot and Gowing 1972) and *C. b. bartonii* (Reid 1977). Bean and Huner (1978) found canned pet food more effective than cut fish bait for catching crayfish. Other factors which might have affected trapping efficiency for crayfish are mesh size, fishing time, nutritional condition and health, and presence or absence of other crayfish in and around traps.

The hand-collection method is thought to be much less biased (excluding young-of-the-year) than baited traps, as it depends less on the differential activity of various age groups and sex (Brown and Bowler 1977). Hand-capture typically yielded a wider range of crayfish sizes in Ball Creek and Pinnacle Branch, and was a better indicator of the overall population structure. Unfortunately, this method fails to sample all habitats equally (i.e. larger rocks and fallen trees cannot be turned); thus, numbers of larger adults, "berried" females, and females bearing young elude capture and inevitably are underestimated. Westman et al. (1978) recommend electrofishing for quantitative sampling of crayfish, but the small size of first-order Coweeta streams prohibited efficient use of electrofishing gear.

One or more biological characteristics may explain the greater size of trapped males versus females in September. Females "in berry" or carrying young tend to be reclusive (Mason 1975, Reid 1977, Brown and Bowler 1977, Hamr and Berrill 1985), and are typically burrowed under large rocks in Coweeta streams and Craig Creek. Hamr and Berrill (1985) believe that mature female *C. b. bartonii* do not molt and increase in size until after they release their young (late August to early October in their study). Sampling in late October or November might have yielded a greater number of large females. There was no significant difference in size among sexes for hand- collections at Coweeta.

Mean catch of crayfish per trap night was consistent among Ball Creek study sections, while only two sections differed from each other in Pinnacle Branch. These findings were similar to those of Reid (1977), who recorded mean values ranging from 0.50 to 6.37 crayfish per trap in streams of northern Maine. The significant decrease in catch per trap between

May and September in Ball Creek may have resulted from mortality of adult crayfish associated with reproduction and molting.

The hand-collection method might also be used to obtain relative abundance estimates in Coweeta streams. Few differences in mean catch per hour data were observed between Ball Creek and Pinnacle Branch. The dramatic increase in catch per hour between May and September may have been influenced by greater sampling experience of field personnel, or lower flows that concentrated crayfish. However, I believe that recruitment of a large young-of-year cohort was the primary reason.

Methods used in this study to obtain relative abundance estimates should provide U. S. Forest Service personnel with a reliable way of monitoring significant but not subtle changes in Coweeta crayfish populations over time. Traps were more time-efficient, and probably provide a more consistent means of sampling since they avoid bias problems created when hand-collecting is conducted by personnel with varying degrees of expertise. Hand-collection involves less cost for materials, but more time expended. It yields a more representative sample of young crayfish, and is much more effective in detecting recruitment trends in the population. Both methods require less time and effort than that needed to obtain accurate density estimates. Working in the same reach of Ball Creek, Huryn (pers. comm.) recently computed a mean density of 13 ± 3 *C. b. bartonii* per m^2, and estimated biomass to be 816 ± 294 mg ash-free dry weight per m^2. These estimates were based on a known-area core sampler, but were biased toward small individuals due to inability to sample large rock and boulder habitat frequented by larger crayfish. Reid (1977) reported standing stock estimates for this species (individuals $>$ 10mm CL) in northern Maine streams during August that ranged from 2.2 to 9.5 per m^2, and biomass of 6.6 to 30.5 g per m^2 (live wet weight). These values were obtained from mark-recapture studies by "various combinations of collection methods" (probably traps and hand capture). My data indicate that the Coweeta crayfish population appears generally healthy, exhibiting abundant numbers (9.2 to 29.8 per m^2) of crayfish with readily available cover. If crayfish are to be used for monitoring stream conditions relative to acidification, relative abundance measurements will help to provide appropriate baseline information.

Size-Frequency Distributions

Basic assumptions of size-frequency analysis are that all animals within a size class grow equally in a designated time period, and that cohorts are discrete. Brown and Bowler (1978) claim that the former assumption breaks down at the attainment of sexual maturity for *A. pallipes.* Individual variation in growth increments at each molt, possible limb loss, and reproductive history make the correlation between size and age less distinct over time for these crayfish (Brown and Bowler 1978). Mason (1975) points out that size overlap definitely occurs, even between age I and II to a minor degree, and is compounded in older classes of *Pacifasticus leniusculus trowbridgii.*

