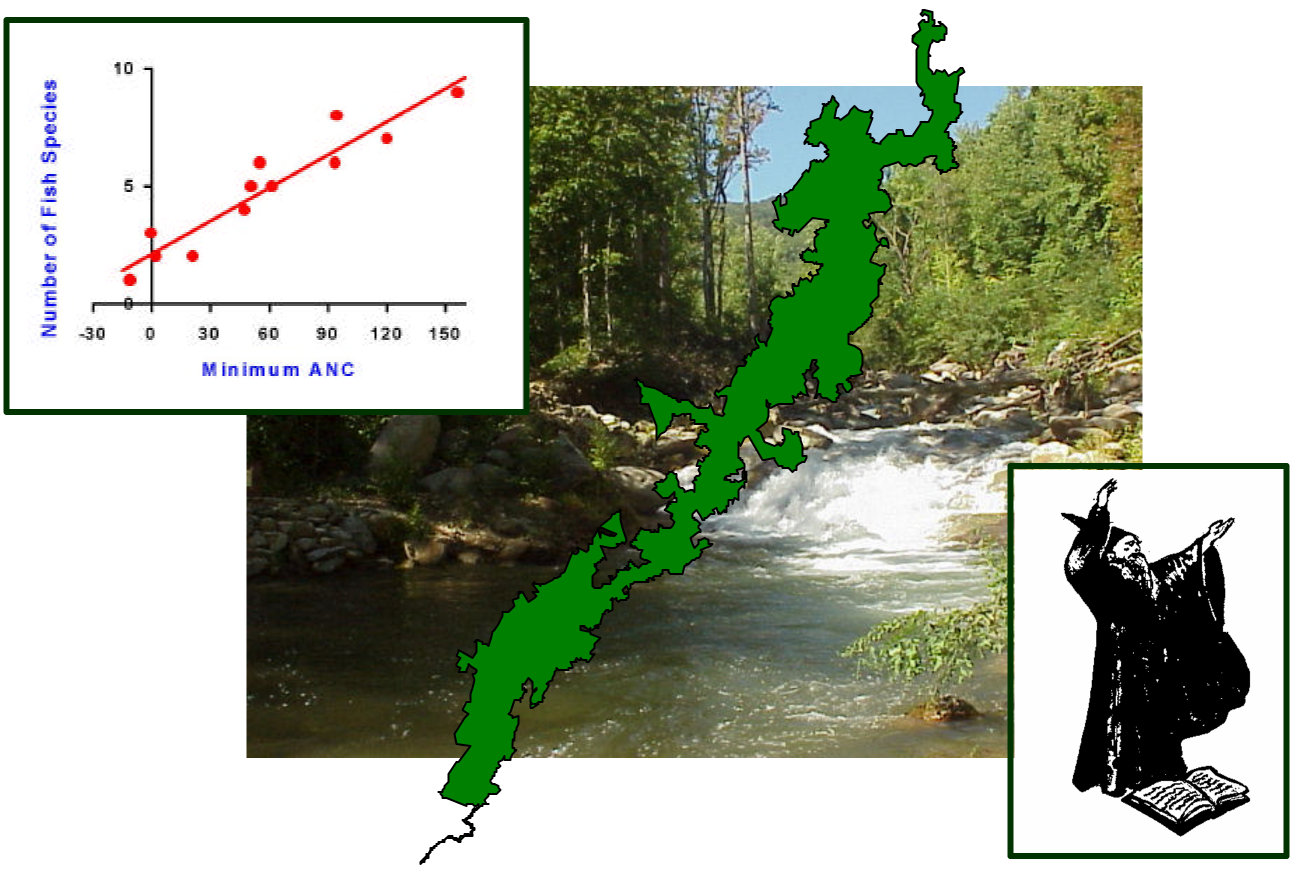
SNP:FISH

Shenandoah National Park: Fish In Sensitive Habitats

Project Final Report - Volume IV

Stream Bioassays, Aluminum Toxicity, Species Richness and Stream Chemistry, and Models of Susceptibility to Acidification



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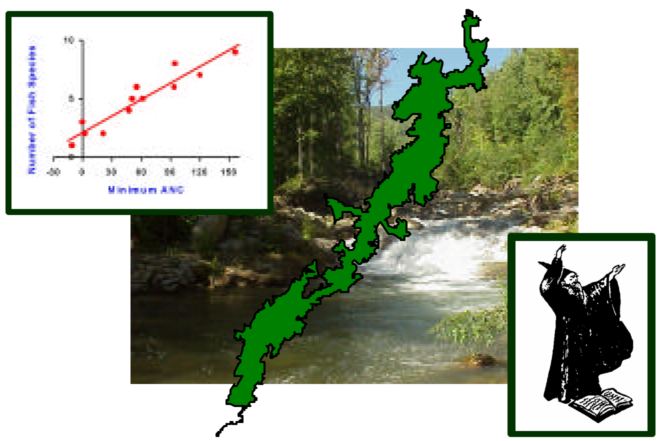

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Chapter 6A

Susceptibility of the early life stages of brook trout, *Salvelinus fontinalis*, and adult blacknose dace, *Rhinichthys atratulus*, to acidification in Shenandoah National Park.

prepared by

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Abstract

To assess the impact of acidification on fish in Shenandoah National Park, two kinds of bioassays were performed using two different fish species. First, mortality bioassays with brook trout (Salvelinus fontinalis) were conducted over a three-year period, in the fall of 1992, 1993, and 1994, and spring of 1993, 1994, and 1995, in each of three Shenandoah National Park streams with differing acid-sensitivities. Brook trout is one of the most acid-tolerant fish species, but like other fish species, its early life stages are more sensitive than adults. There was clear evidence of high mortality rates associated with acid events (abrupt decreases in stream acid neutralizing capacity and pH, together with increases in toxic aluminum concentrations) in the most acid-sensitive stream in two bioassays (fall 1992, spring 1993). Other sources of mortality identified in later bioassays were chronic acidification, drought, flood, and sediment clogging of redds. Second, sub-lethal stress bioassays were conducted with adult blacknose dace (Rhinichthys atratulus) in two Shenandoah National Park streams with differing acid-sensitivities. Because blacknose dace is more sensitive to acidification than brook trout, it was hypothesized that at least sublethal stress would be apparent in adult dace in a stream where acid events had killed young trout; however, no acidification stress as measured by hematocrit changes (an indicator of acid/aluminum stress in other fish species) was detected in a six -month bioassay (July- December, 1994); since no major acid episodes occurred during this period, episodic effects on dace cannot be ruled out.

Background and Introduction

Elevated levels of sulfuric and nitric acid in precipitation have been documented throughout much of Europe and North America. In western Virginia, wet deposition of sulfate is estimated to be 500 eq/ha-yr (Buikema *et al*., 1988), and dry deposition is estimated to be an additional 50-100% that of wet deposition (Shaffer and Galloway, 1982).

Shenandoah National Park (SNP) receives more acid deposition (SO_4^{-2}) than any other U.S. national park (NADP/NTN 1989). Fifty-nine percent of its streams are classified as "sensitive to acidification" with acid neutralizing capacities (ANC's) < 100 *u*eq/L (Herlihy *et al*., 1993). Sensitivity to acidification is determined by local bedrock geology (Chapter 3) (Table 6A-1). In SNP, streams draining catchments with silici-clastic bedrock are most sensitive to acidification (< 25 *u*eq/L ANC); granitic catchments are intermediate (25-75 *u*eq/L ANC), and basaltic catchments are least sensitive (>75 *u*eq/L ANC). Twenty-nine percent of the catchments in SNP are underlain by silici-clastic bedrock; 32% are underlain by granitic bedrock; and 39% are underlain by basaltic bedrock (Gathright, 1976).

Acid deposition and its impact on fish communities has received wide-spread attention in the past 20 years from researchers in the United States, Canada, England, Finland, Sweden and Norway. Fish kills in Norway as early as 1911 were attributed to acidity (Huitfeldt-Kaas, 1922; referenced in Skogheim and Rosseland, 1984), and in the 1920's, decreased production of eggs and fry in Atlantic salmon was linked to acidity (Skogheim and Rosseland, 1984).

In the 1970's researchers linked recruitment failure to abnormally low pH in Ontario lakes (Beamish, 1976), and quantified mortality due to low pH challenges in the laboratory (Trojnar, 1977; Menendez, 1976; Falk and Dunson, 1977). By the 1980's laboratory studies had documented acid-induced mortality of brook trout (*Salvelinus fontinalis*) (Baker and Schofield, 1982; Trojnar, 1977), Atlantic salmon (*Salmo salar*) (Daye and Garside, 1977; Carrick, 1979; Skogheim and Rosseland, 1984), brown trout (*Salmo trutta*) (Skogheim and Rosseland, 1984; Carrick 1979), sea trout (*Salmo trutta*) (Skogheim and Rosseland, 1984) and white suckers (*Catostomus commersoni*) (Baker and Schofield, 1982).

Driscoll et. al. (1980) and Schofield and Trojnar (1980) showed that high aluminum concentrations, made soluble by decreasing pH, were toxic to fish and other aquatic life. The aluminum effects occurred at a pH above the level that was considered toxic to most fish (Wood, 1989). Blacknose dace, rainbow trout, and Atlantic salmon were among the species most sensitive to acid/aluminum stress (Gagen and Sharpe, 1987;

Baker and Christensen, 1991; Haines, 1981), whereas brook trout (*Salvelinus fontinalis*) was among the most tolerant (Skogheim and Rosseland, 1984; Johnson *et al*., 1987; Gagen *et al*., 1993).

Salmonids have been the focus of many bioassays because of their ecological and commercial importance. Research on non-salmonids has included smallmouth bass (*Micropterus dolomieui*) (Kane and Rabeini, 1987; Holtze and Hutchenson, 1989), blacknose dace (*Rhinichthys atratulus*) (Johnson *et al*., 1987), largemouth bass (*Micropterus salmoides*), common shiner (*Notropis cornutus*), white sucker (*Catostomus commersoni*), walleye (*Stizostedion vitreum*), lake whitefish (*Coregonus clupeatormis*) (Holtze and Hutchinson, 1989), mottled sculpins (*Cottus bairdi*) and slimy sculpins (*C. cognatus*) (Gagen *et al*., 1993).

Physiological stress mechanisms

Acid Stress.

Fish in acidified waters encounter special ion balance problems. Adverse effects of decreasing pH occur at around 6.0 for many species of fish. Decline in pH produces rapid effluxes of Na^+ and Cl^- from the gill epithelium. Ions are lost more quickly from the extracellular fluid than the intracellular fluid, causing an osmotic flux of water into cells, including red blood cells. As plasma volume falls, the hematocrit (percent of the blood volume made up by formed elements, including red cells) may increase up to 125%. Thus the reasons for the increased hematocrit are: red blood cell swelling (due to decreased plasma osmotic pressure), decreased plasma fluid volume, plus increased red blood cell release by the spleen (a stress response) (Wood, 1989). The resulting massive increase in blood viscosity makes blood travel through capillaries difficult and strains the heart (Wood, 1989). Circulatory failure due to increased blood viscosity appears to be the cause of death.

Respiratory dysfunction from O_2 delivery or CO_2 release problems were initially thought to cause mortality in acid stress, but oxygen delivery problems do not cause death at realistic pH levels [above 4.0]; even in conditions associated with 50% mortality rates, there is very little lactate (an indicator of oxygen delivery problems) accumulation in the muscles of fish (Wood,1989).

Aluminum Stress:

Aluminum in acidic aquatic environments also compromises ion balance mechanisms in fish. In streams of normal pH (6.0 to 7.0) Al is insoluble and not toxic. At pH's below 5.6, however, Al solubility increases exponentially and becomes extremely toxic to fish (Reader and Dempsey, 1989) (Figure 6A-1). Cationic hydroxides [$Al(OH)^{+2}$] and [$Al(OH)_2^{+}$] predominate at pH's between 5.0 and 5.5, then are largely replaced by free trivalent (aquo) aluminum (Al^{+3}) by pH 4.0. Only inorganic monomeric aluminum (IMA) (also called labile aluminum) is thought to be harmful to fish. This inorganic monomeric fraction comprises cationic aluminum hydroxides, aluminum fluoride complexes, aluminum sulfate complexes and aquo aluminum (Wood, 1989; Driscoll *et al*., 1980). Besides IMA, two other forms of aluminum found in natural waters are organic monomeric (OMA) and acid soluble aluminum (ASA) (Driscoll *et al*., 1980). OMA (also called non-labile) is a single aluminum atom complexed with various organic species. This type of aluminum is highly correlated with dissolved organic carbon concentrations. The ASA group contains polymeric aluminum species. Neither the OMA nor the ASA are thought to be toxic to fish. During acid events (periods of low ANC associated with increases in discharge) when total monomeric aluminum concentrations increase in the stream water, IMA (not OMA) usually makes up the increase (Driscoll *et al*., 1980).

Aluminum raises the net losses of Na^+ and Cl^- above that exhibited by acid stress alone, and sometimes causes gas exchange problems. When salmon and rainbow trout are exposed to 200 ug/L Al at pH 5.0, ATPase activity at the gill epithelium is reduced by 25%; this disruption leads to falling levels of Na^+ and Cl^- in the plasma; carbonic anhydrase activity is also diminished, indicating possible problems with oxygen and carbon dioxide transport in the blood (Potts and McWilliams, 1989). It is generally thought that Al somehow weakens junctions between gill cells, allowing Na^+ to leak out following its concentration gradient (Wood, 1989; Potts and McWilliams, 1989; McDonald *et al*., 1989).

One of the stress effects of aluminum toxicity is an increase in P_{CO2} (partial pressure of carbon dioxide) and a decrease in P_{O2} (partial pressure of oxygen) within the blood plasma. One reason for this may be the precipitation of aluminum hydroxides and/or organic ligand formation on the gill surface, which induces an inflammatory response that thickens and distorts the branchial epithelium, decreasing its transcellular permeability to O_2 and CO_2, yet simultaneously increasing paracellular channel permeability for ion loss (Wood 1989).

Death caused by a lethal aluminum concentration probably results from circulatory failure, secondary to ion balance disruption, plus tissue anoxia.

Effects of Calcium

Calcium (Ca^{2+}) ion ameliorates the toxic effects of acid/aluminum (Potts and McWilliams, 1989; Wood, 1989). Ca^{2+} ions bind to the gill surface and inhibit Na^{+}, Cl^{-} efflux by supporting tight junctions between cells and protecting Na+ pumps from damaging acid/aluminum (Wood, 1989; McDonald, *et al*., 1980; McDonald and Wood, 1981). Rainbow trout held at pH 4.3 for 3 days in 200 ueq/L Ca^{2+} or less experienced significant losses of plasma Na^{+} and Cl^{-}; however, trout held in Ca^{2+} concentrations ~ 300 ueq/L did not show a plasma ion loss.

Acidification is almost exclusively a soft water (Ca^{2+} < 500 ueq/L) problem. Streams with hard water have high enough bicarbonate alkalinity to neutralize any acidity. Thus acidification most often occurs in catchments with relatively low Ca^{2+} concentrations where the risk of acid/aluminum toxicity is high.

In situ testing of sub-lethal stresses

Most experiments on the effects of pH and aluminum on *S. fontinalis* and *R. atratulus* have been done in the laboratory. There are very few field bioassays documenting mortality/survivorship or sub-lethal stress (Johnson *et al*., 1987; Gagen *et al*.,1993; and Rosseland *et al*., 1992). The lack of *in situ* experiments results from difficulties in controlling the interfering variables encountered in the field. Sub-lethal stress in fish is also difficult to measure, compared to the fairly simple determination of survival.

There are four ways sub-lethal stress has been documented in the laboratory: 1) ion loss (Wood, 1988a; b;1989); 2) changes in organosomatic indices (Beamish, 1976; St. Pierre, 1986; Haines, 1981); 3) O_2 transport and delivery problems (Wood, 1988b; 1989; Moyle and Cech, 1988); and 4) hematological fluid volume change (Wood, 1988b; 1989; Moyle and Cech, 1988). Of these four methods, measuring hematological fluid volume change (by hematocrit) may be the best way to measure sub-lethal stress for an *in situ* experiment. Hematocrit is a measure of the formed elements (cells, solids) in the blood and may be determined in the field within 10 minutes after recovery of the specimen from the water. Not only is the determination of hematocrit a quick field technique (given appropriate equipment and technique), but hematocrit also responds rapidly to ion balance problems which occur in fish experiencing aluminum or H^{+} stress (Wood, 1989; Haines, 1981).

Egg and sacfry mortality

Normal survivorship (in the absence of acid stress) for eggs and fry in the laboratory and the field varies among studies, but is typically 70-90%. Trojnar (1977) reported that 82% of brook trout eggs survived from the eyed stage to hatching when incubated at a pH of 5.64, versus 91% when incubated at a pH of 8.07 (aluminum was not measured). Menendez (1976) found that at pH 7.04, 76% of alevins survived at the end of a ninety-day test, versus 53% at pH 5.57 (aluminum was not measured). Cleveland *et al*. (1986) found that of eggs incubated at a pH of 5.5 (and approximately 160 *u*eq/L calcium) with 300 *u*g/L aluminum, only 21% survived the hatching process, versus 65% among those not exposed to aluminum held at the same pH and calcium. Jordahl and Benson (1987) observed 100% mortality of hatchery brook trout embryos held for 125 days in at pH 4.7-5.4, total soluble aluminum 50-460 *u*g/L, and calcium 350-730 *u*eq/L; embryos held at pH 6.1-7.2, total soluble aluminum 10-50 *u*g/L, and calcium 550-930 *u*eq/L, 77 (±10)% survived at the end of 154 days.

Calcium concentrations in some SNP streams is much lower than that reported in many other studies. In streams with silici-clastic bedrock, such as SNP's Paine Run, ambient calcium ranges from 20 to 40 *u*eq/L. In streams with granitic or basaltic bedrock, ambient calcium ranges from 60 to 85 ueq/L or 120 to 150 *u*eq/L respectively. In Paine Run, Ca^{+2} concentrations at baseflow are around 31 *u*eq/L, versus 94 to 176 *u*eq/L during the Johnson *et al*., (1987) bioassay discussed above, 99 to 231 *u*eq/L in the Simonin *et al*., (1993) bioassays, and 350 to 930 *u*eq/L in the Jordahl and Benson (1987). Calcium ameliorates the negative physiological effects of low pH and high aluminum concentrations, especially in the range up to 150µeq/L calcium (reviewed inBulger et al., 1993), so the negative effects of acidification in Paine Run are probably more intense due to locally low calcium values.

Methods

Site descriptions

Paine Run (SNP southern district) is underlain by silici-clastic rock and is extremely sensitive to acidification (ANC < 25 *u*eq/L). Staunton River (central district) is underlain by granitic rock and is intermediate in sensitivity (ANC 25-100 *u*eq/L). Piney River (northern district) is underlain by basaltic rock and is the least sensitive to acidification (ANC 100-300 *u*eq/L). The three catchments are similar in size (Table 6A-1), and each stream contains native populations of both brook trout and blacknose dace.

The three study streams are located in the lower reaches of first order mountain streams (as defined by Moyle and Cech, 1992). General information on the physical and vegetative characteristics are provided in

Table 6A-1. The catchments have steeply-sloped sides and shallow soils, usually ultisols and inceptisols (Hockmannan *et al*., 1979). Forest cover is nearly 100% and is primarily second growth mixed-deciduous hardwoods (see Table 6A-1).

<u>Water chemical composition analyses</u>

Stream water composition data (determined weekly and episodically) for Paine Run, Staunton River and Piney River were taken from data bases kept by the SWAS/FISH project. Both grab samples, collected during baseflow, and samples collected during storm events with ISCO® Model 2900 sequential automated samplers were used in this project. Grab samples were placed on ice and chloroformed (except for aliquots reserved for TMA analyses) in the laboratory within three days of collection. Storm event samples were taken to the laboratory within a week of collection. All samples were stored in polyethylene containers and refrigerated at 4° C until chemical analyses were performed.

pH was measured using a Beckman Psi 21 pH meter and a Corning Calomel Combination pH Electrode. ANC was measured using a two point grant titration, a Beckman Psi 21 pH meter and a Corning Calomel Combination pH Electrode. Ca^{+2} was measured using atomic absorption spectrophotometry. For more information on these techniques see Ryan *et al*. (1989).

TMA was analyzed using the McAvoy *et al.* (1992) adaptation of the automated pyrocatechol violet (PCV) procedure developed by Rogeberg and Henrickson (1985) (see Dennis (1995) for further details). Due to time constraints and cost, we only measured TMA (total monomeric Al) and not the most toxic fraction of Al (inorganic monomeric aluminum (IMA)). IMA has been shown to be the major portion of TMA during events, especially in stream water where organic acids are low (Driscoll, 1980), as in SNP. Therefore, it is likely that the TMA measured during the episodes was mostly IMA and that acute acid/Al toxicity contributed to mortality.

<u>Biological analyses</u>

The study animals:

Brook trout (*Salvelinus fontinalis*) is native to SNP. It is an important game fish and has been the subject of many studies investigating acid/Al toxicity. Eggs and sacfry of most fish species, including brook trout, are more susceptible to acid/Al toxicity than adults. Two strains of brook trout (*Owhi* and *Nashua)* obtained from hatcheries were used to assess the survival of the early life stages (see Methods: *Brook trout egg and sacfry survival assessment*).

Blacknose dace *(Rhinichthys atratulus)* is also native to SNP and is more susceptible to acid and aluminum toxicity than brook trout (Johnson *et al*., 1987). Stress effects due to low pH may first appear between 5.6 and 6.1 (Baker and Christensen, 1991). Blacknose dace captured by electrofishing were used study sub-lethal episodic stress in bioassays in their native streams (see Methods: *Episodic sub-lethal stress assessment*).

Field equipment Design

Two to six cages containing *S. fontinalis* eggs (for the early life stage mortality bioassay) were maintained at Paine Run, Staunton River and Piney River (Figure 6A-2). Cages were also maintained at Paine Run and Piney River to contain *R. atratulus* adults for the sub-lethal stress investigations (not Staunton River due to logistical considerations). Cages were made of a 1.0 m x 0.5 m Rubbermaid® laundry basket fitted with a hinged lid made from .60 cm thick plywood. The cages were attached downstream of a wooden plough which prevented swiftly moving water from disturbing them (Figure 6A-3). Up to two cages were attached to each plough. The ploughs and bioassay cages were placed in pools deep enough to ensure that the bioassay animals would not be exposed to air during low flow. The plough and cage arrangements were located within 50m (200m at Paine Run) of the SWAS/FISH water sampling station.

Brook trout egg and sacfry survival assessment

Eyed-eggs of *S. fontinalis* were obtained from White Sulfur Springs State Hatchery (West Virginia, USA), Paint Bank State Hatchery (Virginia, USA) or Egan State Hatchery (Utah, USA). Hatchery strains are useful because both the parental stock and pre-experimental rearing conditions are standardized, so differences in bioassay survival can more easily be attributed to differences in water quality among streams. They are also disease-free and readily available. The two strains used in this bioassay are the *Nashua*, a fall-hatching strain which originated in Nashua, New Hampshire, USA, and the *Owhi*, a winter-hatching strain derived from a lake on the Owhi Indian Reservation in Canada (Bob Shaffer, director of White Sulfur Springs State Hatchery, pers. comm.). *Nashua* (fall-hatching strain) eggs were used in three fall (1992, 1993, 1994) bioassays and *Owhi* (spring-hatching strain) eggs were used in three spring (1993, 1994, 1995) bioassays.

Before the eggs were introduced into the study streams, they were acclimated to stream temperature by slowly pouring stream water over the egg mass while monitoring its temperature (Charlie Stevens, director of Paint Bank State Hatchery, pers. comm.). When the stream's temperature was reached, 50 or 100 eggs were placed into artificial redds (plastic boxes with approximately 2 cm depth of #4 quartz gravel) of either 1.3 pints (0.470 liters) or 1.4 quarts (1.20 liters) respectively. The redd boxes were made by cutting the sides out of polyethylene containers leaving approximately 1.0 cm on all sides. The sides were then closed with a 1 mm fiberglass mesh attached with 100% silicon caulk. To minimize handling, the eggs were counted by assuming that 25 eggs lined up against a ruler will be 105 mm long (Fiss, 1991). This assumption was confirmed for both the *Nashua* and *Owhi* strains of brook trout. The gravel and eggs were then gently mixed. The artificial redds were then stacked within the cages already in the stream. One thousand to two thousand hatchery eggs were placed in each study stream for each bioassay.

Eyed-eggs devleop into sacfry. Survivorship of either eggs of fry was assessed by removing one or two of the artificial redds each week after introduction to estimate survival to that date; this minimized disturbance to the remaining redds. Removed eggs were discarded to avoid introduction into the stream. Since all eggs had either hatched or died by the end of the bioassays, final survivoship values refer only to fry.

In all, about 15,000 brook trout eyed-eggs (which develop into sac-fry after hatching) were placed into artificial redds for 30-90 days (with the exception of the spring 1995 bioassay, in which massive floods in all three streams destroyed the bioassay equipment and fish 2 days after beginning).

Until recently, most *in situ* studies place the bioassay animals into a chronically acidified stream which is acutely toxic to fish (Gagen and Sharpe, 1987; Rosseland *et al*., 1992; Sharpe *et al*., 1983). Our method gives us the opportunity to document survivorship over a long period of time in episodically acidified streams of moderate toxicity. The Episode Response Project (Baker et al., 1996; Van Sickle et al., 1996) also placed trout and other fish species in espisodically acidified streams.

Episodic sub-lethal stress assessment

Adult blacknose dace were collected from Paine Run and Piney River by electrofishing and then placed in holding containers (six per container). The holding containers were made by cutting the sides out of 2.7 liter polyethylene containers leaving approximately 1.0 inch on each side. Fiberglass mesh (1.4 mm) was then placed over the sides and attached with 100% silicon caulk. The holding containers were placed within the cages described above.

Sub-samples of six dace were sacrificed periodically over six months (July-December 1994) for hematocrit determinations. To increase statistical power, measurements made during baseflow at different times were grouped together under one treatment (more measurements per treatment increases the power of the test).

A statistical power of 95 can be achieved with a sample size of 6 assuming a variance in hematocrit score of 7 (δ = 7) and a difference of 15 points between groups (Δ = 15). Initial field data indicates that the variance estimate is correct for baseflow hematocrit (variance (δ) = 7, n = 8). The estimate of a 15-point difference in hematocrit is reasonable and less than that recorded in stressed versus unstressed rainbow trout and Atlantic salmon (Wood, 1989; Rosseland *et al*., 1992).

$$\text{power} = Z \; \{ - z_{1-\infty/2} + (n^{1/2}\Delta)/ (\delta_1{}^2 + \delta_2{}^2{}_{/k})^{1/2} \} \qquad (1)$$

δ_1 = variance of hematocrit, baseflow
δ_2 = variance of hematocrit, episode
$z_{1-\infty/2}$ = z score of 1 minus one half the type one error
Δ = difference between the samples hematocrit values
n_1 = sample size n_1
k = expected ratio of n_1/n_2

Hematocrit was measured by 1) anesthetizing the animals with MS-222 (tricaine methanesulfonate), 2) removing their tails at the distal end of the caudal peduncle, 3) filling a heparinized capillary tube with 10 ul of blood and centrifuging for 3 minutes 20 seconds at 11,500 rpm. The dace were then be killed by severing the spine at the base of the skull.

This study is the first to measure hematocrit in blacknose dace. The use of the anesthetic MS-222 facilitates blood sampling and is humane; however, MS-222 may affect hematocrit values in another fish species, rainbow trout (Iwama *et al*., 1988; Fredricks *et al*., 1992; Lowe-Jinde and Niimi, 1983; Reinitz and Rix, 1977). To test whether MS-222 significantly affects hematocrit of blacknose dace, hematocrit values of six non-anesthetized versus six anesthetized dace (exposed to 250 mg/l for ten minutes) were compared. A *t*-test indicated no significant difference in hematocrit between the two groups. Therefore, it is unlikely that a 250 mg/L MS-222 exposure will not affect the hematocrit of blacknose dace (exposed for under 10 minutes).

Length and mass of each dace were recorded so that the condition factor (K) of each could be computed as:

$$K= [(\text{mass in grams})/(\text{length in millimeters})^3] \times 10^6 \qquad (2)$$

As all dace were of the same age class, no scaling constant was needed in the formula (LeCren, 1951).

Neither condition factor nor hematocrit changed significantly with time during the course of the bioassays at Paine Run or Piney River; however, seasonal differences may occur (see Discussion).

Statistics

Statview SE + Graphics™ for the Macintosh and Microsoft Excel 4.0 were used for statistical analyses. Student's t-test was used to determine hematocrit differences between streams. Kruskal-Wallis was used to detect significant differences in brook trout embryo/sacfry survivorship and the Dunn procedure was used to compare specific groups (Lee, 1992; Rosner, 1990).

Results and Discussion

Part I: Brook Trout Mortality Bioassays

Six bioassays starting with brook trout eyed-eggs (these develop into sac-fry) took place in each of the three intensive streams: fall, 1992; spring and fall, 1993; spring and fall 1994; and spring 1995. The first two show clear evidence of high mortality associated with episodic acidification; in the third and sixth bioassays, high mortality in all streams resulted from drought or flood, respectively; the fourth and fifth bioassays suggest chronic acidification and sedimentation as sources of mortality (Table 6A-3).

Fall 1992 Trout Bioassay: *episodic acidification mortality*

The fall 1992 brook trout bioassay ran from 11/5/92 to 12/3/92. Fall 1992 was damp, with significant rainfall on the seventeenth and eighteenth days of the bioassay in all three streams, which produced an acidic episode in Paine Run (Figures 6A-5d, 6A-6d and 6A-7d). At the time of the hydrologic/acidic event, the eggs had recently hatched and were extremely sensitive to Al toxicity (Jordahl and Benson, 1987; Baker and Schofield, 1982). Survivorship in Paine Run dropped from 74% to 19% in one week, then continued to decline to 5% the following week (Figures 6A-4 and 6A-5a). The decline in survivorship coincided with a large drop in pH, from 5.68 to below 5.1 (Figure 6A-5b). ANC fell from about 1.5 *u*eq/L to -7.2 *u*eq/L (Figure 6A-5c). Ca^{+2} concentration first increased from 32 ueq/L to a peak of 58 *u*eq/L, then declined (Figure 6A-5c). Total monomeric aluminum (TMA) rose from the baseflow level of 14 *ug/L* to 100 *ug/L* during the event (Figure 6A-5d).

Survivorship in Staunton River also declined sharply during the hydrologic event from 70% to 4% during the fall 1992 bioassay (Figures 6A-4 and 6A-6a). During the bioassay there were rapid fluctuations in pH, ANC and Ca^{+2} concentrations (Figures 6A-6b and 6A-6c). TMA was not measured in Staunton River during this bioassay due to logistical difficulties and equipment failure; however, pH ranged from 6.83 to 6.03, so TMA concentration was probably very low (Drever, 1988; Wood, 1989) (Figure 6A-6b). The lowest ANC value was 55 *u*eq/L, and Ca^{+2} concentrations were 63 to 90 *u*eq/L (Figure 6A-6c).

Survivorship in Piney River never fell below 80% during the fall 1992 bioassay (Figures 6A-4 and 6A-7a). Although discharge events occurred at Piney which were similar in magnitude to those at Paine and Staunton (Figure 6A-7d), the pH ranged only from 7.16 to 6.82 (Figure 6A-7b) (the other two streams experienced fluctuations of 0.7 pH units). ANC's were 160 *u*eq/L to 201 *u*eq/L (Figure 6A-7c). Ca^{+2} concentrations were 122 *ueq/L* to 158 *ueq/L* during the bioassay (Figure 6A-7c).

The mortality observed at Staunton River during the fall 1992 bioassay was probably not due to acid/Al toxicity. The pH never fell below 6.0 so the solubility of Al was near its minimum. Some aluminum may have been released due to cation exchange. Even so, it is unlikely that TMA concentration reached above 25 *u*g/L (the highest concentration observed during the bioassays in Staunton River) and a TMA increase of that magnitude should not induce an acute toxicity resulting in such a high mortality. However, rapid fluctuations in pH (between 6.0 and 7.0) were observed during the bioassay. The rapid pH change may have caused a physiological stress resulting in mortality. However, investigations into the effects of rapid pH fluctuations on survivorship of fish have not yet appeared in the literature. Mortality could also have been due to some factor not measured. It should be noted that storm flow during the fall 1992 event at Staunton River was at least twice the mean peak volume of other events observed in the River (mean daily discharge). Peak discharge of similar magnitude was observed at Piney River during two bioassays and did not result in high mortality.

Spring 1993 Trout Bioassay: *episodic acidification mortality*

The spring 1993 brook trout bioassay ran from 1/8/93 to 3/6/93. The spring was fairly dry with no major events until around day 53 of the bioassay, when large hydrological events occurred in all three streams (Figures 6A-9d, 6A-10d and 6A-11d). At the time of the hydrologic/acidic events, the eggs had recently hatched and were extremely sensitive to Al toxicity (Jordahl and Benson, 1987; Baker and Schofield, 1982). In Paine Run, survivorship declined from 90% on the 39th day to 0% on the 53rd (Figure 6A-9a). pH declined from 5.6 to 4.98 within five hours on the 53rd day, and remained depressed for the remaining four days of the bioassay (Figure 6A-9b). ANC was just above 0 *u*eq/L for most of the bioassay, dropping to -8.0 *u*eq/L on the 53rd day (Figure 6A-9c). Ca^{+2} concentration in Paine Run was about 32 *u*eq/L until the event, then increased to 52 *u*eq/L (Figure 6A-9c). Five hours before the catchment responded to the event, pH was 5.65 and TMA was 12 *u*g/L. The next grab was taken 38.4 hours after the pH minimum (4.98). At that time pH was 5.37 and TMA was 80 *u*g/L (Figure 6A-9d). It is likely that TMA was higher during the pH minimum (due to the pH/aluminum relationship mentioned in the Background and Introduction).

In Staunton River during the spring 1993 bioassay, survivorship was 85% when the bioassay ended (Figure 6A-8 and 6A-10a), despite large pH changes on days 52-56 (Figure 6A-10b). The pH was 6.87 (day 52) to 6.05 (day 56). ANC remained fairly constant to the 53rd day. The lowest measured value was 43 *u*eq/L (day 53) and the highest value was 72 *u*eq/L (day 4) (Figure 6A-10c). Ca^{+2} concentration increased during the event, reaching a high of 81 *ueq/L* (Figure 6A-10c). TMA also increased during the event, from under 10 *u*g/L to 25 *u*g/L (Figure 6A-10d). As with Paine Run, the TMA measurements taken from grab samples and do not give a complete description of TMA's behavior during the bioassay, especially during the event.

Trout survivorship at Piney was also high, and was estimated to be 85% at the end of the bioassay (Figure 6A-8 and 6A-11a). Beginning on the 53rd day, chemical variables responded to the hydrological event. pH was 7.11 to 6.59. The lowest pH (6.59) actually occurred 2 days after the first hydrological response of the catchment (Figure 6A-11b). Ca^{+2} concentration rose from 123 *u*eq/L to 144 *u*eq/L by the third day after the storm response began (Figure 6A-11c). ANC reached a low of 96 *u*eq/L on the 55th day (the day when Ca^{+2} was highest and pH was lowest) (Figure 6A-11c). TMA concentrations were determined by grab sample only (as in Paine and Staunton) due to logistical problems. A sample was obtained when pH was at its lowest point (6.59) and the TMA concentration was 8 *u*g/L. One day later it decreased to 3 *u*g/L (Figure 6A-11d).

Spring 1994 Trout Bioassay: chronic acidification plus sedimentation and/or unidentified sources of mortality.

The spring 1994 bioassay ran from 1/14/95 to 4/17/94. The spring was wet with snow covering the catchments for much of the bioassay. There were multiple events of moderate magnitude in all three streams (Figures 6A-15d, 6A-16d and 6A-17d). Differential survivorship among the streams is less clear than in the two previous bioassays because of a) a steady survivorship decline in all streams after the trout eggs hatched; and b) more heterogeneity in survivorship among redds in the high-ANC stream (Figure 6A-14). Nevertheless, survivorship in the low- and mid-ANC streams was lower than that at the high-ANC stream on most occasions, as well as overall (p= .01; Kruskal-Wallis test, Dunn procedure; Lee, 1992; Rosner, 1990) (Table 6A-2). At the termination of the bioassay (when all trout had been counted), survivorship was 22% and 25% in the low- and mid-ANC streams, and 42% the high-ANC stream (Figure 6A-14 and 6A-15a).

During this spring 1994 bioassay, there were four moderate hydrological events at Paine Run (Figure 6A-15d) which kept the pH of Paine Run below 5.6 for 73 days of the 88-day study (Figure 6A-15b, c). During the spring 1994 baseflow, pH was lower and TMA higher than recorded in the previous bioassays. The first event (days 15-20) lowered ANC below 0 *u*eq/L; the event of days 73-74 caused ANC to drop to -3 *u*eq/L (Figure 6A-15c). TMA reached 112 *u*g/L during the event on the 40th day, coinciding with the lowest pH recorded during the bioassay (5.30). However, this TMA peak (Figure 6A-15d) occurred prior to hatching and did not result in a dramatic decrease in survivorship. Two hours after the TMA peak, pH had increased to 5.40 and TMA decreased to 59 *u*g/L. A smaller increase in TMA (29 *u*g/L to 52 *u*g/L) was observed during the next discrete event of the bioassay (day 52), when pH had dropped from 5.50 to 5.36 (Figure 6A-15d). TMA was not analyzed during the first hydrologic event (days 14 to 20) and the last (day 74) due to time constraints.

At Staunton River during the spring 1994 bioassay, pH depressions in coincided with increased discharge; pH was 6.2-6.83 (Figure 6A-16b,d). ANC never dropped below 45 *u*eq/L (Figure 6A-16c). Ca^{+} was usually between 55 *u*eq/L and 65 *u*eq/L, but jumped up to 72 *u*eq/L during the first pH depression. During subsequent pH depressions, however, Ca^{+2} concentrations in the stream water decreased (Figure 6A-16c). TMA concentrations were 8 *u*g/L to 21 ug/L (Figure 6A-16d).

Piney River discharge during the spring of 1994 was more variable than at the other two rivers (Figure 6A-17d). pH was 6.8 to 7.24, fluctuating with discharge (Figure 6A-17b). ANC never dropped below 121 *u*eq/L (Figure 6A-17c). TMA concentration was below 10 *u*g/L for the most of the bioassay but during an event on the 74th day, TMA reached 20 *u*g/L (Figure 6A-17d).

Thus water chemistry in the low-ANC stream in this bioassay appears sufficient to compromise survivorship, but there is no such explanation for lowered survivorship in the mid- and high-ANC streams. Consultation of the field notes reveals that substantial sediment was present in the artificial redds in the mid- and high- ANC streams during the bioassay, but not the low-ANC stream; sediment was especially heavy in the four redds in the high-ANC stream which had the lowest survivorship. Since sediment can reduce survival by restricting water flow and oxygen exchange in trout redds (Waters, 1995), it probably lowered survivorship in at least the high-ANC stream. It is also possible that the eyed-eggs used in the bioassay were in sub-optimal condition; if so, perhaps the difference between survivorship in the low- versus the high-ANC stream (20%) can be attributed to acidification. Alternately, this bioassay could be considered inconclusive due to low survivorship in the reference (high-ANC) stream.

Fall 1994 Trout Bioassay: sedimentation and/or low calcium or unidentified sources of mortality.

The bioassay ran from 11/12/94 to 12/19/94. Hydrologic conditions were damp at all three streams, with major hydrologic events at Staunton (mid-ANC) and Piney (high-ANC), but not at Paine Run (low-ANC). Differential survivorship among the three streams was again less clear than the bioassays showing episodic acidification (Figures 6A-4 and 6A-8, versus 6A-18). As in the spring 1994 bioassay discussed above, substantial sediment was observed in the redds at the mid-and high-ANC streams, but not the low-ANC stream; this may have reduced survivorship (Waters, 1995). Nevertheless, a Kruskal-Wallis test and the Dunn procedure showed that survivorship after hatching at Paine Run was significantly lower than that at Piney or Staunton ($p= .01$). When the bioassay was terminated, mean Paine Run survivorship of sacfry (two remaining artificial redds) was 8%, versus 45% at Staunton River, and 57% at Piney River (Table 6A-2).

Despite the large difference in survivorship between the high-ANC stream and the low-ANC stream in this bioassay, there is no evidence of a conspicuous acidification challenge to the fish even in the low-ANC stream. During this bioassay, pH was 5.74 to 6.11 at Paine, versus 6.83-6.95 at Staunton, and 6.38-7.12 at Piney; total monomeric aluminum was 12-16 µg/L at Paine, versus 12-19 µg/L at Staunton, and 8-11 µg/L at Piney. There was an unusually low pH value for Piney (6.38); this may have contributed to mortality there, among fish acclimated to higher pH values.

