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Acute Toxicity of an Acid Mine Drainage Mixing Zone to Juvenile Bluegill and Largemouth Bass

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Abstract.—The toxicity of an acid mixing zone produced at the confluence of a stream that was contaminated by acid mine drainage (AMD) and a pH-neutral stream was investigated in toxicity tests with juvenile bluegill *Lepomis macrochirus* and largemouth bass *Micropterus salmoides.* Fish mortalities in instream cages located in the mixing zone, below the mixing zone, and upstream in both tributaries were compared to determine relative toxicity at each site. In all tests and for both species, significantly higher mortality was observed in the mixing zone than at any other location, including the acid stream, which had lower pH (2.9–4.3). The mixing zone was defined chemically by rapid precipitation of dissolved aluminum and iron, which arrived from the low-pH stream, and by the presence of white precipitates, which were attached to the substratum and which extended below the confluence. Possible seasonal changes in mixing zone toxicity were investigated by conducting field tests with bluegill in June, July, and August 1996 and in January 1997 and by conducting field tests with largemouth bass in April and May 1997. Toxicity was not significantly different at the extremes of temperature, pH, and metal concentration that occurred in June and July, as compared with January. Toxicity was significantly lower in August; however, elevated stream discharge during the August test may have disturbed mixing zone characteristics. High toxicity in AMD mixing zones may lower the survival of fishes in streams, reduce available habitat, and impede movements of migratory fish.

Acid mine drainage (AMD) results from the oxidation of sulfide minerals that are exposed to the atmosphere through erosion of coal-mine spoils (Van Breeman 1973; Herrman and Baumgartner 1992). Receiving streams typically have low pH, high conductivity, and high metal and sulfate concentrations (Herlihy et al. 1990). In streams that are severely polluted by AMD (pH <3.5), fish may be absent; in less severe conditions (pH = 4.5–6.0), low abundance and diversity may occur (Harris et al. 1983; Short et al. 1990; Rutherford and Mellow 1994; Saiki et al. 1995). Negative effects on stream biota can result from low pH, dissolved metals (aluminum [Al], iron [Fe], zinc [Zn], manganese [Mn], copper [Cu], or cadmium [Cd]), or from sedimentation that is increased by mining activity (for review see Sorensen 1991; Grippo and Dunson 1996). In addition, AMD-contaminated streams affect neutral streams through the production of acid mixing zones at their confluence (McKnight and Feder 1984).

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In acid mixing zones the pH in both streams changes abruptly, and equilibrium between dissolved and particulate metals carried in the AMD stream is disturbed (Theobald et al. 1963; McKnight and Bencala 1990; Herrman and Baumgartner 1992). The pH below a mixing zone is a function of pH and the respective acidity and alkalinity of the two streams (Herrman and Baumgartner 1992). Dissolved metals such as Al, Fe, and, to a much lesser extent, Cu precipitate in the mixing zone, whereas Zn, Mn, and Cd tend to remain dissolved (Theobald et al. 1963; Chapman et al. 1983; McKnight and Bencala 1989, 1990; Sorensen 1991; Kimball et al. 1995). Mixing zones are often apparent based on the presence of visible metal precipitates that are attached to substratum and that are in the water column that extends below confluences (Theobald et al. 1963; McKnight and Feder 1984). Low diversity and abundance of fishes and invertebrates have been documented in affected streams (Harris et al. 1983; Short et al. 1990; Rutherford and Mellow 1994; Saiki et al. 1995); however, the toxicity of AMD mixing zones to fish has not been studied.

Toxicity in mixing zones that results from acid precipitation rather than from AMD has been demonstrated in Norway with Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* (Rosseland et al. 1992; Poleo et al. 1994; Verbost et al. 1995). Precipitation of Al on fish gills resulted in significantly higher fish mortality in the mixing zone than in the acid stream, which had lower pH and higher concentrations of dissolved Al (Rosseland et al. 1992; Poleo et al. 1994; Witters et al. 1996). Atlantic salmon and brown trout were reported to avoid mixing zones; both reduced capacity to repair damaged gills and altered ion concentrations were found when fish passed through mixing zones repeatedly (Atland and Barlaup 1995; Verbost et al. 1995). Acid mixing zones may impede anadromous fish migration, limit spawning areas, and reduce larval fish survival (Rosseland et al. 1992; Poleo 1995).

In coal-mining regions, many streams in a geographical area can be affected by AMD, and numerous mixing zones may be produced within a single watershed (Herlihy et al. 1990). Several AMD mixing zones may occur in a single stream, potentially decreasing suitable habitat for fish and causing stress when fish repeatedly pass through these areas, thus resulting in lower fish production. Seasonal changes in water chemistry and discharge affect AMD streams, which may in turn affect toxicity of mixing zones (Theobald et al. 1963; McKnight and Feder 1984). Severely contaminated (pH <3.5) AMD streams can contain complex mixtures of toxic metals at elevated concentration; however, disequilibrium conditions in mixing zones may be more toxic to fish. The toxicity of AMD mixing zones located in warmwater streams needs to be evaluated, and seasonal changes in toxicity should be considered.