Size overlap between cohorts of *C. b. bartonii* is also apparent. Reid (1977) separated populations of this species into 3 or 4 age components; however, younger age groups were not easily separated in length-frequency plots. His size ranges (mm CL) of age groups in August were as follows: Age 0, 6.0 to 9.5; Age I, 9.6 to 25.2; Age II, 21.2 to 36.2; Age III, 35.9 to 40.6. Length-frequency histograms constructed by Hamr and Berrill (1985) for *C. b. bartonii* collected in the Ontonabee River (Ontario) in May showed only two obvious age groups, while a Buckslides Creek population appeared to have at least three age groups. Their data from September sampling of both populations suggested three age classes (Hamr and Berrill 1985). Size-frequency data from Coweeta populations indicated at least three (and probably four) age classes in May and September (when annual recruitment is virtually completed). The substantial overlap between histogram peaks may be due largely to the continuous schedule of reproductive events in this population. It is not possible to determine whether or not unequal growth patterns contribute to this overlap, since growth was not monitored.

Carapace Length-Wet Weight Relation

Cambarus b. bartonii from Coweeta streams (both sexes) displayed allometric growth, as weight increased faster than the cube of the length (Ricker 1975). One must consider that weight in crayfish is highly variable, depending on the molt stage, and frequent loss of appendages. Carapace length-wet weight equations in this study were based on data collected throughout the growing season (May through October), and did not include animals missing appendages.

Reid (1977) reported carapace length-wet weight equations for four populations of *C. b. bartonii* in northern Maine:

Little Machias River	$\mathrm{LogW(g)} = -4.0356 + 3.3722\ \mathrm{LogL(mm)}$	$r = 0.98$
Sterling Brook	$\mathrm{LogW(g)} = -3.9279 + 3.3039\ \mathrm{LogL(mm)}$	$r = 0.97$
North Pond Brook	$\mathrm{LogW(g)} = -3.8092 + 3.2051\ \mathrm{LogL(mm)}$	$r = 0.99$
South Fox Brook	$\mathrm{LogW(g)} = -4.3040 + 3.5480\ \mathrm{LogL(mm)}$	$r = 0.99$

Growth in these populations was allometric with no differences in mean weights between sexes. Reid (1977) also found the total length-carapace length relation to be approximately 2 to 1. Equations for Coweeta crayfish appeared to be similar to those in Reid's study.

Life History Observations

Sexually mature (form I) males occurred in all Coweeta collections, indicating that they are present year-round. The overall slight dominance of form I over form II males trapped in 1985 was likely a result of gear selectivity for larger crayfish, although crayfish may be more active when in reproductive form. Reid (1977) believes that this gear bias is conducive to a more representative sample of the proportion of form I to adult form II males, rather than form I males present in the population. Sexually mature females (with full cement glands) were observed in all sampling months. It appears that Coweeta crayfish do not have a well-defined reproductive period, although trap captures indicated that the peak of sexual maturation for both sexes was October. Hamr and Berrill (1983) sampled throughout the year and found that both sexes attained peak reproductive maturity together in September in Ontario. Reid (1977) reported form I males in Maine to be most abundant in August, although his sampling effort did not extend throughout the entire year. Many other species of crayfish exhibit fairly well defined reproductive and molting schedules (Momot 1967, Brown and Bowler 1977, Payne and Price 1983). Most species in the genus *Orconectes* follow a general pattern of fall and winter breeding, winter oviposition, and spring recruitment; molting of males from form I to form II typically occurs in the fall (Payne and Price 1983).

The majority of mating of Coweeta crayfish was expected to occur during or shortly after attainment of sexual maturity, probably in the fall. This event may be postponed by a decrease in stream temperatures until a subsequent post-winter warming, as was the case for the only observed incident of copulation which occurred in late May. My observations on attainment of sexual maturity, copulation, oviposition and appearance of young-of-year suggest that mating of Coweeta crayfish could occur throughout much of the year. The literature suggests *C. b. bartonii* may copulate throughout the year, depending on many factors such as geographic location and localized climatic events. In northern Maine, these crayfish mated following a male molt to form I during July and August (Reid 1977). This was prior to the appearance of "berried" or young-bearing females in August and September, suggesting that this species has no strict schedule for reproductive events. Hamr and Berrill (1985) believe that the majority of *C. b. bartonii* mating in Ontario occurred between April and August, despite witnessing copulation in October (Table 22).

Females crayfish carrying eggs or young were seldom observed at Coweeta, probably due to their secretive behavior at this time of the reproductive cycle (Reid 1977, Hamr and

Table 22. Selected life history parameters for *C. b. bartonii* populations.

