Effect of stream acidification and inorganic aluminum on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York

Barry P. Baldigo and Peter S. Murdoch

Abstract: Juvenile brook trout (*Salvelinus fontinalis*) were exposed in cages to fluctuating chemical conditions in four Catskill Mountain streams during the spring and fall of 1989 and the spring of 1990. Specific chemical constituents and characteristics of acidic episodes that correlated with increased fish mortality were identified. Mortality increased during acidic episodes in one poorly buffered stream when inorganic monomeric aluminum (Al_{im}) concentrations increased; mortality was low in three other streams during acidic episodes of shorter duration and smaller magnitude than measured in the poorly buffered stream. Variation in mortality was attributed primarily to differences in concentrations of both Al_{im} and dissolved organic carbon. Linear and logistic regression analyses indicate that either mean or median Al_{im} concentrations could account for 73–99% of the variability in mortality. Regression analyses suggest that mortality was highly related (in order of importance) to Al_{im}, pH, dissolved organic carbon, calcium, and chloride concentration. Brook trout mortality was also highly related to durations of exposure above 0.225 and 0.250 mg/L Al_{im} during test periods. Characteristics of acidic–Al_{im} episodes that are critical to mortality of caged brook trout appear to be (*i*) Al_{im} concentrations of at least 0.225 ± 0.025 mg/L and (*ii*) exposure to these toxic Al_{im} concentrations for at least 2 days.

Résumé : Des juvéniles d'ombles de fontaine (*Salvelinus fontinalis*) ont été exposés dans des cages à des conditions chimiques fluctuantes dans quatre cours d'eau des monts Catskill pendant le printemps et l'automne de 1989 et le printemps de 1990. Les constituants chimiques spécifiques et les caractéristiques des épisodes acides qui étaient corrélés à une hausse de la mortalité des poissons ont été identifiés. La mortalité a augmenté pendant des épisodes acides dans un cours d'eau à faible pouvoir tampon quand les concentrations d'aluminium inorganique monomère (Al_{im}) augmentaient; la mortalité était faible dans trois autres cours d'eau pendant des épisodes acides de plus courte durée et de moindre ampleur que dans le cours d'eau à faible pouvoir tampon. La variation de la mortalité a été attribuée avant tout à des différences dans les concentrations d'une part d'Al_{im}, et d'autre part de carbone organique dissous. Des analyses de régression linéaire et logistique indiquent que les concentrations moyennes ou médianes d'Al_{im} pouvaient expliquer 73–99% de la variabilité de la mortalité. Les analyses de régression permettent de penser que la mortalité était fortement reliée (par ordre d'importance) à Al_{im}, au pH, au carbone organique dissous, au calcium et au chlore. La mortalité de l'omble de fontaine était aussi fortement liée à la durée de l'exposition au-dessus de 0,225 et 0,250 mg/L Al_{im} pendant les périodes de test. Les caractéristiques des épisodes d'acidité–Al_{im} qui sont critiques pour la mortalité des ombles de fontaine en cage semblent être (*i*) des concentrations d'Al_{im} d'au moins 0,225 ± 0,025 mg/L, et (*ii*) l'exposition à ces concentrations toxiques d'Al_{im} pendant au moins 2 jours.
[Traduit par la Rédaction]

Introduction

The effects of acidic precipitation on aquatic ecosystems in much of the northeastern United States have been well documented (see reviews by Haines and Baker 1986; Baker et al. 1990); however, only a few studies have described stream acidification in the Catskill Mountains (Stoddard and Murdoch 1991; Shultz et al. 1993; Murdoch and Stoddard 1993; Wigington et al. 1996) or documented the effects of chronic and episodic acidification on survival of native brook trout (*Salvelinus fontinalis*) in streams of the region (Murdoch et al. 1991; Van Sickle et al. 1996). In addition, the specific chemical constituents and the magnitude, duration, and frequency characteristics of stochastic concentrations that cause fish mortality in natural streams are also not completely understood (Baker et al. 1990).

Relations between pH and survival of fish in the laboratory and in selected natural systems have been studied over the past three decades (see review by Baker et al. 1990). Relations between mortality of native fish (in situ) and specific chemical constituents (other than pH) and their temporal characteristics in naturally acidified streams elude precise definition, however, because (*i*) water chemistry and toxicity vary with stream discharge and season, and (*ii*) complex interactions among biotic and abiotic factors can alternately increase and (or) decrease the detrimental effects of toxic waters on individual fish (Baker et al. 1990).