The objectives of our research were to investigate the toxicity of an AMD mixing zone formed at the confluence of two warmwater streams and to determine toxicity during the summer, winter, and spring. Juvenile bluegill *Lepomis macrochirus* and largemouth bass *Micropterus salmoides* were used in this study, because they are important sport fish in warmwater streams of the southeastern United States.

Study Site

The study was conducted in an acid mixing zone generated at the confluence of Black Branch (AMD) and Cane Creek (pH neutral), both of which are located in Walker County, Alabama, an area that has been noted for AMD problems since the early 1960s (Hyde 1970: T. 15S, R. 8W, S. 18S). Exposed mine tailings and open portals produce AMD and contaminate Black Branch (8.5 km^2 watershed) with high concentrations of dissolved metals and low pH (<3.0). The Cane Creek watershed above Black Branch (13.7 km^2) was also extensively mined in the early 1900s; however, high buffering capacity (alkalinity 200–300 mg/L) maintains neutral pH (7.0–8.0) and reduces the transport of toxic metals to the confluence. There is no record of restoration of Cane Creek above Black Branch, and the factors responsible for the high alkalinity and pH are unknown. Metal-precipitate plumes produced at the confluence of Black Branch and Cane Creek extend for 100–150 m downstream (depending on discharge), and pH stabilizes (7.0–8.0) 10–15 m below the confluence.

Methods

Experimental design.—Acid mixing zone toxicity to juvenile bluegill and largemouth bass was evaluated using instream cages at four specific field sites. Results were evaluated from tests using bluegill in June, July, and August 1996 and in January 1997 and from tests using largemouth bass in April and May 1997. The Cane Creek site was located in Cane Creek, 20–30 m upstream of the confluence with Black Branch (i.e., mixing zone), the Black Branch site was located 20–30 m upstream of the mixing zone in Black Branch, and the mixing zone site was at the confluence of the two streams. Although discharge was not measured in either stream, the width of Black Branch varied between 2 and 3 m (mean depth ≈0.3 m), whereas the width of Cane Creek was between 4 and 6 m (mean depth ≈0.5 m). In the mixing zone site, the pH ranged from 5.5 to 6.0 during the study. The contribution of Black Branch water to mixing zone cages was calculated based on a ratio of Al concentration (total Al mixing zone : total Al Black Branch). A fourth site was located 15–30 m below the confluence.

Fish cages were constructed of plastic pipe (102 mm in diameter, 400 mm long), with nylon screen (2.7-mm mesh) on each end. An opening (350 mm long, 50 mm wide) was located on top for stocking, observation, and measurements of pH and flow rate. At each site, three cages (two in July) were positioned in the streambed with plastic stakes and were shaded by plastic covers. The site located below the mixing zone was not used in June, whereas in July and August, four and six cages were used, respectively. Ten fish were placed in each cage at the start of each test; however, only one largemouth bass (139 mm = mean total length [TL]) per cage was used during April 1997 because of cage size constraints.

Juvenile bluegill were harvested from aquaculture ponds at Auburn University, and largemouth bass were obtained from a commercial fish hatchery (American Sport Fish, Montgomery, Alabama). Fish were kept in 100-L flow-through aquaria (0.5–1.0 L/min; tests conducted during 1996) or in 115-L, static aquaria (50% water exchange twice per week; tests conducted during 1997). Water quality in flow-through aquaria was as follows: alkalinity, 40 mg/L as $CaCO_3$; hardness, 45 mg/L as $CaCO_3$; temperature, 25–27°C; pH, 7.0; and dissolved oxygen, 6–7 mg/L. Water quality in laboratory aquaria was as follows: alkalinity, 17 mg/L as $CaCO_3$; hardness, 60 mg/L as $CaCO_3$; temperature, 15–20°C; pH, 6.9–7.1; and dissolved oxygen, 7 mg/L. Sizes of bluegill (mean TL $\pm$ SD) were 22.0 $\pm$ 1.6 mm (June and July 1996), 34.1 $\pm$ 4.8 mm (August 1996), and 27.3 $\pm$ 2.5 mm (January 1997). Mean total lengths ($\pm$SD) of largemouth bass were 139.0 $\pm$ 9.3 mm (April 1997) and 29.5 $\pm$ 2.2 mm (May 1997).

Bluegill were fed powdered catfish feed and brine shrimp three to five times per week, and largemouth bass (April 1997) were fed golden shiners *Notemigonus crysoleucas* and earthworms three to four times per week. The largemouth bass used in May 1997 tests were not fed because they were transported to the field site 2 d after they were purchased. Largemouth bass used in April 1997 were held in aquaria for 12 weeks; bluegill were kept in aquaria for 18 weeks before use in January 1997; and bluegill used in June, July, and August were kept 2, 4, and 8 weeks, respectively.