	Month											
Study	J	F	M	A	M	J	J	A	S	O	N	D
Ortmann Virginia (1906)	-	Y	MCY	M	M	M	ME	MEY	MY	MC	MY	M
Reid Maine (1977)	-	-	-	-	-	MFCEY	MFE	MFECY	MFY	M	-	-
Hamr and Berrill Ontario (1985)[1]	MF	MF	MF	MF	MF	MFE	MY	MFY	MF	MFC	MF	MF
Hamr and Berrill Ontario (1985)[2]	-	-	-	-	MF	MF	MFE	MFEY	MFY	M	-	-
Present Study North Carolina	-	-	-	-	MFC	MFE	MF	MFE	MFY	MFY	MY	-
Present Study Virginia	-	-	-	E	-	-	MFE	EY	MF	CY	-	-

[1] Ontonabee River

[2] Buckslides Creek

M = Sexually active males (form I); F = Sexually active females (packed cement glands); C = Copulation; E = Females carrying eggs ("berried"); Y = Females bearing young

Berrill 1985). Field data indicate that females were "in berry" primarily during summer months, and eggs hatched in late summer and fall. Coweeta females with broods were observed as late as November (Huryn pers. comm.). Size-frequency data seem to indicate that broods are recruited into the population earlier than fall, with many small juveniles appearing in September samples. Hamr and Berrill (1985) attempted to thoroughly summarize reproductive behavior on an annual basis for two *C. b. bartonii* populations in Ontario. They found marked dissimilarity in timing of reproductive events between crayfish in the Ontonabee River (a large river) and Buckslides Creek (a small creek). Crayfish carried extruded eggs for approximately one month prior to hatching, and larval individuals were carried (in the laboratory) for approximately two weeks (Hamr and Berrill 1985). Assuming these schedules are similar to those of Coweeta crayfish, young-of-year (Age 0) individuals captured in early September (in Ball Creek and Pinnacle Branch) provide evidence for the presence of "berried" females at least as early and probably earlier than mid-July. Hamr and Berrill (1985) reported that juveniles between 13 and 20mm CL in September in Ontario may have been produced the previous autumn. Coweeta stage III hatchlings were 5.0 to 5.4mm CL, and September catches yielded 20 to 25% juveniles between 6.0 and 13.0mm CL, suggesting that a substantial portion of recruitment occurs earlier in the summer.

Ortmann (1906) suggested that *C. b. bartonii* might spawn in winter in northern areas (Pennsylvania), based on observations of females with broods in February. McManus (1960) also found females carrying young (5.2 to 5.5mm CL) in late February in New York, but disagreed with Ortmann. He suggested that oviposition and hatching occurred in late fall, prior to a decrease in water temperature (to 5.5°C) which immobilized the crayfish and arrested their development.

Predominance of one sex in Coweeta monthly trap samples is likely due to behavioral differences affecting activity patterns, such as reclusiveness of females with eggs and broods (Momot 1967, Brown and Bowler 1977, Reid 1977, Berrill and Arsenault 1982). The significant domination by females in traps only during May might be explained by an intense feeding period prior to oviposition. As summer progressed, trap captures became increasingly male-dominated. Hand-collections of crayfish from Coweeta during May and September yielded sex ratios of approximately 1:1. Brown and Bowler (1977) reported sex ratios of nearly 1:1 for *A. pallipes* from late May through early July. Reid (1977) reported an overall 1:1 sex ratio for *C. b. bartonii*, but significantly more males during August trapping, and attributed it to secretive behavior by "berried" and young-bearing females.

Hand-collecting probably yields sex ratios more representative of the Coweeta population. This method appears to effectively capture sexually immature individuals, unaffected by sexually-oriented behavior. Moreover, collecting by hand would seem to eliminate most sexual bias associated with trapping. Previous studies have attributed male-dominated trap catches of crayfish during some months to cessation of feeding by "berried" females (Taylor 1983), seasonal migrations of females due to gonad maturation (Momot and Gowing 1972, Momot and Gowing 1977), "trap happy" males and "trap shy" females (Brown and Brewis 1978), aggressive behavior by males (Westman et al. 1978), male cannibalism on females (Momot 1967), high female mortality during molt (Momot 1967), as well as reclusive behavior of "berried" and young-bearing females. In California, trap catches were dominated by female *P. l. trowbridgii* during summer months, but this was curtailed by the onset of breeding (Mason 1975). This domination by females was attributed to intensive summer feeding following release of broods, and prior to summer molt. The hand-collection method is more difficult to regulate in terms of sampling effort; however, it tends to eliminate many of the biases associated with a passive capture technique such as trapping, and also allows personnel to make more field observations on the crayfish and their habits.