Watersheds and streams within the Catskill Mountains of southeastern New York (Fig. 1) receive atmospheric precipitation that is highly acidic (Barchet 1991; Stoddard and Murdoch 1991). Elevated atmospheric deposition of sulfuric and nitric acids in Catskill watersheds appears to be the basis for both chronic and episodic acidification in some headwater

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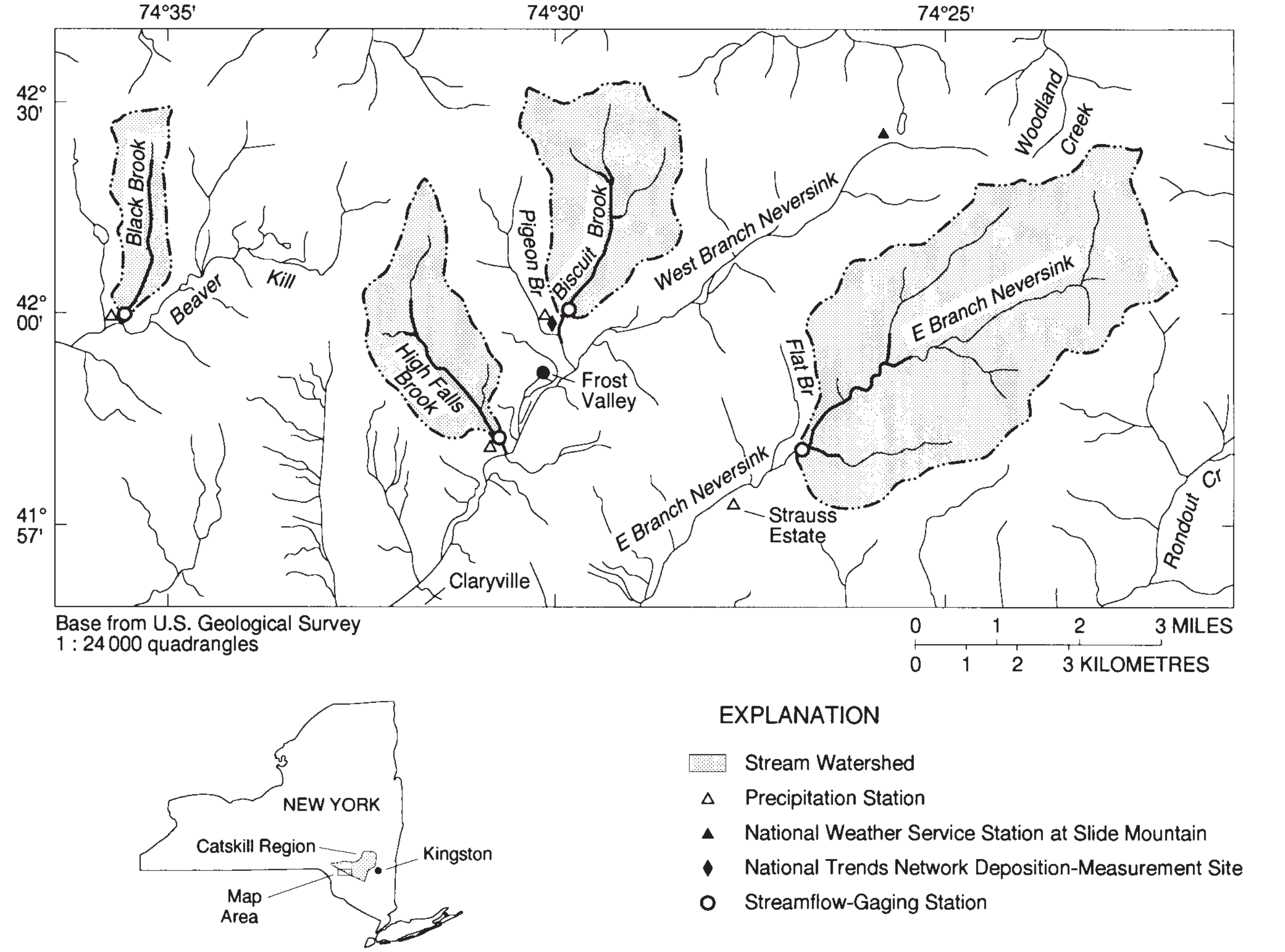
Fig. 1. Location of study area and of the four study streams in the Catskill Mountains of New York State. (Modified from Murdoch et al. 1991, Fig. 2.)