Fish were not fed for 48 h prior to field tests. Thirty-six hours before field tests, fish were randomly separated into four aquaria for pH adjustment. Twenty-four hours before cages were stocked, the pH in each aquarium was adjusted to match the pH of a specific instream (field) site, as follows: 7.0 for Cane Creek, 4.0 for Black Branch, 5.5 for the mixing zone, and 7.0 for below the mixing zone. Adjustment of pH involved dropwise addition of H_2SO_4 in order to gradually lower the pH over 4–5 h while simultaneously monitoring changes with a pH meter (Hanna Instruments, HI 9025C, Woonsocket, Rhode Island). Plastic bags (20 L) containing pH-adjusted water, oxygen, and no more than 100 fish were carried in styrofoam coolers to the study site (3.5-h drive). Unopened bags were placed in the stream in order to allow for temperature adjustment before the cages were stocked. Mortality was determined every half hour, and dead fish were removed immediately. Fish were considered dead when opercular movement stopped, and tests were conducted for 6 h (10 h in May).

Statistics.—Fish mortality at each site was converted into a survival function using time to death for each fish (all cages per site combined; Life Tables SPSS 6.1.2 for Windows 3.1). A Wilcoxon (Gehan) nonparametric test was used to compare survival functions among sites (Gehan 1969), and the same statistic was used to evaluate seasonal changes in toxicity of the mixing zone by comparing survival functions among months at this site. A significance level of 5% was used to test the null hypothesis that all survivor functions were equal.

Water chemistry.—Water velocity was maintained at 3–4 cm/s in each cage by adjusting the orientation of the cages with respect to stream flow; water velocity was measured hourly (Marsh McBirney model 201D). The pH was also measured hourly in all cages. For determination of alkalinity, total hardness, and sulfate, a 1-L water sample was collected from each site during each test in an acid-washed plastic bottle. Water samples were carried from the site in plastic coolers, refrigerated in the laboratory, and analyzed within 10 d. Alkalinity and hardness were determined using a digital titrator (Hach model FF-2, Loveland, Colorado), and sulfate concentration was measured by colorimeter (Hach DR 100). Temperature (Hanna Instruments HI 9025C), dissolved oxygen (YSI model 51B, Yellow Springs, Indiana), and conductivity (YSI model 33) were determined at each site during each test period (2.0–4.0 h).

For metal analysis, filtered (0.45-μm–pore size polycarbonate membrane; Geotech Geopump 2, Denver, Colorado) and unfiltered samples (100 mL each) were collected at each site (1.0–5.0 h) during each field test and were preserved with 1 mL ultrapure HNO_3. Filtration occurred within 30 s after collection. Malfunction of the filter prevented collection of filtered samples during the June test. Water samples were shipped to the U.S. Geological Service, Biological Resources Division, Environmental and Contaminants Research Center (USGS-BRD, ECRC), and metal concentrations were determined using an inductively coupled plasma-mass spectrometer (PE/SCIEX Elan 6000). Mean (N = 2–3) metal concentrations are reported in milligrams per liter plus or minus standard deviation (SD) for samples collected at the same site during the same test period. Comparison of metal concentrations in filtered and unfiltered water samples at each site were used to estimate the proportion of metals that was present as suspended precipitates.

Results

Fish Mortality

No fish in cages located above the mixing zone in Cane Creek died at any time, except during the January 1997 test, when one fish died 1.5 h after the test began. Therefore, experimental conditions and cages probably did not cause mortality but rather confined fish in locations in which water conditions resulted in mortality. No statistically significant differences were observed between the June and July tests, and therefore, results were combined. In all tests with bluegill, significantly ($P < 0.001$) higher mortality was observed in the mixing zone than at any of the other sites (Figure 1).

The toxicity of the mixing zone to bluegill was not significantly ($P = 0.12$) different between June, July, and January; however, the mixing zone in August was significantly less toxic than during other tests ($P < 0.001$). In Black Branch, significantly higher mortality of bluegill occurred in June and July than occurred in any other test ($P < 0.001$).

Mortality of largemouth bass in the mixing zone was significantly higher than at any of the other sites ($P = 0.013$, April; $P < 0.001$, May). In April, the three largemouth bass (mean TL = 139 mm, one per cage) in the mixing zone were all dead after 5.5 h, and no deaths occurred at any other site. In May, mortality in the mixing zone was low (20%) after 6.0 h, possibly because of low water velocity in mixing zone cages (2.0 cm/s between 2.5 and 5.5 h); therefore, the test was extended, and flow rates were returned to 3–4 cm/s until 10.0 h, at which point, all fish in the mixing zone had died. No deaths occurred at any of the other sites (Figure 2).