Calcium concentrations during this time were 28-31 µeq/L at Paine, versus 64-76 µeq/L at Staunton and 128-148 µeq/L at Piney. Perhaps the low calcium values in Paine Run represent sub-optimal rearing conditions. Calcium values in the low-ANC stream (Paine) are quite low, always below the median values for streams in the Episodic Response Project (Wigington et al., 1996). It is also possible that the eyed-eggs used in the bioassay were in sub-optimal condition; if so, this plus sediment could have lowered survivorship. Alternately, this bioassay could be considered inconclusive due to low survivorship in the reference (high-ANC) stream.

Summary Part I:

Four Bioassays Showing Differential Mortality

Differential mortality of trout occurred among the study streams during four of the six bioassays (fall 1992, spring 1993, spring 1994, and fall 1994). In each of these four, trout in Piney River (high-ANC) showed higher survival rates than trout in Paine Run (low-ANC) (Table 6A-2; Figures 6A-4, 6A-8, 6A-14, 6A-18).

The responses of trout in the intermediate-ANC stream, Staunton River, were mixed: they showed survival as low as in Paine Run during the fall of 1992 (Figure 6A-4) but survival similar to that in the high-ANC stream in the spring of 1993 and fall of 1994 (Table 6A-2; Figure 6A-8). In the spring of 1994 the survivorship in Staunton River (Table 6A-2; Figure 6A-14) was intermediate, but not significantly different from that at Paine Run (low-ANC) or Piney River (high-ANC), which differed significantly from each other (Table 6A-2).

Since no habitat characteristics differentiate patterns in the fish communities among the streams, while acid-base status does (Chapter 5A), it seems reasonable to suspect that acid water chemistry lowered survivorship in the low-ANC stream. This appears to be the case in the first two bioassays, in which the most likely source of differential mortality is episodic acidification. Interpretation of the two other bioassays is more complex. Chronic acidification may have contributed to significantly lower survivorship in the low-ANC stream (Paine, Table 6A-2) in the spring 1994 bioassay, while sedimentation probably contributed to low survivorship in the mid- and high-ANC stream. In the fall 1994 bioassay, sedimentation probably contributed to low survivorship in the mid-and high-ANC streams, but not in the low-ANC stream; nevertheless, survivorship was seven times higher in the high- versus the low-ANC stream.

With respect to acidification, it is very difficult to separate episodic effects from chronic effects in terms of their importance for fish, because low ANC streams are both more prone to acid episodes, and have chronically lower pH. It appears that multiple acid episodes occur each year in low ANC streams (Chapters 3 and 4).

The results of the SNP: FISH trout mortality bioassays are in many ways comparable to those obtained by researchers conducting similar experiments in acidified regions of the Adirondacks (Johnson *et al*., 1987; Simonin *et al*., 1993), West Virginia (Jordahl and Benson, 1987), and the three regions studied in the Episodic Response Project (Van Sickle et al., 1996; Baker et al., 1996). However, mortality of the trout sacfry in our study occurred under conditions of higher pH and lower aluminum than in other *in situ* studies. A likely reason for this is very low Ca^{+2} concentrations. In Paine Run, Ca^{+2} concentrations at baseflow are around 31 *u*eq/L, versus 94 to 176 *u*eq/L during the Johnson *et al*. (1987) bioassay discussed above, 99 to 231 *u*eq/L in the Simonin *et al*. (1993) bioassays, and 350 to 930 *u*eq/L in Jordahl and Benson (1987). The median calcium values for 13 streams in the Episodic Response Project were 66-258 *u*eq/L (Wigington et al., 1996). Calcium ameliorates the negative physiological effects of low pH and high aluminum concentrations, especially in the range up to 150 µeq/L calcium (reviewed in Bulger et al., 1993), so the negative effects of acidification in Paine Run are probably more intense due to locally low calcium values. Our results confirm, using *in situ* bioassays, that the hatching/early sacfry period is a sensitive life stage for brook trout with respect to pH and aluminum, and, by comparison, that calcium moderates these effects.

Two Bioassays Showing Mortality due to Drought or Flood

Two other bioassays showed uniformly high mortality in all three streams, rather than differential mortality. During the fall 1993 bioassay, mortality in was very high in all streams within seven days after placement, due to fungal infection (probably *Saprolegnia*) and rapid mortality followed. The low-flow conditions probably allowed the eggs to become infected (Charlie Stevens, Director of Paint Bank Hatchery, personal communication). During that fall, mean daily discharge at each of the streams was substantially lower than had been observed during other seasons (Figures 6A-13a,b,c). Parasitic fungi of Order Saprolegniales are ubiquitous molds in fresh and brackish water. Dead eggs are a rich growth medium for the fungi. Once the disease infects a few eggs it can quickly spread to live eggs in the area (Chacko, 1993). Our experimental design did not include removal of dead eggs from the redds, so the disease probably spread from the dead eggs. The bioassay was terminated after 30 days when all trout had died at Paine Run and Piney River (Figure 6A-12). A substantial discharge event did occur at Paine Run during the fall of 1993, but after the bioassay was terminated.

The spring 1995 bioassay (begun on 1/13/95) was disrupted by a massive hydrologic event, starting on 1/15/95, that occurred in all three streams. Daily discharge during this event was the second highest during the 1003-day period of record at both Paine (238 cfs on 1/15/95, versus the mean of 7 cfs) and Piney (110 cfs on 1/16/95 versus the mean of 11 cfs); it was the fifth highest daily discharge at Staunton (48 cfs on 1/15/95 versus the mean of 9 cfs).

It was clear from inspection after the event that the artificial redds, the cages that contained them, and even the protective protective gear placed upstream, had been agitated substantially during the flood, especially at Paine and Piney. The artificial redds had also trapped much sediment, interfering with the flow of fresh water through the redds (Waters, 1995) following the flood. Subsequent declines in survivorship, similar at all three sites, are probably best attributed to mechanical damage during the flood plus suffocation (due to sediment in the redds) resulting from flood conditions.

During the course of the trout bioassays, mortality was observed from four sources: low discharge, high discharge, sedimentation, and acidification. These are likely to be the major embryo/sacfry mortality sources in SNP headwater streams (Table 6A-3). Two other natural sources of mortality (scouring plus suspension during heavy discharge, and predation), were prevented by the bioassay design.

ANC, pH, aluminum and calcium are highly intercorrelated in nature (reviewed in Bulger et al., 1993). ANC controls pH; the solubility and toxicity of aluminum is pH-dependent; hydrogen ion (its concentration is measured by pH) is directly toxic to fish, as is aluminum; calcium ameliorates acid and aluminum toxicity, and also contributes to ANC. ANC can be used in modeling because of its statistical and mechanistic relationships to the "physiological variables", pH, aluminum and calcium. ANC is readily modeled by MAGIC; the effect of aluminum or pH can be translated from calculated ANC values; use of ANC does not negate the direct importance of the other three.

Summary Part II:

Blacknose Dace Sub-lethal Stress Bioassays

A sub-lethal stress bioassay, using hematocrit as an indicator of acidification stress, was conducted from 7/94 to 12/94. At Paine Run, the hematocrit of 51 (total) dace was measured on nine occasions in the field, versus 29 (total) dace at Piney River on five occasions in the field. The larger sample size at Paine Run is due to extra effort made to sample during an event. Because blacknose dace is more sensitive to acidification than brook trout, it was hypothesized that at least sublethal stress would be apparent in adult dace in a stream where acid events had killed young trout; however, no acidification stress as measured by hematocrit changes (an indicator of acid/aluminum stress in other fish species) was detected in a six -month bioassay (July- December, 1994); since no major acid episodes occurred during this period, episodic effects on dace cannot be ruled out.

Hematocrit values from Piney River and Paine Run are shown in Tables 6A-4 and 6A-5 respectively. The values shown for 8/18/94 at Paine Run were taken during an event when mean daily discharge rose from 0.55 to 22.5 cfs (Table 6A-5). This event lowered pH from above 6.0 to 5.37 (Table 6A-5). TMA rose from its baseflow concentration of around 15 *u*g/L to 39 *u*g/L at the time of lowest pH. A few hours later when the pH had risen to 5.54, TMA was still higher than baseflow levels (42 *u*g/L) (not shown); this event was of no more than moderate intensity at Paine, compared to a maximum recorded Al concentration there of

128 µg/L. Fifteen dace were sampled during the event and their hematocrit values were compared to those taken at baseflow during the same season (on 7/12, 7/22, 9/16). Students t-test showed no significant difference between the groups (p = 0.15). Therefore, sub-lethal stress was not detected by hematocrit changes, although it has been a good indicator of acidification stress in other species (Wood, 1989; Rosseland *et al*., 1992). Hematocrit had never been measured in blacknose dace before, and therefore had never been used as stress indicator in this species. This study indicates that dace do not show a hematocrit response over the range of conditions observed, which were primarily baseflow.

Unfortunately, only one mildly acidic episode occurred during the sub-lethal stress bioassay (pH : 5.37; TMA: 39-42 µg/L) and an ideal investigation would have measurements from many events. Although no *in situ* sub-lethal stress bioassays with blacknose dace are available for comparison, some *in situ* mortality bioassays have been performed; these suggest that the event in Paine did not produce acute sublethal stress in the experimental fish (Simonin *et al*., 1993; Johnson *et al*., 1987). However, a chronic sub-lethal response in the form of lower condition (K) has been detected among dace in SNP (Dennis *et al*., 1995).

Conclusions

The clearest results of this section come from the trout bioassays indicating that episodic acidification can kill fish in low-ANC streams in SNP. By comparing the most sensitive life stage of a tolerant species (brook trout sacfry) to the adults of a more sensitive species (blacknose dace), these studies have been able to indicate the likelihood of acid stress impacts upon fish in SNP. Episodic acidification occurring after trout eggs have hatched can result in significant mortality such as occurred in three Paine Run mortality bioassays discussed above. Since 29% of SNP's catchments are underlain by silici-clastic bedrock similar to Paine Run, a substantial portion of the Park may experience toxic episodes.

Extrapolation of this study's results to natural brook trout populations in SNP should be done with appropriate caution. The design of the bioassays provided for nearly synchronous hatching of test fish in November (*Nashua* strain) or February/March (*Owhi* strain), whereas native trout in SNP spawn from late September to mid-November, and eggs hatch from February to early April. Since hatching is spread over a three-month period, an entire year class would probably not be extirpated by a single acid episode. However, if SNP streams continue to acidify, the frequency of acid episodes will increase as baseflow conditions deteriorate, and greater impacts on trout populations are expected.

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	Paine Run	Staunton River	Piney River
Sensitivity to Acidification	extremely sensitive	sensitive	insensitive
Area (km^2)	12.71	10.56	12.66
Watershed Aspect	western	eastern	eastern
SNP District	South	Central	North
Major Bedrock Type	silici-clastic	granitic	basaltic
Bedrock Geology	91% Hampton 9% Antietham	82% Pedlar 4% Old Rag	68% Catoctin 31% Pedlar
Vegetation Classification	96% CO/P 2% H/YP/CH	48% CO/P 11% RO/BL 37% H/YP/CH	36% CO/P 18% RO/BL 42% H/YP/CH
Site Elevation (m)	417	303	342

Table 6A-1. Physical and vegetative characteristics of the three test watersheds. CO/P represents chestnut oak an pine; RO/BL represents red oak and black locust; H/YP/CH represents hemlock, yellow popular, and cove hardwood. Classifications from Teetor (1988).

	Spring 1994	Fall 1994
Paine Survival	22%	8%
Staunton Survival	25%	45%
Piney Survival	42%	57%
Kruskal-Wallis test	**p= .01**	**p= .01**
Paine vs. Piney	sig.	sig.
Paine vs. Staunton	n.sig.	sig.
Staunton vs. Piney	sig.	n.sig.

Table 6A-2. Survival in two trout bioassays showing chronic acidification mortality (spring and fall,1994); Kruskal-Wallis test and Dunn Procedure comparisons. sig. = significant difference, n.sig. = non-significant difference.

Sources of Mortality Observed	# of Bioassays
Flood/Sedimentation	3
Drought /Fungal Infection	1
Acidification	3

Table 6A-3. Sources of mortality during the six brook trout bioassays. The number of bioassays during which a particular mortality source dominated is indicated by the number in the boxes. See text for individual bioasssay results.

Date	H'crit	s.d.	pH	ANC	Ca	TMA	MDD
7/11-7/12	34	3.46	7.26	314	166	14	1.25
7/25-7/26	34.5	1.7	7.07	316	176	29	1.07
9/21-9/23	31.5	1.87	7.03	243	151	15	1.51
11/7-11/8	29.8	2.28					7.7
12/6-12/7	30.5	2.74					

Table 6A-4. Piney River dace hematocrit and water chemical composition during the sub-lethal stress bioassay (7/95 - 12/95). MDD = mean daily discharge in cubic feet/second. ANC and Ca (*u*eq/L), TMA (*u*g/L).

Date	H'crit	s.d.	pH	ANC	Ca	TMA	MDD
7/5-7/6	36.67	1.97	6.04	9.5	26.2	3	0.22
7/12-7/13	36.4	3.21	6.07	6.9	27.6	11	0.33
7/22-7/23	33.8	5.89	6.18	9.4	24.5	15	0.55
8/18-8/18.6	31.53	4.88	5.37	-3.1	46.8	39	22.69
9/16-9/17	33.33	5.05					0.406
10/9-10/11	31.17	2.08	6.15	13.7	29.4	15	4.6
11/2-11/3	33.67	2.08					
12/5-12/6	28.8	1.79					

Table 6A-5. Paine Run dace hematocrit and water chemical composition during the sub-lethal stress bioassay (7/95 - 12/95). MDD = mean daily discharge in cubic feet/second. ANC and Ca (*u*eq/L), TMA (*u*g/L).

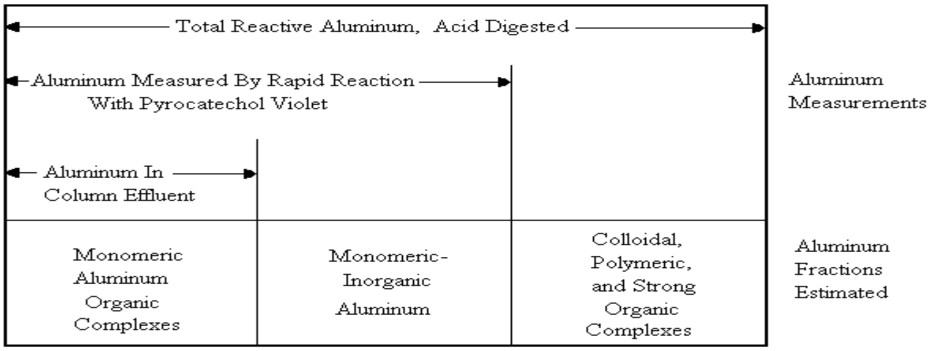


Figure 6A-1. Schematic representation of aluminum fractions (from Driscoll, 1984).

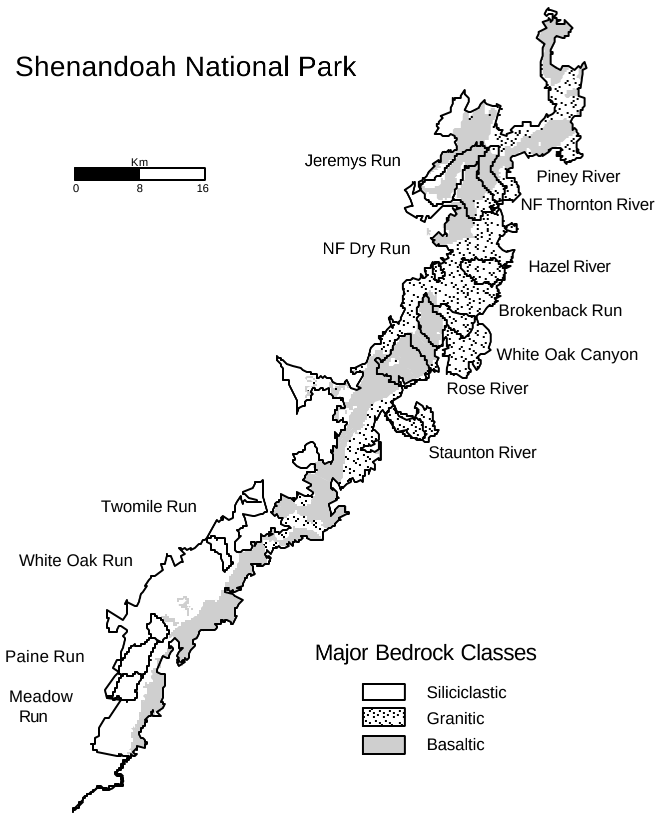


Figure 6A-2. SNP: FISH study sites and catchments in Shenandoah National Park. Intensive sites (Piney River, Staunton River, Paine Run) were gauged for discharge measurements, had continuous stage recorders, and both 8-hour and stage-activated automatic water samplers. Water samples were also collected by staff weekly. These streams were the sites of bioassays, plus fish and habitat surveys in each year of the study (1992-95). Extensive sites were sampled for water chemistry at least quarterly, and fish and habitat surveys were conducted at least once in the study years.

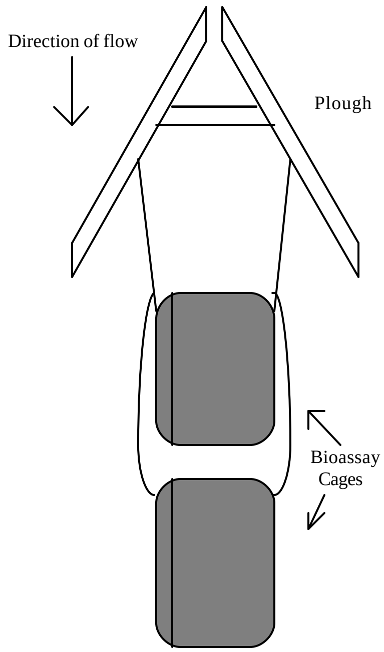


Figure 6A-3. Bioassay field site set up. A wooden plough protects the bioassay cages from high flow.

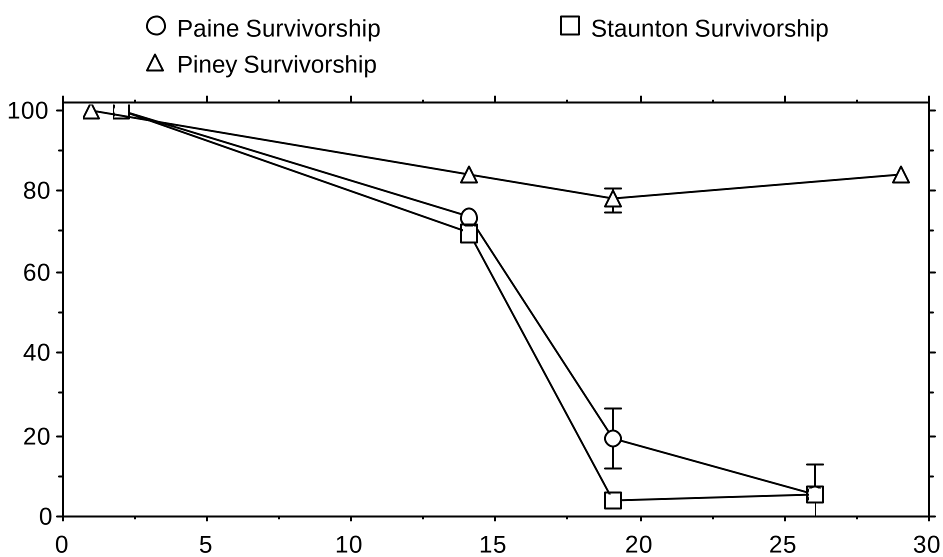


Figure 6A-4. Fall 1992 brook trout bioassay survivorship. Survivorship is estimated by both replicated and unreplicated sub-sample. Survivorship at Paine Run and Staunton River decreased dramatically between the 15th and 19th day of the bioassay. On the 19th day all three of the study streams were experiencing large hydrologic events as a result of recent storms.

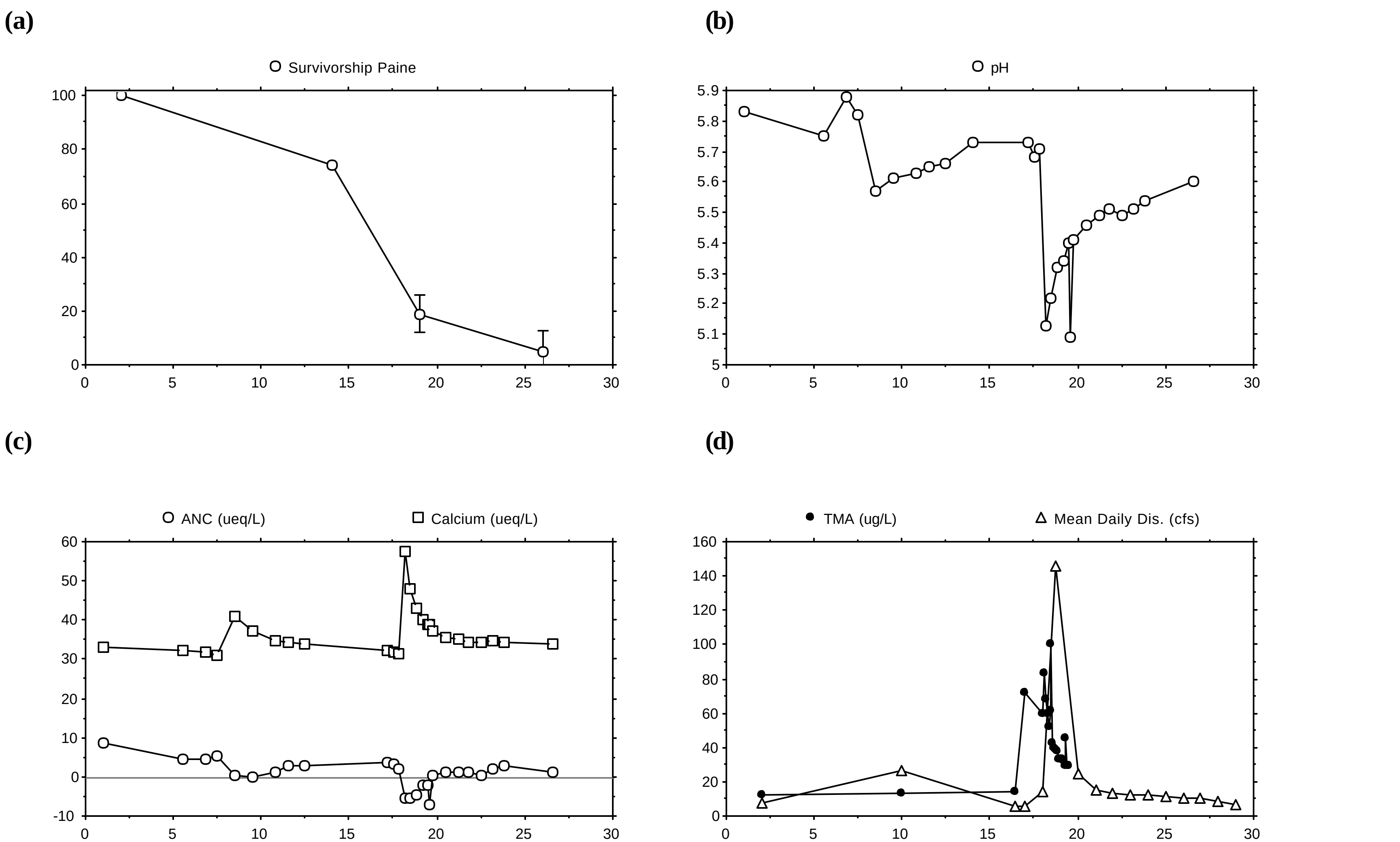


Figure 6A-5. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Paine Run during the fall 1992 bioassay. Survivorship is estimated by both replicated and unreplicated sub-sample.

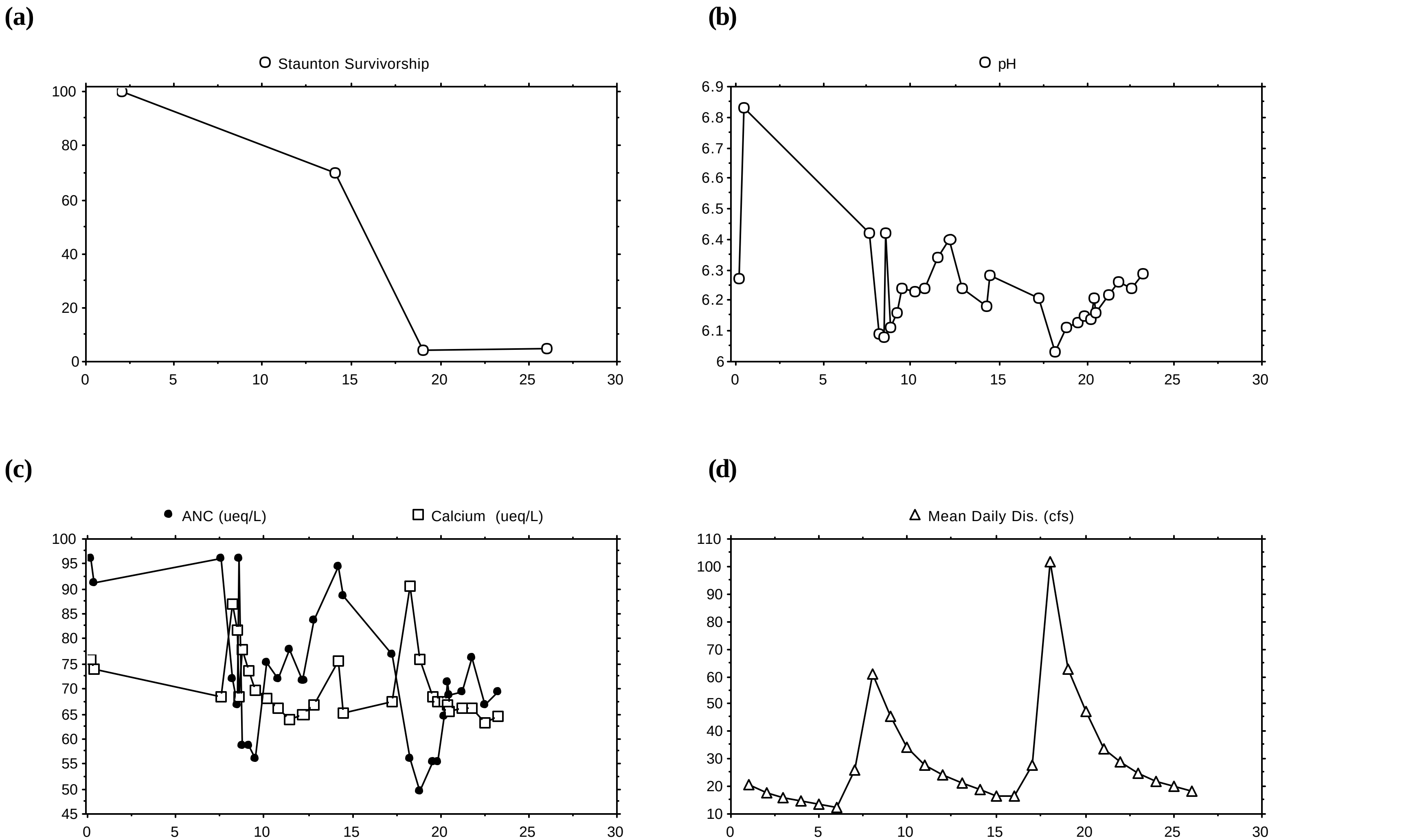


Figure 6A-6. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Staunton River during the fall 1992 bioassay. Survivorship is estimated by both replicated and unreplicated sub-sample.

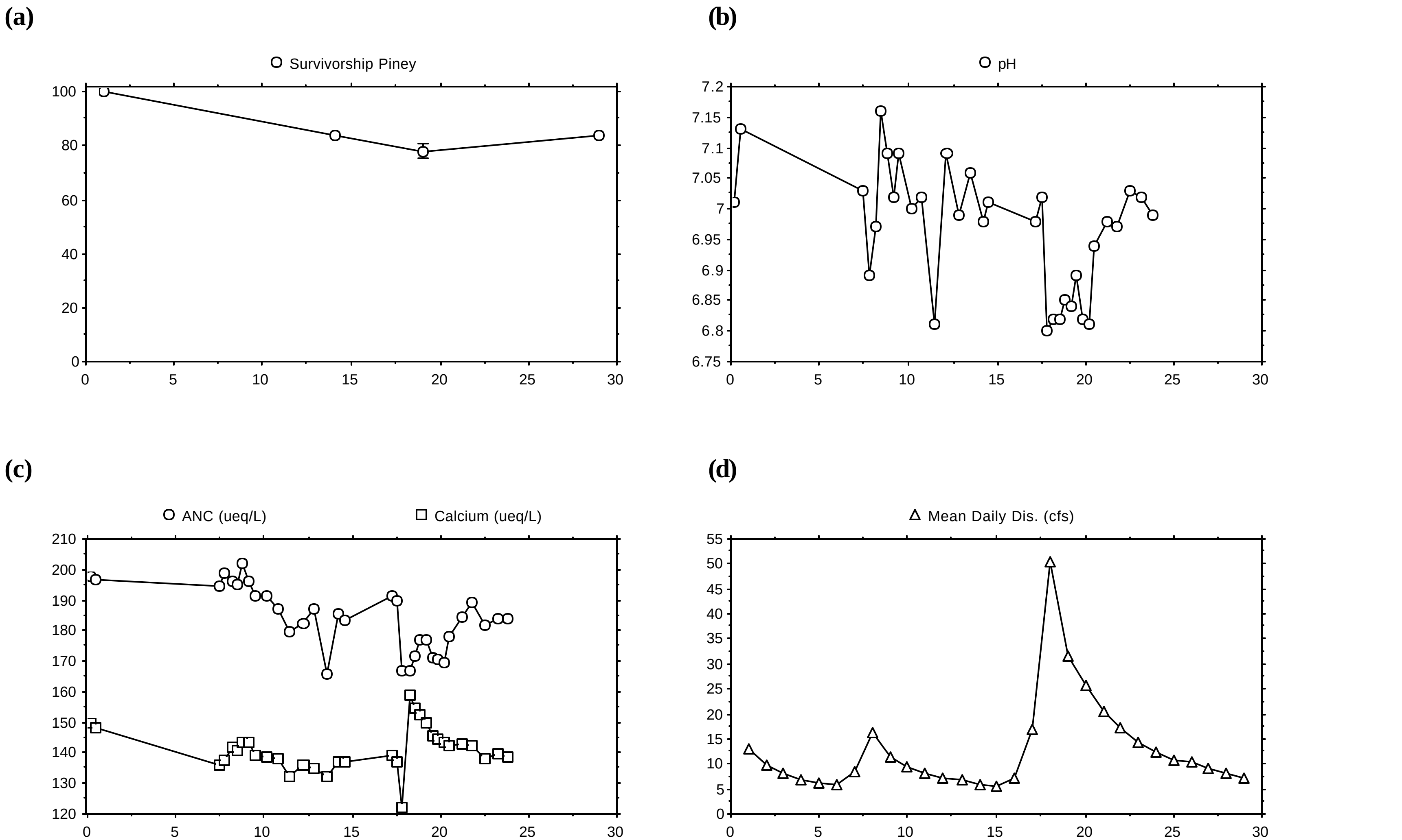


Figure 6A-7. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Piney River during the fall 1992 bioassay. Survivorship is estimated by both replicated and unreplicated sub-sample (except on the 19th day where n = 2).

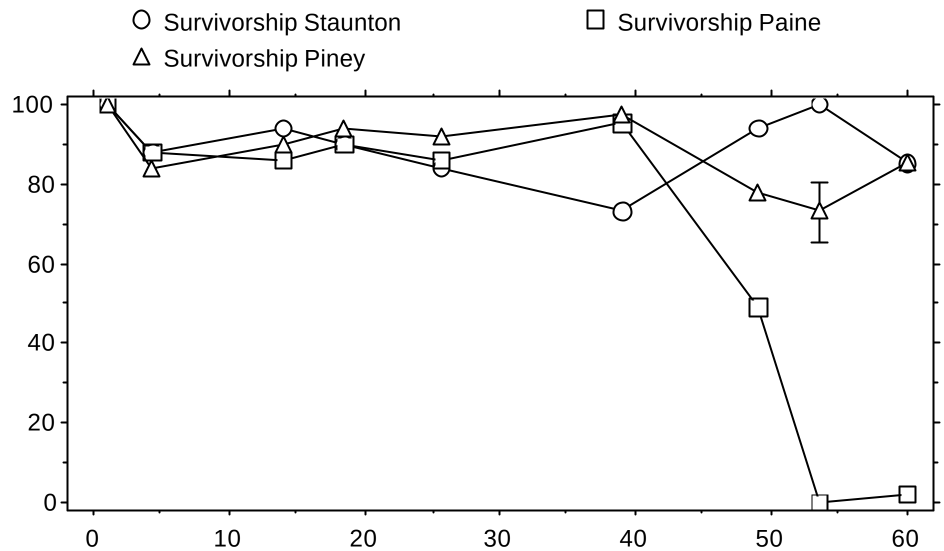


Figure 6A-8. Spring 1993 brook trout mortality bioassay survivorship. Survivorship is estimated by an unreplicated sub-sample (except for Piney River on the 53rd day). This accounts for the apparent increase in survivorship seen, for instance, at Staunton River between the 39th and 49th day from initiation. On the 54th day of the bioassay there was a sharp decline in survivorship at Paine Run. The decline coincided with a large storm which substantially increased discharge at all three of the bioassay streams.

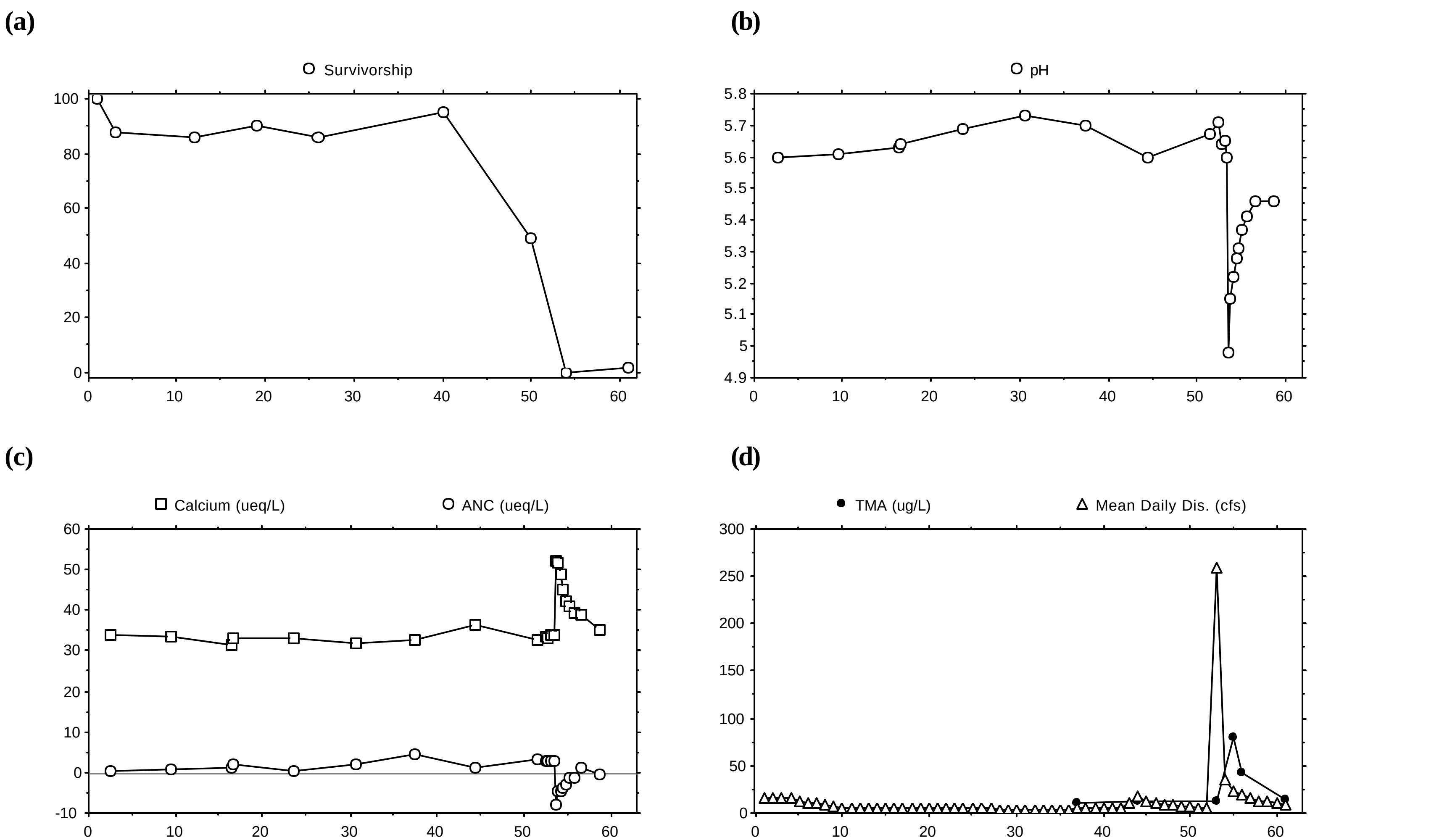


Figure 6A-9. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Paine Run during the spring 1993 bioassay. Survivorship is estimated by an unreplicated sub-sample. On the 54th day of the bioassay there was a sharp decline in survivorship. The decline coincided with a large storm which substantially increased discharge in the stream. pH remains around 5.6 up until day 53 of the bioassay when a large episode occurred and pH plummeted to 4.98. Total monomeric aluminum concentration increased either as a result of cation exchange or increased mineral solubility (Figure 6A-1).

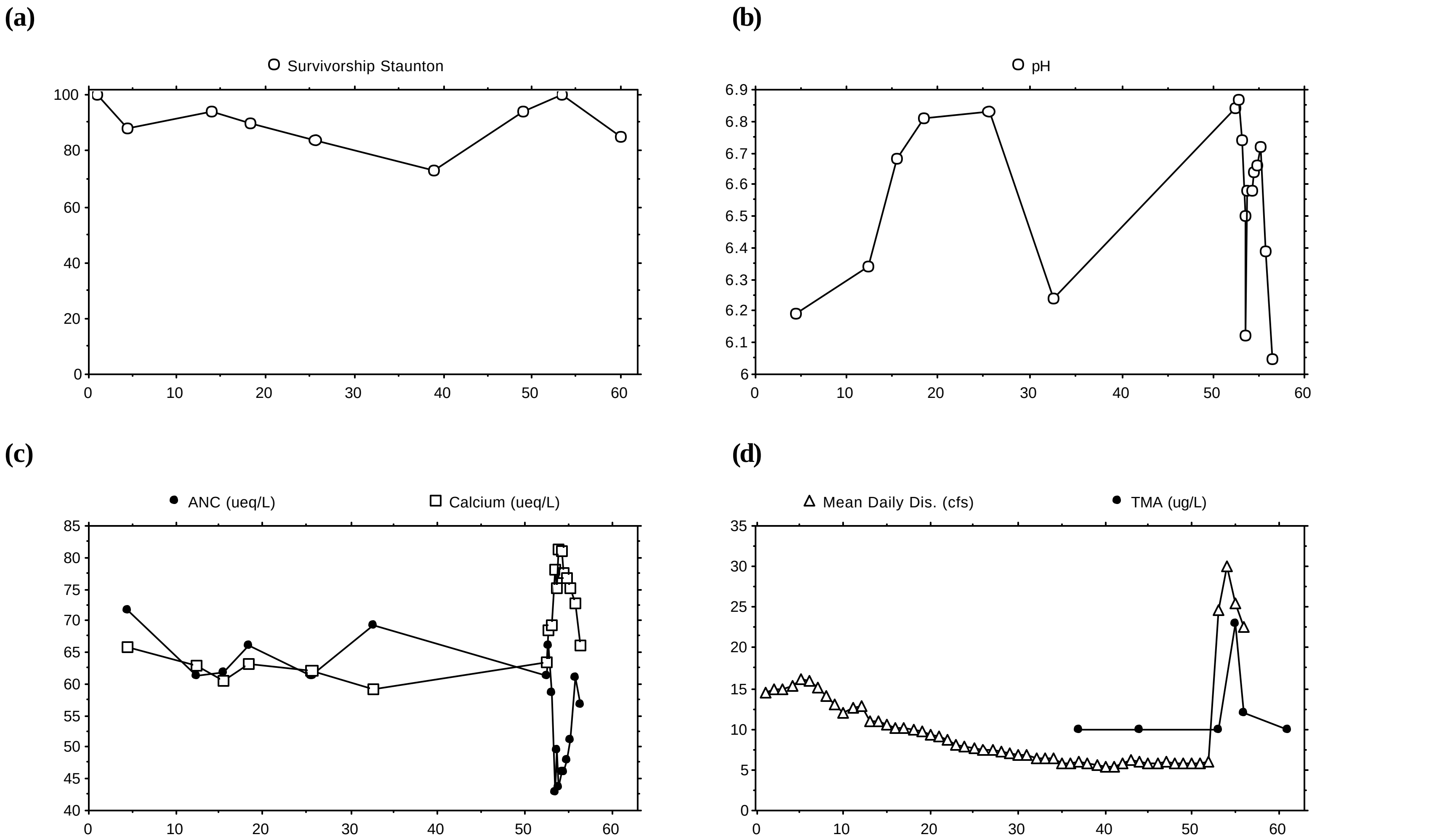


Figure 6A-10. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Staunton River during the spring 1993 bioassay. Survivorship is estimated by an unreplicated sub-sample. Survivorship was high for the duration of the bioassay. The sharp decrease in pH which occurred on the 53rd day coincided with a large storm event. pH did not fall below 6.0 however, so the solubility of aluminum was minimal (see Figure 6A-1). Note that the range of the y-axis for figures (6A-10b) though (6A-10d) is different than the range of the equivalent Paine Run figures.