streams (Murdoch and Stoddard 1993). During 1989, the Catskill region received 21.9 kg/ha of wet NO_3^- deposition and 25.7 kg/ha of wet SO_4^{2-} deposition (Wigington et al. 1996). Although well-buffered soils prevent acidification of most streams throughout the Catskill region, parts of the Neversink River (Fig. 1) and other small headwater systems drain poorly buffered soils and have become acidified (Rich 1934; Stoddard and Murdoch 1991; Shultz et al. 1993). The wide range of acidic conditions that prevail in the Neversink River and adjacent Delaware River basins provide an ideal setting to study how chemical constituents and temporal characteristics affect fish mortality.

In 1988, the U.S. Environmental Protection Agency began the Episodic Response Project (ERP) (Wigington et al. 1993) to document the effects of episodic acidification on aquatic systems. The ERP was designed to describe and quantify the hydrology, chemistry, and biology of 13 headwater streams within mountainous regions of west-central Pennsylvania and in the Adirondack and Catskill mountains of New York state. One objective of the project was to identify which constituents and acidic-episode characteristics (frequency, magnitude, duration) were most highly related to brook trout mortality and to the decline of their natural populations in affected streams. The U.S. Geological Survey (USGS) coordinated ERP field research in the Catskill Mountains, and general results were summarized by Wigington et al. (1993). This paper presents results from the Catskill research and evaluates relations among selected chemical constituents, characteristics of their fluctuating concentrations, and mortality of caged native brook trout in four headwater streams during 1989–1990.

Study sites

The four streams chosen for the ERP investigation in the Catskill Park are about 50 km west of Kingston, N.Y. (Fig. 1). Biscuit Brook and High Falls Brook are tributaries to the West Branch Neversink River at Frost Valley; the East Branch Neversink site is about 5 km east of Frost Valley; and Black Brook is a tributary to the Beaver Kill in the Delaware River basin and is about 8 km west of Frost Valley. The drainage areas for Black Brook, High Falls Brook, Biscuit Brook, and East Branch watersheds are 3.7, 7.1, 9.9, and 23.1 km^2, respectively. The four streams were selected for study on the basis of (*i*) extensive data from long-term chemical monitoring at Biscuit Brook, (*ii*) proximity of a field laboratory (at Frost Valley), and (*iii*) the wide range in base-flow pH, acid neutralizing capacity (ANC), and total dissolved aluminum (Al_{td}) concentrations.

The climate of the Catskill Mountain region is classified as humid continental and is subject to extreme seasonal changes in temperature; the average annual temperature during 1951–1975 at a National Weather Service (NWS) station near the southeastern edge of the Catskill Park was about 9°C (Tornes 1979). The average annual snowfall during the same period was about 173 cm (Tornes 1979). The average annual rainfall during 1950–1982 at the NWS Slide Mountain station, 8 km northeast of Frost Valley (Fig. 1) was estimated to be 157 cm (Murdoch et al. 1991). Precipitation during 1989 at the National Trends Network deposition-monitoring station, adjacent to the Biscuit Brook site, was about 142 cm; volume-weighted pH of precipitation during 1989 and the during the previous 7 years was approximately 4.3 (Barchet 1991; Murdoch and Stoddard 1993). Regional and local precipitation chemistry is described in greater detail by Murdoch et al. (1991) and Barchet (1991). The physiography, geology, and soil classification of the Catskill region are summarized by Murdoch and Stoddard (1993) and Wigington et al. (1996) and discussed in detail by Tornes (1979), Rich (1934), Way (1972), and Ethridge (1977).