Carapace length measurements indicate that crayfish in Coweeta streams attain a larger size at sexual maturity and overall than reported for other populations (Crocker 1957, Reid 1977, Rorer and Capelli 1978, Hamr and Berrill 1985) (Table 23). This might be attributed to a longer growing season for this southern population. Most *C. b. bartonii* in northern Maine become sexually mature during their third summer (age II); some reach maturity late in the second summer of life (age I) after having hatched early the previous year (Reid 1977). Few lived to breed a second time. Hamr and Berrill (1985) suggested sexual maturity was attained

Table 23. Size comparison (CL mm) of adult crayfish from Coweta with other populations of *C. b. bartonii*.

	Male			Female		
Study	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Ortmann (1906)[1]	49	-	-	48	-	-
Crocker (1957)	18.5	36.7	-	-	38.8	-
Reid (1977)	21.4	41.2	30.5	26.4	40.6	31.1
Rorer and Capelli (1978)	20	-	-	20	-	-
Hamr and Berrill (1985)	18[2]	36	26.8	20[2]	36	27
Present Study	26.4	44.5	34.1	26.6[3]	41.8	33.1

[1] Measurements in total length (mm).

[2] Hamr and Berrill (1985) considered the minimum size of adults to be 24mm CL.

[3] One female with internally developing eggs measured 24.8mm CL.

by this species in Ontario at the end of the third summer (age II); however, the majority did not reproduce until the following summer (age III), with only a few surviving into another year. They attribute the longer life cycle of these northern crayfish to a shorter growing season, compared to other species. My size-frequency data suggest that Coweeta crayfish may follow a pattern similar to that described by Reid (1977).

Field observations at Coweeta indicate that many crayfish appeared to molt in late summer and early fall. Although no actual molts were observed, many recently molted specimens were captured in September and October. Momot (1967) suggests that crayfish exoskeletons remain soft for 24h to 36h following the molt. During this period, *C. b. bartonii* would be unusually susceptible to predation and cannibalism, but remains hidden, and thus less available to sampling. Adults of this species in northern Maine molted primarily from July through September, whereas juveniles molted from June through September (Reid 1977). Frequency of male molts of *C. b. bartonii* was highest in September and early October in Ontario; female molts occurred most frequently from late August through early October, but in some cases not before winter (Hamr and Berrill 1985).

Taylor (1985) examined the popular hypothesis that male crayfish alternate between form I and form II throughout the year. He studied a population of *P. spiculifer* in Georgia in which the form II stage may be only a developmental predecessor to form I. Hamr and Berrill (1985) reported that the hypothesized pattern of mature form I individuals molting in synchrony to form II and back to form I by midsummer may be lost in *C. robustus* and *C. b. bartonii*. They believe that timing of the male molt cycle in these species may be based on a strategy of simultaneous occurrence of form I males with mature females, and molting may not occur until September, when cued by an environmental variable such as decreasing temperatures (Hamr and Berrill 1985). Apparently, adult females of these species will molt only once a year following release of young (Hamr and Berrill 1985). My observations of males remaining form I throughout the summer at Coweeta supports the hypothesis of Hamr and Berrill (1985). In widely fluctuating, high altitude streams of the Coweeta basin and similar watersheds, it may be just as maladaptive for crayfish to reduce their potential breeding period by molting in summer to a non-reproductive form (II), as suggested for northern populations (Hamr and Berrill 1985).

It is likely that *C. b. bartonii* in Coweeta streams and Craig Creek remain inactive for 3 to 4 months (December through March) of the year, when water temperatures average less than 10°C (Figure 6). Aiken (cited in Momot and Gowing 1972) observed locomotor activity to decline very rapidly in crayfish as water temperatures dropped below 10°C. Brewis and Bowler (1983) found that molting ceased in *A. pallipes* at temperatures below 10°C. During late October in northern Maine, the species was quite active in one stream at a temperature of 10.0°C, but was inactive and burrowed into stream banks of a nearby stream at temperatures of 5.5°C.

Ortmann (1906) believed that temperature played an important role in the distribution of *C. b. bartonii*, and was the cause of a poorly defined life history schedule of events (mating, breeding, etc.). This species' burrowing habits, prolonged reproductive periods, and winter inactivity are thought to be necessary adaptations, critical to survival in harsh northern climates (Hamr and Berrill 1985). Although southern populations of this species may not be subjected to the extreme winters of more northern climates, Coweeta headwater streams are harsh environments with variable flows, potential for irregular temperature fluctuations, and shortened growing seasons. In this environment, a species with restricted opportunity for breeding might be more vulnerable to poor recruitment or total failure of a year-class due to even one untimely, particularly harsh climatic event. As their large numbers indicate, *C. b. bartonii* seems to be well adapted for life in these high elevation headwater systems.