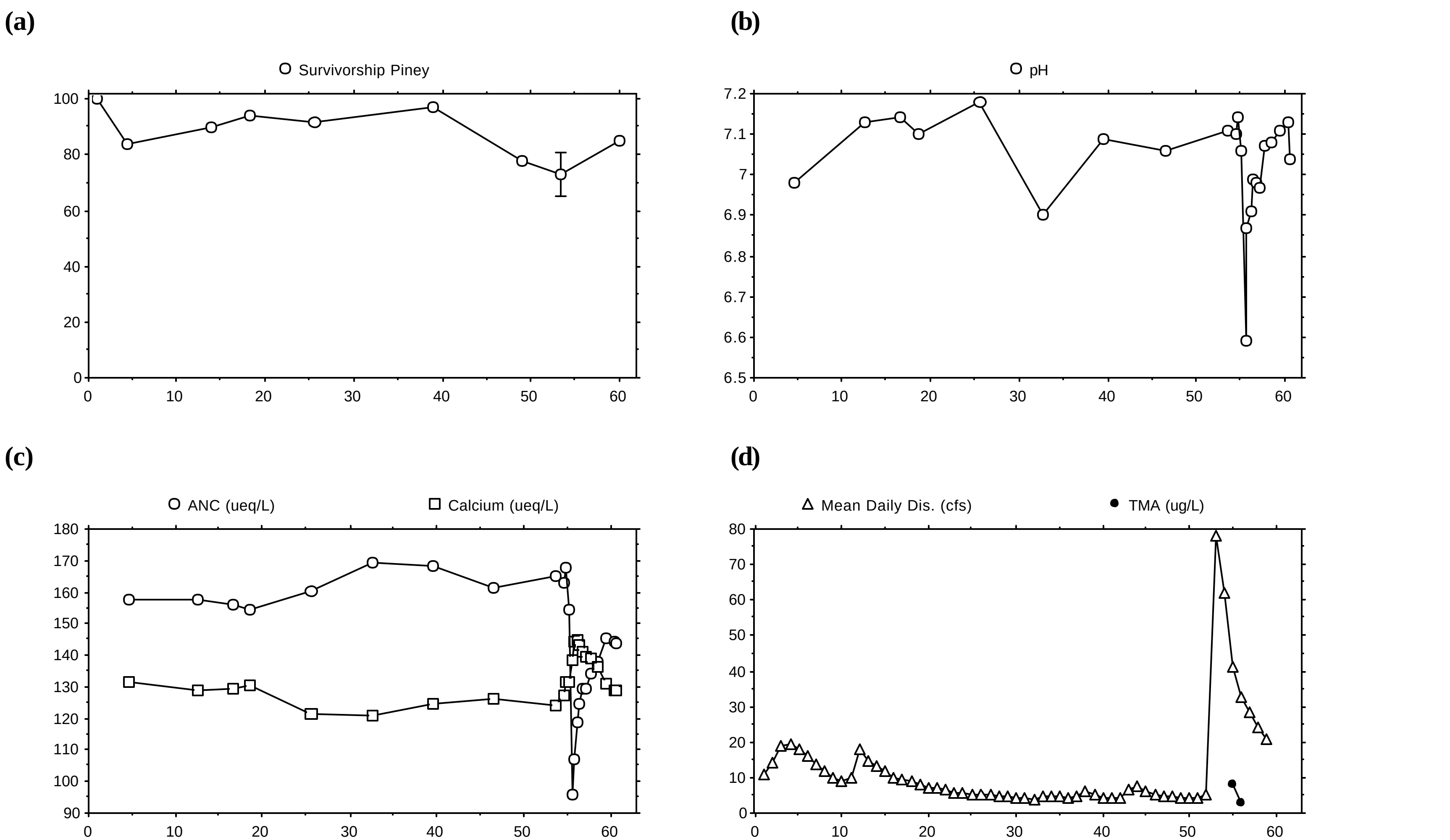


Figure 6A-11. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Piney River during the spring 1993 bioassay. Survivorship is estimated by an unreplicated sub-sample. Survivorship was high for the duration of the bioassay. The sharp decrease in pH which occurred on the 53rd day coincided with a large storm event. pH did not fall below 6.0 however, so the solubility of aluminum was minimal (see Figure 6A-1). Note that the range of the y-axis for figures (6A-11b) though (6A-11d) is different than the range of the equivalent Paine Run figures.

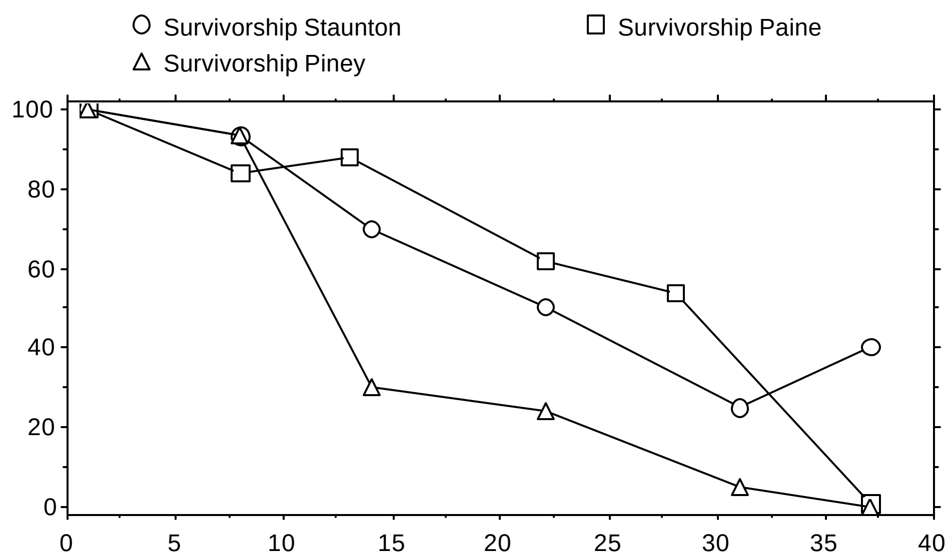
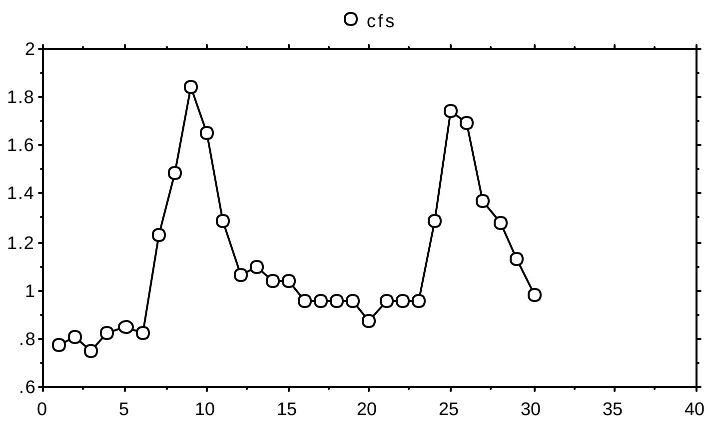
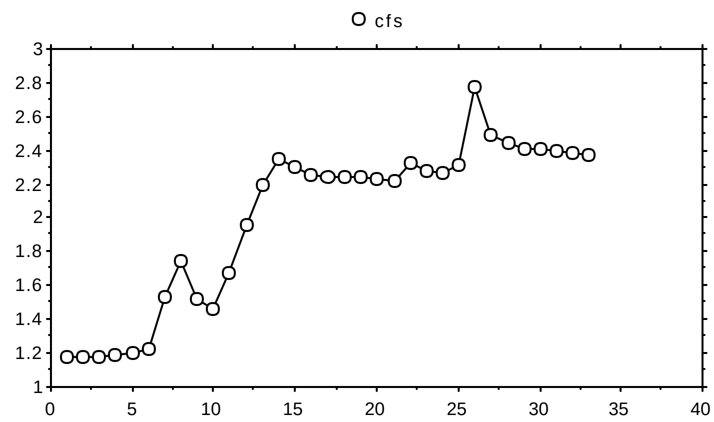


Figure 6A-12. Fall 1993 brook trout mortality bioassay. Survivorship is estimated by an unreplicated sub-sample. The bioassay was terminated after 37 days when survivorship at Paine Run and Piney River reached 0%. On the 7th day of the bioassay it was noticed that embryos in all three streams had become infected by a fungus. The fungus became more prevalent as the bioassay went on and was probably responsible for the mortality observed in all three streams. The infection could have occurred because of abnormally low stream flow during this period. Figures 6A-13a,b,c show mean discharge for each of the streams during the bioassay. Periods of low flow may allow fungi opportunities to attack embryos that they would not have otherwise (Charlie Stevens, director of Paint Bank State Hatchery, personal communication). This mortality bioassay was not considered successful due to the fungi induced mortality.

(a) Staunton River mean daily discharge



(b) Piney River mean daily discharge



(c) Paine Run mean daily discharge

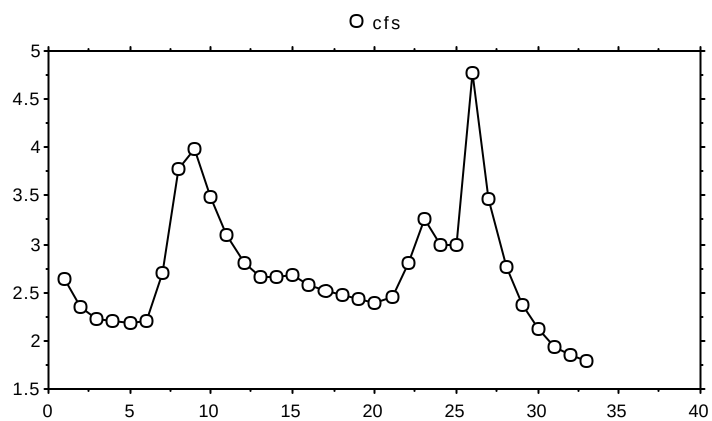


Figure 6A-13. Mean daily discharge from Paine Run (**a**), Staunton River (**b**) and Piney River (**c**) during the fall of 1993. Discharge in all three streams is much lower than was observed during the same period the year before (Figures 6A-9d, 6A-10d and 6A-11d).

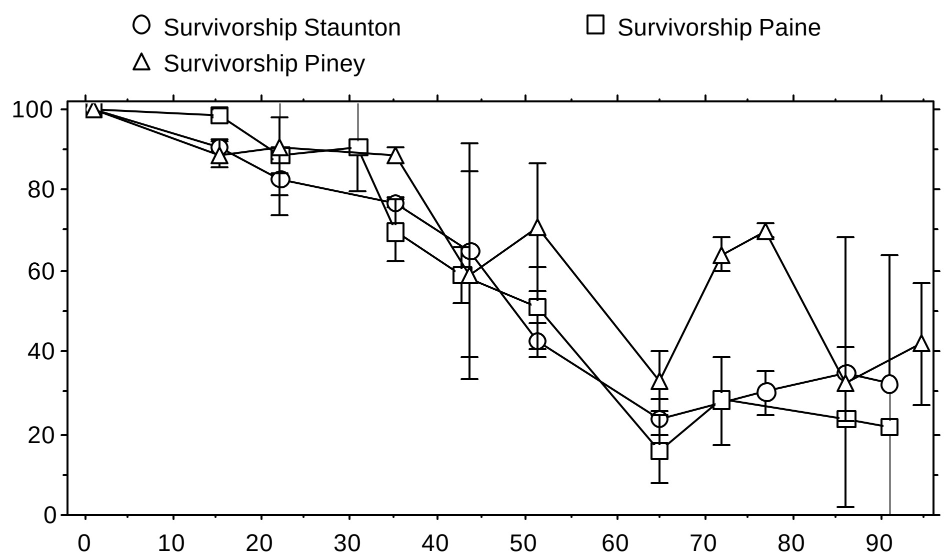


Figure 6A-14. Survivorship during the spring 1994 brook trout mortality bioassay. Survivorship is estimated by replicated sub-samples. Error bars show ± one standard deviation. The bioassay was terminated when the supply of test embryos was exhausted in Paine Run. Kruskal-Wallis and the Dunn procedure showed that survivorship in Paine Run was significantly lower than that Piney River.

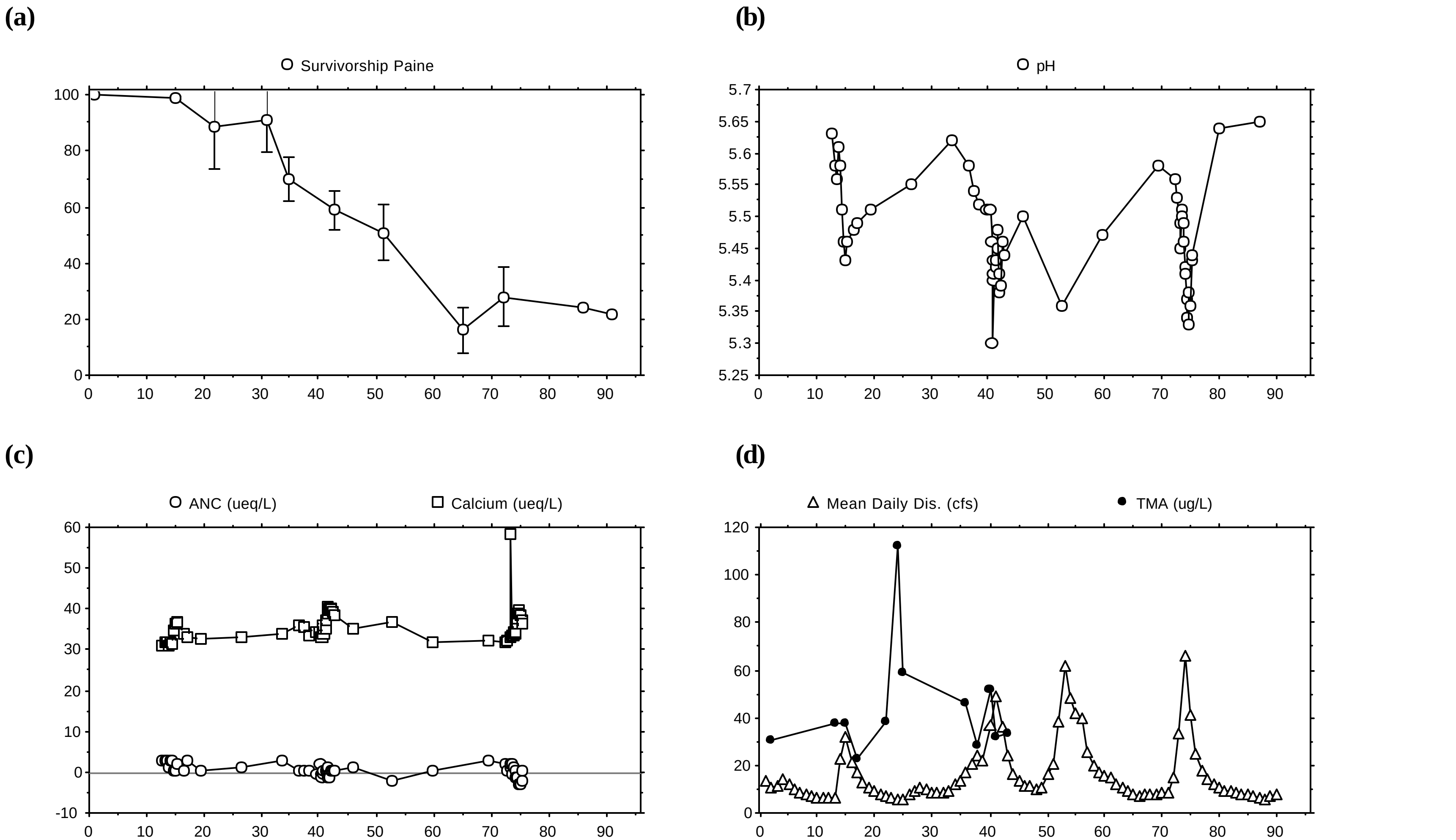
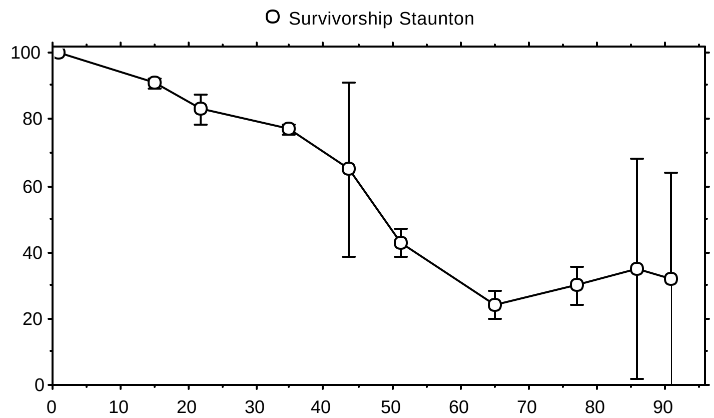
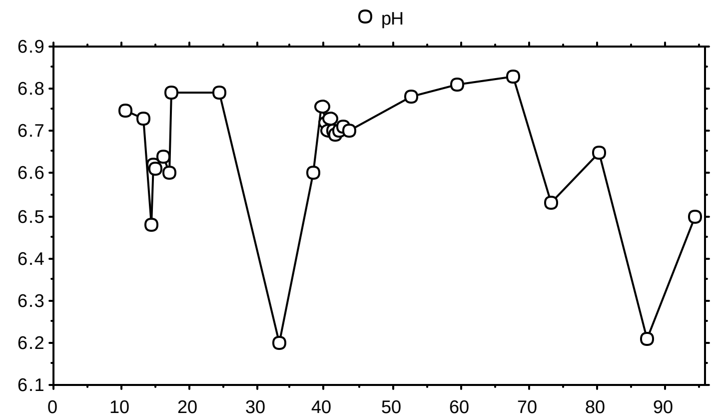


Figure 6A-15. Temporal patterns of (**a**) % survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Paine Run during the spring 1994 bioassay. Survivorship is estimated by a replicated sub-sample.

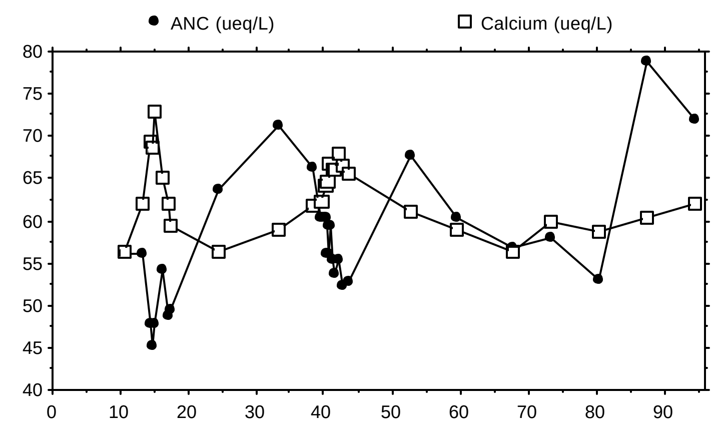
(a)



(b)



(c)



(d)

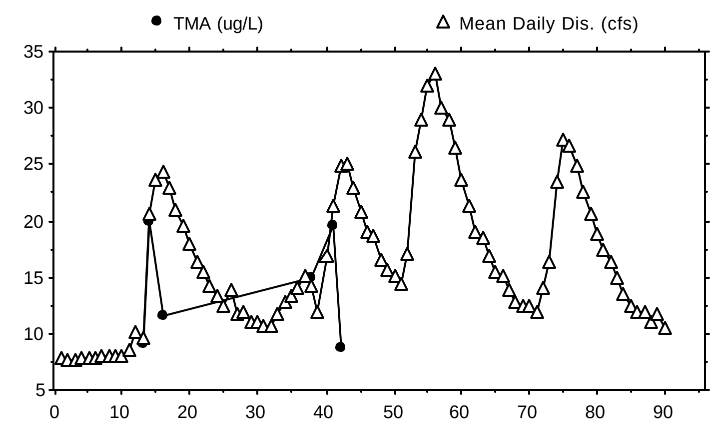


Figure 6A-16. Temporal patterns of (**a**) survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Staunton River during the spring 1994 bioassay. Survivorship is estimated by replicated sub-sample.

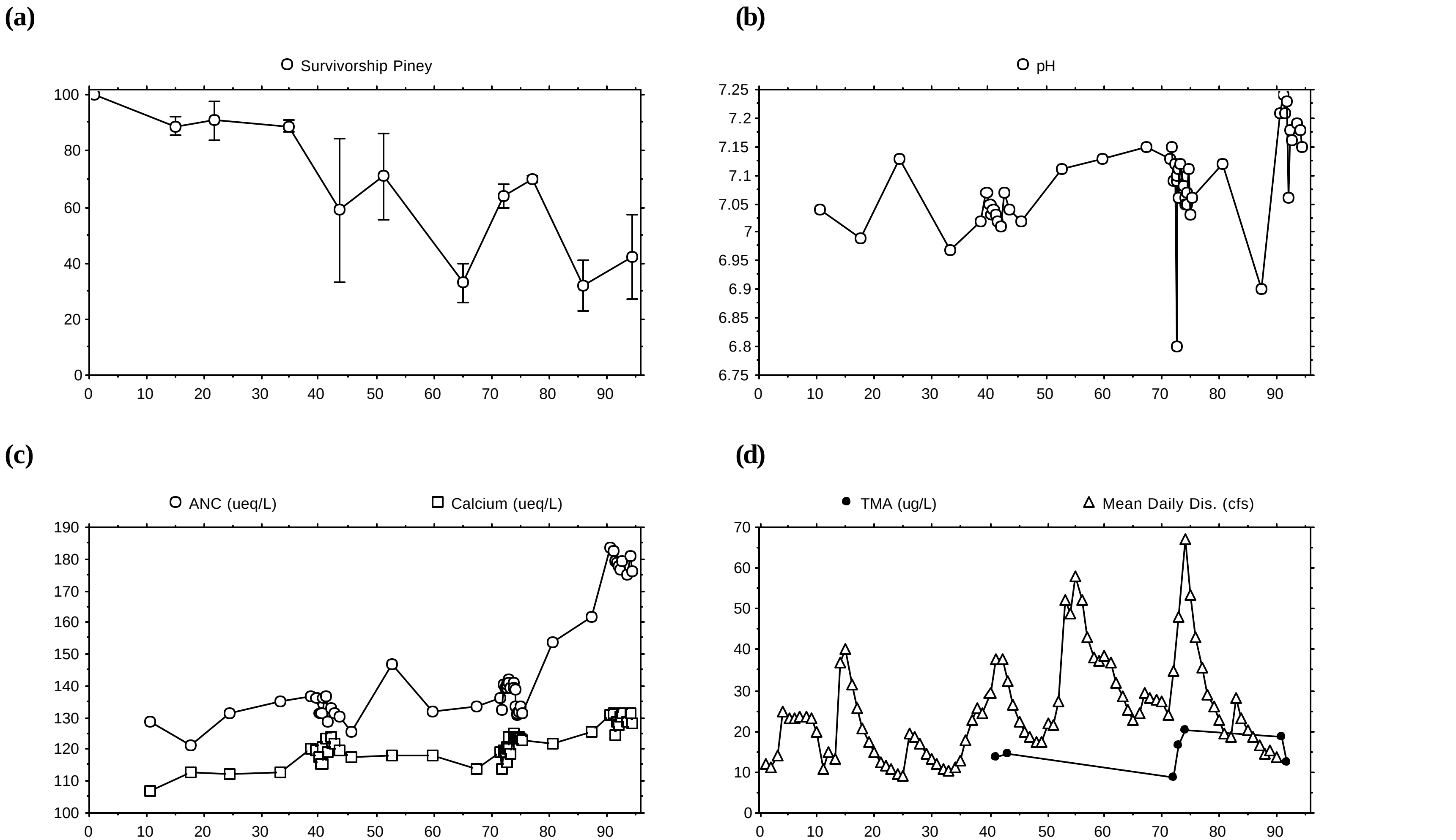


Figure 6A-17. Temporal patterns of (**a**) survivorship, (**b**) pH, (**c**) ANC and Calcium, and (**d**) Mean Daily Discharge and TMA at Piney River during the spring 1994 bioassay. Survivorship is estimated by replicated sub-sample. Survivorship was high for the duration of the bioassay.

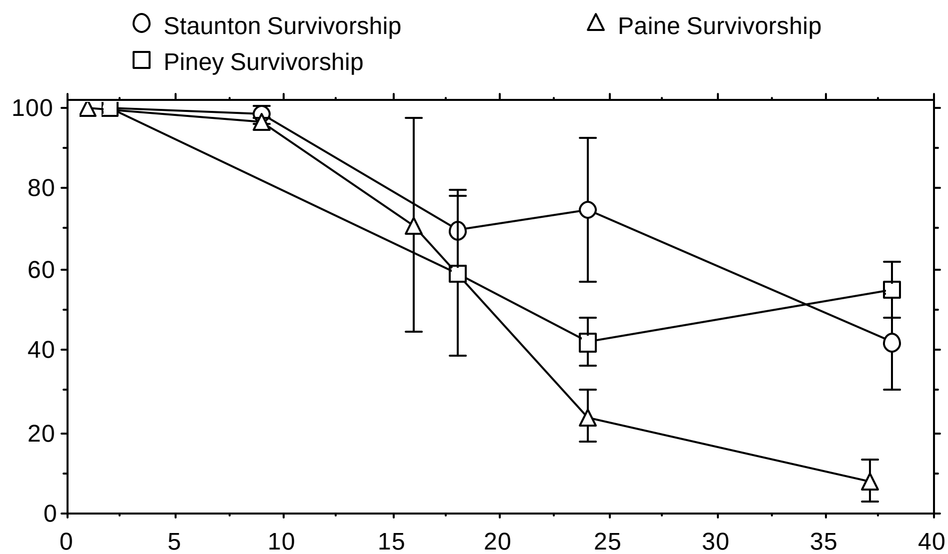


Figure 6A-18. Survivorship during the fall 1994 brook trout mortality bioassay. Kruskal-Wallis and the Dunn procedure showed a significant difference among the streams (p= .0036) and that Paine had significantly lower survivorship than Piney or Staunton.

SNP:FISH
Shenandoah National Park: Fish In Sensitive Habitats
Project Final Report, Volume IV

Chapter 6B

The susceptibility of blacknose dace *(Rhinichthys atratulus)* to acidification in Shenandoah National Park

prepared by

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Abstract

Populations of blacknose dace (*Rhinichthys atratulus*) occur in many northern and southern Appalachian streams. This fish species is often regarded as sensitive to acidification. Blacknose dace populations in some Shenandoah National Park (SNP) streams presently experience pH and aluminum concentrations, which produce deleterious effects on blacknose dace in Ontario and Adirondack streams. The purpose of the present study was to determine if lethal and/or sublethal acidification effects on adult blacknose dace could be detected before conditions have deteriorated sufficiently to cause the extirpation of populations in acidified streams. Water chemistry measurements and bioassays were conducted in three SNP streams differing in acid neutralizing capacity (ANC) over and 14-month period. pH, ANC, total monomeric aluminum, and calcium were analyzed for water samples taken from both base flow conditions and storm events. Comparison of these measurements to related studies indicates that If current atmospheric deposition rates of sulfur remain constant, blacknose dace (and other fish species) populations in SNP may in the future be lost from low-ANC streams

Blacknose dace in low-ANC SNP streams may currently experience sublethal (versus lethal) stress sufficient to lower growth rates. Repeated surveys over time in three streams differing in baseflow ANC indicated perennial differences in condition factor (an indicator of sublethal stress) among dace; dace from the low-ANC stream were in poorer condition on all sampling occasions. Comparison of dace condition factor among 11 streams (of different acid-base status) sampled within a two - week period shows a strong positive relationship between dace condition factor and stream pH. However, survival bioassays did not detect differences in mortality among dace in different streams during baseflow conditions, and whole-body sodium levels (an indicator of sublethal stress in other species) were similar in the three intensively studied streams.

Introduction

Shenandoah National Park (SNP) is located downwind of large sources of sulfur and nitrogen emissions (National Academy of Sciences, 1986). Recent sulfur deposition rates in the SNP are about 10 times the pre-industrial rate (Cosby et al., 1991). Until recently, SNP received more acidic deposition than any other national park in the United States (NADP/NTN, 1989); it is now second only to Great Smoky Mountains National Park (NADP/NTN, 1994). Sulfate is the major anion in precipitation; nitrate concentrations are generally in the equivalent ratio of 1:2 of sulfate (Galloway, 1993 - from SNP precipitation analysis). Wet deposition of sulfate in western Virginia is estimated to be about 500 eq ha^{-1} yr^{-1} (Summers et al., 1986; Buikema et al., 1988). Dry deposition of sulfur is estimated to be from 50% to 100% of the amount of wet deposition (Shaffer and Galloway, 1982; Rochelle et al., 1987).

Chronic stream water acidification has been documented for two streams (Deep Run and White Oak Run) in SNP, monitored weekly since 1979 (Ryan et al., 1989). Projections indicate that 32% to 88% of SNP streams will have ANC (acid neutralizing capacity) values near 0 when stream sulfate concentrations reach steady state (Webb et al., 1989)

The negative effects of acidification on freshwater fish are well documented (Altshuller and Linthurst, 1984; Schindler et al., 1985; Johnson et al., 1987; Schofield and Driscoll, 1987; Matuszek et al., 1991; for extensive reviews of the subject see Haines and Johnson, 1982, and Baker and Christensen, 1991; Van Sickle et al., 1996). Fish responses to acidification occur at the community, population, and/or organismal level. Community effects include subtle or gross changes in species composition. Population effects include changes in body size, density, abundance, total biomass, age-class structure, and extinction. The investigation of organismal responses may provide physiological evidence for impacts from acidification. Organismal responses are reviewed in the next section.

Twenty-eight species of fish in eight families (Table 6B-1) are found in SNP (SNP Fish Management Program). Weekly and/or quarterly chemical analyses of SNP streams since 1979 indicate that acidic conditions occur which may affect the distribution and abundance of some of these fish species. Values for pH of about 5.0 have been observed often in weekly water samples from Paine Run (this report). This value is within or below the "critical" pH threshold ranges for nine fish species found in SNP (Baker and Christensen, 1991; Table 6B-1). "Critical" pH, estimated by averaging the pH thresholds for effects from a variety of separate studies, is defined by Baker and Christensen as the pH below which population effects are likely to occur. Other SNP fish species are apt to be sensitive to present acidic conditions in Park waters, but there is little information on their acid tolerances.

Background

Dissolved aluminum, pH, and calcium are important chemical constituents in determining fish survival in acidified waters (Bergman and Mattice, 1990; Kelso et al., 1990; Parkhurst et al., 1990; Bulger et al., 1993). Aluminum speciation and solubility are pH-dependent. The solubility of aluminum is at a minimum near pH 6.5, and increases dramatically as pH approaches 5.2 (Driscoll, 1989). There are (Figure 6B-1) three main fractions of aluminum in natural waters (Driscoll, 1984). Monomeric aluminum species, especially the labile, or inorganic monomeric fraction (such as Al^{3+} and ionic Al-OH complexes, Table 6B-2), are considered to be most toxic to fish (Driscoll et al., 1980; Baker, 1983).

Numerous studies have examined the toxic effects of aluminum and low pH on fish (Baker and Schofield, 1982; Gunn and Noakes, 1986; Mount et al., 1988; Ingersoll et al., 1990; Parkhurst et al., 1990). Most of the toxicological responses of freshwater fish to acid/aluminum stress fall into two categories: effects on oxygen uptake and transport, and effects on iono-regulation. Impaired iono-regulation has been cited as the key mechanism causing death in acute acid/aluminum stress (Muniz and Leivestad, 1980; McDonald, 1983). However, effects on oxygen and carbon dioxide exchange at the gills also apparently occur at higher aluminum concentrations (Wood, 1989). Calcium ameliorates acid/aluminum toxicity (Graham and Wood, 1981; Brown, 1983; Ingersoll et al., 1990). Fish are more tolerant of low pH levels and high aluminum concentrations in waters with higher calcium concentrations. This may be due to calcium's influence on membrane permeability (Baker and Christensen, 1991).

Many investigations of fish responses to acidic waters have used standard mortality bioassays in the field (Schofield, 1965; Sharpe et al., 1983; Johnson et al., 1987; Kane and Rabeni, 1987; Rosseland et al., 1992; Van Sickle et al. 1996) or laboratory (Cleveland et al., 1986; Siddens et al., 1986; Hunn et al., 1987; Holtze and Hutchinson, 1989). Mortality bioassays are effective methods to document lethal environmental stressors. However, mortality bioassays used alone do not detect sublethal stress before conditions have deteriorated sufficiently to cause mortality.

Other studies have focused on sublethal stress indicators, including: ventilatory rate (Walker et al., 1988; Walker et al., 1991), oxygen uptake and transport (Packer, 1979), ion balance (McWilliams, 1980; Booth et. al., 1988), hormones (Tam et al., 1990; Witters et al., 1991), branchial mucus production (Daye and Garside, 1980; Tietge et al., 1988), various hematological, fluid volume, and cardiovascular parameters, (Neville, 1979; McWilliams, 1980; Wood et al., 1988; Rosseland et al, 1992; and reproduction (St. Pierre et al., 1986).

There have been few field or *in situ* studies of the sublethal effects of acid/aluminum stress, such as that by Rosseland et al., 1992. This is probably due to logistical problems, such as transporting test structures, distance to research sites, the absence of reliable power sources, and the fragility physiological recording equipment. However, *in situ* bioassays are desirable because they approximate natural conditions more closely than laboratory studies, and may produce more realistic results.

One method to determine *in situ* acute sublethal acid/aluminum effects in fish is to measure changes in body sodium concentration, because it responds rapidly to iono-regulatory disturbances**.** Net body sodium loss is the primary cause of death in fish exposed to lethally high acid and toxic aluminum concentrations (McDonald et al., 1989). Net body sodium loss is due to hydrogen and aluminum ion interference with gill ion transport systems and/or paracellular calcium binding sites. This interference results in diminished influx and greatly stimulated efflux of sodium and other electrolytes (Packer and Dunson 1970, 1972; McDonald, 1983; McDonald et al., 1989).

Gagen and Sharpe (1987) and Grippo and Dunson (1991) used body sodium concentrations in both laboratory and field studies to evaluate acute sublethal stress in fish exposed to acidic waters. Gagen and Sharpe observed that substantial net sodium loss occurred in natural settings with high total aluminum concentrations. The body sodium concentration of brook trout confined in cages at an *upstream* test site in Linn Run, a chronically low pH stream in Pennsylvania impacted by acidic precipitation, was found to be inversely correlated with exposure duration (a < 0.01; r = 0.66). The ambient total aluminum concentration at the time of the study for that site was near 450 mg/L and pH was approximately 5.1. Brook trout confined at *downstream* site (circumneutral pH and less than 50 mg/L total aluminum) had significantly greater sodium concentrations at 2-, 16-, and 24-h sub-samples than those at the more acidic upstream site. Brook trout at the *upstream* site experienced a 34% net loss of body sodium, versus less than 17% for fish at the *downstream* site. The two other fish species examined, rainbow trout and brown trout, also showed significant reductions in body sodium concentrations at the upstream test site after one day of exposure (rainbow trout = 35%; brown trout = 40%).

Grippo and Dunson (1991) found that the degree of loss of body sodium in the fathead minnow (*Pimephales promelas*) was a sensitive indicator of acute acid/metal sublethal stress. They exposed test animals for eight hours to acidic mine water at two pHs (4.11 and 3.90; total aluminum equaled 1501 mg/L and 1900 mg/L, respectively). Mean body sodium concentrations per gram wet mass of fish in both exposure groups declined significantly after six and eight hours of exposure (control = time zero). Additionally, fish in the 3.90 pH and 1900 mg/L total aluminum exposure group exhibited significantly lower body sodium concentrations after six and eight hours of exposure than those in the 4.11 pH and 1501 mg/L exposure group.

Ponderal or *condition indices* can be used as measures of fish responses to chronic, sublethal environmental stressors. They can be used to relate the consequences of biochemical and physiological alterations to observed changes in the individual and the population (Goede and Barton, 1990). A widely used condition index is the *condition factor* (Le Cren, 1951; Anderson and Gutreuter, 1983), often expressed as weight/length^3 multiplied by a scaling constant (to make the value more mathematically tractable).

Goede and Barton (1990) discuss the relationship between condition factor and environmental stress at length:

> "A decline in condition factor is usually interpreted as depletion of energy reserves such as stored liver glycogen or body fat. This decline may reflect a change in feeding patterns, which could be a behavioral response to certain stressors (Brown et al., 1987), or an increase in metabolic rate in response to stress (Skadsen et al., 1980; Schreck, 1981, 1982; Barton and Schreck, 1987). A drop in condition factor due to a decline in fat or other body reserves need not be due to stress or strictly starvation. The condition factor, energy reserves, and proximate composition can fluctuate seasonally, reflecting changes in feeding activity and nutrient availability (MacKinnon, 1972; Tyler and Dunn, 1976; Adams et al., 1982), and it can change with physiological development and sexual maturation (Le Cren, 1951; Denton and Yousef, 1976; Strange and Pelton, 1987)."

Objectives and Research Questions

The principal motivation for this study was to investigate the extent to which an acid-sensitive, ecologically important fish species SNP is affected by stream water acidification. The specific objective was to determine if several indicators of acid-stress were affected in the blacknose dace. These indicators were monitored in three study streams differing in ANC, and across a gradient of ANC in 11 streams hosting blacknose dace. The underlying premise was that these indicators would be most affected in the streams most sensitive to acidification. The specific research questions associated with this objective were:

1. How do ANC, pH, monomeric aluminum, and calcium concentrations vary among the three test streams?
2. Are there different survival rates of blacknose dace in the three test streams?
3. Is episodic sublethal stress (measured by change in body sodium concentrations) detectable in blacknose dace in the three test streams?
4. Are there differences in the condition factor of blacknose dace among the three intensive streams?

5. What is the relationship (if any) between dace condition factor and measures of stream chemistry across a gradient of ANC in 11 streams?

Site Descriptions

Site Selection Criteria

The three study streams (Figure 6B-2) were chosen to reflect a range in sensitivity to acidification, based on differences in their dominant bedrock types and ANCs (Table 6B-3). The three principal bedrock types in SNP are basaltic, granitic, and silici-clastic petrological assemblages (Gathwright, 1976). Differences in weathering rates, mineral composition, and soils among these types lead to surface waters with different ANC concentrations. Additional streams were chosen for a synoptic study of dace condition factor. All streams had at least quarterly stream chemistry data for the 3 previous years of this study. Altshuller and Linthurst (1983), Winger et al. (1987), and Knapp et al. (1988) have used the criterion of ANC concentrations of < 200 meq/L for the identification of; Lynch and Dise (1985) used a more stringent ANC concentration of < 100 meq/L for acid-*sensitive* surface waters. ANC concentrations of < 50 meq/L were used by Gibson et al. (1983) and Schindler (1988) to identify *extremely sensitive* surface waters.

The soils of the region are primarily ultisols and inceptisols (Hockmman et al., 1979). Soils within the study region are shallow and rocky, with occasional bedrock exposure. Forest cover is nearly complete. The forests are predominately second growth mixed-deciduous. Air temperature, number of frost-free days, and annual precipitation amounts are fairly uniform across the study region (Cosby et al., 1991).

Materials and Methods

Water Chemistry Analyses

Both grab samples and samples collected during selected storm events with ISCO® Model 2900 sequential automated samplers were used in this study. Grab samples were placed on ice and chloroformed (except for aliquots reserved for aluminum analyses) in the laboratory within three days of collection. Storm event samples were removed from the automated sampler and taken to the laboratory within a week of collection. All samples were refrigerated at 4 °C in polyethylene containers until chemical analyses were performed.

pH, ANC, and calcium concentrations were measured for water samples collected weekly and during storm events following the analytical methods of Ryan et al. (1987).