Water Chemistry

Each experimental period was characterized by no rainfall and stable flow conditions in both streams, except in January, when rain began after 5.0 h, but flow rates did not increase before the test was completed. The mean hourly pH (±SD) in Cane Creek above the confluence was highest in June–July (8.0 ± 0.3) and lowest in January 1997 (7.7 ± 0.2). In Black Branch, the lowest mean hourly pH occurred in June–July 1996 (3.0 ± 0.1), whereas the highest mean hourly pH was in January 1997 (4.1 ± 0.1). Abrupt changes in pH occurred in the mixing zone, and metal-precipitate plumes were visible during all tests. Cages located in the mixing zone received a greater portion of Black Branch water than of Cane Creek water, and variations in water chemistry occurred because of changes in relative contributions from the two streams. Below the mixing zone, mean hourly pH (±SD) stabilized rapidly within 10 m and was highest in July 1996 (7.67 ± 0.03) and lowest in January 1997 (6.29 ± 0.54); however, metal precipitates were present in the water column for over 50 m downstream of the confluence during each test.

In Black Branch, the lowest mean hourly pH (June–July 1996) corresponded to the highest concentrations of Al, Fe, and Mn (Table 1). In July, the concentrations of dissolved (filterable) Zn, Cu, and Cd arriving from Black Branch were low, and these concentrations were not evaluated in later tests (see Discussion; Table 1). Total Al concentrations were highest in June–July 1996 (12.57 ± 0.35 mg/L) and lowest in January 1997 (2.52 ± 0.13 mg/L), whereas maximum Fe concentrations occurred in June–July 1996 (1.36 ± 0.43 mg/L) and minimum concentrations occurred in August (0.48 ± 0.12 mg/L). Aluminum and iron were present almost entirely as dissolved (filterable) metals in Black Branch during all months. Above the mixing zone, Cane Creek had low total Al and Fe concentrations. Highest mean concentrations of Al and Fe in Cane Creek above Black Branch oc-

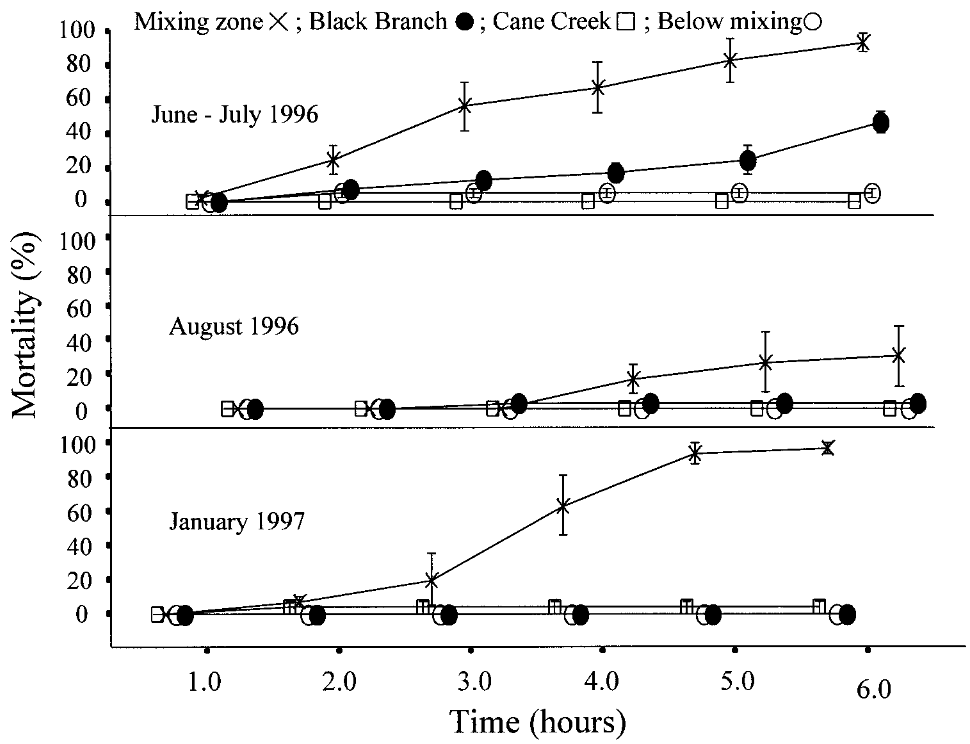

FIGURE 1.—Mean (±SE) mortality of bluegill during exposure tests conducted in June–July and August 1996 and January 1997 at instream sites located in Cane Creek, Black Branch, the mixing zone, and 15–30 m below the confluence (Walker County, Alabama). Mortality was determined at the same time at each site during each test, and 3–6 cages were used at each site.

curred in June–July for Al (0.06 ± 0.04 mg/L) and in August for Fe (0.47 ± 0.13 mg/L).