Effects Of Acid Exposure

Acute Lethality Tests

Toxicity test results suggest that individual adult and juvenile *C. b. bartonii* are very acid tolerant relative to other crayfish and freshwater organisms. Previous studies indicate that adult crayfish other than *C. b. bartonii* are fairly tolerant of acute acid exposure, but exhibit some degree of variability. Reported 96h LC_{50}'s were pH 2.5 and 2.8 for *P. clarki* and *O. rusticus*, respectively (Morgan and McMahon 1982), and pH 2.35 for *O. virilis* (France 1984). The 96h LC_{50} values obtained for *C. b. bartonii* are considerably lower on the pH scale than those found for many fishes and insects. Beamish (1972) reported an LC_{50} of pH 3.9 (100h) for the white sucker (*Catostomus commersoni*). Daye and Garside (1975) determined an LC_{50} of pH 3.5 (167h) for brook trout (*Salvelinus fontinalis*). Rainbow trout (*Salmo gairdneri*) exposed to acid for 96h exhibited an LC_{50} of pH 4.0 to 4.2 (McDonald et al. 1980), and for 167h, pH 4.1 to 4.5 (Graham and Wood 1981). Jagoe et al. (1984) reported 96h median lethal concentrations ranging from pH 4.0 to 4.2 for yearling sunapee char (*Salvelinus alpinus*). Examples of acute acid toxicity to aquatic insects include 96h LC_{50}'s ranging from 3.15 to 4.65 for immatures of nine species of caddisflies, stoneflies, dragonflies and mayflies, with one caddisfly (*Brachycentrus americanus*) exhibiting an LC_{50} at pH 1.5 (Bell and Nebeker 1969). Lechleitner et al. (1985) recorded 96h LC_{50}'s for three species of stonefly nymphs from pH 2.8 to 3.3. My experiments with *C. b. bartonii* yielded LC_{50} values considerably lower (on the pH scale) than all but one of these fish and insect studies, indicating that selected life stages of this crayfish are very tolerant of low pH.

The susceptibility of *C. b. bartonii* from Coweeta streams to acute acid exposure appears to be dependent upon size or perhaps life cycle stage. Supporting data were reported for *O. virilis* by France (1984), who obtained 96h LC_{50} values of pH 2.35 for adults, and pH 2.95 and pH 3.70 for juveniles and stage III independent hatchlings, respectively. Similar size-related results have been reported for freshwater fishes (Fromm 1980). The mechanism for acid toxicity in fish and crayfishes is thought to be similar; 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 a relatively greater ion exchange (surface) area for smaller individuals, and an increased resistance with physiological maturation (Schofield 1976, France 1984), possibly due to a lower metabolic rate. These findings stress the importance of including several life history stages in acid toxicity investigations. It is quite possible that additional experiments would show specific periods of *C. b. bartonii*'s reproductive cycle, particularly early life stages such as hatchlings and eggs, to be more susceptible to reduced pH than the three cohorts studied (Appelberg 1984).

Despite wide variation in fiducial limits, the observed similarity in LC_{50}'s between Coweeta and Craig Creek adult crayfish further substantiates the tolerance for acid by this species, and indicates no apparent effects of Coweeta stream pH on acid tolerance, to date. Water chemistry data indicate that stream pH values in Ball Creek and Pinnacle Branch average about 6.51 (5.60 minimum) and 6.70 (4.90 minimum), respectively, on an annual basis (W. Swank pers. comm.), while pH of Craig Creek was measured at 7.1 to 7.2 at collection sites. My data demonstrate no greater tolerance to laboratory acid exposure by Coweeta as opposed to Craig Creek crayfish. In spite of the geographical and long evolutionary segregation, the physiological response to pH of crayfish from these two populations remains the same. However, these results do not preclude the possibility of adaptation to continued exposure to reduced stream pH by Coweeta or other crayfish.

Influence Of Temperature On Acid Tolerance

Minimal research has been conducted on the effects of temperature on aquatic invertebrate sensitivity to potential toxicants. Data from experiments with fish are contradictory (Cairns et al. 1975), but pollutants are usually assumed to be more toxic at higher temperatures near the organism's maximum thermal tolerance (Sprague 1970). Cairns et al. (1975) concluded that temperature is more of a factor in acute toxicity than in long term exposure. Coweeta crayfish exhibited differences in resistance to acute acid toxicity with a 5° or 6°C change in water temperature. Direct comparisons of acute LC_{50} values for *C. b. bartonii* to those of other crayfish species should consider differences in experimental temperatures, since it appears that temperature does influence acid toxicity (Morgan and McMahon 1982, France 1984).