Total monomeric aluminum was analyzed using the McAvoy et al. (1992) adaptation of the automated pyrocatechol violet (PCV) procedure first developed by Røgeberg and Henrickson (1985). An auto-analyzer

(Technicon II, Tarrytown, NY), a manifold employed to mix samples and reagents for the reaction of PCV and Al, and a colorimeter equipped with a 50-mm flow cell and a 590-nm filter were used in this method of determination of monomeric aluminum. The 50-mm flow cell was used to obtain a greater detection limit than that possible using a standard 15-mm flow cell. Two sets of six standards (0, 20, 40, 60, 80, and 100 mg/L monomeric aluminum) bracketed each analytical run. The detection limit was approximately 10 mg/L.

Biological Analyses

Experimental Animals

The blacknose dace, *Rhinichthys atratulus* (family Cyprinidae; order Cypriniformes), ranges from the Atlantic, Great Lakes, Hudson Bay, Mississippi, and upper Mobile Bay drainages from Nova Scotia to Manitoba and south to northern Georgia and northern Alabama (Page, 1991). Blacknose dace are typically found in small, usually cool, gravely or rocky headwaters, creeks, and small rivers of high to moderate gradient. They are common in many areas, particularly in slower runs and pools of mountain and spring-fed streams. Of the three subspecies, *R. a. atratulus* occurs in SNP. Adult length is 40 to 100 mm (Lee, 1980). The breeding season is from late spring through mid-summer. The blacknose dace is an important native forage fish and is found in most of the streams in SNP. Baker and Christensen (1991) estimated the "critical" pH range for the blacknose dace (from 7 studies) to be 5.6 to 6.2.

Fish Capture

Adult blacknose dace native to the test stream were collected by electrofishing at 400 -600 V DC using a Smith-Root Model 12 POW electrofisher. After capture, fish were placed in a bucket and observed for approximately 30 minutes. Fish not vigorously swimming after this time were released and not tested. Fish collected for condition factor analysis were anaesthetized in MS-222, measured, and allowed to recover until swimming vigorously before release back into the stream; incidental mortality due to handling was less than 0.05%.

Fish Exposure Cages

Polyethylene containers (3.2 liter) with four 7 x 12 cm. openings (screened with 1.4 mm fiberglass mesh) held fish during exposure periods. The container design was modeled after Johnson et al. (1987). Six containers were placed in a cage constructed of a plastic laundry basket with a hinged plywood top painted with brown polyurethane (to retard degradation). The cages were placed behind plywood ploughs that served to control water velocity and provide protection from stream debris (Figure 6B-3). Two replicate experimental units were used in each of the three test streams. The experimental units were located in pools within 200 meters of the water-sample collection and hydrological monitoring stations.

<u>Survival (or Mortality) Bioassays</u>

Survival bioassays were conducted from early October 1992 to late November 1993 (the experiment was not performed at Piney River during the fall of 1992 due to time constraints in building the cages). Survival of the experimental animals was checked at weekly (usually) intervals. The median number of fish at each site was 36 per bioassay. Tested fish were released after a 20-40 day bioassay; fish did not loose weight during the bioassay, and some fish gained weight, indicating that the exposure cages permitted at least some feeding. The numbers of dace tested in each stream were 288 in Paine, 195 in Staunton, and 181 in Piney. During the 14-month period, there were 8 bioassays each at Paine and Staunton, and 6 at Piney.

<u>Whole Body Sodium Bioassay</u>

The procedure for this bioassay followed Grippo and Dunson (1991). Adult blacknose dace were collected from each of the test streams during late April 1993. The fish were placed in the experimental cages and allowed to acclimate to the test conditions for approximately one week. Each following week (for a total of nine weeks) a random subsample of 4-6 fish was removed from one exposure cage and immediately killed by a sharp blow to the head. Subsamples of fish were to be collected during large storms but only one such storm occurred during the experimental period; it was not sampled due to the fact that the storm occurred overnight. Sampled fish were transferred to the laboratory in preweighed glass jars in a chilled container. Fish were then weighed, dried at 90°C for at least 24 h, and weighed again, then dissolved in concentrated reagent-grade nitric acid. The resultant solutions were diluted with deionized water (1:99) and then analyzed for sodium concentration with a Thermo Jarrell Ash AA/AE Spectrophotometer Model Smith-Hiefrje 22.

<u>Condition Factor</u>

For comparisons among the three intensive streams, condition factors were calculated for samples of fish collected from each stream by electrofishing during four 2- or 3-day sampling periods (Staunton River was sampled only three times, omitting December 1993) in June 1993, September 1993, October 1993 and December 1993. Sample sizes were 49-255 dace per stream per sampling date. A Fulton-type (Anderson and Gutreuter, 1983) condition factor was calculated as the weight (to the hundredth gram) divided by the cube of the total length (snout to the posterior-most portion of the tail) in mm multiplied by a scaling constant of the value 10^6.

For comparisons among a larger group of streams, a second set of dace collections was made in 11 streams in the last week of July 1994. Sample sizes were 25-51 dace per stream. The purpose of collecting nearly simultaneously in all streams was to avoid seasonal effects on K, as demonstrated in the multiple surveys in the intensive streams (Figure 6B-17, showing highest condition in June) and in Chapter 5D. For example, the data on dace length and weight presented in Chapter 5D were collected in June-August 1994, spanning times of reproduction, post spawning, and summer growth, all of which might affect condition of fish; dace were clearly in reproductive condition in June, 1994, when dace in Brokenback Run were sampled, and showed the heaviest weights of dace in streams sampled that summer (Table 5D-3 and Chapter 5 text). Thus females carrying eggs would be sampled in June, but not August. Water chemistry data for this analysis included 15 quarterly (spanning the previous 3.5 years, the maximum life span of blacknose dace; Reed and Moulton, 1973) streamwater samples for each stream collected between January 1991 and July 1994. The quarterly samples were collected in all streams during the last weeks of January, April, July and October, with the exception of two samples collected in the first week of February rather than the last week of January.

In addition to the three "intensive" streams (Paine, Staunton, Piney) and five "extensive" streams (Meadow, Twomile, White Oak, Brokenback, North Fork Dry), time during the July 1994 sampling period permitted the collection and analysis of dace from three additional streams, for which quarterly stream chemistry data from UVA's SWAS program were also available; these data were incorporated into the relationship between stream ANC and blacknose dace condition factor. These three streams (Hazel, Rose, and North Fork Thornton) are labeled "SWAS" below. The reason for their inclusion was that quarterly chemistry for at least 3.5 years (the maximum life span of blacknose dace; Reed and Moulton, 1973) for each was on hand (as for the 3 intensive and 5 extensive streams) and they also provided the opportunity to include higher ANC streams in this that analysis. Thus 11 streams with water chemistry and dace condition factor were available for the stepwise linear regression analysis described under "Statistics" below.

Eleven streams from which dace were collected in July, 1994

SNP: FISH intensive	SNP: FISH extensive	SWAS
Paine	Meadow	Hazel
Staunton	Twomile	Rose
Piney	While Oak	North Fork Thornton
	Brokenback	
	North Fork Dry	

Statistics

SPSS for Windows Release 6 was used for statistical analyses. Survival bioassay results were analyzed using the KAPLAN-MEIER procedure with the log rank test to determine if the survival functions (rates) of each stream were equal. ANCOVA models evaluating stream effects for both the body sodium bioassay and condition factor survey (controlling for differences in weight and length, respectively) were generated using the GENERAL FACTORAL ANOVA procedure. Evaluation of all pairwise stream-effect comparisons for both the body sodium bioassay and condition factor survey was planned *a priori*. Simple linear contrasts with an error rate set at 0.05 were performed to evaluate the stream-effect pairwise comparisons. The experiment-wise error rate for all linear contrasts for both ANCOVA models was 0.143.

Staview II was the statistical package used for stepwise multiple linear regression analysis using dace condition and water chemistry from 11 streams. The candidate water chemistry variables were the median, minimum, and maximum values for the concentrations of ANC, conductivity, calcium, sodium, nitrate, sulfate, potassium, silica, chloride, and hydrogen ion, from water chemistry samples collected quarterly (15 occasions) from January 1991 to July, 1994. Theses values for each stream were used as the independent variables in a stepwise multiple linear regression, with condition factor (K) of the dace from the same stream as the dependent variable. Separate analyses were performed for the a) the three intensive streams, b) the three intensive plus five extensive streams, and c) the intensive and extensive streams plus three additional streams for which water chemistry and blacknose dace condition data had been collected.

Results

Water Chemistry Analyses

Weekly and episodic ANC, pH, and concentrations of total monomeric aluminum and calcium for test streams during the survival bioassay (10/1/92 to 11/23/93) are shown in Figures 4 to 8, respectively. ANC is plotted versus pH for each stream during the same time period in Figure 6B-9; calcium is plotted versus ANC in Figure 6B-10. Figure 6B-11 shows box plots of aluminum values during the dace bioassay for all three streams. There was no aluminum data from late November, 1992 to early March, 1993 due to equipment failure.

There were substantial differences in ANC among the streams. Using the acidification-sensitivity classifications of Lynch and Dise (1985), Gibson et al. (1983), and Schindler (1988), Piney River may be considered to have been relatively *insensitive*, Staunton River *sensitive*, and Paine Run *extremely sensitive* to acidification during the experimental period.

The variation in ANC among the streams was reflected in stream differences of pH, storm-event total monomeric aluminum, and calcium concentrations. Base flow calcium concentrations during the bioassay averaged 146-, 70-, and 34 µeq/L for Piney River, Staunton River, and Paine Run, respectively. Piney River pH was never in Baker and Christensen's critical pH range for blacknose dace (5.6 to 6.2) during the experimental period. pH values for Staunton River were in the upper part of this range (the minimum pH during the experimental period was 6.03) on several occasions but the total time that pH was below 6.2 was only ten days. Paine Run pH, on the other hand, *remained* in this critical range for all except *two* samples for the entire experimental period. Storm events produced pH depressions as low as 4.98 in Paine Run.

During this bioassay, total monomeric aluminum during storm events was highest at Paine Run (102 µg/L). Figures 6b and 7 show several unusually high concentrations of total monomeric aluminum for Staunton River and Piney Rivers relative to the associated pH values (Figure 6B-5). These high aluminum concentrations all come from samples collected during storm events. There are little data concerning changes in total monomeric aluminum concentrations during storm events for circumneutral and high ANC streams. One explanation is that these high concentrations may be due to the increased particulate load associated with storm run-off; the PCV method may not be completely monomer-specific (Santore, pers. comm.). The solubility of aluminum in waters of high ANC and the sensitivity of the PCV method to particulate aluminum are complex issues and deserve further attention.

Biological Analyses

Survival Bioassay

Percent survival of blacknose dace during the 14 month experimental period was high (generally >90%) in all bioassays, with only two instances of high mortality in individual bioassay cages at Piney River (the reference, or high-ANC, site) attributed to siltation following high flow events. Kaplan-Meier survival analysis indicated no difference in median survival time between Staunton River (intermediate ANC) versus Paine Run (low ANC).

Body Sodium

Stream ANC, pH, total monomeric aluminum, and calcium concentrations varied little during the experimental period (Figures 12-15), and can be described as base flow conditions during the nine-week bioassay. The results of the ANCOVA indicated significant stream and week differences. Pairwise linear contrasts testing the stream effect revealed that Paine Run and Staunton River dace body sodium concentrations were both significantly higher than those of Piney River ($p<0.0005$ and $p<0.005$. respectively). Paine Run whole body sodium concentrations were significantly higher than those in Staunton River ($p< 0.01$).

Condition Factor - Seasonal pattern in the intensive streams.

Mean condition factors for all sampling dates in the intensive streams (Paine, Staunton and Piney) are shown in Figure 6B-17. Summary descriptive statistics of mean weights, lengths, and length-adjusted condition factors of all fish samples are given in Tables 5-7. Results of an ANCOVA comparing condition factors (adjusted for differences in length) among all streams for the first three sampling dates indicated a significant stream effect ($p < 0.000$). Significance values and sample sizes of simple linear contrasts of all pairwise stream comparisons are shown in Table 6B-8. The results of a second ANCOVA comparing length-adjusted condition factors of Paine Run (mean = 8.18, S.D. = 0.80, n = 50) and Piney River fish (mean = 8.93, S.D. = 0.74, n = 49) on a fourth sampling date indicated that Paine Run condition factors were significantly lower ($p < 0.000$). Thus, differences among intensive streams over time were apparent.

Condition Factor - Water chemistry and dace condition factor differences among 11 streams

In the three analyses discussed below, pH minimum (Table 6B-8) was the first variable entered into the multiple stepwise regression, with r-squared values of 0.818 to 0.970, explaining 81.8% to 97% of the variance in the data. Since additional variables explained only a few percent of the variance, the results are presented as simple linear regressions using only pH minimum; this was the form of the relationship entered into the predictive model discussed in Chapter 7.

Condition factors for blacknose dace collected July, 1994 in the three intensive streams are shown in Figure 6B-18, together with the results of the regression analysis which yielded Figure 6B-18. Despite the inclusion of only three streams, the regression is highly significant ($p = 0.0001$).

Condition factors for blacknose dace collected July 1994 in the three intensive plus five extensive streams are shown in Figure 6B-19, together with the results of the regression analysis, which yielded Figure 6B-19. The same pattern appears as in Figure 6B-18, with a highly significant p value (0.0001).

Condition factors for blacknose dace collected July, 1994 in the three intensive, five extensive, and three additional SWAS streams are shown in Figure 6B-20, together with the results of the regression analysis which yielded Figure 6B-20. The addition of three additional streams yields a similar pattern as in Figures 18 and 19. The greater scatter among values in upper range of minimum pH may indicate that above some pH value above about 6.0, factors other than pH minimum may affect condition factor.

Discussion

Comparison Of Results To Related Studies

Schofield and Driscoll (1987) found that blacknose dace transplanted from Moss Lake inlet (pH 6.5 - 7.1; total monomeric aluminum 5 -73µg/L) to Merriam Lake outlet (pH 4.4 - 4.8; total monomeric aluminum 296 - 499 µg/L) suffered 100 % mortality after 28 days of exposure. The median survival time was 2.7 days. Johnson et al. (1987) observed, using a series of *in situ* bioassays within the North Branch of the Moose River basin, that adult blacknose dace experienced 100 % mortality after 144 hours of exposure in waters of pH as high as 5.32. Inorganic monomeric aluminum concentrations as low as 20 µg/L (pH = 4.96) were associated with 70 % mortality after 144 hours. (Driscoll and Bisgoni, 1984, determined on a sample of 321 Adirondack surface waters that the inorganic monomeric fraction made up 56 % of total monomeric aluminum species. It should be noted that during storm events, inorganic monomeric aluminum species form an increasingly larger fraction of total dissolved aluminum as flow increases (Kretser et al., 1991). On the other hand, adult blacknose dace in waters with pH as low as 5.12 and inorganic monomeric aluminum concentrations as high as 150 µg/L experienced only 0 to 40 % mortality after 144 hours.

Simonin et al. (1993) examined the effects of acidic storm events on the survival of adult blacknose dace. They found, in two bioassays conducted in Bald Mountain Brook and Seventh Lake Inlet, that after 24 days of acclimation to base flow conditions, adult blacknose dace experienced 0 % mortality in water of median pH 5.86 and median inorganic monomeric aluminum 42 µg/L (peak concentration = 146 µg/L) and 5 % mortality in water of median pH 5.47 and median inorganic monomeric aluminum concentration of 64 µg/L (peak concentration = 118 µg/L). These bioassay results are consistent with the results of the ERP, which found the blacknose dace were absent from streams with median high flow pH < 5.2 and inorganic-monomeric aluminum > 100µg/L (Baker et al., 1996).

Schofield and Driscoll (1987) observed that blacknose populations occurred only in lakes in the Adirondack region with summer base flow pH above 6. Kretser et al. (1989), as reported in Simonin et al. (1993), presented survey data of 1469 Adirondack waters, which showed that blacknose dace, occurred at a minimum (annual) pH of 5.59. Paine Run summer base flow pH was about 6 during the experimental period. The winter and early spring base flow minimum was between 5.5 and 5.6. Thus, pH throughout the year in Paine Run is near the limits of occurrence for blacknose dace populations in the Adirondack region. The Episodic Response Project (Van Sickle et al. 1996) found (also in the Adirondacks) that bioassay exposures of blacknose dace to >200µg/L inorganic monomeric aluminum for more that about 4 days resulted in substantial mortality. Conditions did not approach that severity of exposure in this study.

The results of the condition factor comparisons among the three intensive streams indicated that the mean length-adjusted condition factor of fish from Paine Run was about 11 % lower than the Piney and Staunton River values. No studies exist reporting changes in condition factor of blacknose dace during acidification. However, dramatic changes were associated with a 12 % reduction in lake trout condition factor in a study by Schindler (1987). The growth of 2-6 year old fish and annual survival rate had declined significantly by year 8 of the study. Larval recruitment had failed completely. Stomachs of trout analyzed during the last analyzed year of the study were always empty, indicative of the very low amount of food resources. Substantial changes had also occurred in much of the other lake biota. Key organisms in the food web were eliminated from the lake at pH as high as 5.8. There was no successful recruitment by any other species of fish (six species) in the lake. Crayfish, leeches, and the mayfly Hexagenia, previously abundant in the lake, were extirpated. These changes were found to be caused by the hydrogen ion alone, and not by the secondary effect of aluminum toxicity, because the lake itself was acidified, whereas the source of aluminum in most instances is catchment soil (Driscoll, 1989). The range of total monomeric aluminum concentration observed in lake 223 was only 7 to 36 µg/L, far less than the concentrations that have caused mortality among fishes during episodic events.

Integration of Biological Results

The *egg* and *larval* life-history stages of fish are generally more sensitive to acid/aluminum stress than the adult stage. For example, studies indicate that brook trout eggs and fry experience mortality in a low-ANC versus high-ANC stream in SNP (MacAvoy and Bulger, this report). Recruitment failure is often cited as a primary mechanism leading to fish population declines due to acid/aluminum stress (Jensen and Snevik, 1972; Peterson et al., 1982; Mills et al., 1987). Differences in mortality rates of the egg and larval life-history stages of blacknose dace may presently occur among the three test streams despite the fact that no differences in acidification-related mortality were detectable for adults.

In addition to differences in acidification-related water chemistry, several other environmental factors may affect fish condition. This list of environmental factors includes differences in: thermal and drought stresses, nutrient concentrations, primary productivity, and intra- and interspecific competition. Analysis of weekly temperature data measured during the survival bioassay show that the mean and minimum temperatures of the three streams are within 1 C, and that the maximum temperatures are within 3 C. Measurements of stage height recorded weekly throughout the course of the study indicate that stream flow is much more variable during much of the year in the mid- and high ANC streams than in the low ANC stream. Summer flows were found to be very similar in all streams. Schindler et al. (1985) observed that phytoplankton production, rates of decomposition, and nutrient concentrations did not decrease in Lake 223 during eight years of acidification; these two factors were not responsible for the dramatic decline observed in lake trout condition. Currently, the only evidence concerning competition effects on condition for this study are observations made of the effort required to catch fish during electrofishing. Roughly the same amount of time was needed to catch a prescribed number of fish for the low ANC stream as for the high ANC stream; this time was about half of that required for the mid-ANC stream. Although the evidence presented is certainly imperfect, it does suggest, at least indirectly, that the environmental factors discussed above are not primarily responsible for the poorer condition of Paine Run blacknose dace.

There were substantial differences in among stream sodium concentrations during the 14-month survival bioassay period. Base flow concentrations ranged seasonally from approximately 21 to 23 µeq/L in Paine Run, from 60 to 70 µeq/L in Staunton River, and from 80 to 120 µeq/L in Piney River. Subsequently, the gradient in sodium concentration between a fish and its environment varied among the three streams. This gradient was approximately 3 to 3.5 times lower for fish from Staunton River and 4 to 6 times lower for fish from Piney River than for fish from Paine Run. The difference in this gradient between Staunton River and Piney River was relatively insignificant; fish from Piney River experienced on average a gradient 1 to 2 times lower than fish from Staunton River. Since acidification interferes with ion balance, the body sodium results are the opposite of what one might expect: the dace in the lowest-ANC stream had the highest sodium level, despite higher blood versus stream concentration gradients in sodium, plus the effects of chronic and acute acidification.

The energy costs to fish for active iono-osmoregulation can be substantial (Farmer and Beamish, 1969; Bulger, 1986). Paine Run dace, exposed to a steeper iono-osmotic gradient, probably devote a larger proportion of their metabolic resources to iono-osmoregulation, at the expense of somatic growth. We can speculate that their higher body sodium concentration may be the result of physiological overcompensation. This, coupled with the fact that Paine Run dace are more likely to suffer gill damage and subsequent passive loss of osmolytes to the environment due to periodic pH depressions, may at least partially explain why Paine Run dace are in poorer condition than those from Staunton and Piney Rivers.

Future Considerations

Webb et al. (1989) used a simple linear model to relate changes in streamwater base cation concentrations to changes in concentrations of SO_4^{2-} as streams in the mountains of western Virginia approach steady state with respect to *atmospheric* SO_4^{2-} deposition. Their estimates of potential acidification were made assuming base cation increase factors equal to 0.4 and 0.8 times the expected SO_4^{2-} increase. The median future ANC loss was estimated as 90 and 30 µeq/L, respectively, for the two assumed factors. Using the conservative estimate of 30 µeq/L, it is interesting to consider what sorts of changes might occur to pH, aluminum concentrations, and the population of blacknose dace in Paine Run and streams with similar ANC.

The median ANC for Paine Run during the 14 month experimental period of this investigation was 1.9 µeq/L (n = 179). It is possible to roughly estimate the corresponding changes in pH and total monomeric aluminum concentrations that would occur given a 30 ueq/L reduction in ANC, by first regressing pH with ANC, using the values of these parameters measured during the experimental period. One can then reduce the present median ANC by 30 µeq/L and solve the regression equation (pH = 0.0467[ANC] + 5.48; R^2 = 0.86) to obtain an estimate of pH associated with the reduced ANC. Doing so produces a median pH estimate of 4.18 for Paine Run. Although this is a crude estimate of the potential equilibrium pH and does not consider the buffering effects of aluminum and organic acids at very low pH, this value is well below the 5.5 to 6.0 threshold range estimated by Mills and Schindler (1986) below which many cyprinid species are negatively impacted. In all instances in similar studies adult blacknose dace (and many other species) have experienced significant mortality at pH values higher than 4.18.

Following a similar procedure of that above for pH and total monomeric aluminum (except using a second-order polynomial equation: total monomeric aluminum = $31.51[pH]^2 - 411.79[pH] + 1345.9$; R^2 = 0.62) a median pH of 4.18 corresponds to an estimated median total monomeric aluminum concentration of 175 µg/L. In light of the results of Schindler et al. (1987), a base flow total monomeric aluminum concentration of 175 µg/L could be associated with significant population declines in certain species of fish. Given that fry have been found to be more sensitive than adults (Johnson et al., 1987), significant blacknose dace population effects can be expected to occur if such changes in ANC and aluminum were to take place in Paine Run and other low ANC streams in SNP.

Conclusions

pH and total monomeric aluminum concentrations were observed in Paine Run that have produced deleterious organismal, population, and community effects in other localities. Significant and perennial differences in condition factor were found to exist between the low-ANC versus the high-ANC stream. Differences in condition factor of the magnitude found in this study have been associated with profound deleterious population effects in other studies. Comparison of dace collected nearly simultaneously in 11 streams show a strong relationship between minimum pH and condition factor. No acidification-related differences in survival of adult blacknose dace were found among the three test streams during 14 months of investigation. Paine Run dace had whole-body sodium concentrations which were elevated relative to fish from Piney and Staunton Rivers. Perhaps adult blacknose dace use energy in the low-ANC streams that might be diverted to growth and higher condition factor in non-acidified streams.

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Fish Species of Shenandoah National Park N = 28 + 2 rare forms (one of which is a hybrid)			“critical pH” from Baker & Christensen (1991)
COMMON NAME	*LATIN NAME*	FAMILY	
American Eel	*Anguilla rostrata*	Anguillidae	
Mtn. Redbelly Dace	*Phoxinus oreas*	Cyprinidae	
Rosyside Dace	*Clinostomus funduloides*	Cyprinidae	
Longnose Dace	*Rhinichthys cataractae*	Cyprinidae	
Blacknose Dace	*Rhinichthys atratulus*	Cyprinidae	5.6 to 6.2
Central Stoneroller	*Campostoma anomalum*	Cyprinidae	
Fallfish	*Semotilus corporalis*	Cyprinidae	
Creek Chub	*Semotilus atromaculatus*	Cyprinidae	5.0 to 5.4
Cutlips Minnow	*Exoglossum maxillingua*	Cyprinidae	
River Chub	*Nocomis micropogon*	Cyprinidae	
Bluehead Chub	*Nocomis leptocephalus*	Cyprinidae	
Common Shiner	*Luxilus cornutus*	Cyprinidae	5.4 to 6.0
Northern Hogsucker	*Hypentelium nigricans*	Catostomidae	
Torrent Sucker	*Thoburnia rhothocea*	Catostomidae	
White Sucker	*Catastomus commersoni*	Catostomidae	4.7 to 5.2
Margined Madtom	*Noturus insignis*	Ictaluridae	
Brook Trout	*Salvelinus fontinalis*	Salmonidae	4.7 to 5.2
Brown Trout	*Salmo trutta*	Salmonidae	4.8 to 5.4
Tiger Trout*	*Salmo X Salvelinus*	Salmonidae	
Rainbow Trout	*Oncorhynchus mykiss*	Salmonidae	4.9 to 5.6
Mottled Sculpin	*Cottus bairdi*	Cottidae	
Rock Bass	*Ambloplites rupestris*	Centrarchidae	4.7 to 5.2
Smallmouth Bass	*Micropterus dolomieui*	Centrarchidae	5.0 to 5.5
Largemouth Bass	*Micropterus salmoides*	Centrarchidae	
Redbreast Sunfish	*Lepomis auritus*	Centrarchidae	
Pumpkinseed	*Lepomis gibbosus*	Centrarchidae	
Johnny Darter	*Etheostoma nigrum*	Percidae	
Tesselated Darter	*Etheostoma olmstedi*	Percidae	
Fantail Darter	*Etheostoma flabellare*	Percidae	
Greenside Darter*	*Etheostoma blennioides*	Percidae	

*rare (total of 6-10 individuals collected)

Table 6B-1. The fish species of Shenendoah National Park and (where available) the "critical pH" value reported for each by Baker and Christensen (1991).

hydrolysis reaction	log K°
$AL^{3+} + H_2O = ALOH^{2+} + H^+$	-4.99
$AL^{3+} + 2H_2O = AL(OH)^{2+} + 2H^+$	-10.13
$AL^{3+} + 4H_2O = AL(OH)^{2+} + 4H^+$	-22.16
$2AL^{3+} + H_2O = AL(OH)^{2+} + 2H^+$	-7.69

Table 6B-2. Hydrolysis reactions and equilibrium constants for the aquo aluminum ion (from Lindsay and Whitehall, 1989).

	Paine Run	Staunton River	Piney River
Sensitivity to Acidification	extremely sensitive	sensitive	insensitive
Area (km^2)	12.71	10.56	12.66
Watershed Aspect	western	eastern	eastern
SNP District	South	Central	North
Major Bedrock Type	silici-clastic	granitic	basaltic
Bedrock Geology	91% Hampton 9% Antietham	82% Pedlar 4% Old Rag	68% Catoctin 31% Pedlar
Vegetation Classification	96% CO/P 2% H/YP/CH	48% CO/P 11% RO/BL 37% H/YP/CH	36% CO/P 18% RO/BL 42% H/YP/CH
Site Elevation (m)	417	303	342

Table 6B-3. Physical and vegetative characteristics of the three intensive test watersheds. CO/P represents chestnut oak and pine; RO/BL represents red oak and black locust; H/YP/CH represents hemlock, yellow poplar, and cove hardwood. Classifications from Teetor (1988).

Label	mean (g/mmex3 x 10ex6)	standard error	no. cases
entire population	9.6	0.03	1301
Paine Run	9.05	0.05	484
6/16/93	9.49	0.06	249
9/17/93	8.45	0.11	101
10/30/93	8.98	0.16	84
12/4/93	8.19	0.11	50
Staunton River	10.08	0.08	322
6/17/93	10.34	0.07	191
9/18/93	9.5	0.11	57
10/30/93	9.86	0.22	74
Piney River	9.83	0.05	495
6/18/93	10.24	0.08	255
9/19/93	9.22	0.08	103
10/31/93	9.85	0.09	88
12/5/93	8.91	0.11	49

Table 6B-4. Length–adjusted condition factors: means, standard errors, and samples sizes for each of the sampling dates.

Label	mean (mm)	standard error	no. cases
entire population	54.2	0.5	1301
Paine Run	50.7	0.7	484
6/16/93	50.4	0.9	249
9/17/93	56	1.5	101
10/30/93	47.6	1.6	84
12/4/93	46.3	1.6	50
Staunton River	61.9	1.2	322
6/17/93	64.7	1.4	191
9/18/93	64.2	2.8	57
10/30/93	53	2.7	74
Piney River	52.7	0.8	495
6/18/93	51.6	1.2	255
9/19/93	57	1.7	103
10/31/93	52.1	1.8	88
12/5/93	50.1	2.1	49

Table 6B-5. Lengths: means, standard errors, and samples sizes for each of the sampling dates.

Label	mean (g)	standard error	no. cases
entire population	2.13	0.06	1301
Paine Run	1.47	0.07	484
6/16/93	1.53	0.1	249
9/17/93	1.79	0.18	101
10/30/93	1.2	0.13	84
12/4/93	0.95	0.13	50
Staunton River	3.28	0.18	322
6/17/93	3.6	0.26	191
9/18/93	3.37	0.45	57
10/30/93	2.4	0.28	74
Piney River	2.03	0.09	495
6/18/93	2.13	0.13	255
9/19/93	2.23	0.22	103
10/31/93	1.84	0.2	88
12/5/93	1.43	0.2	49

Table 6B-6. Weights: means, standard errors, and samples sizes for each of the sampling dates.

stream comparison	combined sample size (total number of fish)	significance value
Paine *vs.* Staunton	756	$p<0.001$
Paine *vs.* Piney	880	$p<0.001$
Staunton *vs.* Piney	768	$p<0.1$

Table 6B-7. Sample sizes and linear contrasts of all pairwise comparisons of length-adjusted condition factors for fish in the three intensive streams.

STREAM	pH minimum	pH min date	mean K (adjusted)	n
Intensive streams				
PAINE	5.18	30-Jul-91	8.169	39
STAUNTON	6.29	30-Jan-91	9.689	43
PINEY	6.75	28-Apr-92	10.259	48
Extensive streams				
MEADOW	4.97	30-Jul-91	8.087	28
TWOMILE	5.37	30-Jul-91	8.514	31
WHITE OAK	5.73	28-Jul-92	8.775	25
BROKENBACK	6.25	30-Jan-91	9.844	51
NORTH FORK DRY	6.28	28-Jan-92	9.284	48
SWAS streams				
HAZEL	6.23	29-Jan-92	9.181	41
ROSE	6.59	29-Jan-92	9.768	42
NORTH FORK THORNTON	6.71	28-Jul-92	9.489	47
TOTAL				443

Table 6B-8. Blacknose dace condition factors in 11 streams in July 1994, with pH minimum recorded during the previous 3.5 years, its date, and samples sizes.

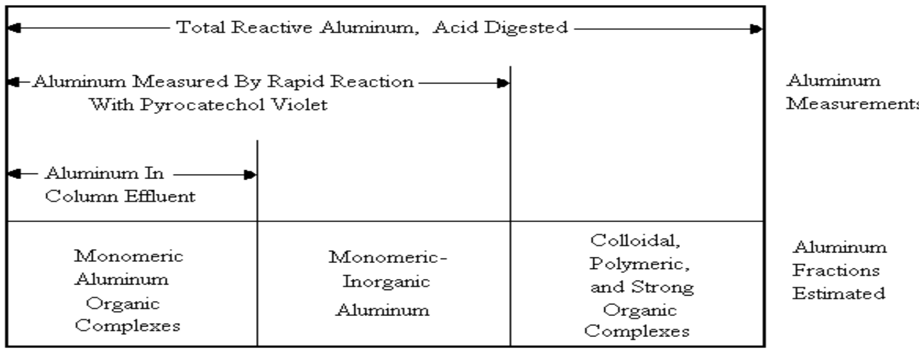


Figure 6B-1. Schematic representation of aluminum fractions (from Driscoll, 1984).

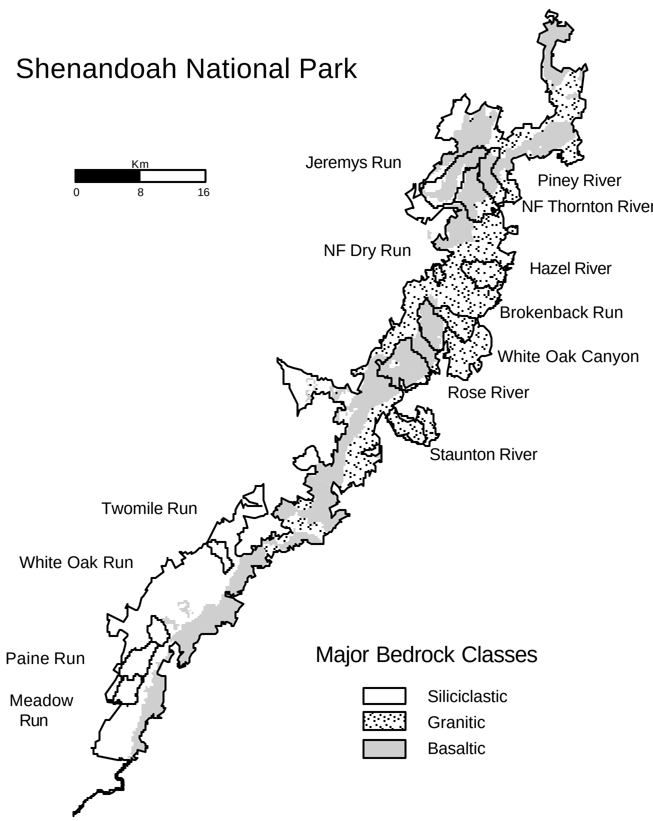


Figure 6B-2. SNP: FISH study sites and catchments in Shenandoah National Park. Intensive sites (Piney River, Staunton River, Paine Run) were gauged for discharge measurements, had continuous stage recorders, and both 8-hour and stage-activated automatic water samplers. Water samples were also collected by staff weekly. These streams were the sites of bioassays, plus fish and habitat surveys in each year of the study (1992-95). Extensive sites were sampled for water chemistry at least quarterly, and fish and habitat surveys were conducted at least once in the study years.

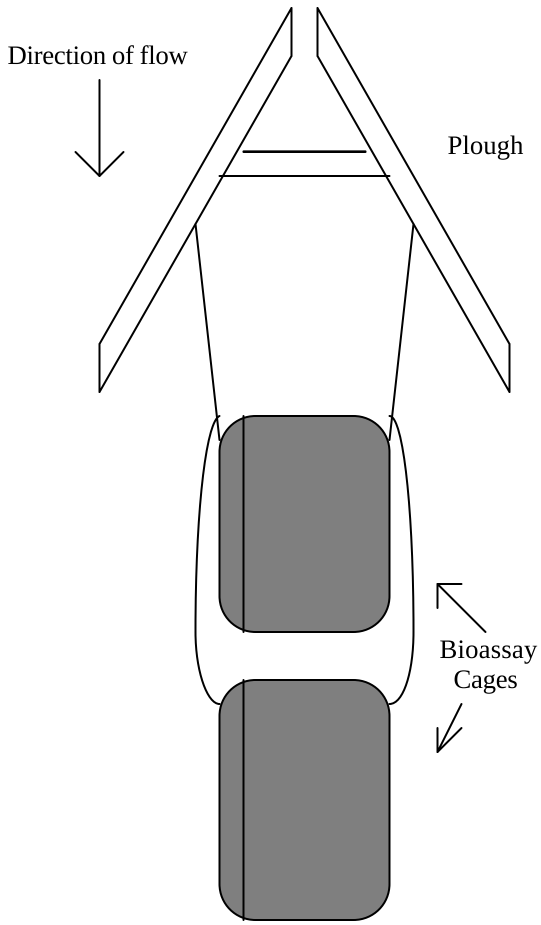


Figure 6B-3. Bioassay field site set-up. A wooden plough protects the bioassay containers with cages inside from high flow (Overhead view), while the slot (center top) admits stream flow even under low-flow conditions. Multiple bioassay cages could be placed in each of the two large containers shown, and could be withdrawn individually with minimal disturbance to the remaining cages. The two wings of the plough are about 1.5 m long. The ploughs were heavily built and weighted with about 100 kg of stone from the stream; they were also chained to upstream trees.

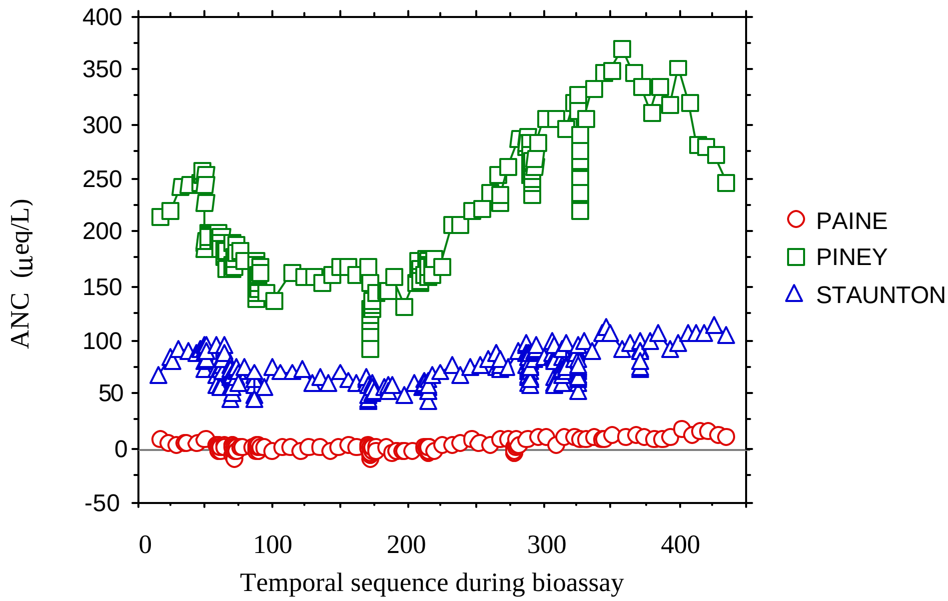


Figure 6B-4. ANC concentration during the dace survival bioassay, from 10/1/92 to 11/23/93.

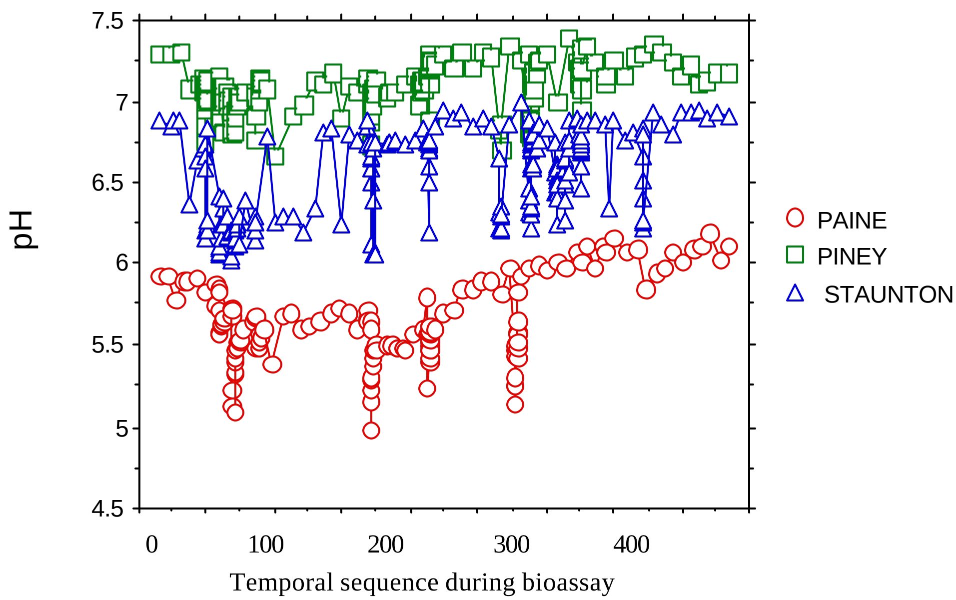


Figure 6B-5. pH during the dace survival bioassay, from 10/1/92 to 11/23/93.