In the mixing zone, more variation in pH (5.5 ± 0.5) and metal concentration was evident than was noted in Black Branch or Cane Creek, and the contribution of Black Branch was higher than that of Cane Creek in all test months, based on the ratio of Al concentrations in the two streams (88 ± 2.8% Black Branch water). The amount of dissolved Al relative to the total Al was variable among tests (Figure 3). During July, dissolved concentrations of Al were higher in the mixing zone than were total concentrations of Al (filtered Al = 2.2 mg/L; total Al = 1.3 mg/L), which indicated sampling or analytical errors (Figure 3). Slower precipitation of Fe was observed in the mixing zone in January than was observed during other months (total Fe = 1.06 ± 0.01 mg/L; dissolved Fe = 0.76 ± 0.02 mg/L; 72% dissolved; Figure 3), and less precipitation of Fe was also evident below the mixing zone in January.

At the site below the mixing zone, pH stabilized rapidly (7.67 ± 0.03 in July and 6.29 ± 0.54 in January). In contrast to the mixing zone, dissolved Al concentrations below the mixing zone were low and did not vary substantially among tests, which indicated that precipitation of dissolved Al occurred before Al precipitates arrived at cages located below the mixing zone (mean filterable Al = 0.152 ± 0.039 mg/L over all months). In addition, monthly variation in total Al concentrations in Black Branch and the mixing zone (see above) was not evident below the mixing zone (mean total Al = 1.23 ± 0.18 mg/L over all months; Figure 3).

Virtually all of the Mn was dissolved (filterable) in each stream; however, in Black Branch, Mn concentration was highest in July 1996 (6.09 ± 0.43 mg/L) and lowest in January 1997 (1.48 ± 0.07 mg/L), whereas in Cane Creek, Mn concentrations were low in all months (<0.25 mg/L; Figure 3). Manganese existed almost entirely in dissolved forms both in and below the mixing zone during all tests (mixing zone, mean % dissolved Mn = 96.5 ± 8.0; below mixing zone, mean % dissolved Mn = 95 ± 6.6; Figure 3). The higher contribution of water from Black Branch was responsible for higher Mn con-

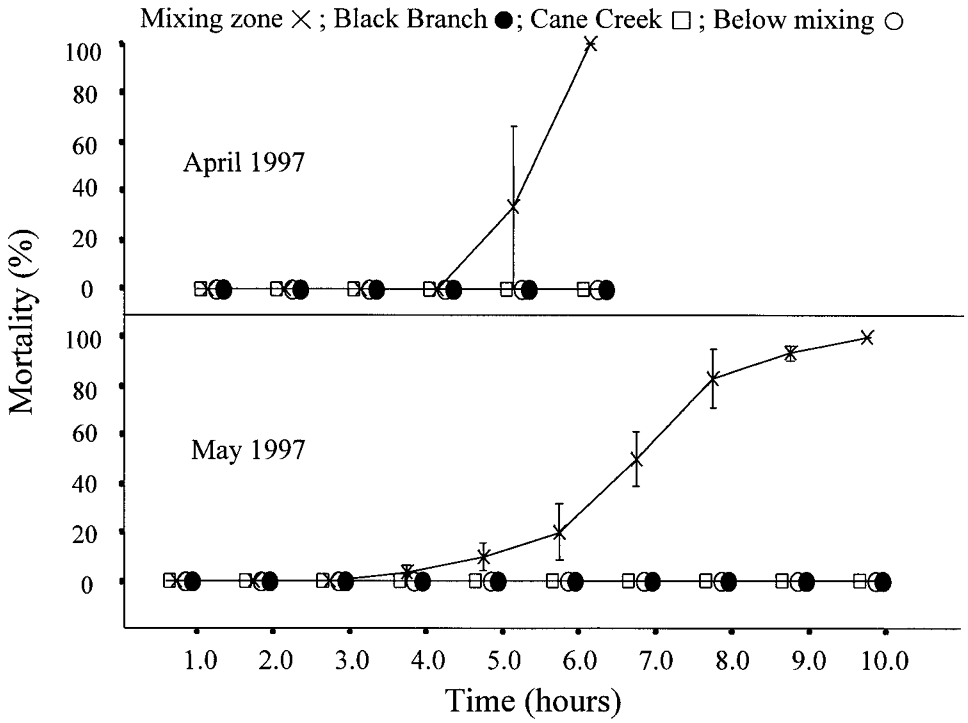

FIGURE 2.—Mean mortality (±SE) of largemouth bass in the mixing zone located at the confluence of Cane Creek and Black Branch (Walker County, Alabama) during exposure tests conducted in April and May 1997. During both April and May tests, no fish died in any cages located in Cane Creek, Black Branch, and below the mixing zone. Three cages were located at each site. In April, one fish was placed in each cage, and in May, 10 fish were placed in each cage.

centrations at the mixing zone site, and further dilution of mixed water by Cane Creek resulted in lower Mn levels below the mixing zone (Figure 3).