Many low-order watersheds of the Neversink River basin have steep gradients, thin soils, and extensive bedrock outcrops that provide rapid runoff, short residence time for subsurface water, and negligible buffering; thus, they are moderately to extremely acidic (Murdoch et al. 1991). Black Brook, a headwater stream in the adjacent Delaware River basin, was the primary reference stream in this study; ANC was well above zero at all times. High Falls Brook acted as a second reference stream because it underwent no periods of negative ANC, although ANC often approached zero. Biscuit Brook was selected as an episodically acidified site because ANC was generally above zero during base flow but often below zero during high flows. The East Branch Neversink was a chronically acidified stream; ANC values seldom exceeded zero, even during base-flow periods.

Methods

Water quality and discharge on the four streams (Fig. 1) were monitored from November 1988 through June 1990. Stream water was sampled weekly at each station and more frequently during changes in flow and during toxicity tests. Juvenile brook trout, native to the West Branch of the Neversink River and Pigeon Brook, were exposed (in cages) to natural conditions at each site for periods as long as 36 days during the spring and fall of 1989 and the spring of 1990. Field operations and quality-assurance procedures summarized here are described in detail by Wigington et al. (1993). Stream stage was measured and recorded every 15 min at each site by a submersible pressure transducer and electronic data logger. Discharge was measured twice monthly and used to generate stage-to-discharge ratings and 15-min discharge data for each stream in accordance with standard USGS methods (Rantz 1983). At each site, data loggers were programmed to instruct automated samplers to collect water samples at specified increments of rising and falling stage during periods of changing discharge. Routine water samples were collected weekly or biweekly from each site and filtered and preserved within 24 h; automated samples were generally processed within 3 days of collection.

From 50 to 80 routine water samples were collected at each stream during 1989 and during the first 5 months of 1990. A variable number of additional water samples were collected automatically at each stream during periods of high discharge and during toxicity tests. Total monomeric aluminum (Al_{tm}) and organic monomeric aluminum (Al_{om}) measurements were made on only about 10% of the routine samples because analytical costs and processing times were prohibitive. The actual numbers of routine Al_{tm}, Al_{om}, and inorganic monomeric aluminum (Al_{im}) measurements per stream were from 0 to 18 because the established criterion for processing monomeric Al aliquots was a water pH less than 5.5, which was seldom met at Black Brook and High Falls Brook.

The mean, median, minimum, maximum, and standard deviation (SD) for most chemical constituents were calculated for each site from the routine water samples. Statistics for chemical constituents during toxicity tests were obtained from all water samples collected during respective periods of exposure at each site. Statistics for monomeric-aluminum species (Al_{tm}, Al_{om}, and Al_{im}) at Black Brook were estimated from the annual flow record and the relations between the concentration of each of the three species and discharge because (*i*) no monomeric Al analyses were conducted on routine samples from this site; (*ii*) measured monomeric Al concentrations were low, typically near detection limits at this site; and (*iii*) only 10 monomeric Al analyses were performed at Black Brook.

We present results of 20 toxicity tests that used only native brook trout from a common source (West Branch of the Neversink River) and a small tributary (Pigeon Brook) (Fig. 1). The mortality of caged fish was measured at each stream during three periods: April 4 through June 24, 1989; October 4 through November 30, 1989; and March 9 through May 6, 1990. Tests were terminated and restarted after mortality exceeded either 20% at Black Brook or 90% at the other three sites; therefore, the exposure durations ranged from 11 to 36 days. Young-of-the-year brook trout (age 0+) were used in all tests; during spring periods, tested individuals were almost age 1+.

In each set of tests, brook trout were collected, placed into a large holding chamber at High Falls Brook for 24–48 h, then selected randomly for tests at each of the four study streams. For each test and stream, 20 or 21 brook trout were separated into three to five plastic, screen-sided jars that were in turn placed in cages near streamflow-gauging sites in accordance with techniques described by Johnson et al. (1987). Fish survival was checked and recorded every day or two during testing. Fish lengths ranged from 41 to 91 mm and averaged 71.5 ± 7.5 mm (mean ± SD). Fish weighed from 1 to 6 g and averaged 2.8 ± 0.9 g. Total lengths and mass did not differ ($p \leq 0.0003$) among streams or test periods, but mean lengths were 4–6 mm greater during spring tests than during fall tests.