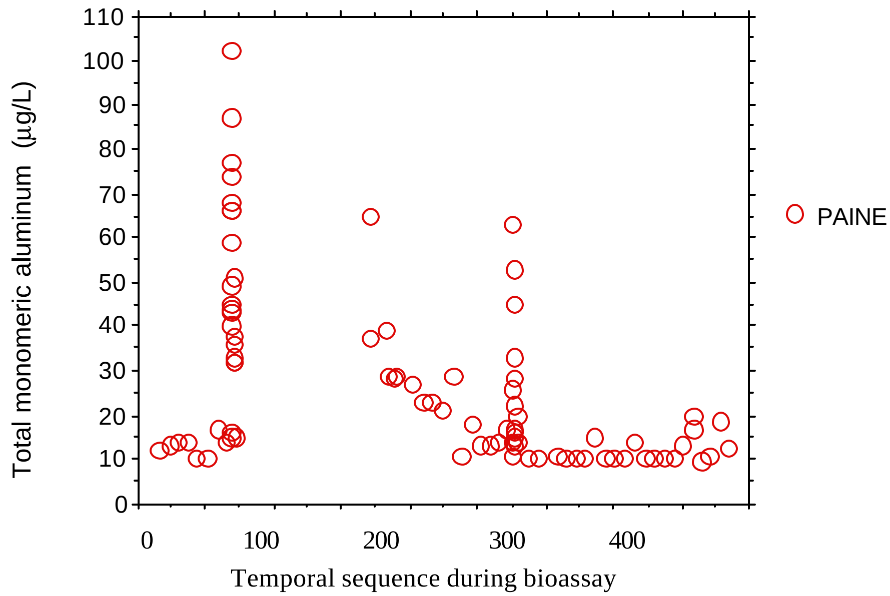


Figure 6B-6a. Paine Run total monomeric Al (µg/L) during dace survival bioassay, from 10/1/92 to 11/23/93. Note that Figures 6B-6a, 6B-6b and 6B-7 have different vertical ranges.

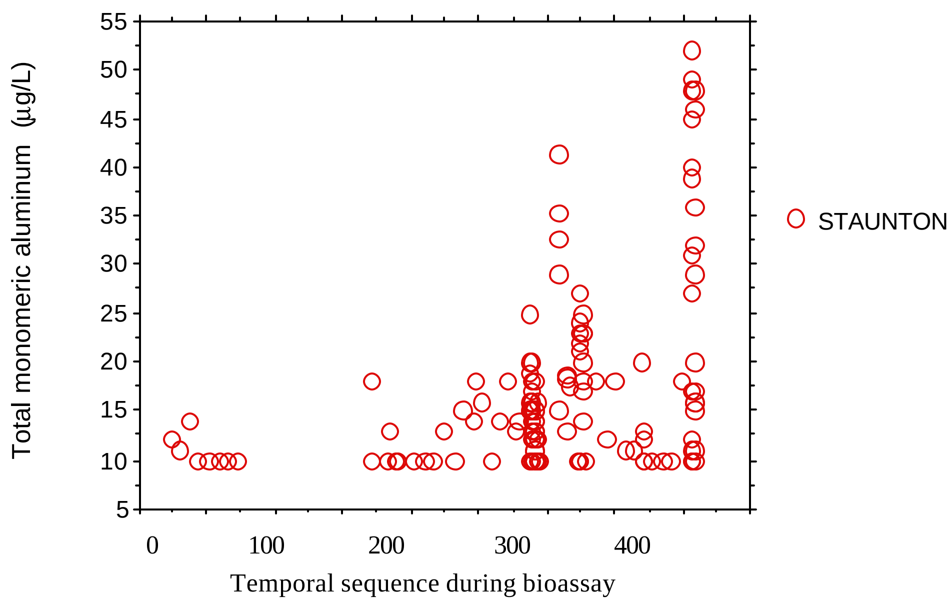


Figure 6B-6b. Staunton River total monomeric Al (µg/L) during dace survival bioassay, from 10/1/92 to 11/23/93. Note that Figures 6B-6a, 6B-6b and 6B-7 have different vertical ranges.

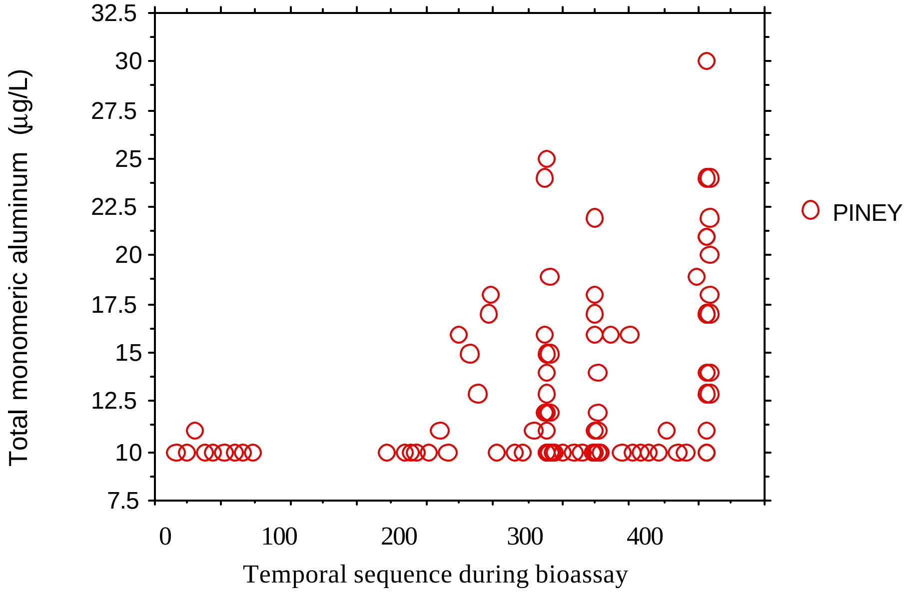


Figure 6B-7. Piney River total monomeric Al (µg/L) during dace survival bioassay, from 10/1/92 to 11/23/93. Note that Figures 6B-6a, 6B-6b and 6B-7 have different vertical ranges.

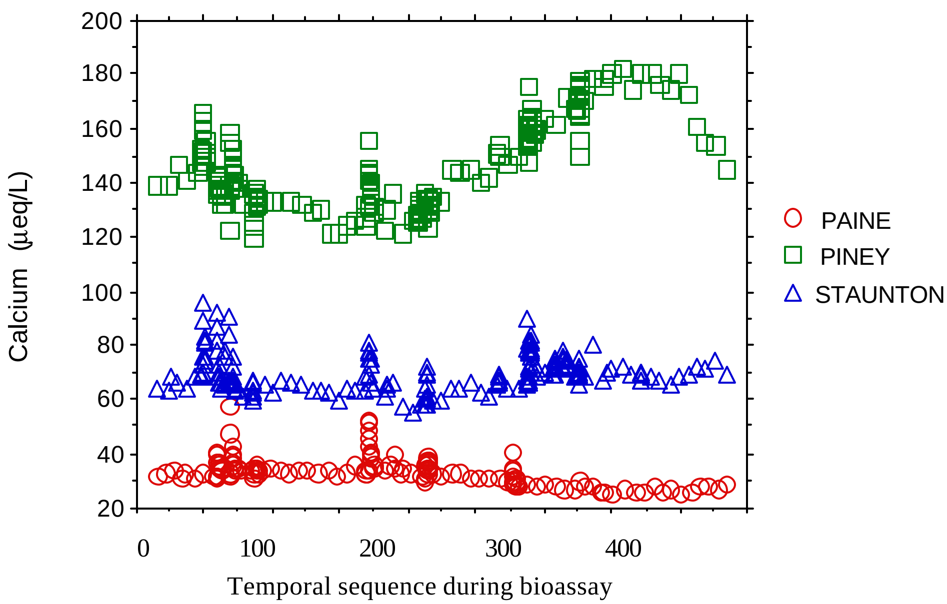


Figure 6B-8. Calcium concentrations during the dace survival bioassay, from 10/1/92 to 11/23/93.

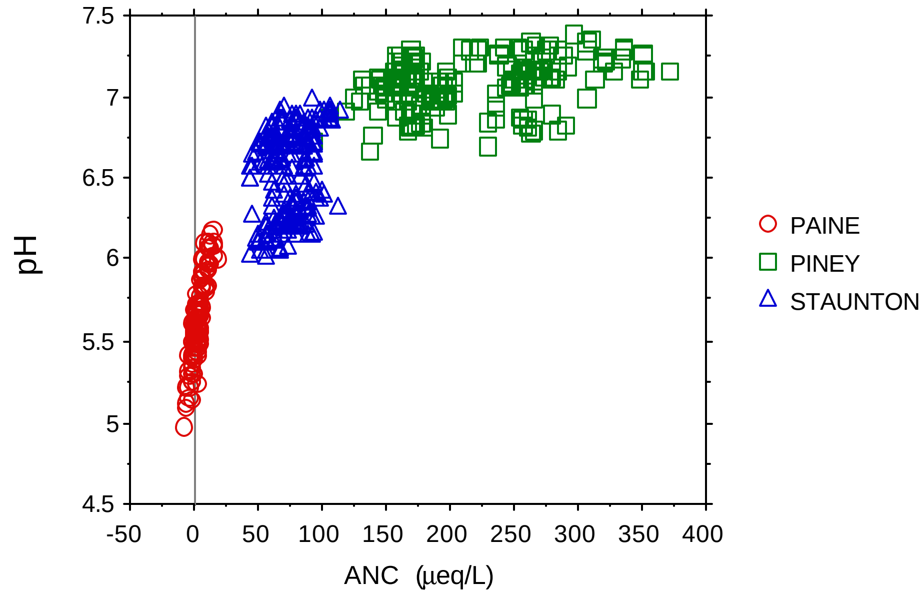


Figure 6B-9. pH versus ANC during the dace bioassay from 10/1/92 to 11/23/93, for all three streams.

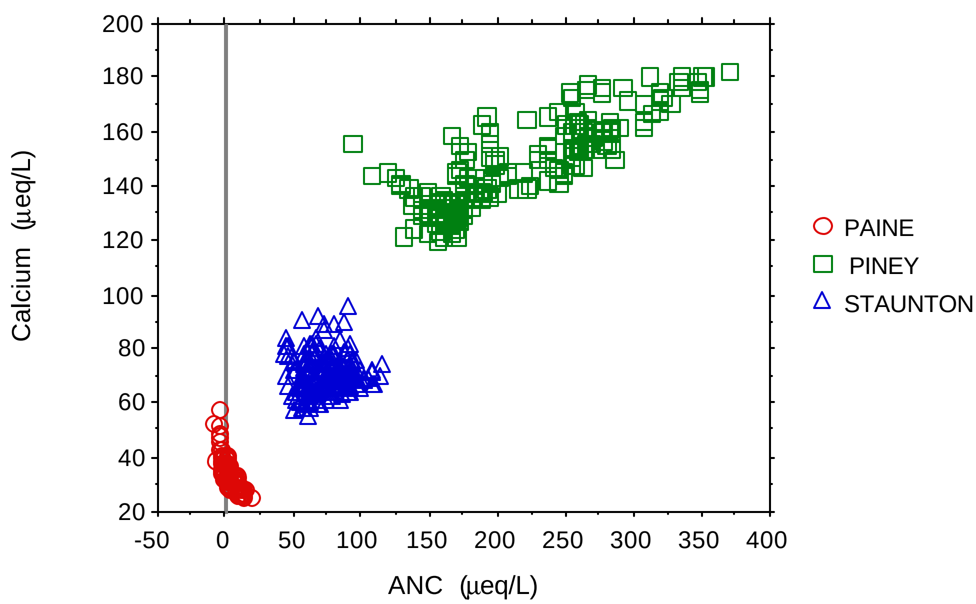


Figure 6B-10. Calcium versus ANC during the dace bioassay from 10/1/92 to 11/23/93, for all three streams.

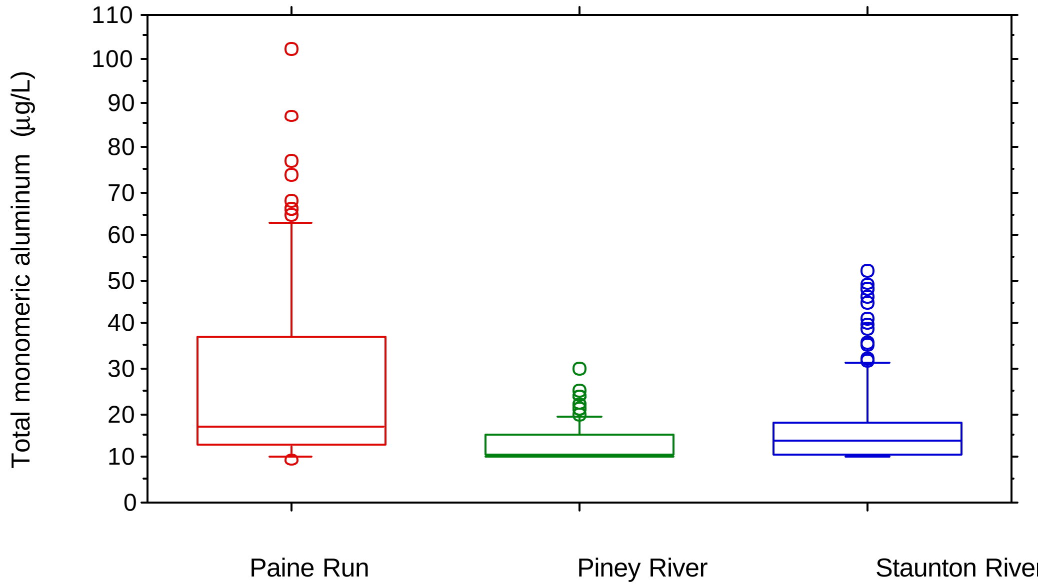


Figure 6B-11. Box plots of aluminum values during the dace bioassay, from 10/1/92 to 11/23/93, for all three streams. Shown are 10th, 25^{th,}, 50th, 75th, and 90th percentiles as horizontal lines, and values beyond these percentiles are shown as circles.

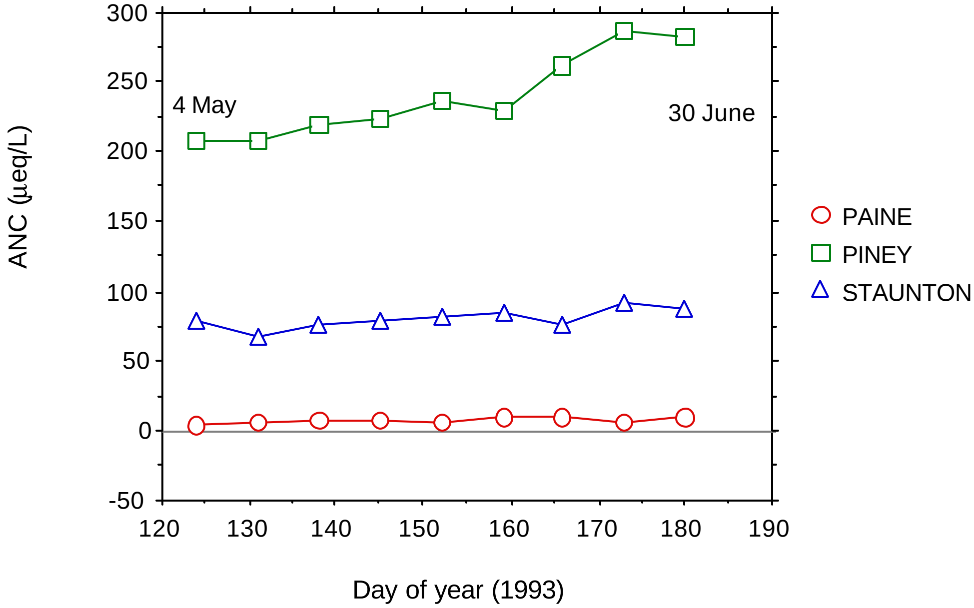


Figure 6B-12. ANC during body sodium bioassay.

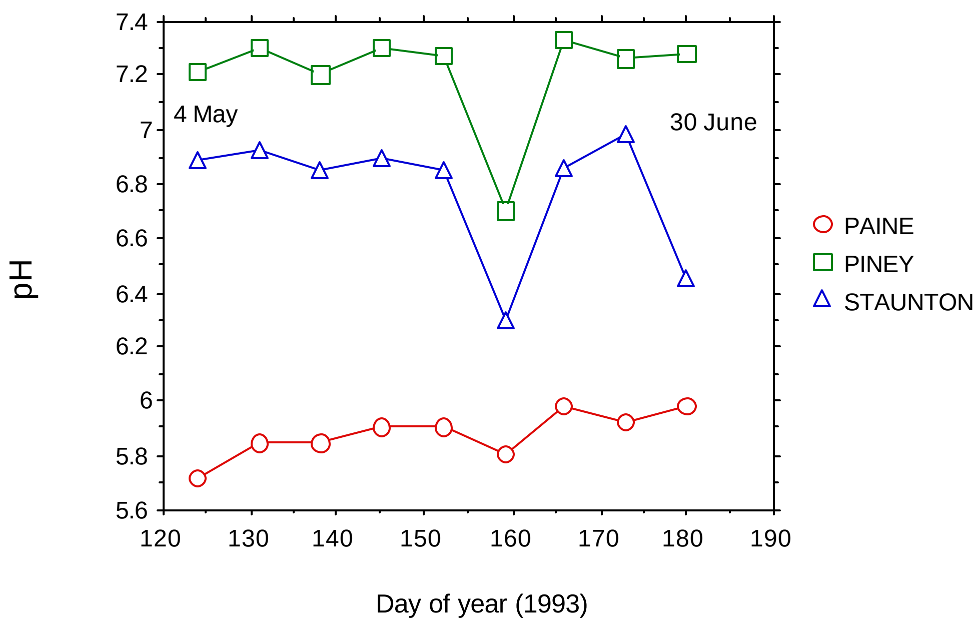


Figure 6B-13. pH during body sodium bioassay.

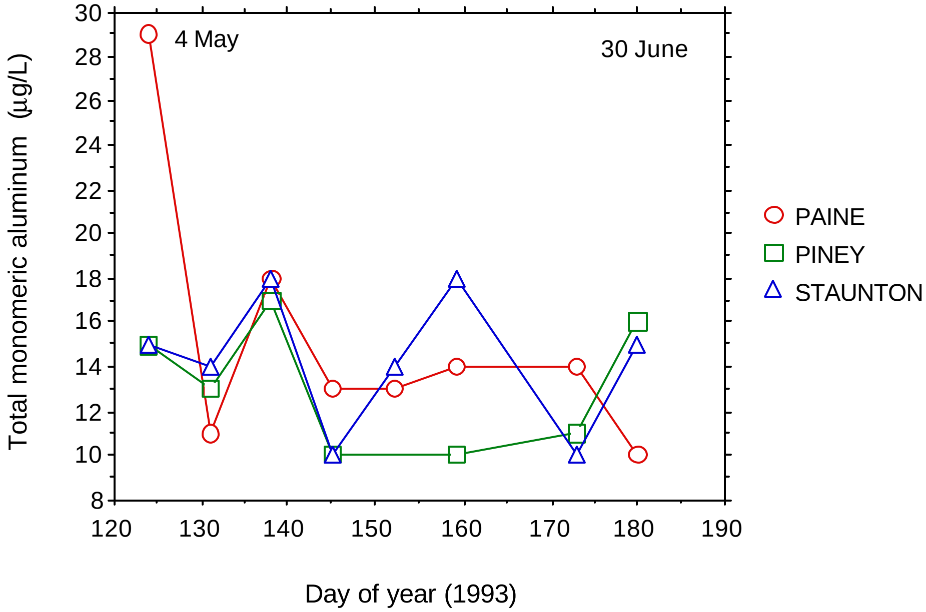


Figure 6B-14. Total monomeric aluminum during body sodium bioassay

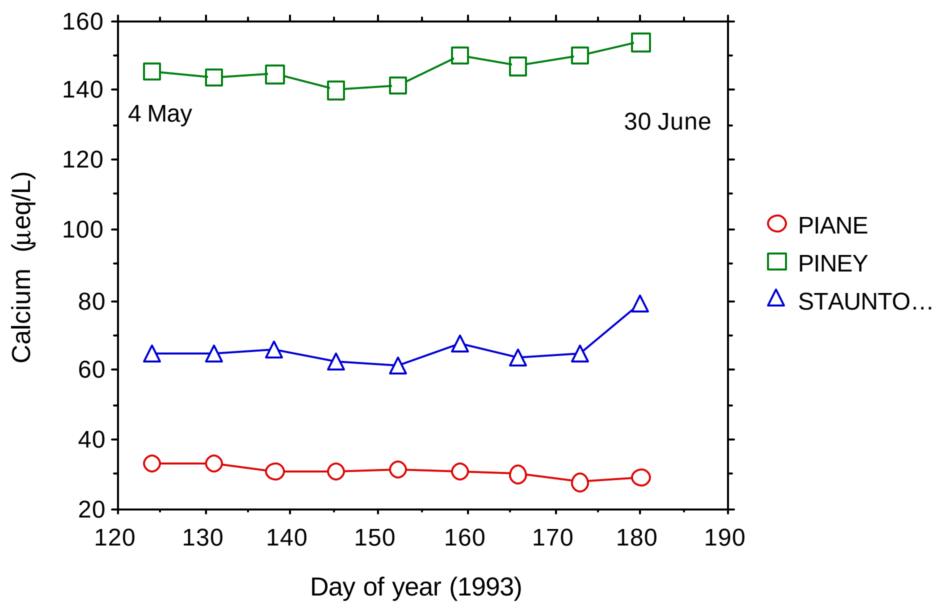
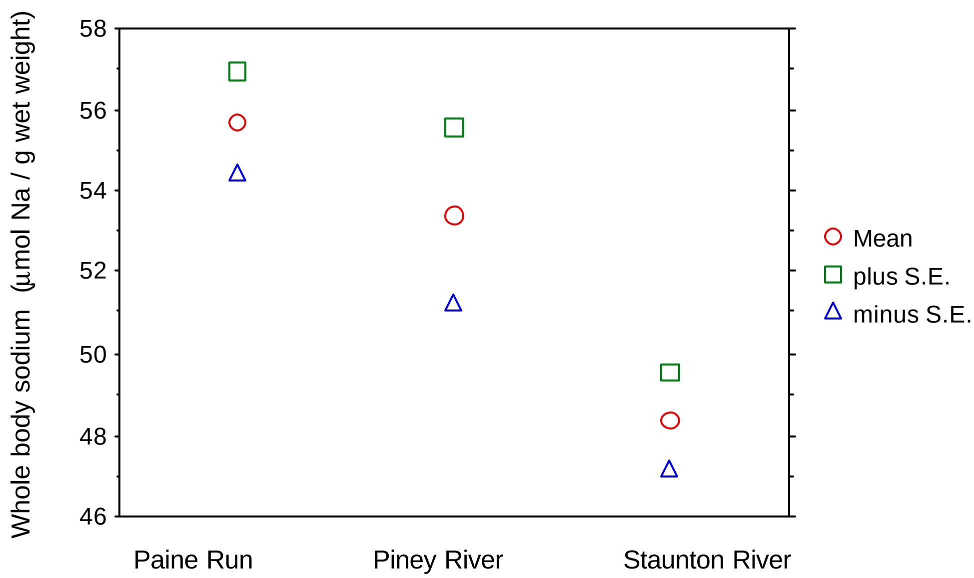


Figure 6B-15. Calcium during body sodium bioassay.



stream comparison	**combined sample size (total number of fish)**	**significance value**
Paine *vs.* Staunton	87	p<0.01
Paine *vs.* Piney	89	p<0.0005
Staunton *vs.* Piney	88	p<0.005

Figure 6B-16. Whole body sodium concentrations expressed as µmol/g wet weight of blacknose dace during the 9-week body sodium bioassay. The center point of each trio of points represents the mean; the two other points of each trio are the mean plus or minus the standard error. Shown below in table form are the sample sizes and linear contrasts of all pairwise comparisons of whole body sodium for fish in the three intensive streams. Samples sizes were 44, 43, and 45, for Paine (low ANC), Staunton (mid ANC), and Piney (high ANC) respectively.

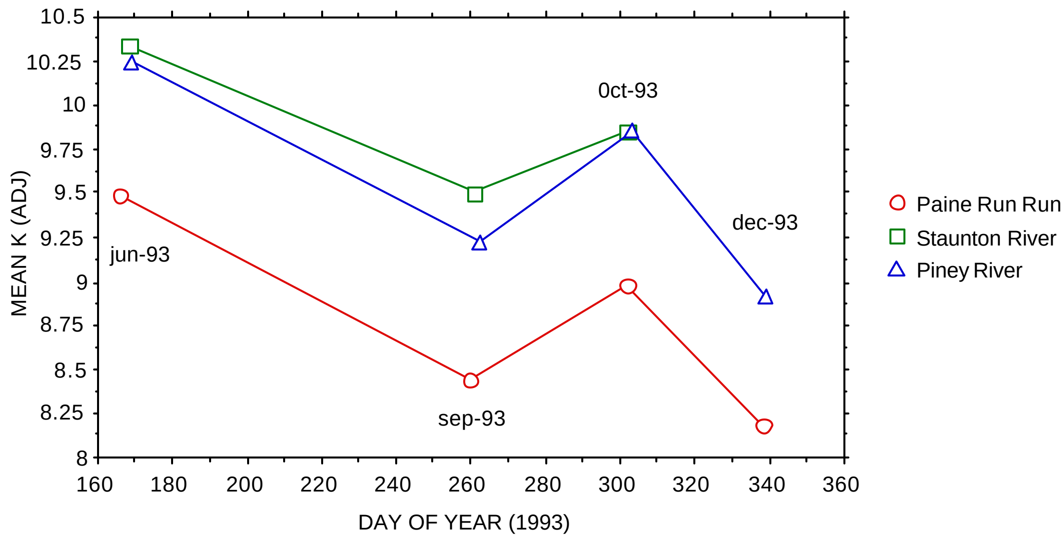
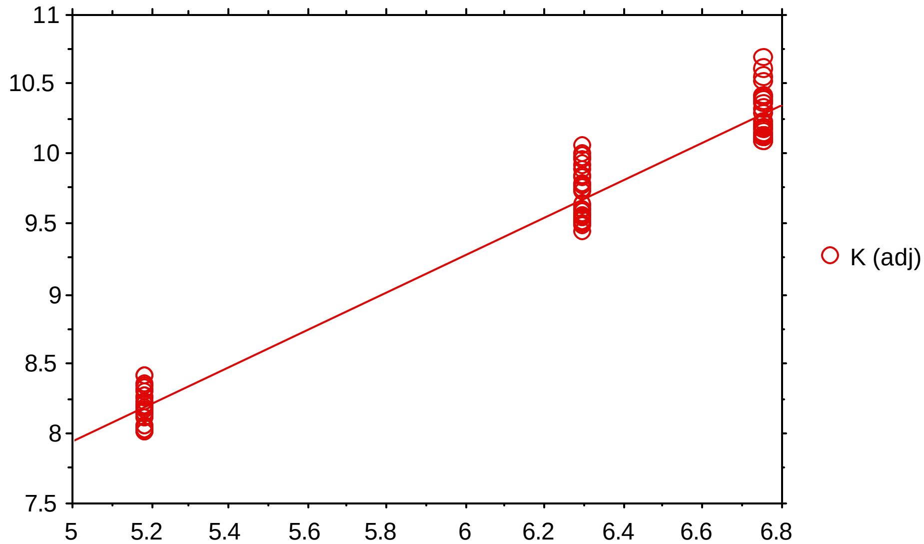


Figure 6B-17. Condition factors (K adj.) on four sampling dates in 1993, showing seasonal effects on K and differences among streams with differing ANC. Means, standard errors, dates and sample sizes (grand total is 1301 dace) are shown in Table 6B-4; statistical comparisons are shown in Table 6B-7.



Simple Regression X 1 : pH minimum Y 1 : K (adj)

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
130	.985	.97	.97	.154

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	97.858	97.858	4142.059
RESIDUAL	128	3.024	.024	p = .0001
TOTAL	129	100.882		

No Residual Statistics Computed

Simple Regression X 1 : pH minimum Y 1 : K (adj)

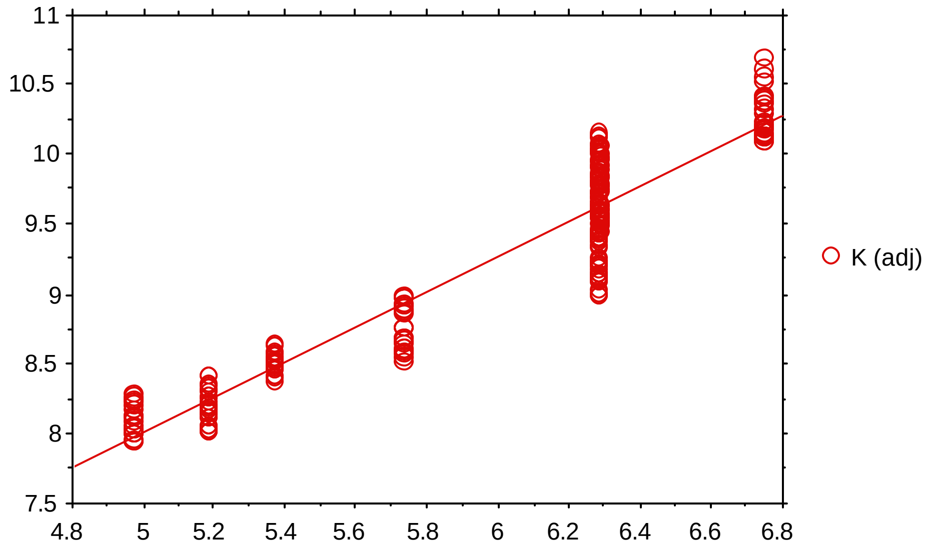
Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	1.252				
SLOPE	1.337	.021	.985	64.359	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	9.417	9.47	9.421	9.466
SLOPE	1.296	1.378	1.303	1.371

Figure 6B-18. Three “intensive” streams. Plot and regression table for blacknose dace condition factor (K) in July ‘94, versus lowest pH among 15 quarterly stream samples (Jan. ‘91-Jul. ‘94).



Simple Regression X 1 : pH minimum Y 1 : K (adj)

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
313	.956	.913	.913	.229

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	171.42	171.42	3265.813
RESIDUAL	311	16.324	.052	p = .0001
TOTAL	312	187.744		

No Residual Statistics Computed

Simple Regression X 1 : pH minimum Y 1 : K (adj)

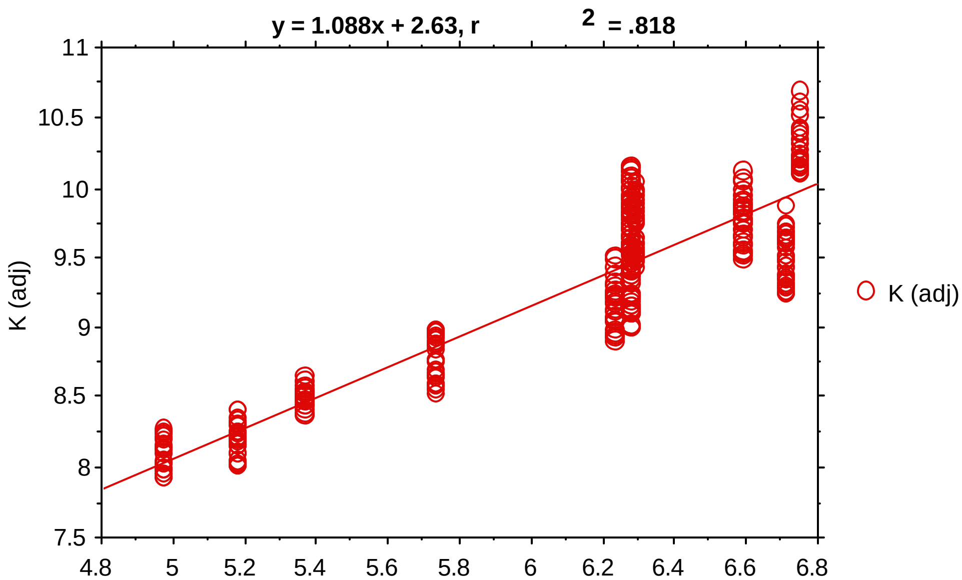
Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	1.704				
SLOPE	1.26	.022	.956	57.147	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	9.192	9.243	9.196	9.239
SLOPE	1.216	1.303	1.223	1.296

Figure 6B-19. Five "extensive" and three "intensive" streams. Plot and regression table for blacknose dace condition factor (K), versus lowest pH among 15 quarterly stream samples (Jan. '91-Jul. '94).



Simple Regression X 1 : pH min (91-94,qt) Y 1 : K (adj)

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
443	.905	.818	.818	.291

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	167.575	167.575	1985.056
RESIDUAL	441	37.229	.084	p = .0001
TOTAL	442	204.804		

No Residual Statistics Computed

Simple Regression X 1 : pH min (91-94,qt) Y 1 : K (adj)

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	2.63				
SLOPE	1.088	.024	.905	44.554	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	9.268	9.322	9.272	9.318
SLOPE	1.04	1.136	1.047	1.128

Figure 6B-20. Three additional, plus three "intensive" and five "extensive" streams. Plot/regression table for blacknose dace condition factor (K), versus lowest pH among 15 quarterly stream samples (Jan. '91-Jul. '94).

SNP:FISH
Shenandoah National Park: Fish In Sensitive Habitats
Project Final Report, Volume IV

Chapter 6C

Stream chemistry and fish species richness in Shenandoah National Park

prepared by

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Abstract

The number of fish species (1-9 species) in 13 streams in Shenandoah National Park SNP) is very strongly related (r-squared = 0.816, p = 0.0001) to the minimum Acid Neutralizing capacity (ANC) observed in weekly/quarterly water samples taken over a seven-year period. ANC derives ultimately from weathering bedrock minerals. Different bedrock types in SNP yield streams with differing ANC values, and the streams with lowest ANC host fewest species of fish. ANC controls and is negatively correlated with hydrogen ion (measured by pH) and aluminum concentrations (both toxic to fish), and is positively correlated with calcium (Ca) concentration (which ameliorates acid and aluminum toxicity). Fish species differ in their acid-stress sensitivity. Acidification reduces species richness by eliminating the most acid-sensitive species first, so bedrock geology is linked mechanistically to acid toxicity and species richness in SNP. All SNP streams drain into one of three rivers, each of which hosts 25 of 28 species of fish found in SNP, so biogeographic barriers are unlikely to contribute substantially to the pattern of species distributions across the ANC gradient.

Introduction.

Shenandoah National Park (SNP) offers the opportunity to examine the effects of water chemistry on fish species richness in a restricted geographic area receiving uniform acidic deposition, with a common fish species pool, but having different bedrock types yielding streams with very different acid-base status.

The loss of biodiversity is a major global concern, and North America is no exception (Studds, 1991). For example, about 20% of Virginia's freshwater fish species (about 40 species of about 200 species) are at risk, largely due to human activities (Jenkins and Burkhead, 1993). Numerous studies have shown that fish species richness (the number of fish species) is positively correlated with pH (Rago and Wiener, 1986). Lowering pH can reduce species richness by eliminating sensitive species (Matusek and Beggs, 1988; Schindler et al., 1989; Minns et al., 1990). In the Adirondacks, there is a significant positive correlation between lake pH (and ANC) and the number of fish species present in a lake (Kretser et al., 1989). Further, of the 53 species of fish recorded in the Adirondack Lakes Study (Kretser et al., 1989), half [26 species] are absent from lakes with pH of less than 6.0 [5.97].

The Southeast region of the United States hosts a rich diversity of fish species. There are about 950 freshwater fish species in North America (Jenkins and Burkhead, 1993), of which about 485 species can be found in the U.S. Southeast, and about 350 species can be found in the Southern Appalachian Mountains (SA) south of the Roanoke and New Rivers (Walsh et al., 1995). The total numbers of fish species by state in the region is impressive : 107 in Maryland, 164 in West Virginia, 199 in North Carolina, 210 in Virginia, plus the richest state freshwater fish fauna in the country, 307 in Tennessee (Jenkins and Burkhead, 1993). The absence of glaciation and a relatively warm climate, together with general latitudinal effects and abundant rainfall (35-100 inches per year), contribute to this regional diversity (Adams and Hackney, 1992); for probably the same reasons, habitat and intraspecific genetic diversity is also high (SAMAB, 1996). Thus, from a biodiversity point of view, the U.S. Southeast is a unique national resource.

Elevated levels of sulfuric and nitric acids in precipitation have been documented throughout much of Europe and North America, including Virginia. Wet deposition of sulfate in western Virginia is estimated to be about 500 eq ha^{-1} yr^{-1} (Summers et al., 1986; Buikema et al., 1988). Dry deposition of sulfur is estimated to be from 50% to 100% of the amount of wet deposition (Shaffer and Galloway, 1982; Rochelle et al., 1987). Recent sulfur deposition rates in the SNP are about 10 times the pre-industrial rate (Cosby et al., 1991).

Shenandoah National Park (Virginia, U.S.A.) is located downwind of the principal sources of sulfur and nitrogen emissions in the U.S. (National Academy of Sciences, 1986); Shenandoah National Park (SNP) receives more acid deposition (SO_4^{-2}) than any other U.S. national park (NADP/NTN 1989). Fifty-nine percent of SNP streams are classified as "sensitive to acidification" with acid neutralizing capacities (ANC's) < 100 *u*eq/L (Herlihy *et al*., 1993). Sensitivity to acidification is determined by local bedrock. In SNP, streams draining catchments with silici-clastic bedrock are most sensitive to acidification (< 25 *u*eq/L ANC); granitic catchments are intermediate (25-75 *u*eq/L ANC), and basaltic catchments are least sensitive (>75 *u*eq/L ANC). Twenty-nine percent of the catchments in SNP are underlain by silici-clastic bedrock; 32% are underlain by granitic bedrock; and 39% are underlain by basaltic bedrock.

Weekly and/or quarterly chemical analyses of SNP streams since 1979 indicate that acidic conditions occur which may affect the distribution and abundance of some of these fish species. Values for pH have been observed as low as 4.98 for Paine Run (Chapter 3). This value is within or below the "critical" pH threshold ranges for ten fish species found in SNP (Baker and Christensen,1991; Table 6C-1). "Critical" pH, estimated by averaging the pH thresholds for effects from a variety of separate studies, is defined by Baker and Christensen as the pH below which population effects are likely to occur. Other SNP fish species are apt to be sensitive to present acidic conditions in Park waters, but there is little information on their acid tolerances.

Since Shenandoah National Park contains streams with low ANC, and receives substantial acid deposition (Webb et al., 1989), the fish species richness of at least some of its streams may be affected. The purpose of this study is to determine the relationship between fish species richness and acid-base status of streams.

Material and Methods

SNP (established in 1936) traverses a 112-km segment of the Blue Ridge Mountains in northern Virginia, is 5-10 km wide, and occupies more than 300 square miles. Elevation is about 1200-4050 feet above sea-level (Gathwright, 1976). SNP is primarily forested, and precipitation ranges from about 45-60 inches per year (Lynch, 1987). About 50 streams drain SNP. Those on the west side of SNP drain into the Shenandoah River, which flows northeastward to the Potomac River, which turns southeastward to Chesapeake Bay. Those on the east side of SNP flow into either the Rappahannock or James Rivers, also flowing southeastward into Chesapeake Bay.

There is considerable overlap among the fish faunas of the three drainages to which SNP streams contribute (Jenkins and Burkhead 1993). Specifically, of the 28 fish species (Table 6C-1) found in SNP (SNP Fisheries Management Program, 1994), 25 occur in all three drainages. Two species (fantail darter, central stoneroller, Table 6C-1) are absent from the Rappahannock drainage, and one species (johnny darter, Table 6C-1) is absent from the Potomac and Rappahannock drainages.

Study streams.

The streams used in this analysis include the three SNP:FISH intensive sites (Paine, Staunton and Piney), and the five extensive sites (Meadow, Twomile, White Oak, Brokenback, and North Fork Dry/Shaver Hollow); at least seven years of stream chemistry (from the SWAS Project and this project) exist for these streams, plus fish survey data from this project.

In addition to the three "intensive" streams (Paine, Staunton, Piney) and five "extensive" streams (Meadow, Twomile, White Oak, Brokenback, North Fork Dry) quarterly stream chemistry from the UVA's SWAS program plus fish community surveys from SNP's Fisheries Management Program were also available for five streams; these data were incorporated into the relationship between stream ANC and fish species richness. These five streams (Hazel, Madison, Jeremy's, Rose, North Fork Thornton) are labeled "SWAS-FMP" below. The reason for their inclusion was that quarterly chemistry for at least seven years for each was on hand, and reliable fish community surveys had been performed by SNP staff; thus they met the criteria for inclusion in the regression models for species richness and ANC, and they also provided the opportunity to include higher ANC streams in this analysis.

Study Streams included in the analyses.

SNP: FISH intensive	**SNP: FISH extensive**	**SWAS-FMP**
Paine	Meadow	Hazel
Staunton	Twomile	Madison
Piney	While Oak	Jeremy's
	Brokenback	Rose
	North Fork Dry	North Fork Thornton

Statistical analysis.