Assuming that water temperature influences acid toxicity to crayfish in Coweeta streams in the same manner as observed in laboratory experiments, one can speculate about ecological implications. Populations of this species probably are subjected to the most severe acid exposure during spring snowmelt when water temperatures are relatively low (Jeffries et al. 1979, DeWalle et al. 1983). Coweeta streams , however, do not experience acidic meltwater from a large snowpack (J. Webster pers. comm.), and mean annual precipitation is distributed uniformly throughout the year (Swank and Douglass 1977). A review of stream chemistry data for Ball Creek and Pinnacle Branch showed that periods of depressed pH values occurred mostly during winter months. These periods (particularly February and March) coincide with periods of highest and most variable flow, and might be affected by rain or snowfall events which seldom show pH measurements above 4.50 (Swank and Douglass 1977). Regardless of the cause of depressed pH in Coweeta streams, it appears that some of the most acidic conditions have occurred when crayfish are more tolerant due to reduced temperatures, and the younger and perhaps more acid-sensitive life stages are not present. However, this does not preclude the possible occurrence of severe episodic inputs of acid (such as storm events) during periods of elevated stream temperatures.

Physiological Investigations

Hemolymph Ion Regulation

The significant decrease in hemolymph sodium levels following acid exposure was consistent with reported sodium decreases in aquatic insects (Lechleitner et al. 1985), fish (Fromm 1980, McWilliams 1980), and most other crayfish (Shaw 1960, Morgan and McMahon 1982, McMahon and Morgan 1983, Appelberg 1985, Wood and Rogano 1986), although Hollett et al.(1986) found no change in adult *C. robustus*. Reduction of Na^+ has been attributed to a decrease in the uptake rate of Na^+ across gills, probably caused by a disturbance of the Na^+/H^+ exchange mechanism (Shaw 1960, Appelberg 1985). Increased or normal passive efflux of Na^+ would also contribute to the loss (Morgan and McMahon 1982). Swinehart and Cheney (1984) suggest that acid exposure causes ionoregulatory failure in chinook salmon (*Oncorhynchus tshawytscha*) and freshwater mussels (*Anodonta californiensis*) through primary amine loss, thus destroying the integrity of membranes and membrane processes.

Recent work by Wood and Rogano (1986) indicates that physiological responses of crayfish (*O. propinquus*) to acid exposure are quite different from those of fish. Specifically, response of crayfish in soft water ($[Ca^{++}] = 0.20 \text{mequiv} \times L^{-1}$) resembled that of rainbow trout in hard water; high overall influx of acid equivalents, severe acidosis and relatively small Na^+ loss (but considerably greater than Cl^- loss). In addition, crayfish in softwater displayed inhibited ion influxes during exposure. Conversely, fish suffered decreased ion levels primarily due to increased ion efflux during acute exposure, but due mostly to uptake inhibition over the long term (McDonald et al. 1983, Hobe et al. 1984 cited in Wood and Rogano 1986). Wood and Rogano (1986) also suggest that gill permeability to passive ion loss is significantly more acid resistant in crayfish than in fish, but direct H^+ penetration of the carapace is a problem to crayfish. Whether by uptake inhibition or increased efflux, the decrease in $[Na^+]$ from *C. b. bartonii* hemolymph implicates a disturbance in ion regulatory abilities as one probable mode of sublethal toxic action. Such physiological stress which might weaken Coweeta crayfish in a natural situation may also increase susceptibility to disease, parasites or predators; or this stress may induce behavioral changes, further affecting activities such as feeding or the reproductive process.