Mean water temperature (Table 2) was 26.5 ± 1.3°C (SD) in summer tests (June–August 1996), 7.3 ± 1.7°C in winter (January 1997), and 18.4 ± 0.1°C in spring (April–May 1997). Dissolved oxygen was above 7.0 mg/L at all sites in all months. Alkalinity in Cane Creek above the mixing zone was highest during summer (360 mg/L as $CaCO_3$) and lowest during winter (250 mg/L as $CaCO_3$; Table 2). Below the mixing zone, alkalinity followed the same trend as it did in Cane Creek above the mixing zone; however, it was depressed by contribution of Black Branch water, which had no detectable alkalinity (Table 2).

TABLE 1.—Mean concentrations (range in parenthesis, mg/L) of filterable metals in water samples collected during exposure of bluegill to acid mine drainage (AMD) during July 1996. Sites were located in Cane Creek (pH neutral), Black Branch (AMD), and 15–30 m below their confluence (Walker County, Alabama). Water samples were collected two times during the 6 h test.

Metal	Sites		
	Cane Creek	Black Branch	Below mixing
Al	0.14 (0.09–0.12)	13.2 (13.1–13.2)	0.22 (0.20–0.22)
Fe	0.07 (0.06–0.08)	1.8 (1.7–1.8)	[a]
Zn	0.07 (0.06–0.08)	0.33 (0.31–0.35)	0.02 (0.01–0.03)
Mn	0.18 (0.17–0.18)	6.5 (6.4–6.6)	0.51 (0.44–0.58)
Cu	0.01 (0.01)	0.02 (0.02–0.03)	0.01 (0.01)
Cd	0.001 (0.001)	0.002 (0.002)	0.001 (0.001)

[a] Below detection limit.

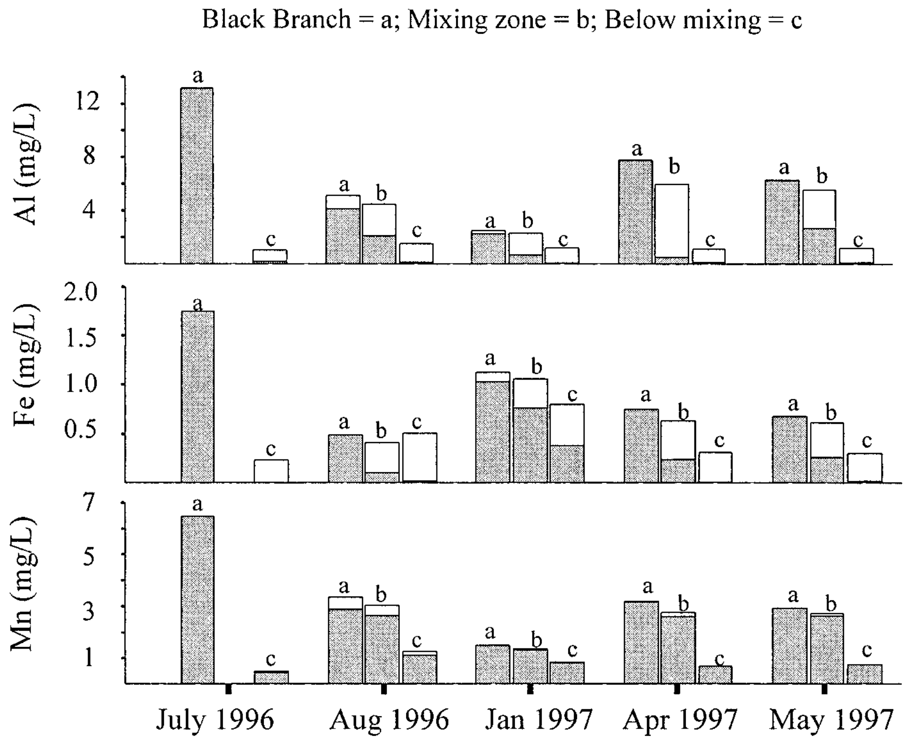

FIGURE 3.—Concentrations of Al, Fe, and Mn during exposure tests with bluegill (July and August 1996 and January 1997) and largemouth bass (April and May 1997). The first bar in each month is Black Branch **(a),** the next is the mixing zone **(b),** and the last is below the mixing zone **(c)** (Walker County, Alabama). The top of each bar indicates the total metal concentration (mg/L), and the shaded portion indicates the filterable metal.

Discussion

This study is the first to demonstrate acute toxicity to warmwater stream fishes of an AMD mixing zone. Fish mortality in the mixing zone was significantly higher than it was at any other site in each test, and mortality reached 96–100% in every month except August. Toxicity in this mixing zone could have been caused by precipitation of Fe or Al (or both) on fish gills, by reduced pH (5.0–6.0), or by rapidly changing pH that resulted from the varying contributions of the two streams. The reduced pH in the mixing zone was probably not a factor, because fish held in Black Branch were exposed to lower pH (<4.3) and had significantly lower mortality than did fish in mixing zone cages. Rapidly changing the pH between 5.0 and 6.0 could result in toxicity; however, in other field and laboratory studies of acid mixing zones, pH has been controlled, and precipitating metals were identified as being responsible for the mortality of Atlantic salmon and brown trout (Poleo et al. 1994; Witters et al. 1996). In studies conducted in Norway, streams acidified by acid precipitation were examined with pH variation (4.8–6.5) in the mixing zone that was similar to that used in our study, and the mortality of salmonids was related to precipitation of Al on gills (Rosseland et al. 1992).