A stepwise multiple regression (using the Statview II statistical package) was performed, using fish species richness as the dependent variable, and the median, minimum, and maximum values for each of the water chemistry values as potential predictor variables.

Results and Discussion.

There was a highly significant ($p<0.0001$) relationship between Minimum ANC (during the seven-year period of record) and fish species richness among the 13 streams, such that the streams having the lowest ANC hosted the fewest species. The first variable entered into the stepwise multiple regression, Minimum ANC (recorded during the period of record), showed the highest predictive capacity of any of the variables. Its r-squared value (0.816) indicates that it alone accounts for approximately 82% of the variance in fish species richness (Table 6C-3, Figure 6C-1).

Additional variables added little explanatory value. Consequently, the results of the regression of fish species richness versus water chemistry are presented as a simple regression using only minimum ANC as the predictor for fish species richness in SNP. The numbers of fish species and minimum ANC for each stream are shown in Table 6C-2. The regression relationship between fish species richness and Minimum ANC was incorporated into the predictive model discussed in Chapter 7.

Simple linear regressions were also performed on the two subsets of the 13-stream data set at the suggestion of a reviewer. These two subsets were a) the three SNP: FISH intensive streams (Table 6C-4, Figure 6C-2), and b) the eight SNP: FISH streams including both the three intensive streams and the 5 extensive streams (Table 6C-5, Figure 6C-3). The same pattern is revealed in all three figures (6C-1, 6C-2, 6C-3). The lack of statistical significance in Table 6C-4 ($p=.0579$) is attributed to the small number of streams included.

It is very difficult to separate episodic effects from chronic effects in terms of importance for fish species richness because low ANC streams are both more prone to acid episodes, and have chronically lower pH. However, we can say that those SNP streams which host fewer fish species are those likely to have significant aluminum pulses during acid episodes (Chapters 6 A and 6B).

The Episode Response Project (ERP) was a two-year interdisciplinary study which evaluated the occurrence and biological effects of episodic acidification in the northeastern United States (Wigington et al., 1996). It monitored water chemistry and its effects on brook trout (young of the year and yearlings) and other fish species in four Adirondack streams, four Catskills streams, and five Appalachian Plateau streams in Pennsylvania. All streams had physical habitats judged suitable for fish survival and reproduction, and all had indigenous fish populations in at least part of the stream system (Baker et al., 1996). Twelve of the 13 ERP streams had chemical conditions judged suitable for most fish species during low flow (median pH 6-7; inorganic Al< 60µg/L) (Baker et al. 1996). Those which had moderate to severe acid episodes had significantly higher fish mortality during bioassays versus non-acidic streams (Van Sickle et al. 1996). The concentration of inorganic Al (highly toxic to fish) was the chemical variable most strongly related to mortality in the four test species (brook trout, mottled sculpin, slimy sculpin and blacknose dace). Since Al, pH and Ca concentrations are highly intercorrelated in nature (Kretser et al., 1989; Bulger et al., 1993; Bulger et al., 1995), pH and Ca provided only modest additional information, and only in the case of brook trout (Van Sickle et al., 1996). ERP streams with high fish mortality during acid episodes also had lower brook trout density and biomass, and lacked more acid-sensitive species.

The results of the SNP: FISH study support the conclusion that fish species richness is strongly related to acid-base status of streams, and hence to local bedrock geology. It is expected that if acidification of SNP streams continue, there will be losses of species in low-ANC streams at higher rates than in mid-ANC or high-ANC streams. It should be noted that the blacknose dace population in Meadow Run has been in decline for some years, and now is the first documented loss attributable to acid precipitation in SNP (Atkinson pers. communication)

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Fish Species of Shenandoah National Park

N = 28 + 2 rare spp (one of which is a hybrid)

COMMON NAME	*LATIN NAME*	FAMILY	SNP Drainages Hocutt & Wiley 1986 (Potomac) Shenandoah	Rapp'k	James
American Eel	*Anguilla rostrata*	Anguillidae	Ma	Ma	Ma
Mtn. Redbelly Dace	*Phoxinus oreas*	Cyprinidae	IP	IP	N
Rosyside Dace	*Clinostomus funduloides*	Cyprinidae	N	N	N
Longnose Dace	*Rhinichthys cataractae*	Cyprinidae	N	N	N
Blacknose Dace	*Rhinichthys atratulus*	Cyprinidae	N	N	N
Central Stoneroller	*Campostoma anomalum*	Cyprinidae	N		N
Fallfish	*Semotilus corporalis*	Cyprinidae	N	N	N
Creek Chub	*Semotilus atromaculatus*	Cyprinidae	N	N	N
Cutlips Minnow	*Exoglossum maxillingua*	Cyprinidae	N	N	N
River Chub	*Nocomis micropogon*	Cyprinidae	N	N	N
Bluehead Chub	*Nocomis leptocephalus*	Cyprinidae	IP	IP	N
Common Shiner	*Luxilus cornutus*	Cyprinidae	N	N	N
Northern Hogsucker	*Hypentelium nigricans*	Catostomidae	N	N	N
Torrent Sucker	*Thoburnia rhothocea*	Catostomidae	N	IP	N
White Sucker	*Catastomus commersoni*	Catostomidae	N	N	N
Margined Madtom	*Noturus insignis*	Ictaluridae	N	N	N
Brook Trout	*Salvelinus fontinalis*	Salmonidae	N	NI	N
Brown Trout	*Salmo trutta*	Salmonidae	I	I	I
Tiger Trout*	*Salmo X Salvelinus*	Salmonidae			
Rainbow Trout	*Oncorhynchus mykiss*	Salmonidae	I	I	I
Mottled Sculpin	*Cottus bairdi*	Cottidae	N	N	N
Rock Bass	*Ambloplites rupestris*	Centrarchidae	I	I	I
Smallmouth Bass	*Micropterus dolomieui*	Centrarchidae	I	I	I
Largemouth Bass	*Micropterus salmoides*	Centrarchidae	IP	IP	N
Redbreast Sunfish	*Lepomis auritus*	Centrarchidae	N	N	N
Pumpkinseed	*Lepomis gibbosus*	Centrarchidae	N	N	N
Johnny Darter	*Etheostoma nigrum*	Percidae	N		
Tesselated Darter	*Etheostoma olmstedi*	Percidae	N	N	N
Fantail Darter	*Etheostoma flabellare*	Percidae	N		N
Greenside Darter*	*Etheostoma blennioides*	Percidae	N*		

*rare (6-10 ind)

N Native
I Introduced
NI Regarded as native, but maybe introduced
IP Regarded as introduced, but maybe native
Ma Marine or euryhaline

Table 6C-1. The fish species of Shenandoah National Park Streams.

STREAMS	MINIMUM ANC (μeq/L)	# OF FISH SPECIES
SNP:FISH streams in analysis		
PAINE (Intensive)	-0.6	3
STAUNTON (Intensive)	50.3	5
PINEY (Intensive)	120.3	7
MEADOW (Extensive)	-11.4	1
NORTH FORK DRY (Extensive)	21.1	2
TWOMILE (Extensive)	2.8	2
WHITE OAK RUN (Extensive)	1.9	3
BROKENBACK (Extensive)	47.8	3
SWAS-FMP streams in analysis		
HAZEL (FMP)	55.3	6
MADISON (FMP)	61.1	5
JEREMY'S (FMP)	93.7	6
ROSE (FMP)	94.4	8
NORTH FORK THORNTON (FMP)	156.2	9

Table 6C-2 Minimum ANC and number of fish species (species richness) for the study streams. (Minimum ANC recorded during period of record - at least 7 years - by SWAS project and SNP:FISH project; number of fish species from SNP:FISH project - intensive or extensive - or from SNP Fisheries Management Olan - FMP).

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
13	.931	.868	.856	.95

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	65.142	65.142	72.124
RESIDUAL	11	9.935	.903	p = .0001
TOTAL	12	75.077		

No Residual Statistics Computed

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	2.204				
SLOPE	.045	.005	.931	8.493	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	4.035	5.196	4.142	5.089
SLOPE	.034	.057	.036	.055

Table 6C-3. Simple linear regression. X-variable is minimum ANC recorded from weekly samples in 7-year period ending in 1995. Y-variable is number of fish species in 13 streams.

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
3	.996	.992	.983	.257

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	7.934	7.934	120.201
RESIDUAL	1	.066	.066	p = .0579
TOTAL	2	8		

No Residual Statistics Computed

Note: 10 cases deleted with missing values.

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	3.141				
SLOPE	.033	.003	.996	10.964	.0579

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	3.115	6.885	4.063	5.937
SLOPE	-.005	.071	.014	.052

Table 6C-4. Simple linear regression. X-variable is minimum ANC recorded from weekly samples in 7-year period ending in 1995. Y-variable is number of fish species in 3 streams (intensive).

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
8	.903	.816	.785	.885

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	20.798	20.798	26.536
RESIDUAL	6	4.702	.784	p = .0021
TOTAL	7	25.5		

No Residual Statistics Computed

Note: 5 cases deleted with missing values.

Simple Regression X $_1$: Minimum ANC (period of record) Y $_1$: Number of fish...

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	2.095				
SLOPE	.04	.008	.903	5.151	.0021

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	2.484	4.016	2.642	3.858
SLOPE	.021	.059	.025	.055

Table 6C-5. Simple linear regression. X-variable is minimum ANC recorded from weekly samples in 7-year period ending in 1995. Y-variable is number of fish species in 8 streams (intensive plus extensive).

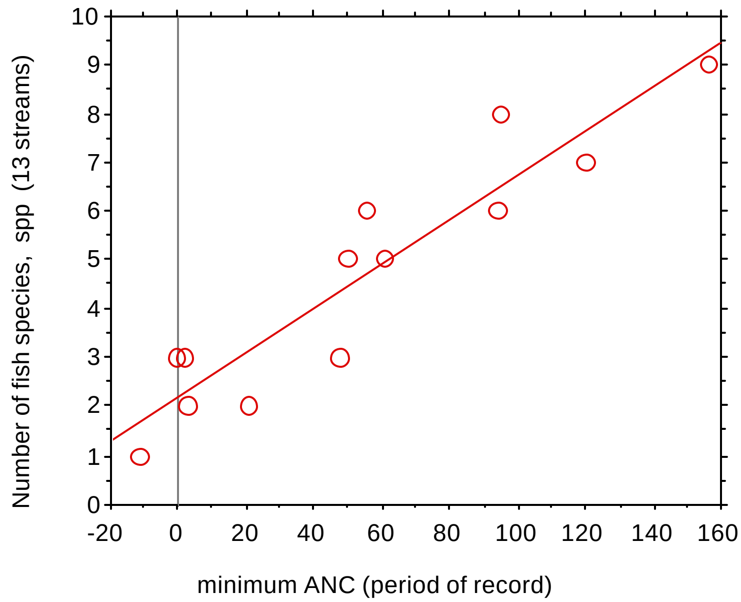

Figure 6C-1. Thirteen streams, with Meadow Run (lowest ANC) showing only one species, reflecting recent loss of Blacknose Dace.

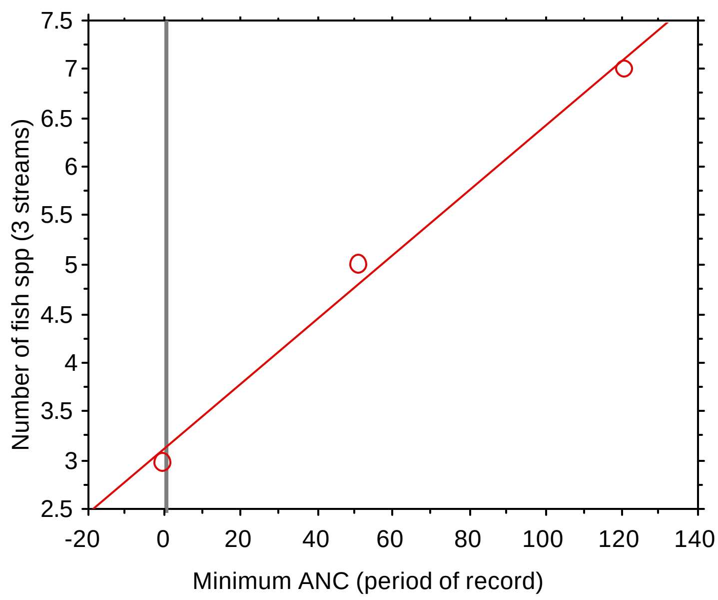

Figure 6C-2. Relationship between Minimum ANC and species number in the three intensive streams.

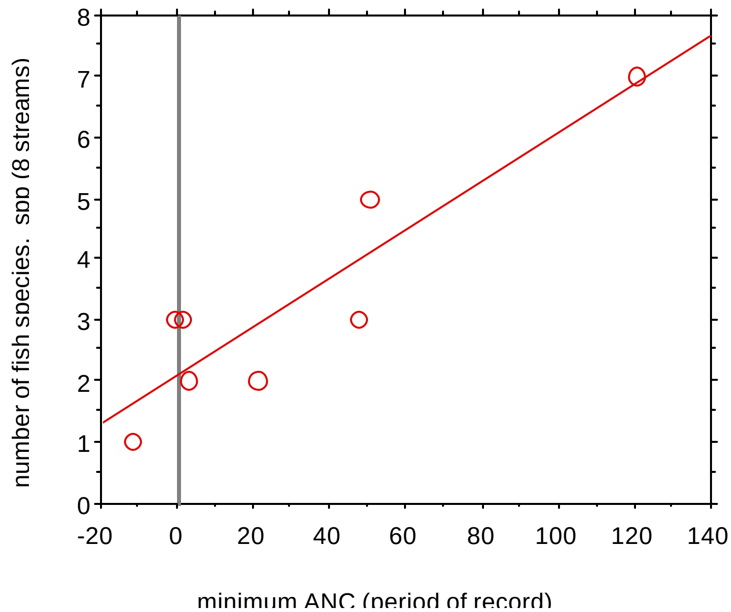

Figure 6C-3. Eight streams (intensive and extensive), with Meadow Run (lowest ANC) showing only one species, reflecting recent loss of Blacknose Dace.

SNP:FISH
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Chapter 7
Modelling the Biological Effects of Water Quality Changes in the Streams of the FISH Catchments

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Introduction

The important findings of the FISH project concerning the biological effects of changing water quality were incorporated into a computer simulation framework. The framework employs user-friendly PC- based computer programs that simulate changes in water quality and resultant biological effects. The framework is based around the MAGIC model (described in the methods section below). The MAGIC model is well known, has been extensively tested and is accepted as a good tool for long-term forecast of water quality changes. The results of the FISH project (described in previous chapters of this report) provide robust relationships between biological effects and both long- and short-term changes in water quality. The modelling framework integrates the results of the FISH project with the simulation capability of MAGIC to provide a tool for long-term forecasts of biological effects.

Two approaches for incorporation of biological effects into MAGIC were pursued:

1) modification of MAGIC to simulate storm episodes and calculate extent of biological effects based on simulated storm chemistry; and

2) development of empirical relationships for predicting biological effects which use standard outputs from MAGIC (and thus require no modification of the MAGIC model).

The conceptual basis of each approach is discussed first. Details of the models, methods and examples of applications for each study site follow.

Approach 1: Modification of the MAGIC computer code to include episode and biological effects modules.

The results of the FISH project demonstrate that transient, short-lived changes in the water quality of streams (such as those that frequently accompany storm discharges or snowmelt) can have profound biological effects. Thus, in order to simulate biological responses to water quality changes, a model might reasonably be expected to be able to simulate short-term or episodic changes in water quality. A common approach to modelling storm or snowmelt changes in water quality uses a two component mixing model. The basic concept of the two component mixing model involves combining a quickflow component of stream flow with a baseflow (or pre-storm) component of stream flow to provide a simulation of the chemical characteristics of stormflow in streams. The proportion of each component mixed at any time in any event is usually called the mixing ratio (ratio of volumes of quickflow to baseflow components). To simulate an episode using this conceptual approach, a model must have compartments that can be identified with each component and the temporal resolution sufficient to resolve the short-term changes in mixing ratio that occur during a storm event.

MAGIC, however, was originally conceived and constructed to provide estimates of the long-term changes in monthly average or annual average stream water chemistry. The structure and philosophy of the original model explicitly ignored short-term changes in stream water chemistry such as those that occur during storms or snowmelt episodes. The shortest time period considered in the model is monthly response. The spatial resolution of MAGIC is similarly not conceptually appropriate for implementation of a two-component mixing module. As originally conceived, the model used a highly aggregated representation of the soils within a catchment. There are no compartments in the model, which can be conceptually identified with the source area for the quickflow component. Therefore, in adding an episodic module based on the concept of the mixing of two components, a number of simplifying assumptions must be made. The assumptions and the conceptual limitations they place on this approach to coupling biological effects to MAGIC are discussed next. A more complete description of the MAGIC model and the modifications to the code adopted for this approach are given below in the methods section.

First, the structure of MAGIC produces only monthly or annual average values of water quality variables. It is therefore necessary to assume that annual average (or monthly average) stream chemistry is the base flow or pre-storm chemistry for use in the mixing model. In fact, the pre-storm chemistry of streams changes on a day-to-day basis as a function of discharge, time since last storm, and other factors. That daily scale of variability is not available from MAGIC. Stormflow can only be generated in the modified model by adding a quickflow component to an average baseflow component that does not change from day-to-day within a given month (or year). The severity of an event may therefore be over- or under estimated because the storm mixture can only be calculated using average baseflow chemistry.

Second, the structure of MAGIC does not have the spatial detail to include a compartment that represents the source of quickflow. Therefore, the chemistry of the quickflow component must be specified as an input to the model. A similar problem exists in field studies. It is usually not possible to identify the actual source areas of quickflow. What is usually done in empirical studies is to use appropriate mathematical techniques to decompose the measured stormflow chemistry into estimated quickflow and baseflow component chemistries. Thus, the requirement that quickflow chemistry must be specified a priori for the model is not inconsistent with current field studies and presents no conceptual difficulties. In practice, however, obtaining the estimated quickflow characteristics from observations is difficult and expensive.

Third, the lack of a quickflow source area compartment in the model means that changes in the composition of the quickflow cannot be linked to changes occurring in the simulated soils. Therefore, the user must specify time sequences of the historical and future changes in the chemistry of the quickflow component. It is not likely that the chemistry of the quickflow component remains the same as the catchment soils acidify or as atmospheric deposition changes. Very little is known, however, about the time course of changes in chemistry of quickflow. Indeed, quickflow chemistry is usually defined by a mathematical decomposition technique and future (or past) stormflow chemistry would be needed to produce a temporal sequence of changes in quickflow chemistry (thus obviating the need for a temporal sequence of quickflow chemistry with which stormflow can be predicted). The specification of changes in quickflow chemistry is thus largely problematic.

Fourth, the mixing ratio (volumes of quickflow to baseflow components in a storm) is needed for the model simulation of storm response. In the field, this ratio varies throughout the course of a storm response: starting at zero, rising quickly to some peak value and then declining more slowly back to zero as discharge subsides. There are tracer techniques based on isotopes or conservative tracers that allow calculation of the mixing ratio and its variation throughout a storm. These values and variations, however are different from storm to storm and are greatly affected by the severity of the storm. The structure of MAGIC as amended to include the episode module cannot explicitly account for variation in mixing ratio during an event.

Fifth, during storm episodes the aluminum concentrations in streams are not controlled by equilibrium thermodynamic reactions. Total inorganic monomeric aluminum at any point in an event is a function of a number of non-equilibrium processes. Aluminum dynamics in MAGIC are based on assumed equilibrium with a solid form of aluminum trihydroxide. The episode module, therefore, must be calibrated to produce the correct concentrations of inorganic monomeric aluminum during simulated episodes. In the field, these concentrations vary with source of the quickflow, intensity of storm, etc., making selection of a calibrated "effective" solubility of aluminum problematic.

Sixth, the two component mixing module added to MAGIC has no temporal frame of reference. That is, the model mixes baseflow and quickflow components and calculates the resulting chemistry of the mixture, but does not specify the length of time that the simulated stormflow persists in the stream channel. The temporal resolution of MAGIC does not allow such considerations.

Finally, in coupling the simulated episode chemistry to the biological effects module, the computer model does not take into account spatial and temporal factors that may negate the assumed effects of the simulated water quality. These factors include: the presence of suitable habitat for the species (and the presence of the species in the stream); the existence of refugia within real systems (which may provide protection against the full effects of the toxic exposure); the timing of the episode (toxic effects in real systems only occur if the sensitive life stage is present); or the exposure-duration characteristics of the toxic response (the model does not simulate the duration of an episode).

Considering all of these conceptual limitations, the modified MAGIC computer code produced for the FISH project can best be described as a preliminary approach to the difficult task of simulating episodic stream chemistry and resultant biological effects. Using the assumptions required by the modified model's conceptual foundation, the model does allow simulation of the mixing of two components of streamwater, and estimation of the biological effects of the resultant mixture. However, the simulated episode and its biological effects must be considered hypothetical.

That is, the model will mix two components in a given ratio (which is constant for the simulated storm), where one component is a monthly or yearly average (the output of MAGIC's long-term simulation) and the other component has assumed chemical characteristics (which may or may not be empirically constrained by observations). Furthermore, the aluminum mobilized in the simulated event must be empirically calibrated and the model does not specify the duration of the episodic chemistry in the stream channel. The biological effects simulated as a result of exposure to this stormflow mixture must be prefixed by a list of assumptions: if suitable habitat exists, if the species is present, if the species is in the sensitive life stage, if no refugia exist, if the episodic chemistry persist long-enough to produce the deleterious effect, - then this episodic chemistry will produce this level of biological effect. Rather than simulate any given real storm, selection of appropriate parameter values in the episode module will allow the user to examine a "typical" or "reference" storm event.

Therefore, this approach (utilizing a modified MAGIC model that incorporates episodic and biological effects modules) is probably best used as an heuristic tool for understanding the complex interactions involving long-term changes in baseflow chemistry, episodic chemical changes in streams and potential biological effects. The model can be very effectively used in a series of "what if" exercises designed to address any number of questions about episodic response and biological effects. This is the great strength of this modification of the MAGIC model. Given a baseline scenario, comparisons of the direction of change and magnitudes of change in predicted biological effects can be examined for different values of a single parameter or for different combinations of values of multiple parameters.

Approach 2: Development of empirical relationships for biological effects using standard MAGIC outputs.

The MAGIC model was developed to simulate the long-term changes in average water quality in response to long-term changes in atmospheric deposition. The concern when the model was developed was to provide a tool that would simulate long-term trends in water quality while ignoring short-term fluctuations about the trends. The model has been widely tested and found to be reliable for these uses (see MAGIC description below). The model has also been widely used in policy and assessment activities both in the U.S. and abroad (see also below). It seems desirable, then, to use this tested and accepted version of MAGIC for estimating biological effects investigated in the FISH project. In this approach, the results of the FISH project were integrated "off line" to develop an empirical scheme for estimating biological effects that interfaces directly with outputs of the original model (as opposed to modifying the computer code to interface with the detailed results of the FISH project).

Use is made of the very strong correlation that exists between average annual stream alkalinity and the short-term changes that occur in water quality variables in response to storm or snowmelt discharge. That is, streams that have a high average annual alkalinity are not likely to display episodic acidic responses regardless of the intensity of a storm. On the other hand, as average annual alkalinity declines, episodic acidification responses that affect biota are observed to increase in severity (as average annual alkalinity declines minimum storm alkalinity and pH also decline and maximum storm aluminum concentrations increase). This suggests that an empirical scheme can be constructed based on average alkalinity that will classify a stream's biological status without having to explicitly model the episodic response of the stream (with the associated difficulties discussed in Approach 1 above).Based on the results of the FISH project, such a classification system was adopted for the streams of SNP using four classes that encompass the range of biological effects observed in the FISH study sites.

"Not Acidic"

Streams in this classification have an average annual alkalinity greater than 50 μeq/L. At this level of average annual alkalinity there is essentially no likelihood of storm induced acid episodes. Stream alkalinity remains above zero in all seasons and flow regimes. As a result there are no observations of problems related to acid toxicity on biota in these streams.

"Indeterminate"

Streams in this classification have an average annual alkalinity between 20 and 50 μeq/L. In this range of average annual alkalinity streams may or may not experience episodic acidification during storms. The occurrence of episodic acidity depends on a number of hydraulic and physical/chemical characteristics that cannot be readily predicted (such as: the ratio of storm- to baseflow which is a function of the geomorphology of the catchment and storm severity; the occurrence of springs or minor alkaline tributaries that can buffer storm events at these levels of annual alkalinity; etc.). Observed biological effects are also variable depending on whether or not acidic episodes occur. This is a problematic category. It is difficult to have much confidence about estimates of biological responses in streams included within this category. In general, membership in this category is transient as a stream moves from "not acidic" to the next category "episodically acidic" during acidification (or in the other direction during recovery from acidification).

"Episodically Acidic"

Streams in this classification have an average annual alkalinity in the range 0-20 μeq/L. At these low levels of average annual alkalinity storm induced acid episodes are always observed (albeit at different frequencies of occurrence from stream to stream). Streams in this category will lose sensitive species of fish and will thus display reduced species richness. There are measurable sub-lethal stresses on individuals of more acid-tolerant species in streams in this category.

"Chronically Acidic"

Streams in this classification have an average annual alkalinity less than 0 μeq/L. In order for a forested, headwater stream in the mountains of Virginia to have an annual average alkalinity less than 0 μeq/L, it must have a negative alkalinity for most of the year, not just during storm events. As a result, the biological community of streams in this category is severely affected. Loss of even acid-tolerant species occurs in these streams and these streams have very low species richness. Mortality due to acid stress is common.

Methods

The MAGIC Model

The potential effects of sulfur deposition on surface water quality have been well-studied throughout the United States, particularly within EPA's Aquatic Effects Research Program (AERP), a component of the National Acid Precipitation Assessment Program (NAPAP). Major findings were summarized in a series of State of Science and Technology Reports (e.g., Sullivan 1990, Baker et al. 1990) and the final NAPAP policy report, the 1990 Integrated Assessment (NAPAP 1991). The major tools available for evaluating the potential response of aquatic resources to changes in atmospheric deposition of sulfur are mathematical models. One of the prominent models developed to estimate acidification of lakes and streams is MAGIC (Model of Acidification of Groundwater In Catchments, Cosby et al., 1985a-c). MAGIC was the principal model used by the National Acid Precipitation Assessment Program (NAPAP) scientists in assessment of potential future damage to lakes and streams in the eastern United States (NAPAP 1991, Thornton et al. 1990). The validity of the model has been confirmed by comparison with estimates of lake acidification inferred from paleolimnological reconstructions of historical lake changes in pH (Sullivan et al. 1991, 1996) and with the results of several catchment-scale experimental acidification and deacidification experiments (e.g., Cosby et al. 1995, 1996).

Model Description.

MAGIC is a lumped-parameter model of intermediate complexity, developed to predict the long-term effects of acidic deposition on surface water chemistry. The model simulates soil solution chemistry and surface water chemistry to predict the monthly and annual average concentrations of the major ions in these waters. MAGIC consists of: 1) a section in which the concentrations of major ions are assumed to be governed by simultaneous reactions involving sulfate adsorption, cation exchange, dissolution-precipitation- speciation of aluminum and dissolution-speciation of inorganic carbon; and 2) a mass balance section in which the flux of major ions to and from the soil is assumed to be controlled by atmospheric inputs, chemical weathering, net uptake and loss in biomass and losses to runoff. At the heart of MAGIC is the size of the pool of exchangeable base cations in the soil. As the fluxes to and from this pool change over time owing to changes in atmospheric deposition, the chemical equilibria between soil and soil solution shift to give changes in surface water chemistry. The degree and rate of change of surface water acidity thus depend both on flux factors and the inherent characteristics of the affected soils.

Cation exchange is modelled using equilibrium (Gaines-Thomas) equations with selectivity coefficients for each base cation and aluminum. Sulfate adsorption is represented by a Langmuir isotherm. Aluminum dissolution and precipitation are assumed to be controlled by equilibrium with a solid phase of aluminum trihydroxide. Aluminum speciation is calculated by considering hydrolysis reactions as well as complexation with sulfate and fluoride. Effects of carbon dioxide on pH and on the speciation of inorganic carbon are computed from equilibrium equations. Organic acids are represented in the model as tri-protic analogues. First-order rates are used for retention (uptake) of nitrate and ammonium in the catchment. Weathering rates are assumed to be constant. A set of mass balance equations for base cations and strong acid anions are included. Given a description of the historical deposition at a site, the model equations are solved numerically to give long-term reconstructions of surface water chemistry (for complete details of the model see Cosby et al., 1985 a-c, 1989).

Magic has been used to reconstruct the history of acidification and to simulate the future trends on a regional basis and in a large number of individual catchments in both North America and Europe (Lepisto et al., 1988; Whitehead et al., 1988; Cosby et al., 1989, 1990, 1996; Hornberger et al., 1989; Jenkins et al., 1990a-c; Wright et al, 1990, 1994; Norton et al., 1992).

Model implementation.

Atmospheric deposition and net uptake-release fluxes for the base cations and strong acid anions are required as inputs to the model. These inputs are generally assumed to be uniform over the catchment. Atmospheric fluxes are calculated from concentrations of the ions in precipitation and the rainfall volume into the catchment. The atmospheric fluxes of the ions must be corrected for dry deposition of gas, particulates and aerosols and for inputs in cloud/fog water. The volume streamflow of the catchment must also be provided to the model. In general, the model is implemented using average hydrologic conditions and meteorological conditions in annual or seasonal simulations, i.e., mean annual or mean monthly deposition, precipitation and streamflow are used to drive the model. The model is not designed to provide temporal resolution greater than monthly. Values for soil and streamwater temperature, partial pressure of carbon dioxide in the soil and streamwater and organic acid concentrations in soilwater and streamwater must also be provided.

As implemented in this project, the model is a two-compartment representation of a catchment. Atmospheric deposition enters the soil compartment and the equilibrium equations are used to calculate soil water chemistry. The water is then routed to the stream compartment, and the appropriate equilibrium equations are reapplied to calculate streamwater chemistry.

Once initial conditions (initial values of variables in the equilibrium equations) have been established, the equilibrium equations are solved for soil water and streamwater concentrations of the remaining variables. These concentrations are used to calculate the streamwater output fluxes of the model for the first time step. The mass balance equations are (numerically) integrated over the time step, providing new values for the total amounts of base cations and strong acid anions in the system. These in turn are used to calculate new values of the remaining variables, new streamwater fluxes, and so forth. The output from MAGIC is thus a time trace for all major chemical constituents for the period of time chosen for the integration. Details of the numerical integration and a computer code for implementing the model are given by Cosby et al. [1984a].

Calibration Procedure.

The aggregated nature of the model requires that it be calibrated to observed data from a system before it can be used to examine potential system response. Calibration is achieved by setting the values of certain parameters within the model that can be directly measured or observed in the system of interest (called “fixed” parameters). The model is then run (using observed and/or assumed atmospheric and hydrologic inputs) and the outputs (stream water and soil chemical variables, called “criterion” variables) are compared to observed values of these variables. If the observed and simulated values differ, the values of another set of parameters in the model (called “optimized” parameters) are adjusted to improve the fit. After a number of iterations, the simulated-minus-observed values of the criterion variables usually converge to zero (within some specified tolerance). The model is then considered calibrated. If new assumptions (or values) for any of the fixed variables or inputs to the model are subsequently adopted, the model must be re-calibrated by re-adjusting the optimized parameters until the simulated-minus-observed values of the criterion variables again fall within the specified tolerance.

The calibration procedure requires that soils, stream and atmospheric deposition data are available for each stream. There are, however, no measurements of either soil properties or atmospheric deposition at any of the FISH study streams. The requisite data have been estimated for each site by: a) using extrapolations of measurements for deposition at other sites within SNP; and b) assigning soil properties based on landscape classification of the streams and using soils data from similar landscape classifications.

These estimates of the fixed parameters and deposition inputs are subject to uncertainties so a "fuzzy" optimization procedure was implemented for calibrating the model. The fuzzy optimization procedure consisted of multiple calibrations of each catchment using random values of the fixed parameters drawn from the observed possible range of values, and random values of deposition from a range including uncertainty about the extrapolated values. Each of the multiple calibrations began with (1) a random selection of values of fixed parameters and deposition, and (2) a random selection of the starting values of the optimized parameters. The optimized parameters are then adjusted using the Rosenbrock (1960) algorithm to achieve a minimum error fit to the target variables. This procedure is undertaken ten times for each stream. The final calibrated model is represented by the ensemble of parameter values and variable values of all of the successful calibrations.

Calibrations are based on volume weighted mean annual fluxes for a given period of observation. The length of the period of observation used is not arbitrary. Model output will be more reliable if the annual flux estimates used in calibration are based on a number of years rather than just one year. There is a lot of year-to-year variability in atmospheric deposition and catchment runoff. Averaging over a number of years reduces the likelihood that an “outlier” year (very dry, etc.) is the primary data on which model forecasts are based. On the other hand, averaging over too long a period may remove important trends in the data that need to be simulated by the model. For the study here, the model was calibrated using all 3 years of data available from the FISH study sites (1993-1995 calendar years) to provide the volume weighted annual flux estimates for runoff chemistry. The same period of time was used for calculating the average annual atmospheric deposition using the observed data from deposition monitoring site at North Fork Dry Run (see below).

Modifications for the FISH project

The MAGIC model as described above was modified for the FISH project by including new subroutines to calculate the chemistry of a mixture of the stream baseflow with a quickflow component. The baseflow chemistry is that simulated by the unmodified MAGIC model (monthly or annual average concentrations of all major ions and alkalinity in the stream). The changes to the model allowed the introduction of a quickflow component (which explicitly contains as variables the same major ions as are in the MAGIC model) and provided the necessary numerical solution algorithms to calculate the chemistry of the mixture of baseflow and quickflow components.

Subroutines were added to allow the user to specify as inputs to the model: 1) the composition of the quickflow component and the variation of this composition throughout the course of the simulation; and 2) the mixing ratio and the non-equilibrium aluminum and organic acid characteristics of the storm flow. Subroutines were also added to allow user to request output that consists of: 1) comparative tables of quickflow, baseflow and stormflow chemistry for any period in the simulation; 2) the effects of the stormflow, baseflow or quickflow chemistry on the biological community.

Specifically, the biological effects represented are the expected number of species in the stream (based on simulated alkalinity), the condition factor of black-nosed dace (based on simulated pH), and the percentage of brook trout sac-fry mortality expected (based on inorganic monomeric aluminum concentrations). These biological effects relationships are described in Chapter 6 of this report and are summarized here as used in MAGIC.

The number of species in the stream is calculated using the linear equation

$$\text{number of species} = 0.033 * ANC_{min} + 2.2 ,$$

where ANC_{min} is the episodic minimum ANC calculated from the episodic chemistry routine.

The dace condition factor is calculated using the linear equation

$$\text{condition factor} = 1.28 * pH_{max} + 0.6 ,$$

where pH_{max} is the pH calculated by the episodic chemistry routine.

Trout sac-fry survival is calculated using the algorithm

$$\text{\% survival} = 100\% \text{ if total inorganic monomericAl} < 20\ \mu g/L ,$$

$$\text{\% survival} = 0\% \text{ if total inorganic monomericAl} > 80\ \mu g/L ;$$

if total inorganic monomeric Al is between 20 and 80 μg/L, survival is calculated using the linear equation

$$\text{\% survival} = 100*(80 - \text{total Al})/60 ,$$

where the total inorganic monomeric Al concentration (μg/L) is calculated by the episodic chemistry routine.

The input and output subroutines are interactive. The user can change the values of quickflow components or stormflow parameters from the keyboard. The year and/or month (either in the future or in the past) for which the stormflow and biological effects calculations are done can be selected from the keyboard and the results immediately displayed. It is thus possible to make immediate comparisons (for example): 1) of the effects of the same storm parameters in different years (or months) when the baseflow was different; 2) the effects of different storm parameters at the same time of year: 3) the effects of specific ionic differences in the composition of the quickflow component; etc.

The subroutines were interfaced with the original MAGIC routines in the following manner. The codes for the original MAGIC model (written in FORTRAN) were not altered. The same inputs and outputs are needed for this modified code (modifications also written in FORTRAN). The simulation for a particular site is run for the hindcast and forecast periods exactly as with the original MAGIC model. Once these simulations are complete, the model passes through the simulated stream water chemistry data that are stored on file and calculates the stormflow chemistry for each period of time in the simulation using the specified storm parameters and quickflow chemistry. The resultant simulated stormflow chemistry values are stored on an additional file. Then for each period of time for which stormflow (and therefore baseflow and quickflow) chemistry are stored, the program calculates and stores the predicted biological effects on yet another file. The user then has access to tabular and graphical output for all variables at all times of the simulation. If the user wishes to change storm parameters or quickflow concentrations, these changes can be entered at the keyboard and new stormflow chemistry and biological effects are calculated without having to re-run the MAGIC simulations themselves. On currently available PC computer equipment these calculations are exceedingly fast (a few seconds for simulation of 250 or more years).

The user has the option of saving any combination of storm parameters and quickflow concentrations on an external file that can be stored, transported and accessed at a subsequent model session. This feature avoids the need to re-enter parameter sets that the user wishes to save (i.e., "calibrated storm parameters", bets guesses, etc.). The files on which storm parameters are stored are physically distinct from the standard MAGIC parameter files so that different storm parameter files can be mixed or matched with different MAGIC catchment calibration parameter sets.

There is no “User’s Manual” for the original MAGIC model. The model was designed to be interactive. All parameters are accessible through menu driven display screens. Labels (and physical units) for inputs, outputs and parameters are also displayed on-screen. The user is directed to start the model and learn through interactive sessions using the built in example parameter sets. The same philosophy was adopted for the modifications implemented for the FISH project.

Additional requirements for the application of MAGIC to the FISH sites.

There were two practical problems concerning availability of input data that had to be solved before MAGIC (either the original or modified version) could be applied to the FISH sites and calibrated for use in long-term simulation. The general solution to these problems is described here. Details of the calibration procedure and the final applications to each site are described later in this chapter.

Information about the physical and chemical characteristics of the soils in each of the study catchments is needed for application of MAGIC. The FISH project did not support collection of soils data and there were no pre-existing data on soils in any of the FISH catchments. Appropriate data do exist, however, for soils in other forested headwater catchments in western Virginia. These data were extrapolated to the FISH site using a landscape classification scheme based on bedrock geology. The dominant bedrock type in each of the FISH catchments was identified from bedrock geology maps. The dominant bedrock type of the catchments for which existing soils data were available was also identified. All soil pits in a given classification were used to derive mean and ranges of parameter values needed for application of MAGIC. The landscape classification scheme and the soils data used for the FISH catchments are described in detail in the methods section (below).

Atmospheric deposition to each FISH catchment is needed for calibration and application of MAGIC. The model requires input fluxes of the base cations, sulfate, nitrate and ammonia. The fluxes must be total atmospheric deposition. There are two monitoring sites maintained by SWAS in the SNP where wet deposition is measured on a weekly basis - White Oak Run (WOR) in the southern part of SNP, and North Fork Dry Run (NFDR) in the central part of SNP. The data from both sites provide coverage for the entire period of the FISH project. The data from NFDR was adopted for the modelling exercises of the FISH project because the site was more centrally located relative to the three

FISH catchments. Estimates or observations of dry deposition and cloud/fog deposition to each site are also needed for the model. These data were obtained from atmospheric monitoring activities at NFDR and Big Meadows in SNP. Details of the atmospheric inputs are described in detail in the methods section (below).

Data Sources

Stream Water Quality Data.

The stream water quality data used (and the methods of sampling and analysis) are described elsewhere in this report. Those measured stream concentrations and discharges were used to construct: 1) average annual runoff fluxes; 2) volume weighted annual average concentrations; and 3) input/output mass balance budgets. These were calculated for each major ion in the streams for each year of the study as well as for the entire three year period (Tables 7-1 A,B and C). The three-year averages were used in the calibration of MAGIC for each site. (The source of the data on input fluxes used in Tables 7-1 A,B and C is described below in the atmospheric deposition section). For this project alkalinity is defined as the charge balance alkalinity:

$$\text{alkalinity} = \text{SBC} - \text{SAA}$$

where SBC is the sum of base cation concentrations (calcium, magnesium, potassium, and sodium) and SAA is the sum of acid anion concentrations (sulfate, nitrate and chloride), all concentration in μeq/L. Volume-weighted annual mean concentrations for all three sites for the period 199xxtoxxx were used for the calibration and modelling exercises in this project.

Atmospheric Deposition.

Atmospheric deposition used for this assessment must be the **total** deposition flux of the major ions to each catchment. Total deposition consists of three components:

$$\text{Total deposition} = \text{wet deposition} + \text{dry deposition} + \text{cloud/fog deposition.}$$

Wet deposition is the flux of ions occurring in precipitation. Dry deposition results from particulate and gaseous fluxes. Cloud and fog inputs can also important at all sites. Wet deposition and precipitation amounts measured at North Fork Dry Run (Table 7-2) were used for the FISH study catchments.