Calcium is probably the most important and abundant cation in the crayfish (and crustaceans in general), and is stored primarily in the exoskeleton as $CaCO_3$ (Huner et al. 1976, Greenaway 1985). As the crayfish molts to allow growth, large amounts of Ca^{++} must also be accumulated from the environment to form and harden the exoskeleton during postmolt. The animal is usually in a state of dynamic Ca^{++} equilibrium during intermolt. While preparing to molt (premolt), Ca^{++} is resorbed from the "old" exoskeleton, and stored at several sites in the body including the gastrolith (McWhinnie 1962), hepatopancreas (Huner et al. 1976) and intracellular compartments of muscle (Wood and Rogano 1986). Much Ca^{++} may be lost from the body via transport across gills (Greenaway 1985). Upon ecdysis, recalcification is immediately critical, and accomplished not only through environmental sources such as water, food and even the shed exuvia, but also by mobilization of internal stores (Adegboye 1983, Greenaway 1985). Metabolism of Ca^{++} in crayfish is an active and essential process, highly dependent upon environmental sources, and easily susceptible to environmental disturbances (McWhinnie 1962, Adegboye 1983, Greenaway 1985, Wood and Rogano 1986). During my laboratory experiments, which were conducted in "soft" water (38 to 44 mg/l alkalinity), "environmental" sources of Ca^{++} were minimal, causing crayfish to rely heavily on internal stores. Since it appears that this ion acts as an important buffer to acidosis, it is probable that acid tolerance by *C. b. bartonii* was equal to or greater than might be expected in Ball Creek or Pinnacle Branch which exhibit much lower alkalinities (0.5 to 2.6 mg/l, W. Swank pers. comm.).

It appears that environmental acidification (depending on severity) can have an effect on crayfish physiology. The observed increase in hemolymph [Ca^{++}] for *C. b. bartonii* in the present study contrasts with decreases reported for fish (Fromm 1980, McDonald et al. 1980), but is consistent with significant increases in [Ca^{++}] in intermolt adults of some other crayfish species exposed to reduced pH (Morgan and McMahon 1982, Wood and Rogano 1986). Appelberg (1986) suggested that a slight decrease in total hemolymph Ca^{++} of *A. astacus* exposed to pH 4.0 and 5.0 could be attributed directly to pH levels or physiological differences within the intermolt stage, since plasma Ca^{++} rose when these specimens were exposed to pH 3.7. When interpreting and comparing pH effects on internal ion regulation in this study and others, ion composition of laboratory water may be important. Uptake rate of Na^{+} (discussed above), Cl^{-}, and Ca^{++} depends on environmental concentrations (Shaw 1960, Adegboye 1983, Greenaway 1985). Increasing hemolymph Ca^{++} levels and decreasing Na^{+} levels (discussed above) in fairly "soft" water indicate interference of ion regulatory mechanisms and is probably one mode of acid toxicity in *C. b. bartonii.* However, hemolymph ion concentration determinations demonstrated that these measurements are prone to high variability and experimental error.

Suggestions of interspecific differences in acid tolerance by crayfish are based primarily on geographical distributions with respect to environmental pH (Berrill et al. 1985, Hollett et al. 1986). Results from studies conducted by Hollett et al. (1986), utilizing physiological parameters ($[Na^{+}]$, $[Ca^{++}]$), illustrate greater tolerance of low pH by *C. robustus* as opposed to *O. rusticus.* Berrill et al. (1985) believe the genus *Cambarus* generally is more tolerant than *Orconectes.* My observations of acid-induced physiological disturbances in Coweeta crayfish ($pH = 2.62$, 20°C) versus results of Hollett et al. (1986) for *C. robustus* ($pH = 3.8$, 15°C) may reflect interspecific differences in acid tolerance, or may be attributed to differences in pH exposure levels and water temperature. LC_{50} values obtained in the present study lend support for greater tolerance by *Cambarus* species.

Carapace Calcium Experiments

Several studies have postulated an internal buffering mechanism in crayfish (Malley 1980, Morgan and McMahon 1982, Wood and Rogano 1986) as well as in the crab, *Cancer productus* (deFur et al. 1980), which responds to an internal state of hemolymph acidosis induced by environmental acidification. Uptake inhibition of Ca^{++} in acidified soft water has been observed in postmolt *O. virilis* (Malley 1980, Malley and Chang 1985), and net Ca^{++} losses to the environment concomitant with elevated hemolymph levels were reported for *O. propinquus* (Wood and Rogano 1986), substantiating the hypothesis of mobilization of internal $CaCO_3$ stores. In my experiments, *C. b. bartonii* carapaces showed no signs of Ca^{++} loss during acute acid exposure. Carapace $[Ca^{++}]$ was unexpectedly higher in acid-exposed versus reference animals, but such wide 95% confidence intervals indicate variability was so high that these data are probably of little value. A 4 day acid exposure might not be sufficient to expect mobilization of exoskeleton $CaCO_3$ stores. Perhaps during acute acid episodes, more readily mobilized Ca^{++} stores such as the gastrolith (McWhinnie 1962), intracellular muscle compartments (Wood and Rogano 1986) or hepatopancreas (Huner et al. 1976) are utilized for buffering purposes, and a chronic exposure is necessary to mobilize calcium from the exoskeleton. It is interesting to note percent Ca^{++}, measured in *C. b. bartonii* carapaces, was much lower than the 20.0 to 26.6% range reported for other species (Huner et al. 1976). Appelberg (1979) found a decreasing Ca^{++} content in carapaces of *A. astacus* with decreasing pH, but he observed molting individuals under chronic exposure.