When low pH Black Branch water containing elevated concentrations of dissolved metals arrived in the mixing zone, the pH increased abruptly, and Fe and Al precipitated rapidly into colloidal and particulate forms. Manganese did not precipitate, was at lower concentration in the mixing zone than in Black Branch, and, therefore, was probably not the cause of mortality in the mixing zone. However, metal species were not determined, and changes in Mn speciation between Black Branch and the mixing zone may have increased toxicity. Changes in Mn speciation are suspected to increase rainbow trout *Oncorhynchus mykiss* mortality in hatcheries that use hypolimnetic reservoir water that is rich in reduced Mn (Nix and Ingols 1981); however, water in this system is extremely soft, which accentuates the toxicity of many divalent metal ions. The concentrations of Zn and Cu were below the concentration that is lethal to 50% of test organisms

TABLE 2.—Mean (SD in parentheses) water quality characteristics at each site during toxicity tests with bluegill during June, July, and August 1996, and January 1997; and with largemouth bass in April and May 1997. The mixing zone was at the confluence of Cane Creek (pH 7–8) and Black Branch (acid mine drainage). The site below the mixing zone was 15–30 m below their confluence (Walker County, AL). Metals (Ca^{2+}, Mg^{2+}, Na^{+}) concentrations are from filtered samples.

Analysis	Cane Creek	Black Branch	Mixing zone	Below mixing
June–Aug 1996 (N = 3; metals N = 2; conductivity N = 1)				
Temperature °C	26.5 (1.3)	25.13 (2.5)	25.3 (1.16)	25.5 (0.7)
Alkalinity[a]	361 (65)	0	57 (37)	292 (68)
SO_4^{2-} (mg/L)	1,206 (273)	576 (266)	730 (108)	965 (161)
Ca^{2+} (mg/L)	151 (15)	62 (29)	91 (56)	130 (11)
Mg^{2+} (mg/L)	139 (5)	33 (20)	78 (67)	118 (24)
Na^{+} (mg/L)	73 (3)	21 (7)	42 (29)	63 (13)
Conductivity[b]	1,550	1,000	1,200	1,450
January 1997 (N = 1)				
Temperature °C	5	8	7	9
Alkalinity[a]	215	0	20	141
SO_4^{2-} (mg/L)	1,100	340	520	740
Ca^{2+} (mg/L)	139	25.7	35.1	76.3
Mg^{2+} (mg/L)	116	13.0	20.9	59.2
Na^{+} (mg/L)	59	14.6	18.5	33.8
Conductivity[b]	1,500	360	400	1,050
April–May 1997 (N = 2)				
Temperature °C	18.5 (1.2)	18.4 (1.0)	18.3 (0.9)	18.4 (1.1)
Alkalinity[a]	269 (15.6)	0	5.5 (0.7)	197.5 (40.3)
SO_4^{2-} (mg/L)	1,400 (282)	540 (28)	740 (85)	1,340 (368)
Ca^{2+} (mg/L)	165 (7.1)	56.3 (2.9)	70.7 (3.2)	146 (9.9)
Mg^{2+} (mg/L)	152.8 (10.3)	26.7 (1.2)	44.9 (0.8)	131 (14)
Na^{+} (mg/L)	84.9 (8.0)	21.1 (0)	30.5 (5.0)	74.1 (10.1)
Conductivity[b]	1,150 (70)	435 (17.7)	525 (35.4)	1,275 (459)

[a] (mg/L as $CaCO_3$).
[b] (μS/cm).

(LC50) during 96 h for bluegill at similar pH and water hardness and were probably not acutely toxic (Zn 96-h LC50 = 10 mg/L; Cu 96-h LC50 = 1.1 mg/L; Sorensen 1991).

Seasonal differences in water chemistry were observed, and these may explain changes in toxicity of Black Branch water but they do not explain lower toxicity in the mixing zone during August, as compared with other months. In Black Branch, solubility differences of dissolved metals may have resulted from differences in pH (3.0 for June–July, 4.1 for January), temperature (25–5°C), seasonal changes in dissolved organic material, or different characteristics of eroding mine waste and other watershed minerals that released ions into the stream. Although concentrations of dissolved Al, Fe, and Mn varied among test months, the behavior of the three in the mixing zone was similar, except for Fe. Precipitation of Fe was reduced in January, and higher concentrations of dissolved Fe were present within and below the mixing zone. Lower precipitation of Fe in January may have resulted from lower temperatures.