There are no observations of dry deposition or cloud/fog deposition for any stream included in this study.

Estimates of dry deposition fluxes and cloud/fog deposition fluxes of sulfate and nitrate have, however, been published for two sites in the Shenandoah National Park. Dry deposition has been estimated for Big Meadows, VA, a part of the National Dry Deposition Network (NDDN) established in 1986. The network currently operates as a component of the Clean Air Status and Trends Network (CASTNet). Clarke et al. (1997) describe the 50 station CASTNet dry deposition network and give estimates of dry deposition for the year 1991. Estimates of annual cloud/fog deposition fluxes of are available for North Fork Dry Run for the years 1986-1987 as part of the output of the Mountain Cloud Chemistry Project (MCCP; Vong et al., 1991). The periods of time covered by the MCCP and CASTNet estimates do not directly overlap the period included in this study (1993-1995), but are sufficiently close in time that the data can be reliably used.

These dry deposition flux and cloud/fog deposition flux estimates were extrapolated (both is space and time) to the site at NFDR where wet deposition was measured. This allowed calculation of total deposition for NFDR for the period 1993-1995 (Table 7-2). The ratio of estimated total deposition to the observed wet deposition was then calculated for sulfate, nitrate and ammonium ions. These ratios (called the dry deposition factors) were then used for all three streams in this study to calculate total deposition of sulfate, nitrate and ammonium from the estimated wet deposition data. The dry deposition factors used were: 2.0 for sulfate, 2.5 for nitrate and 2.5 for ammonium.

Deposition History.

Given current total deposition for each stream, the modelling required a temporal sequence of how that deposition has varied historically for the period over which anthropogenic deposition to the streams has been important. That is, the total historical loading to a stream and the temporal pattern of that loading must be provided to the model. The MAGIC model is sensitive to assumptions about historical deposition. The pattern of the historical deposition determines the total loading of acidic deposition that the site has received, and thus affects how the model simulates responses to future changes in loading.

Such long-term, continuous historical deposition data do not exist. The approach adopted for this project was to use historical emissions as a surrogate for deposition. The emissions for each year in the historical period are normalized to emissions in the reference year (the year for which observed data are available). This produces a sequence of scalar numbers (scale factors) that have a value of 1.0 for the reference year. Values of the scale factor for other years are (by definition) the fractions of reference year emissions that occurred in that year (e.g., if emissions in 1950 were 86% of what they were in the reference year, then the scale factor for 1950 is .86).

Using this scaled sequence of emissions, historical deposition was estimated by multiplying the total deposition measured (or estimated) for 1994 (the reference year used in this project) by the emissions scale factor for any year in the past to obtain deposition at that year in the past. An implicit assumption is that the relationship between emissions and deposition is unchanged over time. Thus, if emissions in 1950 were 86 % of the emissions in the reference year, then deposition in 1950 is assumed to be 86% of deposition in the reference year.

A key assumption in this procedure is that the "source" area for the emissions used to scale deposition at a site can be correctly identified. Emissions data for SO2 are available on a state-by-state basis back to the turn of the century. In NAPAP, yearly emissions from all states comprising an EPA administrative region were added together to produce a "regional" emissions history. All sites for modelling that were situated within a particular EPA region used that regional emissions history. That approach was adopted for this project and deposition histories for SO4, NO3 and NH4 for the streams were scaled using the scaled sequence of SO2 emissions in the EPA mid-Atlantic region (Figure 7-1).

Landscape Classification.

The forested mountain watersheds in western Virginia are geochemically more sensitive to the effects of acidic deposition than other landscape categories that comprise the southern Appalachian region (such as agricultural lands, etc.). Within the stratified sub-group of forested mountain watersheds, however, there is a still a range of sensitivity to acidic deposition and the landscape can be further stratified. This stratification provides the landscape classes needed to associate soils properties with each of the selected streams (a prerequisite for model calibration).

The landscape classes used here are based on an analysis of stream water quality in relation to watershed geology. The geographic area covered includes the Blue Ridge Mountains and the Ridge and Valley physiographic provinces of western Virginia. Within these provinces there are a number of distinct bedrock types that can be used to classify the streams (each stream was assigned to a landscape class based on the dominant bedrock type within its catchment). Previous analyses by Lynch and Dise (1985) and Webb et al. (1994) have established that a close relationship exists between stream water quality and bedrock type for catchments in the mountains of Virginia. In particular, Webb et al. (1994), identified six geological classes that served to account for spatial variation in alkalinity among streams in the same region (Figure 7-2). Of the six classes described by Webb et al. (1994), three were used for the three study catchments in the FISH project: Piney Run lies predominantly on Blue Ridge basaltic bedrock; Staunton River lies predominantly on Blue Ridge granitic bedrock; and Paine Run lies predominantly on the Blue Ridge siliciclastic bedrock.

Soil properties.

Soil data for use in the model application were available for a total of 14 sample sites located in four forested mountain watersheds in western Virginia. Soil data for three of the watersheds were obtained in 1986 and 1987 through the Direct-Delayed Response Program (DDRP), a component of the National Acid Precipitation Assessment Program. The methods of soil sampling and analysis used for the DDRP are described in Church et al., 1992. Soil data for the additional watershed were obtained through the SWAS program using methods closely comparable to those of the DDRP.

The soil sample sites were assigned to each of the geologically defined landscape classes. The number of soil sample sites available for each class were: Blue Ridge siliciclastic (9); Blue Ridge granitic (3); and Blue Ridge basaltic (3). The soils data for the individual soil horizons at each sampling site were aggregated based on horizon, depth and bulk density to obtain single vertically aggregated values for each site (Table 7-3). The soil parameters used in the model included soil depth, bulk density, pH, cation-exchange capacity, and exchangeable bases on the soil (calcium, magnesium, potassium, and sodium).

Results

Calibrations at the FISH sites

All three streams included in this project were successfully calibrated. Good fits were obtained for the model applied to each of the study streams (as measured by comparisons of simulated and observed variable values, Table 7-4). Errors between simulated and observed annual volume-weighted average concentrations were all less than 3 μeq/L for the calibration period. Simulation of soil variables was equally good with errors between simulated and observed exchangeable cation concentrations all less than 0.2% (Table 7-4). These results were obtained using the average parameter set (see below) derived from the "fuzzy calibration" procedure.

Multiple Calibrations and the Average Parameter Set

Ten calibrations were attempted for each stream as part of the "fuzzy calibration" procedure and at least nine successful calibrations were achieved for each stream. For each stream, all of the successful calibration parameter sets can be used for hindcast and forecast simulations. For example, if a given stream had 10 successful calibrations, the parameter sets for each of those calibrations could be used in the model to reconstruct the historical changes in stream water quality (based on the assumed historical deposition pattern) and to forecast the future changes in water quality (based on three assumed future deposition scenarios, see below). There are thus 10 possible values for each water quality variable in every year of the historical reconstruction of this stream, and 10 possible values of each variable for each year of each of the future deposition scenarios applied for this stream. These ranges of possible past and future responses represent the uncertainty (or confidence) intervals associated with simulation of each variable.

The results discussed below are based on simulations using the **average** values of the optimized and fixed parameters (average values of all successful calibration parameter sets; Table 7-5). The use of the average parameter set for each stream assures that the simulated responses are neither over- nor underestimates. Rather, the simulation based on average values of parameters approximates the most likely behavior of each stream (given the assumptions inherent in the model and the data used to constrain and calibrate the model). The full ensembles of calibrated parameters for each stream have been archived and can be use to derive uncertainty estimates for the simulations.

Episode Simulations

The modified code developed to simulate storm episodes was implemented for each site using the average calibrated parameter set. As discussed above, the selection of a single set of episode parameters for a stream is problematic. Storm events vary widely in intensity, and duration with resultant differences from storm to storm in mixing ratios and source areas (i.e., ionic concentrations) for the quickflow component. Therefore, a "typical" or "reference" set of episode parameters must be chosen for use in comparative simulations of storm responses. Two "reference" episodes are examined for this report. Both use the same model compartments for quickflow and baseflow components, and both assume aluminum solubility in the episode to be the same as in stream baseflow. The two "reference" episodes differ in the assumed mixing ratio, and the magnitude of the difference was selected (as described below) to bracket the range of episode intensity observed at the study sites.

The concentrations of ions in the quickflow component used in the episode simulations were set equal to the annual volume-weighted average concentrations of wet deposition in the year for which the episode was simulated. The concentrations of the baseflow component were set equal to the volume weighted annual average stream chemistry simulated by MAGIC in the year for which the episode was simulated. It was observed in the course of the FISH field work that episodes on the Staunton River would sometimes produce negative alkalinities and sometimes would not. The mixing ratios for the two reference episodes were chosen such that episodes simulated for Staunton River in 1994 a) would or b) would not produce negative alkalinities. The mixing ratios used were 0.2 (20% quickflow and 80% baseflow) for the low intensity reference storm that would not produce negative alkalinity; and 0.8 (80% quickflow and 20% baseflow) for the high intensity storm that would produce negative alkalinity in the Staunton River simulations. These mixing ratios bracket a range of storm intensities likely to occur in SNP streams.

Hindcast Water Quality

The calibrated model for each site was used to reconstruct the history of water quality changes that have occurred at each stream as a result of changing atmospheric deposition. The pattern of responses differs among the streams.

Historical acid anion concentrations were similar for all three streams: Piney River (Figure 7-3A), Staunton River (Figure 7-3B), and Paine Run (Figure 7C). However, the historical base cation concentrations were much higher in Piney River producing a higher historical alkalinity in that stream (and reflected in the higher base cation weathering estimates derived for Piney River, Table 7-5). The increases in acid anion concentrations (as acidic deposition increased) in each stream are largest for Paine Run (Figure 7C), the stream with the lowest historical base cation concentrations and the lowest estimated base cation weathering rates. The historical reconstruction for Paine Run suggest that the stream has lost almost all of its alkalinity as a result of acidic deposition inputs to the catchment. The simulated losses of alkalinity from Piney River (Figure 7A) and Staunton River (Figure 7C) are much smaller. The decline in annual average pH of the streams has been most dramatic for Paine Run, with the rate of decline accelerating as the alkalinity approached zero.

The upward spikes in acid anion and base cation concentrations (and the resultant depression in alkalinity and pH) that occur between 1989 and 1994 are the result of increased NO3 and base cation leaching from the catchments following forest defoliation by the gypsy moth. This "gypsy moth effect" was incorporated in the model simulations by allowing a transient decline in the nitrate uptake parameterization of the model. The effect was assumed to begin in 1989 when the moth was first identified within the catchments. The decline in NO3 uptake was assumed to be highest in the first year of the simulated infestation and decays linearly back to complete retention of NO3 within 10 years. The base cation increases result from cation exchange processes in the model in response to the increased concentration of acid anions as NO3 is mobilized and leached from the soils. The "gypsy moth effect" as included in these simulations is complete by 1998 and is not assumed to re-occur in the forecast scenarios.

Episodic Water Quality Changes

The reference episodes defined for this report produced the desired range of episodic responses at Staunton River. The lower mixing ratio (0.2) did not produce an acid episode in Staunton River in 1994 (Table 7-6A) while the higher mixing ratio (0.8) did (Table 7-6B). Storms of both intensities produced acid episodes in 1994 in Paine Run, while neither storm produced episodic acidity in Piney River in 1994 (Tables 7-6A,B). The simulations suggest that none of the streams were historically susceptible to episodic acidification.

The episode simulations suggest that the largest changes in water quality during episodes occur in Piney River (as measured by alkalinity depressions; Tables 7-6A,B), and the smallest changes occur in Paine Run. This is primarily a dilution effect, the higher overall ionic strength of Piney River will be diluted more when mixed with the lower ionic strength quickflow component. However, the most relevant water quality variable with respect to biological response of the streams is not the magnitude of the alkalinity depression, but the minimum alkalinity that occurs in the stream. Large declines in alkalinity (and ionic strength) can be tolerated as long as the lowest alkalinity reached is above zero.

Forecast Results

Forecast Scenarios.

Three scenarios of future acidic deposition were considered in this study: constant deposition at 1994 levels and two levels of reduced deposition (40% and 70% reductions from 1994 levels). The scenarios are based only on changes in sulfate deposition, all other ions in deposition for each stream are assumed to remain constant into the future at 1994 levels. For each scenario, simulations were run for fifty years into the future (1994 - 2044). For the two scenarios assuming reduced deposition, the sulfate deposition reductions were implemented linearly over 20 years (1994 - 2014), with constant sulfate deposition at the reduced level assumed for the final 30 years of simulation (2014 - 2044).

Output from the future simulations are examined at two times, the years 2015 and 2044. The former represents the responses of the streams immediately after the completion of the deposition reduction and might be considered the “direct” effect of the assumed reduction. The latter year is included to examined any “delayed” effects that might occur as a result of continued deposition at the reduced levels.

Forecast Water Quality

The forecasts for all three streams suggest that future water quality in these streams will not improve under any of the three simulation scenarios (Figures 7-4 A,B,C). Aside from this general statement, the responses of the three streams are distinct.

Future acid anion concentrations in Paine Run and Piney River behave similarly (Figures 7-4 A,C): concentrations increase under constant deposition, stay approximately the same under a scenario of 40 % reduction, and decline with a 70% reduction. While the pattern is similar, the magnitude of the acid anion concentration responses in Paine Run is larger. Acid anion concentrations in Staunton River (Figure 7-4 B) change the least, and a 70% reduction is necessary to prevent further increases in acid anion (e.g., SO4) concentrations in that stream. These results are consistent with the fact that the Staunton River catchment apparently has the highest sulfate adsorption capacity of the three catchments (see input/output budgets - Tables 7-1A,B,C; and see calibrated SO4 adsorption parameters - Table 7-5).

Future base cation concentrations in these streams also behave similarly among the streams (Figures 7-4 A,B,C). The base cation responses are driven by the changes in acid anion concentrations. In general, if acid anion concentrations increase, the base cation concentrations also increase, but not as much. The degree to which base cations respond to acid anions depends on the base saturation of the soils. Low base saturation soils are not capable of producing large quantities of base cations in response to increases in acid anion concentrations. This effect is seen in the comparison between Paine Run (Figure 7-4C) and Piney River (Figure 7-4A). The magnitudes of acid anion changes for the three scenarios is much greater for Paine Run than for Piney River, yet the base cation responses are approximately the same. Base saturation is lower in Paine Run than Piney River (Table 7-4) and the base cation response per unit acid anion change is smaller.

The net effect of the differential responses of acid anions and base cations in each stream is reflected in the simulated future alkalinity concentrations for all three streams (Figures 7-4 A,B,C). For Staunton River and Piney River, there is little forecast difference in alkalinity in either stream for any of the future deposition scenarios. This relative insensitivity of alkalinity (despite large changes in acid anion concentrations) is due to the ability of the soils in these catchments to exchange base cations readily (i.e., they have a relatively high base saturation). This cation exchange process buffers against alkalinity losses as acid anion concentrations increase. As acid anion concentrations decline, however, the exchange process also buffers against alkalinity increases (base cations that used to be exported to the stream with acid anions are used to restore lost base saturation on the soils when acid anions decrease, in effect keeping the alkalinity constant).

The alkalinity responses of Paine Run (Figure 7-4C) are very different from the other two streams. There are clear differences in the alkalinity responses of Paine Run to the different forecast scenarios. The soils in Paine Run have a low base saturation and the stream water alkalinity is thus less well buffered against changes in acid anion concentrations. In that the relatively large changes in alkalinity in Paine Run are also occurring near zero alkalinity, there are large changes in pH forecast for this stream. For both constant and 40% deposition reductions, large losses of alkalinity are forecast for this stream. In fact, these two scenarios produce chronic acidity in Paine Run within 20 years. The simulation results suggest that a 70% reduction in deposition is necessary to maintain the current water quality in Paine Run.

Forecast Episodic Water Quality Changes

Episodic water quality changes for the two reference storm episodes show a similar pattern across the watersheds. Future baseflow and episode alkalinity in Piney River and Staunton River for less intense storms are relative insensitive to the assumed future deposition reduction (Figure 7-5A). For Paine Run, however, the episode simulations suggest that both 40% and 70% reductions in deposition will reduce and perhaps remove the episodic response of this stream to less intense storms (mixing ratio = 0.2).

For the more intense storms (mixing ratio = 0.8), all three streams show important differential response to the three assumed future deposition scenarios (Figure 7-5B). For Piney River and Staunton River, constant deposition results in acidic episodes in response to intense storms. The forecast simulations suggest that episodic acidification in response to intense storms will be eliminated from both of these streams with a 70% deposition reduction. For Paine Run, however, even a 70% reduction will not eliminate episodic acidification in response to intense storms.

Biological Response Classification

Based on simulated average annual alkalinity values, the three streams can be assigned to one of the four proposed biological effects classifications for each year in the hindcast and forecast (Table 7-7). The classifications of Piney River and Staunton River do not change either historically or in response to any of the future deposition scenarios. Paine Run, however, is seen to be affected both in the past and in the future. For Paine Run, the future classifications suggest that 70% reductions are necessary to maintain the current status of this stream (episodically acidic). Both constant deposition and 40% reductions will result in this stream becoming chronically acidic within 20 years. Deposition reductions of greater than 70% would be needed to restore this stream to its pre-deposition biological effects classification.

Discussion

Approach 1: Modification of the MAGIC computer code.

As discussed in the Introduction of this chapter, the use of the modified MAGIC model in comparative and speculative simulation exercises is probably the most important and productive use of the modified computer code. In that the modifications did not actually incorporate physically-based mechanisms for simulating the stormflow mixture, the application of these codes requires calibration based on observed field data. Such data are not currently available for many streams. The episodic sampling done as part of the FISH project provides some information about the possible values of the storm parameters required by these modifications (mixing ration, aluminum solubility, etc.). Even with the intense level of effort expended on these sites, however, the parameters in this rather simple code cannot be tightly constrained. Thus, projections of biological effects based on individual simulated storms must be considered highly imprecise at present. However, the use of the modified model in speculative exercises can lead to a better understanding of the dynamics of storms and their potential effects on biota in streams. The modified model almost certainly will raise more questions than it answers, but these questions can be used to guide future research efforts and can be used to help design sampling schemes that will lead to an improved understanding of stormflow in streams.

The use of "reference episodes" in conjunction with MAGIC long-term simulations can provide a framework for assessment of the potential effects and benefits of future deposition scenarios. As demonstrated in this report, these "reference episodes" can be used as indices in **comparative evaluations** of the changes in episodic responses of streams that are likely to accompany changing deposition.

Approach 2: Development of empirical relationships for biological effects.

It can be noted that the biological effects classification scheme proposed here classifies Staunton River as "not acidic'. In the field experiment, the stream was observed to produce acidic episodes during certain events, but not others (i.e., the stream perhaps should be classified as "episodically acidic"). This points out an important limitation in the classification approach. How often and under what conditions must episodes be acidic before biological effects become measurable and pronounced and the stream should be classified as truly "episodic"? In developing a classification scheme, the objective is to capture the important biological effects for a large number of systems. In any classification scheme, individual cases may be found that do not conform. In setting the alkalinity upper limit for the "episodically acidic" class, our informed judgement was that episodes were not only possible but **highly likely** when average annual alkalinity is below 20 μeq/L. The possibility was recognized that, under extreme storm events, a stream like Staunton River (with an annual average alkalinity above 20 μeq/L) may produce occasional acidic episodes, but these are **rare** (not common) episodes and the biota are little affected. The inclusion of the "indeterminate" class was intended to express uncertainty about the range of average annual alkalinities over which episodes are or are not **likely**. Again, our informed judgement was that, while episodes are possible in streams with average annual alkalinities above 50 μeq/L, they are **highly unlikely**.

A second objection to this proposed classification scheme could be focused on the choice of averaging period for the observed alkalinity. The classification proposed in this project is based on average annual alkalinity. The classification could also be based on the minimum monthly average alkalinity observed during the year, for instance. In general, however, there are good correlations among the various measures of alkalinity than can be defined for a stream. This scheme is based on annual averages explicitly so that the output of the MAGIC model can be directly coupled to the classification scheme.

Conclusions

The coupling of the results of the FISH project with the MAGIC model has allowed an examination of the likely history and future of the three study streams. While the conclusions are expressed in terms of water quality changes, these changes can be directly linked to biological effects using the relationships described elsewhere in this report.

The model simulations of the three FISH project study streams suggest that annual average water quality in these streams is sensitive to both past and future changes in deposition. The historical reconstructions suggest that all streams have been affected by acidic deposition in that acid anion concentrations have increased in all streams and alkalinity has declined in all streams. The future scenarios suggest that at least 70% reductions in sulfate deposition are necessary to preserve the status quo of these streams. That is, a 70 % reduction will prevent further deterioration in annual average water quality. Reductions of greater than 70% are apparently necessary to reverse the losses of alkalinity in these streams and to start the recovery of lost water quality.

The model simulations of episodic water quality in the streams show a similar pattern. Historical episodes in all three streams would not have resulted in negative alkalinities. Acidic episodes are common in 1994 in Paine Run and occasional in Staunton River. In response to constant future deposition all three streams will experience more severe episodic depressions of alkalinity, with Paine Run producing acidic episodes in response to less severe storms and both Staunton River and Piney River producing episodic acidification in response to sever storms. Reductions of 70% in sulfate deposition will prevent acidic episodes in all three streams (except in Paine run under the most intense storms).

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Table 7.1 A Piney River Watershed

Stream Output Fluxes, Stream Volume Weighted Annual Averages and Catchment Input/Output Mass Balances

OUTPUT FLUXES

date		Discharge cm	Ca eq/ha	Mg eq/ha	Na eq/ha	K eq/ha	NH4 eq/ha	SO4 eq/ha	Cl eq/ha	NO3 eq/ha	SBC eq/ha	SAA eq/ha	Calk eq/ha	H eq/ha
12/93		65.5	870.1	735.0	460.1	46.5	0.0	477.1	187.6	300.1	2111.6	964.8	1146.8	0.6
12/94		74.4	940.4	789.7	531.5	45.8	0.0	542.9	210.2	207.8	2307.4	960.9	1346.5	0.7
12/95		83.8	1170.0	957.7	612.4	59.3	0.0	591.6	270.6	205.4	2799.3	1067.6	1731.7	0.8
	Total	223.7	2980.5	2482.3	1604.0	151.5	0.0	1611.5	668.4	713.3	7218.2	2993.3	4225.0	2.0
93-95	3 yr ave	74.6	993.5	827.4	534.7	50.5	0.0	537.2	222.8	237.8	2406.1	997.8	1408.3	0.7

VOLUME WEIGHTED ANNUAL CONCENTRATIONS

date		Discharge m	Ca meq/m3	Mg meq/m3	Na meq/m3	K meq/m3	NH4 meq/m3	SO4 meq/m3	Cl meq/m3	NO3 meq/m3	SBC meq/m3	SAA meq/m3	Calk meq/m3	H meq/m3	pH
12/93		0.65	132.9	112.2	70.3	7.1	0.0	72.9	28.7	45.8	322.5	147.3	175.2	0.1	7.05
12/94		0.74	126.4	106.1	71.4	6.1	0.0	72.9	28.2	27.9	310.0	129.1	180.9	0.1	7.04
12/95		0.84	139.6	114.2	73.1	7.1	0.0	70.6	32.3	24.5	333.9	127.4	206.6	0.1	7.03
93-95	3 yr ave	0.75	133.2	111.0	71.7	6.8	0.0	72.0	29.9	31.9	322.6	133.8	188.8	0.1	7.04

INPUT OUTPUT BALANCES

	Discharge m	Ca meq/m2	Mg meq/m2	Na meq/m2	K meq/m2	NH4 meq/m2	SO4 meq/m2	Cl meq/m2	NO3 meq/m2	SBC meq/m2	SAA meq/m2	Calk meq/m2	H meq/m2
output fluxes 3 year average (93-95)	0.75	99.3	82.7	53.5	5.1	0.0	53.7	22.3	23.8	240.6	99.8	140.8	0.1
input fluxes 3 year average (93-95)	1.08	8.3	2.9	3.5	5.4	31.1	94.7	5.9	54.0	51.2	154.6	-103.4	44.5
net fluxes (out-in) 3 year average (93-95)	-0.33	91.1	79.8	50.0	-0.3	-31.1	-41.0	16.4	-30.2	189.4	-54.8	244.2	-44.5

Table 7.1 B Staunton River Watershed

Stream Output Fluxes, Stream Volume Weighted Annual Averages and Catchment Input/Output Mass Balances

OUTPUT FLUXES

date		Discharge cm	Ca eq/ha	Mg eq/ha	Na eq/ha	K eq/ha	NH4 eq/ha	SO4 eq/ha	Cl eq/ha	NO3 eq/ha	SBC eq/ha	SAA eq/ha	Calk eq/ha	H eq/ha
12/93		70.7	450.5	199.3	414.9	69.9	0.0	316.9	171.7	71.2	1134.6	559.8	574.8	1.7
12/94		65.4	413.9	180.3	390.1	64.7	0.0	287.6	156.7	17.3	1049.1	461.6	587.5	1.6
12/95		73.5	569.4	267.7	443.8	83.6	0.0	309.1	185.2	16.1	1364.4	510.4	854.0	1.8
	Total	209.6	1433.8	647.2	1248.8	218.2	0.0	913.5	513.6	104.6	3548.0	1531.8	2016.3	5.1
93-95	3 yr ave	69.9	477.9	215.7	416.3	72.7	0.0	304.5	171.2	34.9	1182.7	510.6	672.1	1.7

VOLUME WEIGHTED ANNUAL CONCENTRATIONS

date		Discharge m	Ca meq/m3	Mg meq/m3	Na meq/m3	K meq/m3	NH4 meq/m3	SO4 meq/m3	Cl meq/m3	NO3 meq/m3	SBC meq/m3	SAA meq/m3	Calk meq/m3	H meq/m3	pH
12/93		0.71	63.7	28.2	58.7	9.9	0.0	44.8	24.3	10.1	160.6	79.2	81.3	0.2	6.62
12/94		0.65	63.3	27.6	59.7	9.9	0.0	44.0	24.0	2.6	160.5	70.6	89.9	0.2	6.62
12/95		0.74	77.4	36.4	60.3	11.4	0.0	42.0	25.2	2.2	185.5	69.4	116.1	0.2	6.61
93-95	3 yr ave	0.70	68.4	30.9	59.6	10.4	0.0	43.6	24.5	5.0	169.3	73.1	96.2	0.2	6.62

INPUT OUTPUT BALANCES

	Discharge m	Ca meq/m2	Mg meq/m2	Na meq/m2	K meq/m2	NH4 meq/m2	SO4 meq/m2	Cl meq/m2	NO3 meq/m2	SBC meq/m2	SAA meq/m2	Calk meq/m2	H meq/m2
output fluxes 3 year average (93-95)	0.70	47.8	21.6	41.6	7.3	0.0	30.5	17.1	3.5	118.3	51.1	67.2	0.2
input fluxes 3 year average (93-95)	1.08	8.3	2.9	3.5	5.4	31.1	94.7	5.9	54.0	51.2	154.6	-103.4	44.5
net fluxes (out-in) 3 year average (93-95)	-0.38	39.5	18.7	38.1	1.9	-31.1	-64.3	11.2	-50.5	67.1	-103.5	170.6	-44.4

Table 7.1 C Paine Run Watershed

Stream Output Fluxes, Stream Volume Weighted Annual Averages and Catchment Input/Output Mass Balances

OUTPUT FLUXES

date		Discharge cm	Ca eq/ha	Mg eq/ha	Na eq/ha	K eq/ha	NH4 eq/ha	SO4 eq/ha	Cl eq/ha	NO3 eq/ha	SBC eq/ha	SAA eq/ha	Calk eq/ha	H eq/ha
12/93		44.7	159.2	280.6	100.8	212.5	0.0	482.7	108.4	149.6	753.2	740.7	12.6	13.8
12/94		41.5	139.7	249.6	90.9	192.0	0.0	471.6	96.1	85.0	672.1	652.7	19.5	11.7
12/95		47.1	155.9	269.7	107.4	226.5	0.0	530.1	126.1	64.7	759.5	720.9	38.6	12.4
	Total	133.4	454.8	800.0	299.1	631.0	0.0	1484.4	330.6	299.3	2184.8	2114.2	70.6	37.9
93-95	3 yr ave	44.5	151.6	266.7	99.7	210.3	0.0	494.8	110.2	99.8	728.3	704.7	23.5	12.6

VOLUME WEIGHTED ANNUAL CONCENTRATIONS

date		Discharge m	Ca meq/m3	Mg meq/m3	Na meq/m3	K meq/m3	NH4 meq/m3	SO4 meq/m3	Cl meq/m3	NO3 meq/m3	SBC meq/m3	SAA meq/m3	Calk meq/m3	H meq/m3	pH
12/93		0.45	35.6	62.7	22.5	47.5	0.0	107.9	24.2	33.4	168.3	165.5	2.8	3.1	5.51
12/94		0.42	33.6	60.1	21.9	46.2	0.0	113.6	23.1	20.5	161.9	157.2	4.7	2.8	5.55
12/95		0.47	33.1	57.2	22.8	48.1	0.0	112.5	26.7	13.7	161.1	153.0	8.2	2.6	5.58
93-95	3 yr ave	0.44	34.1	60.0	22.4	47.3	0.0	111.3	24.8	22.4	163.8	158.5	5.3	2.8	5.55

INPUT OUTPUT BALANCES

	Discharge m	Ca meq/m2	Mg meq/m2	Na meq/m2	K meq/m2	NH4 meq/m2	SO4 meq/m2	Cl meq/m2	NO3 meq/m2	SBC meq/m2	SAA meq/m2	Calk meq/m2	H meq/m2
output fluxes 3 year average (93-95)	0.44	15.2	26.7	10.0	21.0	0.0	49.5	11.0	10.0	72.8	70.5	2.4	1.3
input fluxes 3 year average (93-95)	1.08	8.3	2.9	3.5	5.4	31.1	94.7	5.9	54.0	51.2	154.6	-103.4	44.5
net fluxes (out-in) 3 year average (93-95)	-0.63	6.9	23.8	6.5	15.6	-31.1	-45.2	5.1	-44.0	21.6	-84.1	105.8	-43.3

Table 7.2 Atmospheric Deposition measured at North Fork Dry Run

Wet Deposition Fluxes, Volume Weighted Annual Averages in Wet Deposition and Total Deposition Fluxes

INPUT FLUXES (Wet Deposition Only)

date		ppt cm	Ca eq/ha	Mg eq/ha	Na eq/ha	K eq/ha	NH4 eq/ha	SO4 eq/ha	Cl eq/ha	NO3 eq/ha	SBC eq/ha	SAA eq/ha	Calk eq/ha	H eq/ha
12/93		99.8	86.0	29.2	35.2	69.5	119.1	491.9	59.9	214.1	339.1	766.0	-426.9	466.6
12/94		95.0	86.7	30.6	33.5	57.0	138.9	458.2	54.1	205.1	346.8	717.4	-370.6	403.0
12/95		128.2	75.4	27.1	36.5	35.3	115.3	470.5	63.5	228.4	289.6	762.4	-472.9	466.9
	Total	323.0	248.1	87.0	105.3	161.8	373.3	1420.6	177.6	647.6	975.5	2245.8	-1270.4	1336.5
93-95	3 yr Ave	107.7	82.7	29.0	35.1	53.9	124.4	473.5	59.2	215.9	325.2	748.6	-423.5	445.5

VOLUME WEIGHTED ANNUAL CONCENTRATIONS (In Wet Deposition)

date		ppt m	Ca meq/m3	Mg meq/m3	Na meq/m3	K meq/m3	NH4 meq/m3	SO4 meq/m3	Cl meq/m3	NO3 meq/m3	SBC meq/m3	SAA meq/m3	Calk meq/m3	H meq/m3	pH
12/93		0.998	8.6	2.9	3.5	7.0	11.9	49.3	6.0	21.5	34.0	76.8	-42.8	46.8	4.33
12/94		0.950	9.1	3.2	3.5	6.0	14.6	48.2	5.7	21.6	36.5	75.5	-39.0	42.4	4.37
12/95		1.282	5.9	2.1	2.9	2.8	9.0	36.7	5.0	17.8	22.6	59.5	-36.9	36.4	4.44
93-95	3 yr Ave	1.077	7.7	2.7	3.3	5.0	11.6	44.0	5.5	20.1	30.2	69.5	-39.3	41.4	4.38

	Ca	Mg	Na	K	NH4	SO4	Cl	NO3	SBC	SAA	Calk	H
Dry Deposition Factors	1	1	1	1	2.5	2	1	2.5				1

TOTAL DEPOSITION (wet deposition times dry deposition factor)

		ppt m	Ca meq/m2	Mg meq/m2	Na meq/m2	K meq/m2	NH4 meq/m2	SO4 meq/m2	Cl meq/m2	NO3 meq/m2	SBC meq/m2	SAA meq/m2	Calk meq/m2	H meq/m2
93-95	3 yr Ave	1.08	8.3	2.9	3.5	5.4	31.1	94.7	5.9	54.0	51.2	154.6	-103.4	44.5

Table 7.3 Soils characteristics measured in Shenandoah National Park

Characteristics for individual soil pits and descriptive statistics for all pits on common geologic classes

Characteristics of individual pits (vertically aggregated using depth and bulk density to weight individual horizon values)

Watershed	Geologic Class	Site	Depth cm	Bulk Density kg/m3	pH	CEC meq/kg	Exchangeable Cations Ca meq/kg	Mg meq/kg	Na meq/kg	K meq/kg	Exchangeable cations as % of CEC Ca %	Mg %	Na %	K %	BS %
Moormans River	Basaltic	2	180	1458	5.58	117.2	38.0	30.0	0.5	0.8	32.4	25.6	0.4	0.7	59.1
Moormans River	Basaltic	3	123	1368	5.08	110.1	10.9	34.6	0.5	0.8	9.9	31.4	0.4	0.7	42.5
Moormans River	Basaltic	4	55	1165	4.88	95.9	10.5	3.2	0.2	0.9	10.9	3.4	0.2	1.0	15.5
Lower Lewis Run	Siliciclastic	1	55	1311	4.63	124.5	1.6	0.8	0.1	1.7	1.3	0.6	0.1	1.3	3.3
Lower Lewis Run	Siliciclastic	2	151	1536	4.82	42.9	1.8	2.4	0.1	1.3	4.2	5.5	0.2	3.0	12.8
Lower Lewis Run	Siliciclastic	3	84	1296	4.43	81.8	0.7	1.2	0.0	1.8	0.8	1.4	0.0	2.2	4.5
White Oak Run	Siliciclastic	1	131	1300	5.13	88.1	7.9	6.3	0.4	1.9	9.0	7.2	0.4	2.2	18.8
White Oak Run	Siliciclastic	2	50	1300	4.55	152.7	0.6	0.8	0.5	3.8	0.4	0.5	0.3	2.5	3.7
White Oak Run	Siliciclastic	3	101	1501	4.37	115.8	0.3	0.5	0.4	1.3	0.3	0.4	0.4	1.2	2.2
White Oak Run	Siliciclastic	4	95	1300	4.73	114.6	1.7	2.5	0.5	2.1	1.5	2.2	0.4	1.8	5.8
White Oak Run	Siliciclastic	5	55	1300	4.50	152.7	0.5	0.7	0.4	2.5	0.3	0.5	0.2	1.6	2.7
Moormans River	Siliciclastic	1	50	1273	4.90	62.5	1.0	0.3	0.0	1.1	1.7	0.4	0.0	1.7	3.9
North Fork Dry Run	Granitic	1	107	863	5.04	91.0	4.0	1.3	0.0	1.0	4.4	1.5	0.3	1.1	7.2
North Fork Dry Run	Granitic	2	134	1011	4.95	60.8	1.0	3.6	0.0	1.1	1.7	6.0	0.3	1.8	9.8
North Fork Dry Run	Granitic	3	144	1096	5.13	86.7	2.4	6.4	0.0	3.0	2.8	7.4	0.3	3.4	13.9

Summary statistics for all pits within a geologic class

Used For	Geologic Class		Depth cm	Bulk Density kg/m3	pH	CEC meq/kg	Exchangeable cations as % of CEC Ca %	Mg %	Na %	K %	BS %
Piney River	Basaltic	Mean	119	1330	5.18	107.7	17.7	20.1	0.3	0.8	39.0
	(n = 3)	Max	180	1458	5.58	117.2	32.4	31.4	0.4	1.0	59.1
		Min	55	1165	4.88	95.9	9.9	3.4	0.2	0.7	15.5
		Std.Dev.	63	150	0.36	10.9	12.7	14.8	0.1	0.2	22.0
Paine Run	Siliciclastic	Mean	86	1346	4.67	103.9	2.2	2.1	0.2	1.9	6.4
	(n = 9)	Max	151	1536	5.13	152.7	9.0	7.2	0.4	3.0	18.8
		Min	50	1273	4.37	42.9	0.3	0.4	0.0	1.2	2.2
		Std.Dev.	37	98	0.25	38.1	2.8	2.5	0.2	0.6	5.6
Staunton River	Granitic	Mean	128	990	5.04	79.5	2.9	5.0	0.3	2.1	10.3
	(n = 3)	Max	144	1096	5.13	91.0	4.4	7.4	0.3	3.4	13.9
		Min	107	863	4.95	60.8	1.7	1.5	0.3	1.1	7.2
		Std.Dev.	19	118	0.09	16.3	1.4	3.1	0.0	1.2	3.4

Table 7.4 Calibration results: goodness of fit for the calibrated model

Comparison of simulated and observed annual average concentrations for the calibration year (1994).

Stream Variables (concentrations in ueq/L)

	Piney River			Staunton River			Paine Run	
	Simulated	Observed		Simulated	Observed		Simulated	Observed
Ca	134	133	Ca	69	68	Ca	34	34
Mg	111	111	Mg	31	31	Mg	60	60
Na	72	72	Na	60	60	Na	22	22
K	7	7	K	10	10	K	47	47
NH4	0	0	NH4	0	0	NH4	0	0
SO4	71	72	SO4	42	44	SO4	109	111
Cl	30	30	Cl	24	24	Cl	25	25
NO3	32	32	NO3	5	5	NO3	24	22
SBC	323	323	SBC	170	169	SBC	163	164
SAA	132	134	SAA	72	73	SAA	157	159
Calk	191	189	Calk	98	96	Calk	6	5
pH	7.1	7.0	pH	6.8	6.6	pH	5.1	5.6

Soil Variables (Exchangeable cations in %)

	Piney River			Staunton River			Paine Run	
	Simulated	Observed		Simulated	Observed		Simulated	Observed
Ca	17.7	17.1	Ca	2.9	2.9	Ca	2.2	2.2
Mg	20.1	20.1	Mg	5.0	5.0	Mg	2.1	2.1
Na	0.4	0.3	Na	0.3	0.3	Na	0.3	0.2
K	0.8	0.8	K	2.1	2.1	K	2.0	1.9
Base Saturation	38.9	39.0	Base Saturation	10.3	10.3	Base Saturation	6.6	6.4
pH	5.5	5.2	pH	5.0	5.0	pH	4.6	4.7

Table 7.5 Calibration results: values of fixed and optimized parameters

Fixed parameter values are set before calibration using observed stream and soil data.
Optimized parameter values are adjusted during calibration so that simulated and observed stream and soil chemistry match.