Molting Experiment

Although no significant difference was detected among survival times of molting and intermolt specimens during severe acid exposure, my results indicate molting *C. b. bartonii* are more susceptible to reduced pH. Survival times were greater for intermolt crayfish in five of the six tests, indicating apparent biological significance. All six molting individuals, including one which survived acid exposure, failed to complete the molt (remained soft). The lone molting individual which survived may have been near completion of it's molt, thus enabling it to tolerate the acid stress. Appelberg (1979) regards molting second only to hatching as the most critical period in the life of crayfish. He observed no molting mortality associated with laboratory exposure to a pH range of 4.55 to 7.30; however, depressed pH before and during molt shortened premolt, and reduced carapace rigidity and dry weight. Specimens subjected to pH 4.55 remained very soft, as I observed with Coweeta crayfish. Malley (1980) reported that nonmolting *O. virilis* survived 10 days at pH 4.0, whereas postmolt specimens suffered mortality. In addition, crayfish subjected to pH 5.0 progressed more slowly through molt cycle stages than at pH 6.0. France (1983) reported reduced carapace rigidity due to acid stress in a wild population of the same species. Although reduced Ca^{++} uptake probably is a chief cause of acid-related unsuccessful molts, it may not be severe enough to cause mortality (Malley 1980). The combined effect of this acid-induced phenomenon with other disturbances to ion balance, as well as behavioral and ecological problems, is a potentially lethal situation for molting crayfish. A severe decrease in stream pH during the molt probably would leave Coweeta crayfish (particularly early life stages) vulnerable to a variety of predators and physical injuries.

Conclusions

Coweeta populations of *C. b. bartonii* appear healthy at this time. Observations and population estimates indicate that these high elevation streams contain relatively large numbers of crayfish. Histograms of size-class structure demonstrate a strong 1985 year-class. These crayfish have adapted well to life in unpredictable, and often vulnerable stream systems, through what appears to be a fairly continuous reproductive schedule.

All three life stages of crayfish tested were quite resistant to acid exposure. However, the observed trend of decreasing acid tolerance in younger individuals leads me to believe that earlier life history stages (eggs, stage I and II hatchlings) may be more sensitive to reduced pH. Effects on the reproductive cycle, such as egg loss from pleopods by "berried" females, may be an important consideration when speculating about this species' ability to cope with increasing acidification. In addition, my study and work on other crayfish species demonstrated significant acid-induced physiological effects at reduced pH that may well influence reproductive success and survival. Acid exposures under laboratory conditions were severe relative to natural conditions at Coweeta, but laboratory studies never incorporate all interacting variables and additive and synergistic stressors that might accompany episodic low pH events. The observed relationship between water temperature and acid tolerance of crayfish may be important in warmer regions such as the southeastern United States, as acid deposition intensifies. Higher temperatures than those recorded in first-order Coweeta streams may pose greater acid-related problems for populations of crayfish and other aquatic organisms. Preliminary acid exposures with molting crayfish implicate this stage as perhaps a sensitive period in the life cycle. Acid-induced behavioral modifications to daily activities such as feeding, predator avoidance, or during mating have not been well studied, and may prove to be important. Though Coweeta crayfish exhibited a greater tolerance for reduced pH than reported for other crayfish species, results of this study suggest that insidious sublethal effects could become a problem if these streams follow the same pattern as observed in previously acidified regions of the world.

The ever-increasing threat of acidification to poorly buffered Coweeta streams, and the ecologically important role that populations of *C. b. bartonii* play in these aquatic systems, suggests that periodic monitoring of the crayfish fauna would facilitate the recognition of long-term, large-scale changes that may indicate an overall degradation of these ecosystems. However, based on their high tolerance to reduced pH, I would conclude that *C. b. bartonii* is not an effective indicator of gradual pH changes in these streams. Although this study demonstrated difficulties associated with obtaining accurate estimates of crayfish abundance, it appears that successful monitoring can be accomplished using relative abundance on a catch per unit effort basis. Baited traps or hand capture (more sensitive to recruitment) would provide a suitable method to detect significant annual changes in relative abundance and structure, with reasonable sampling effort.

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