Understanding the toxicity of an acid mixing zone throughout the year is essential to the proper management of an impacted fishery (Rosseland et al. 1992). Our study is the first to address toxicity of an acid mixing zone at several extremes of temperature, pH, and flow rate. Rosseland et al. (1992) suggested that acid mixing zones may be more toxic and may have a greater extent at combined low-temperature high-flow periods, but this was not observed in our study. Acute toxicity in the mixing zone resulted in high mortality (96%) in both conditions: low flow, low pH (3.0 in Black Branch) and high temperature (25°C) in June and July and also in higher pH (4.3 in Black Branch), higher flow, and lower temperature (7°C) in January. In addition, toxicity below the mixing zone was low (5% or less) in all tests.

Mortality in Black Branch was lower than in the mixing zone, despite the higher concentrations of dissolved metals and the lower pH. Stable pH and equilibrium between dissolved and particulate metals in Black Branch may explain the lower mortality observed at this site, compared with the mixing zone. However, fish were not observed in Black Branch during this study and could not be collected in stream sampling during the year in which this study was conducted (E. R. Irwin, unpublished data). Fish were present in Cane Creek; therefore, their notable absence in Black Branch implies that toxicity restricted fish presence. Increased oxygen consumption of bluegill has been reported at low pH (3.0–4.0), and prolonged exposure may lead to death (Pegg and Jenkins 1976). Longer tests than those used in our study (6 h) are needed to demonstrate the toxicity of Black Branch water above the mixing zone.

In the mixing zone, both dissolved Al and Fe precipitated, and the amount and rate of precipitating Al and Fe may be important in determining toxicity and survival time of fish. Acid mixing zones with precipitating Al are toxic to salmonids, and accumulation of Al precipitates on gills can impede respiration and can potentially kill fish (Rosseland et al. 1992; Poleo 1995; Witters et al. 1996). In addition, the accumulation of precipitating iron has been described on the gills of brook trout *Salvelinus fontinalis* (Sykora et al. 1972).

Changes in stream discharge following a rain event have been documented to change instream pH and, consequently, the behavior of dissolved metals in streams (DeWalle et al. 1995). In our study, rainfall prior to the August 1996 test resulted in increased discharge, which disturbed mixing zone characteristics and possibly led to pulses of low- or high-pH water, with reduced amounts of precipitating metals. In addition, lower flow rates in mixing zone cages likely reduced the delivery of precipitating metals to largemouth bass during May 1997, thereby reducing mortality until flow rates were returned to 3–4 cm/s after 5.5 h. These results indicate that toxicity in this mixing zone may be localized.

Downstream from initial mixing of Black Branch and Cane Creek, acute toxicity decreased quickly. Higher and stable pH (6.3–7.6), lower concentration of metals, and minimal precipitation of dissolved metals corresponded to low mortality ($<5\%$) below the mixing zone. The capacity of Cane Creek to buffer water from Black Branch was important in determining the downstream extent of pH instability and metal precipitation (thereby reducing the area in which mixing zone toxicity occurred).

High alkalinity in Cane Creek rapidly raised the pH of acidic water from Black Branch and resulted in pH that was greater than 6.3 below the mixing zone in all months. A rapid pH increase in AMD mixing zones can cause rapid precipitation of Al-hydroxysulfates ($AlOHSO_4$), thereby removing Al from the water column (Herrman and Baumgartner 1992). If the receiving-stream alkalinity is low, pH below the confluence will likely be lower, precipitation of Al will continue further downstream, and the extent of mixing zone toxicity will increase (Theobald et al. 1963; McKnight and Bencala 1989, 1990; Herrman and Baumgartner 1992). These conditions have been reported in Alabama (W. Cartwright, Alabama Department of Industrial Relations, personal communication), Colorado (Theobald et al. 1963), and Europe (Herrman and Baumgartner 1992).

Mixing zones could have important consequences for stream ecosystems, despite the limited extent of acute toxicity. Low pH and precipitating Al cause fish to move downstream or to abandon attempts to move upstream, thereby affecting the movements of fishes in areas in which mixing zones occur (Atland and Barlaup 1992). In addition, researchers investigating acid mixing zones suggest that, if mixing zones are used for spawning or stocking fry, high mortality may occur (Rosseland et al. 1992).

The Appalachian region of the United States contains many streams that are contaminated by AMD, and mixing zones likely occur in these watersheds (Herlihy et al. 1990). Neutral streams, which buffer inputs from AMD streams, may contain many mixing zones along their length, thus requiring moving fish to repeatedly pass through these toxic areas. Therefore, local fish faunas may be affected, and distribution and toxicity of other AMD mixing zones should be evaluated. In addition, relationships between precipitating metals and toxicity should be quantified. The effects of AMD mixing zones on fish communities should be evaluated through regular sampling of fish populations in order to determine temporal differences in species richness, abundance, and distribution. The negative effects that AMD mixing zones have on these fish may affect local citizens by reducing stream recreation as well as the aesthetic value and economic potential of these streams.

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