Soil Fixed Parameters

Units	Parameter	Piney	Staunton	Paine
m	Depth	1.2	1.3	0.9
fraction	Porosity	0.5	0.5	0.5
kg/m3	Bulk Density	1334	989	1346
meq/kg	CEC	108	80	104
ueq/L	SO4 half sat.	100	100	100
log10	KAl	8.8	8.8	8.8
log10	Organic Acid pK1	2.64	2.64	2.64
log10	Organic Acid pK2	5.66	5.66	5.66
log10	Organic Acid pK3	5.94	5.94	5.94
atm	pCO2	0.006	0.006	0.006
deg C	Temperature	8	8	8
meq/m2/yr	Weathering Cl	16	11	5
meq/m2/yr	Uptake Ca	5.5	5.5	5.5
meq/m2/yr	Uptake Mg	1.5	1.5	1.5
meq/m2/yr	Uptake K	4	4	4
meq/m2/yr	Uptake SO4	2.5	2.5	2.5
% of input	Uptake NH4	100	100	100
% of input	Upake NO3	100	100	100

Soil Optimized Parameters

Units	Parameter	Piney	Staunton	Paine
meq/kg	SO4 Max. Adsorp.	9	20	11
meq/m2/yr	Weathering Ca	67	33	4
meq/m2/yr	Weathering Mg	56	13	11
meq/m2/yr	Weathering Na	48	37	4
meq/m2/yr	Weathering K	3	5	13
%	Initial Exchangeable Ca	18.3	3.4	2.4
%	Initial Exchangeable Mg	20.6	5.3	2.5
%	Initial Exchangeable Na	0.4	0.4	0.4
%	Initial Exchangeable K	0.8	2.2	2.2
%	Initial Base Saturation	40.1	11.2	7.6
log10	Ca-Al Selectivity Coeff.	4.3	3.2	0.3
log10	Mg-Al Selectivity Coeff.	3.9	1.5	1
log10	Na-Al Selectivity Coeff.	2.2	0.9	-1.6
log10	K-Al Selectivity Coeff.	-1.9	-3.9	-3.1
mmol/m3	Organic acid	100	85	74

Stream Fixed Parameters

Units	Parameter	Piney	Staunton	Paine
m/yr	Discharge	0.75	0.7	0.44
%	Relative area	0.5	0.5	0.5
log10	Organic Acid pK1	2.64	2.64	2.64
log10	Organic Acid pK2	5.66	5.66	5.66
log10	Organic Acid pK3	5.94	5.94	5.94
atm	pCO2	0.0006	0.0006	0.0006
deg C	Temperature	8.5	8.5	8.5

Stream Optimized Parameters

Units	Parameter	Piney	Staunton	Paine
mmol/m3	Organic acid	10	10	10
log10	KAl	9	9	9

Table 7.6 A **Results of episode simulations for selected years for the FISH catchments with episode chemistry based on mixture of 20% quickflow with 80% baseflow (mixing ratio = 0.2)**

Baseflow component for the episode mixture is annual average simulated streamflow values.
Quickflow component for episode mixture is the annual average wet deposition.

	year	Sum Base Cations (SBC) ueq/L Baseflow	Episode	Sum Acid Anions (SAA) ueq/L Baseflow	Episode	Alkalinity (SBC-SAA) ueq/L Baseflow	Episode	Alkalinity depressi (Epsd-Bsflw) ueq/
Piney River	1854	242	198	33	28	209	170	-39
	1994	323	262	132	119	191	143	-48
constant	2044	320	260	132	119	188	141	-47
40% reduction	2044	300	244	107	96	193	148	-45
70% reduction	2044	287	234	92	81	195	153	-42
Staunton River	1854	138	114	28	24	110	90	-20
	1994	170	139	72	72	98	67	-31
constant	2044	182	149	92	88	90	61	-29
40% reduction	2044	172	141	80	74	92	67	-25
70% reduction	2044	166	136	71	65	95	71	-24
Paine Run	1854	91	77	30	26	61	51	-10
	1994	163	134	157	140	6	-6	-12
constant	2044	166	137	198	173	-32	-36	-4
40% reduction	2044	146	121	152	132	-6	-11	-5
70% reduction	2044	134	111	125	108	9	3	-6

Table 7.6 B **Results of episode simulations for selected years for the FISH catchments with episode chemistry based on mixture of 80% quickflow with 20% baseflow (mixing ratio = 0.8)**

Baseflow component for the episode mixture is annual average simulated streamflow values.
Quickflow component for episode mixture is the annual average wet deposition.

	year	Sum Base Cations (SBC) ueq/L Baseflow	Episode	Sum Acid Anions (SAA) ueq/L Baseflow	Episode	Alkalinity (SBC-SAA) ueq/L Baseflow	Episode	Alkalinity depressi (Epsd-Bsflw) ueq/
Piney River	1854	242	63	33	13	209	50	-159
	1994	323	80	132	82	191	-2	-193
constant	2044	320	79	132	82	188	-3	-191
40% reduction	2044	300	75	107	64	193	11	-182
70% reduction	2044	287	72	92	51	195	21	-174
Staunton River	1854	138	42	28	12	110	30	-80
	1994	170	49	72	70	98	-21	-119
		182						
constant	2044	172	51	92	74	80	-23	-103
40% reduction	2044	166	49	80	58	86	-9	-95
70% reduction	2044	166	48	71	46	95	2	-93
Paine Run	1854	91	33	30	12	61	21	-40
	1994	163	47	157	87	6	-40	-46
constant	2044	166	48	198	95	-32	-47	-15
40% reduction	2044	146	44	152	73	-6	-29	-23
70% reduction	2044	134	42	125	57	9	-15	-24

Table 7.7 Classification of streams in bilogical effects categories based on simulated annual average alkalinity.

(Alkalinity ranges that define each class are given in the text)

	year	**Piney River**	**Staunton River**	**Paine Run**
	1854	not acidic	not acidic	not acidic
	1994	not acidic	not acidic	**episodically acidic**
constant	**2015**	not acidic	not acidic	**chronically acidic**
40% reduction	**2015**	not acidic	not acidic	**episodically acidic**
70% reduction	**2015**	not acidic	not acidic	**episodically acidic**
constant	**2044**	not acidic	not acidic	**chronically acidic**
40% reduction	**2044**	not acidic	not acidic	**chronically acidic**
70% reduction	**2044**	not acidic	not acidic	**episodically acidic**

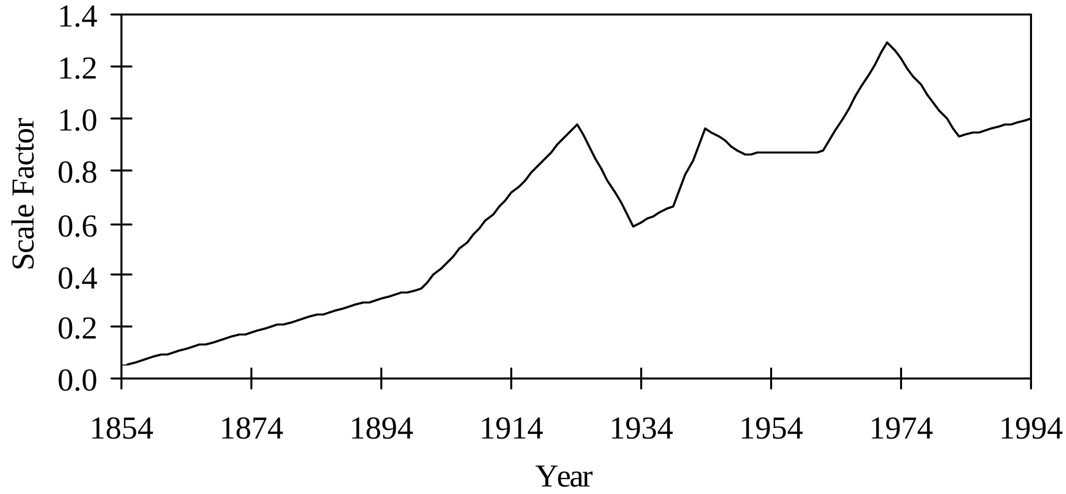

Figure 7-1 Scaled historical emissions used to set hindcast deposition sequences of sulfate, nitrate and ammonium.

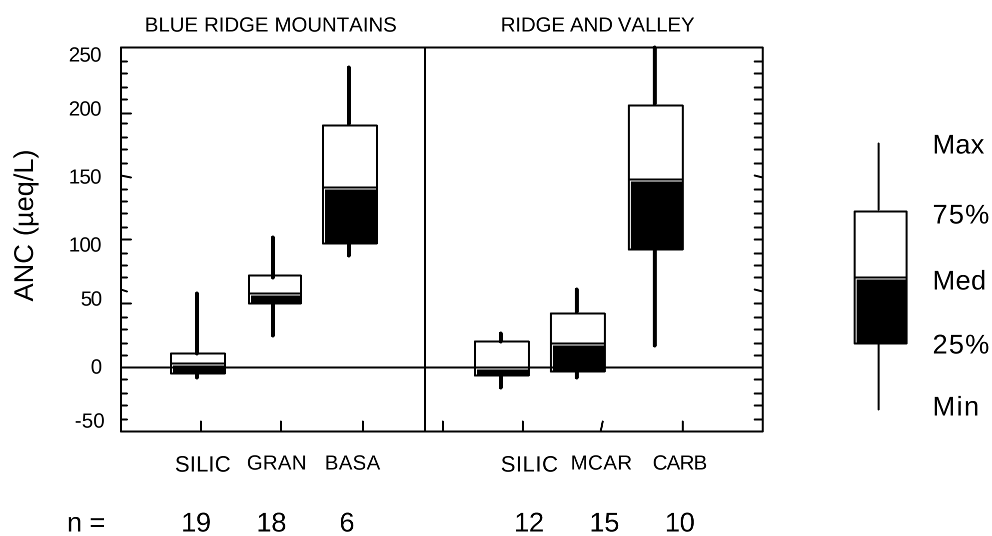

Figure 7-2 Range, nmedian, and interquartile distributions for 1998-1993 springtime acid-neutralizing capacity (ANC) concentrations for VTSSS long-term monitoring sites, grouped by watershed response classes defined by physiography and bedrock geology (from Webb et al., 1994). SILI = siliciclastic; GRAN = granitic; BASA = basaltic; MCAR = minor carbonate; and CARB = carbonate. Note that Valey and Ridge siliciclastic and minor carbonate c;asses were combined for the present analysis. Watersheds in the minor carbonate class are primarily siliclastic with limited limestone inclusions.

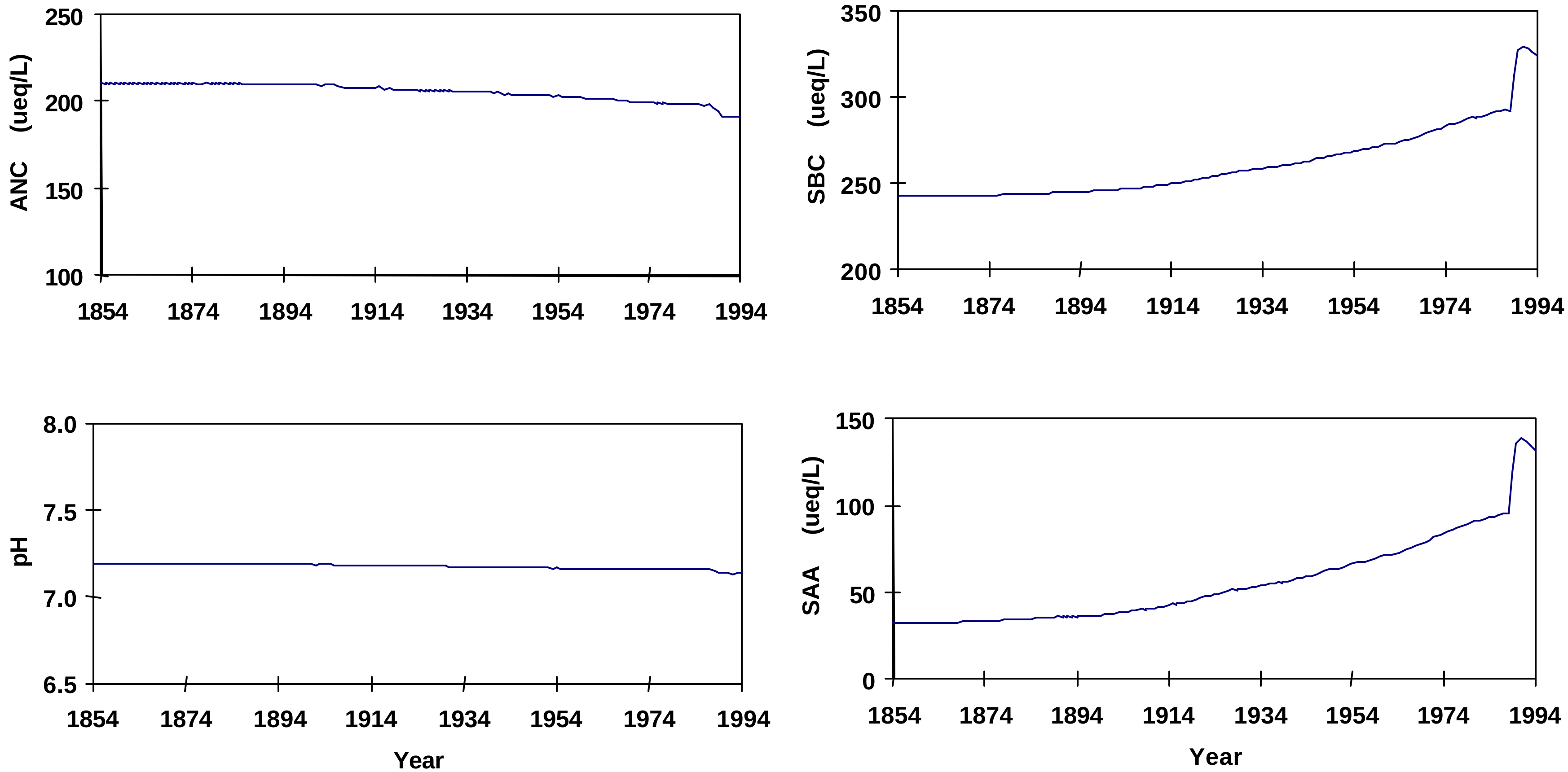

Figure 7-3A Hindcast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Piney River.

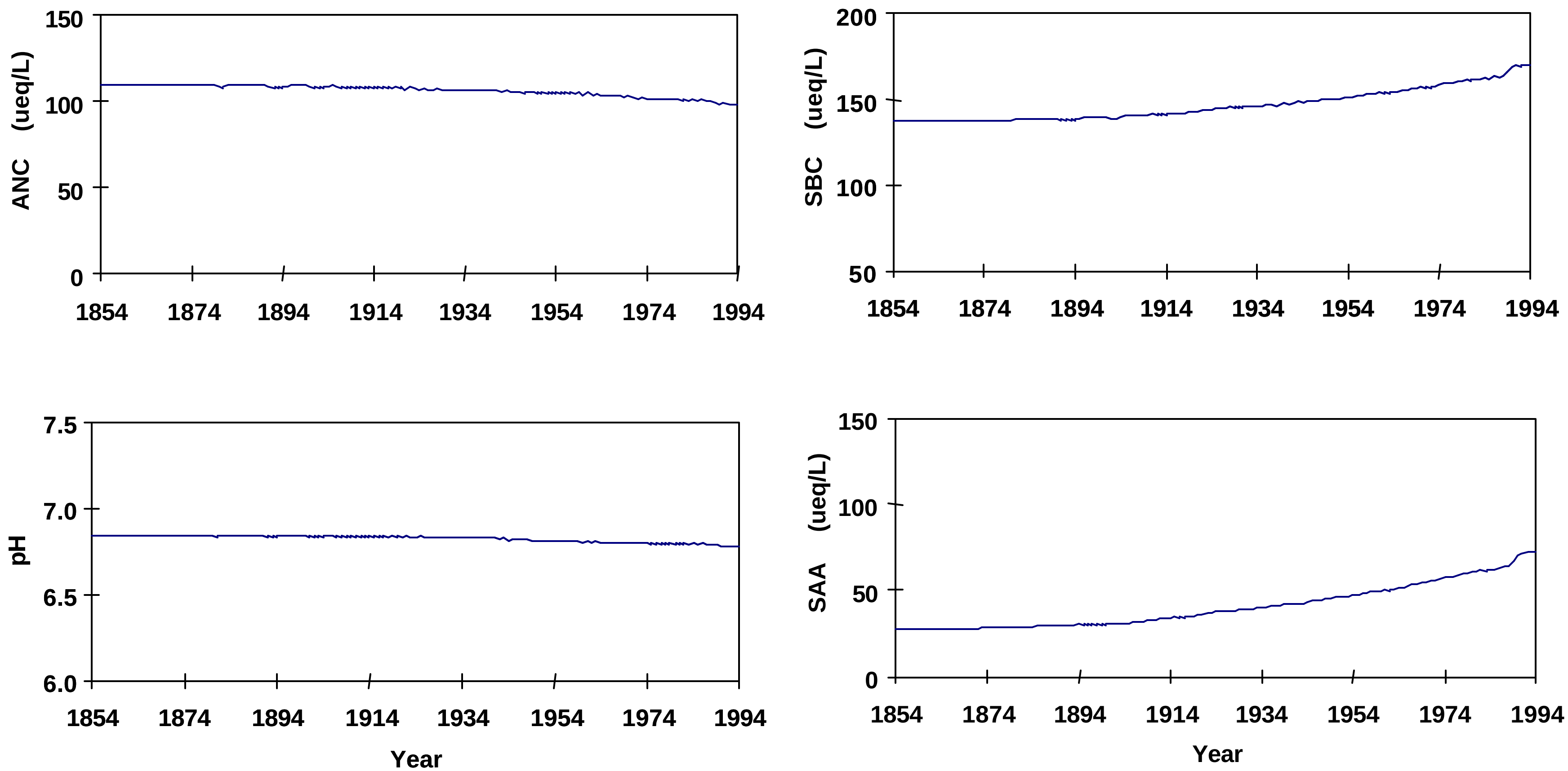

Figure 7-3B Hindcast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Staunton River.

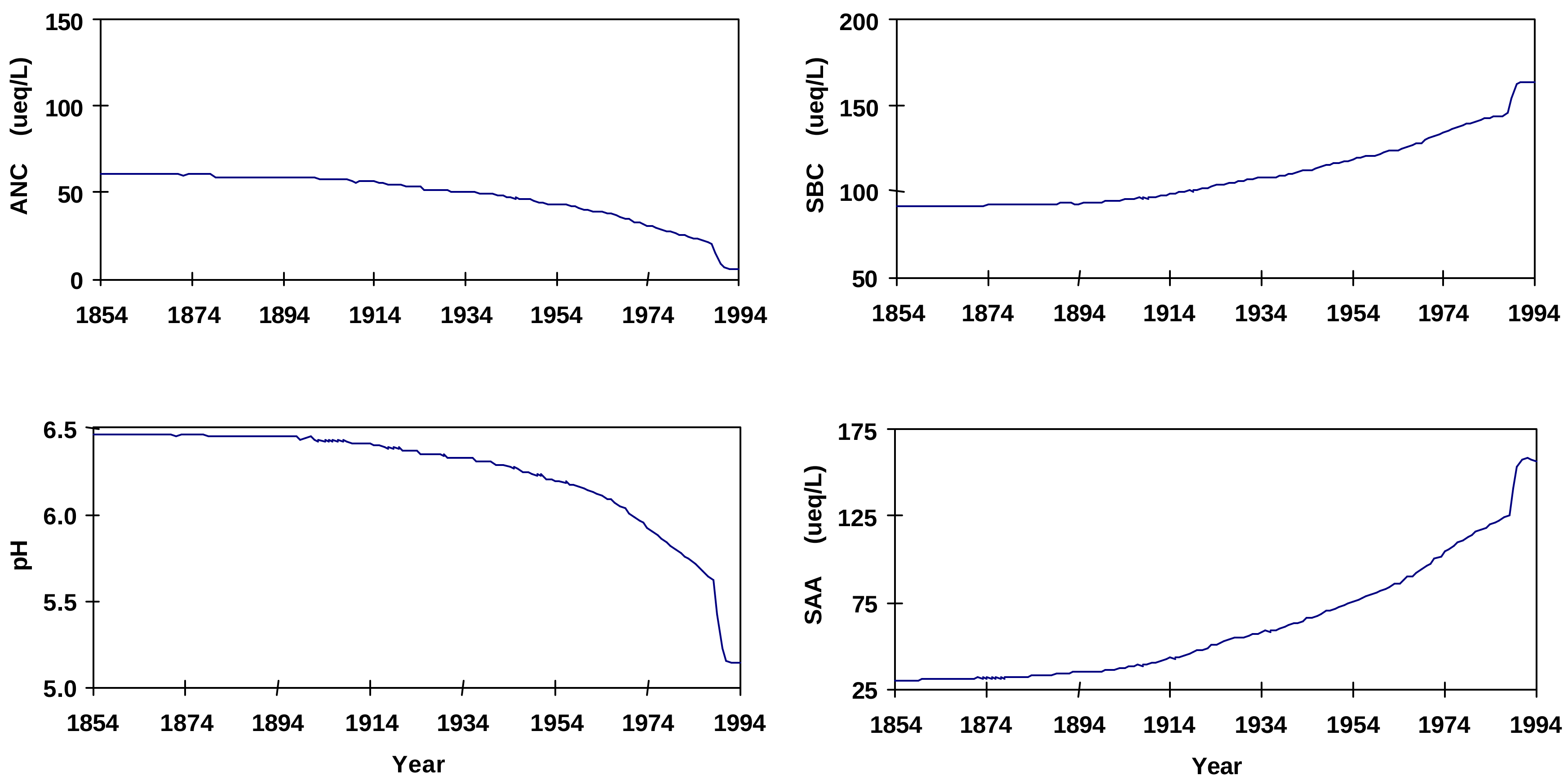

Figure 7-3C Hindcast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Paine Run.

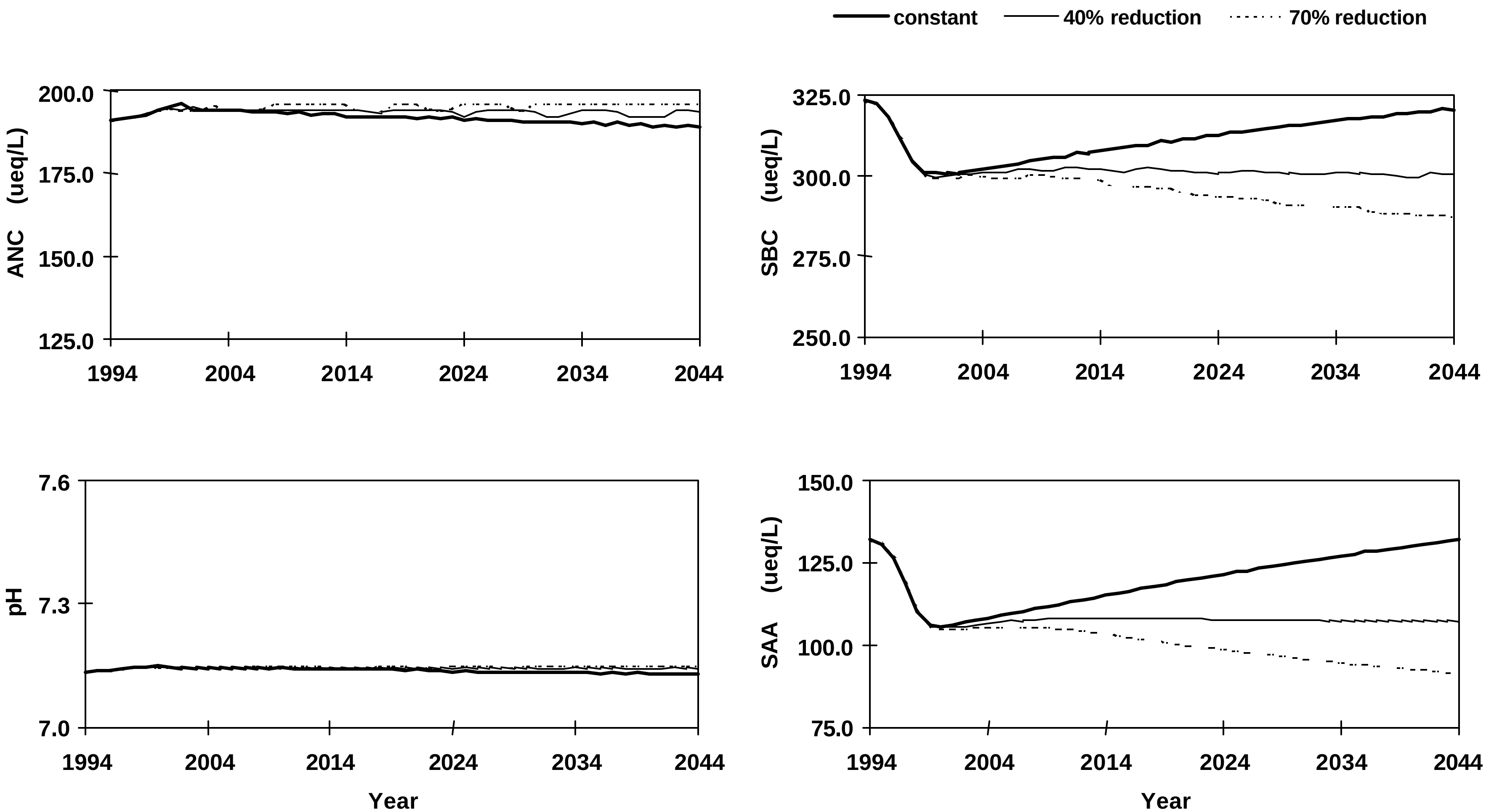

Figure 7-4A Forecast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Piney River. Three forecasts are shown. One scenario assumes constant deposition of all ions from 1994 into the future. The two alternate scenarios simulate the effects of 40% and 70% reductions in sulfate deposition over 20 years (from 1994 to 2014) followed by constant deposition at the new level for 30 more years. The initial downward trends in all scenarios between 1994 and 1998 results from recovery from the gypsy moth defoliation (see text). The trends following 1998 are affected only by the trends and changes in atmospheric deposition of sulfate.

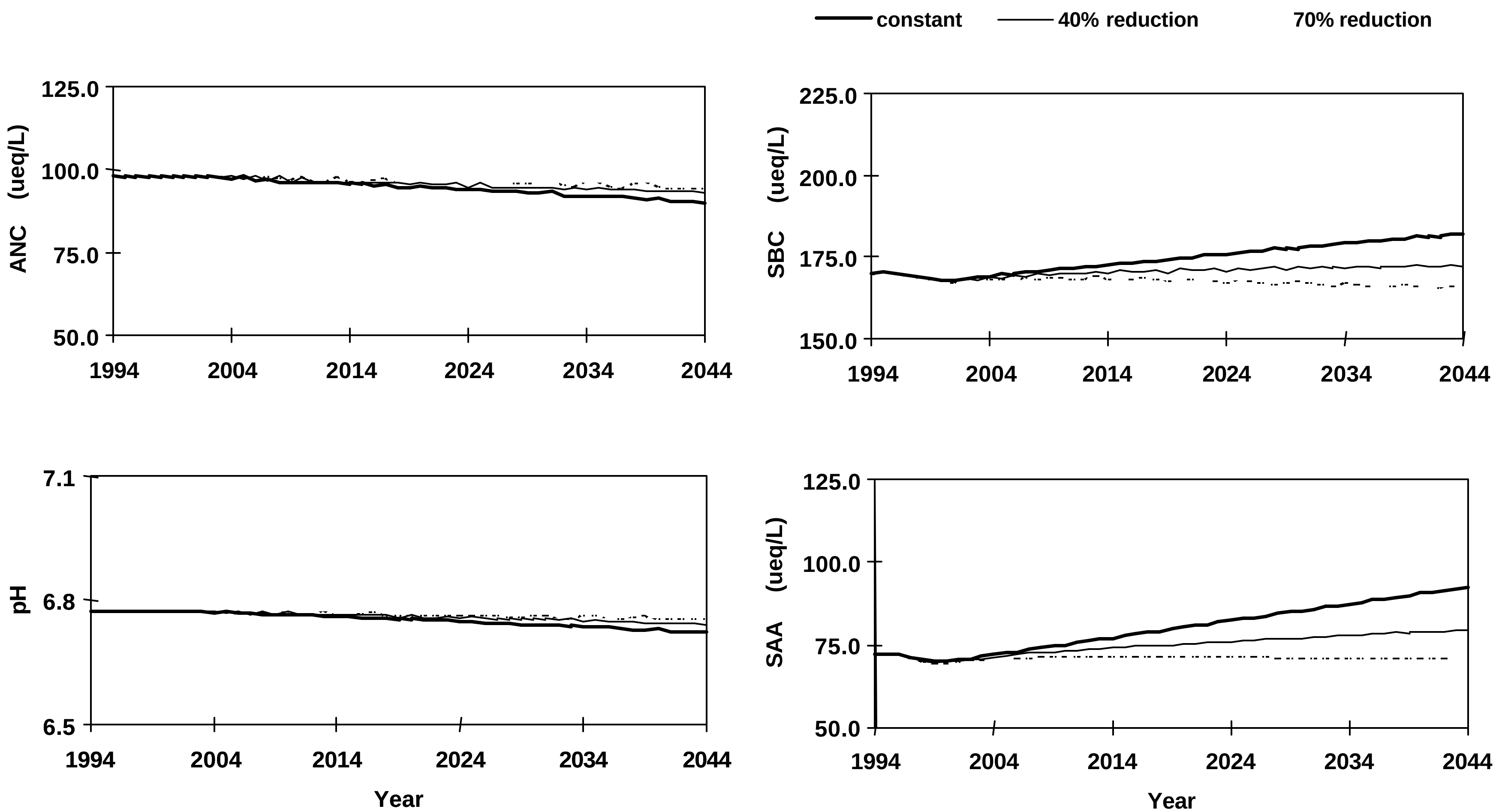

Figure 7-4B Forecast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Staunton River. Three forecasts are shown. One scenario assumes constant deposition of all ions from 1994 into the future. The two alternate scenarios simulate the effects of 40% and 70% reductions in sulfate deposition over 20 years (from 1994 to 2014) followed by constant deposition at the new level for 30 more years. The initial downward trends in all scenarios between 1994 and 1998 results from recovery from the gypsy moth defoliation (see text). The trends following 1998 are affected only by the trends and changes in atmospheric deposition of sulfate.

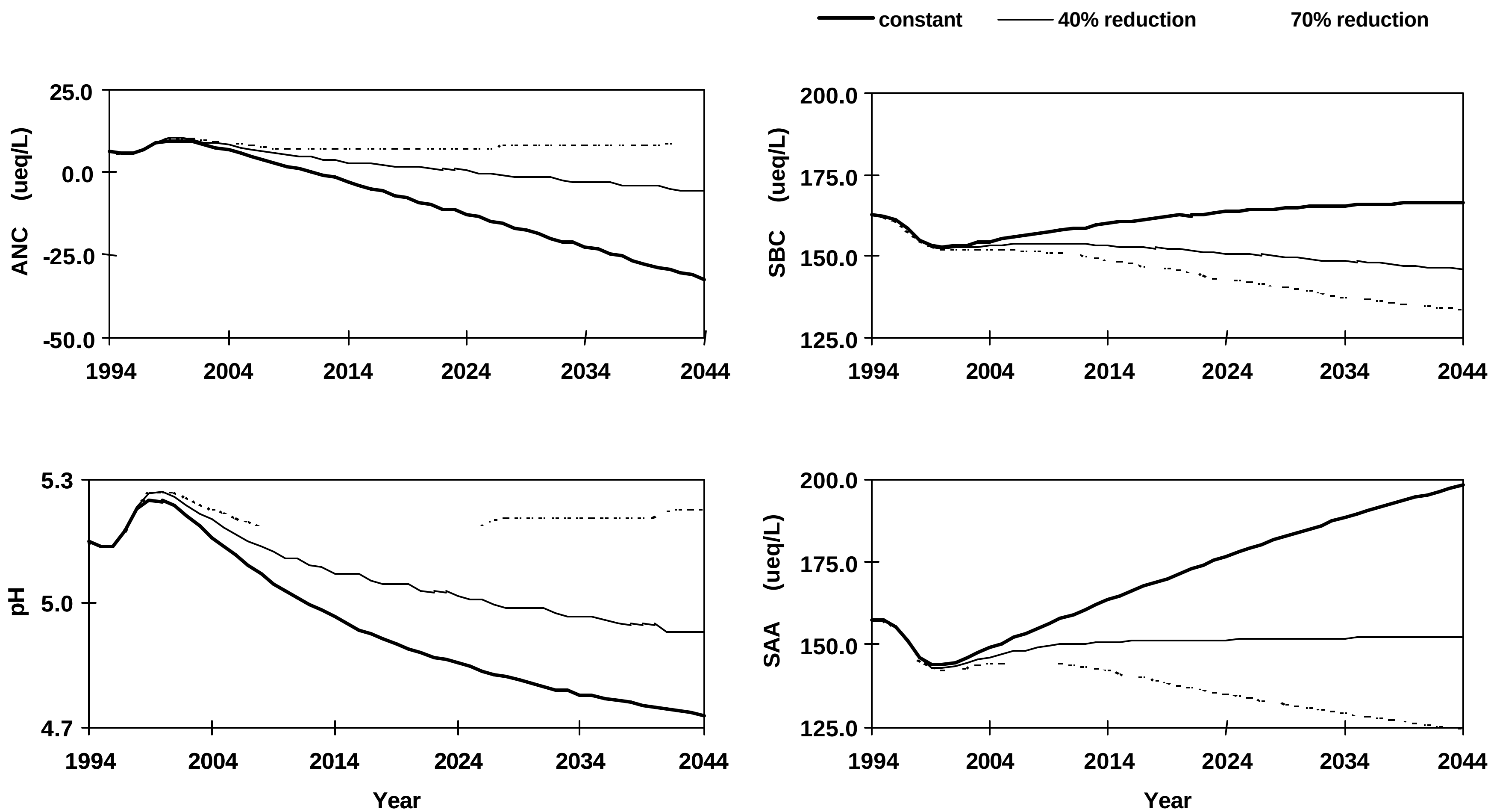

Figure 7-4C Forecast values of ANC, the sum of base cations (SBC = Ca + Mg + Na + K), the sum of acid anions (SAA = SO_4 + NO_3 + Cl) and pH for Paine Run. Three forecasts are shown. One scenario assumes constant deposition of all ions from 1994 into the future. The two alternate scenarios simulate the effects of 40% and 70% reductions in sulfate deposition over 20 years (from 1994 to 2014) followed by constant deposition at the new level for 30 more years. The initial downward trends in all scenarios between 1994 and 1998 results from recovery from the gypsy moth defoliation (see text). The trends following 1998 are affected only by the trends and changes in atmospheric deposition of sulfate.

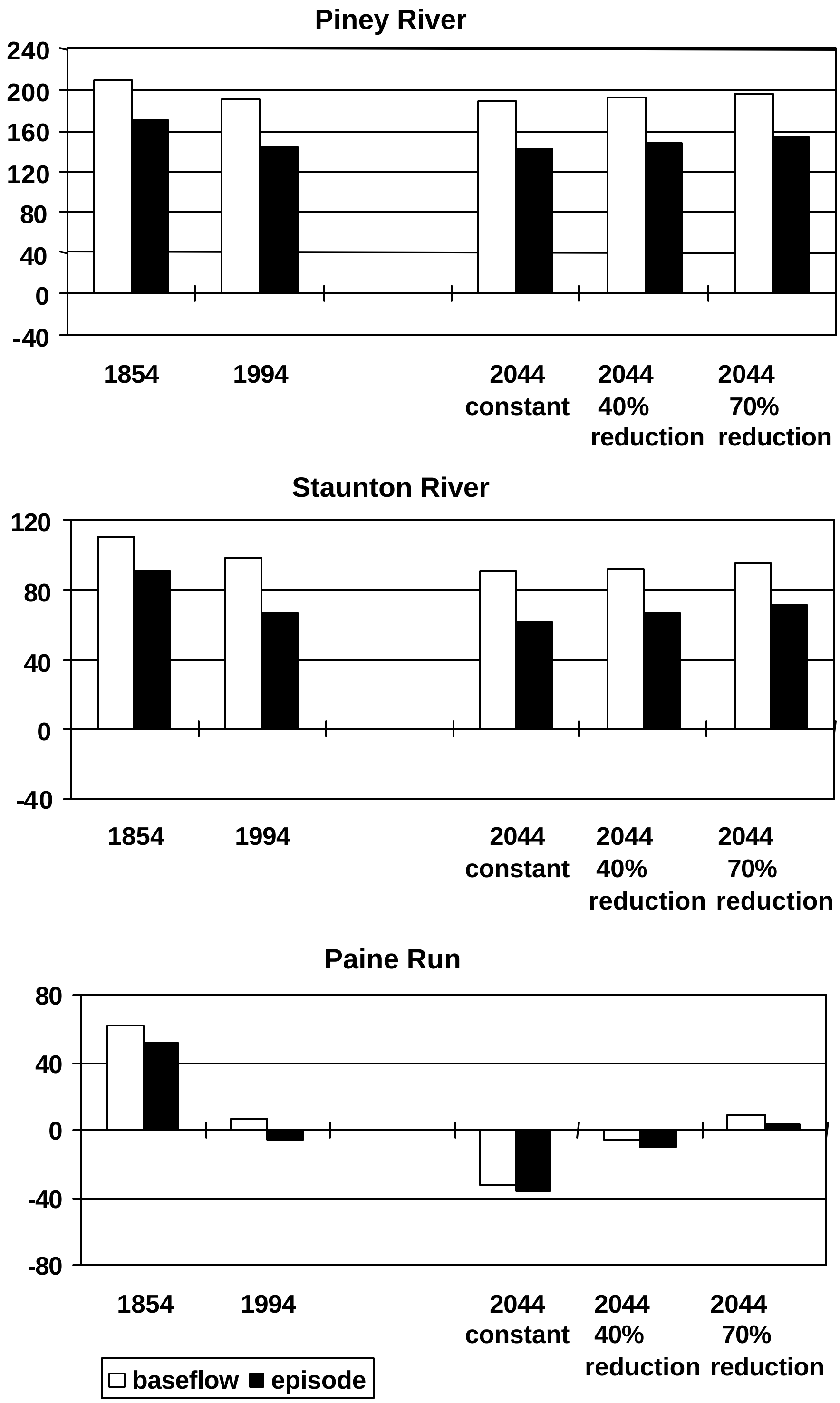

Figure 7-5A Baseflow and episodic ANC (μeq/L) for selected years of the three forecasrt scenarios, using a mixing ratio of 0.2 (20% quickflow, 80% baseflow).

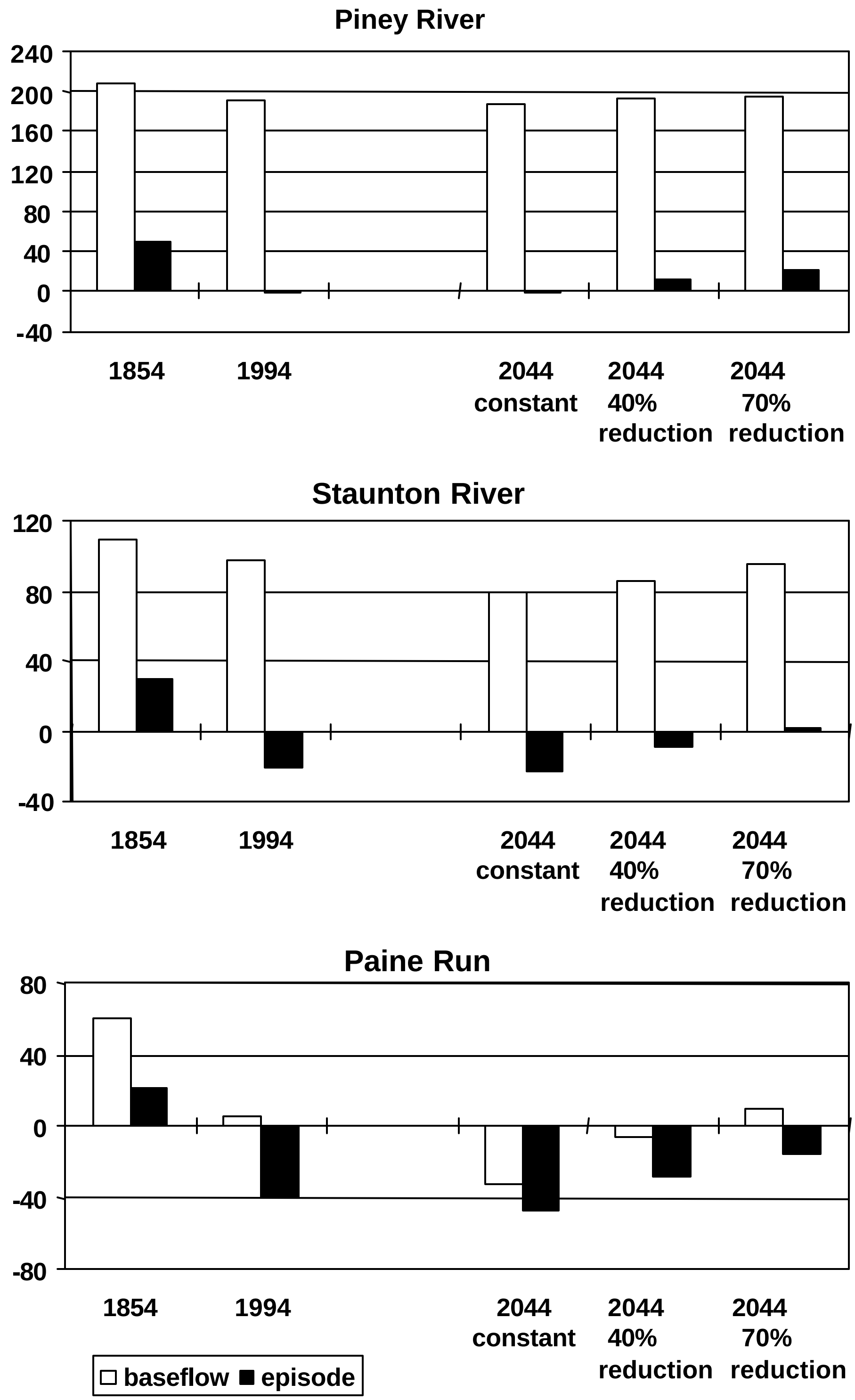

Figure 7-5B Baseflow and episodic ANC (µeq/L) for selected years of the three forecasrt scenarios, using a mixing ratio of 0.8 (80% quickflow, 20% baseflow).