Simple linear and logistic regression analyses were used to define relations between brook trout mortality (percent mortality at the end of each test) and estimates of mean pH, Al_{td}, Al_{tm}, Al_{om}, Al_{im}, Ca^{2+}, NO_3^-, SO_4^{2-}, Cl^-, and dissolved organic carbon (DOC) concentrations from all water samples collected at each stream during toxicity-test periods. Constituent means (and their median, maximum, and minimum statistics) that were related to percent mortality ($p \leq 0.05$) and those potentially related to either Al_{im} concentration or to fish sensitivity were then analyzed through stepwise multiple regression techniques. In stepwise (forward and backward) multiple regression analyses, independent regressors were judged to be potentially important and thus useful predictors of brook trout mortality if, after inclusion in the equation, the standard deviation of the residuals (SDR; equivalent to the root mean square error), decreased by at least one unit and the F values remained significant ($p \leq 0.05$). The rank or importance of each constituent to brook trout mortality level was gauged by (*i*) the probability of a legitimate association; $p \leq 0.05$ (significant t and F values), (*ii*) the coefficient of determination (r^2) or the adjusted r^2 for multiple-linear and logistic regressions, and (*iii*) the SDR.

Analyses of relations between statistics for individual chemicals and mortality levels assume that single constituents affect brook trout mortality independent of other constituents, whereas multiple-regression analyses assume that more than one variable affects fish mortality. All

Table 1. Physical characteristics and concentrations of selected constituents for the four streams in the Catskill Mountains, N.Y., 1989–1990.

(A) Geographic characteristics

	Black Brook	High Falls Brook	Biscuit Brook	East Branch Neversink
Gauge latitude (N)	42°00′42″	41°58′40″	41°59′43″	41°58′01″
Gauge longitude (W)	78°36′13″	71°31′21″	74°30′05″	74°26′54″
Gauge elevation (m)	681	590	490	651
Drainage area (km^2)	3.65	7.10	9.84	23.1
Quadrangle	Seager	Claryville	Claryville	Peekamoose

(B) Water quality characteristics

	Black Brook				High Falls Brook				Biscuit Brook				East Branch Neversink			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
pH	6.53	0.32	5.44	7.04	6.48	0.34	5.40	7.08	5.99	0.34	4.97	6.50	4.85	0.16	4.27	5.35
ANC	95.8	38.1	17.1	175.9	87.4	36.8	11.5	171.8	24.1	12.1	–6.3	53.1	–8.1	6.9	–45.3	17.5
Al_{im}	0.006	na	0	0.072	0.017	0.018	0	0.08	0.077	0.066	0	0.243	0.211	0.081	0	0.639
Al_{tm}	0.046	na	0.028	0.179	0.073	0.003	0.010	0.203	0.125	0.079	0.015	0.363	0.262	0.085	0.051	0.746
Al_{om}	0.026	na	0.031	0.114	0.056	0.016	0.020	0.182	0.062	0.020	0.017	0.141	0.060	0.032	0.016	0.310
Al_{td}	0.061	0.086	0.002	0.539	0.043	0.072	0.001	0.773	0.055	0.046	0.002	0.332	0.217	0.080	0.016	0.737
Ca^{2+}	4.51	0.57	2.73	5.90	4.13	0.60	1.87	5.55	2.77	0.27	1.96	3.96	1.38	0.16	0.75	2.39
DOC	1.99	1.59	0.66	8.07	2.06	1.14	0.28	7.96	2.01	0.92	0.34	7.51	2.31	1.14	0.29	11.67
Mg^{2+}	0.60	0.08	0.38	0.80	0.55	0.07	0.28	0.73	0.61	0.06	0.38	0.77	0.62	0.08	0.34	0.97
Na^{+}	0.29	0.12	0.13	0.89	0.32	0.06	0.18	1.42	0.34	0.09	0.19	0.87	0.34	0.06	0.16	2.00
K^{+}	0.27	0.10	0.18	1.05	0.25	0.07	0.13	0.90	0.24	0.06	0.18	0.83	0.27	0.08	0.17	0.65
SO_4^{2-}	6.36	0.49	4.80	7.04	6.14	0.53	4.38	7.00	6.12	0.43	4.69	7.00	5.54	0.27	4.12	6.28
NO_3^{-}	3.01	0.99	0.59	6.69	2.45	1.11	0.99	6.59	2.61	1.21	0.69	7.99	2.25	1.10	0.63	6.57
Cl^{-}	0.57	0.08	0.19	0.82	0.58	0.08	0.26	0.83	0.62	0.08	0.35	0.80	0.55	0.05	0.36	0.82
Si	2.29	0.34	0	3.19	2.26	0.44	0.96	3.71	2.15	0.39	1.00	2.94	2.28	0.41	0.87	2.85
Flow	0.13	0.14	0.01	1.68	0.28	0.28	0.03	2.38	0.34	0.51	0.02	10.02	1.41	2.57	0.10	28.27

Note: Most physical information is from Murdoch et al. (1991). Flow values (m^3/s) were derived from hourly 1989 flow data. Chemical data were calculated from weekly water samples collected from January 1989 to June 1990. Values of Al_{tm}, Al_{om}, and Al_{im} at Black Brook were estimated from calculated hourly data. Concentrations of all chemical constituents, except acid neutralizing capacity (ANC) in microequivalents per litre and pH, are in milligrams per litre. DOC, dissolved organic carbon; Al_{im}, inorganic monomeric aluminum; Al_{tm}, total monomeric aluminum; Al_{om}, organic monomeric aluminum; Al_{td}, total dissolved aluminum. na, not available.

analyses assume that the residuals are normally distributed and their variances are homeoscedastic. Although these assumptions are often strained, even when normalized or transformed data are used, the analyses rank the importance of specific constituents to fish mortality, and identify interactions among constituents that could indirectly affect both water toxicity and brook trout sensitivity. Logistic regression analyses assume that mortality data are binomially distributed and that the observed response of fish mortality to a linear toxicant gradient asymptotically approaches the 0 and 100% level for low and high toxicant concentrations, respectively.

Hourly Al_{im} concentrations were estimated for each site (during test periods) to assess the effect of episodic increases in Al_{im} concentration on fish mortality. These data were calculated from mean hourly discharge and the relations between stream discharge and either (*i*) measured Al_{im} concentrations or (*ii*) an intermediate factor, such as pH, that is highly correlated with both discharge and Al_{im}. The calculated hourly Al_{im} record for each stream and each exposure period was used to summarize magnitude, duration, and frequency statistics for Al_{im} concentrations that fluctuated (Al_{im} episodes) in relation to periods of base flow and episodic acidification. In this paper, the term Al_{im} episode refers to a rapid and often sustained increase in ambient concentration of Al_{im} in stream waters; it always occurs in conjunction with acidic episodes and discharge events. Regression analyses were then used to relate percent brook trout mortality to the magnitude and temporal-Al_{im} statistics.

Results

Stream hydrology and chemistry

The mean hourly discharge of each of the four streams during the study period ranged from 0.13 to 1.41 m^3/s (Table 1) and is positively related to watershed area. Large fluctuations in discharge occurred throughout toxicity-test periods at each site (Fig. 2).

The degree of acidification (stream pH) generally reflected ANC values across study sites. Mean ANC in Black Brook and High Falls Brook was about 90 μequiv./L and never dropped below 10 μequiv./L. Mean ANC in Biscuit Brook was 24.1 μequiv./L; negative ANC values occurred only during very high flows. Mean ANC for the East Branch Neversink River was –8.1 μequiv./L; severe pH and ANC depressions were recorded frequently in this stream throughout the study. Mean pH ranged from a low of 4.85 in the chronically acidified stream (East Branch Neversink) to a high of 6.53 at the primary reference stream (Black Brook). Water pH was seldom below 6.0 at Black Brook and at the second reference stream (High Falls Brook), rarely below 5.5 at the episodically acidified stream (Biscuit Brook), and rarely exceeded 5.0 at the chronically acidified East Branch Neversink. Concentrations of Al_{im} averaged 0.006 and 0.017 mg/L at Black Brook

Fig. 2. Calculated and measured inorganic monomeric aluminum (Al_{im}) concentrations, stream discharge, stream pH, and cumulative brook trout mortality observed during toxicity tests at (A) Black Brook, (B) High Falls Brook, (C) Biscuit Brook, and (D) the East Branch Neversink River, 1989–1990. Most graphs depict more than one toxicity test per season; start and end dates are in Table 2.

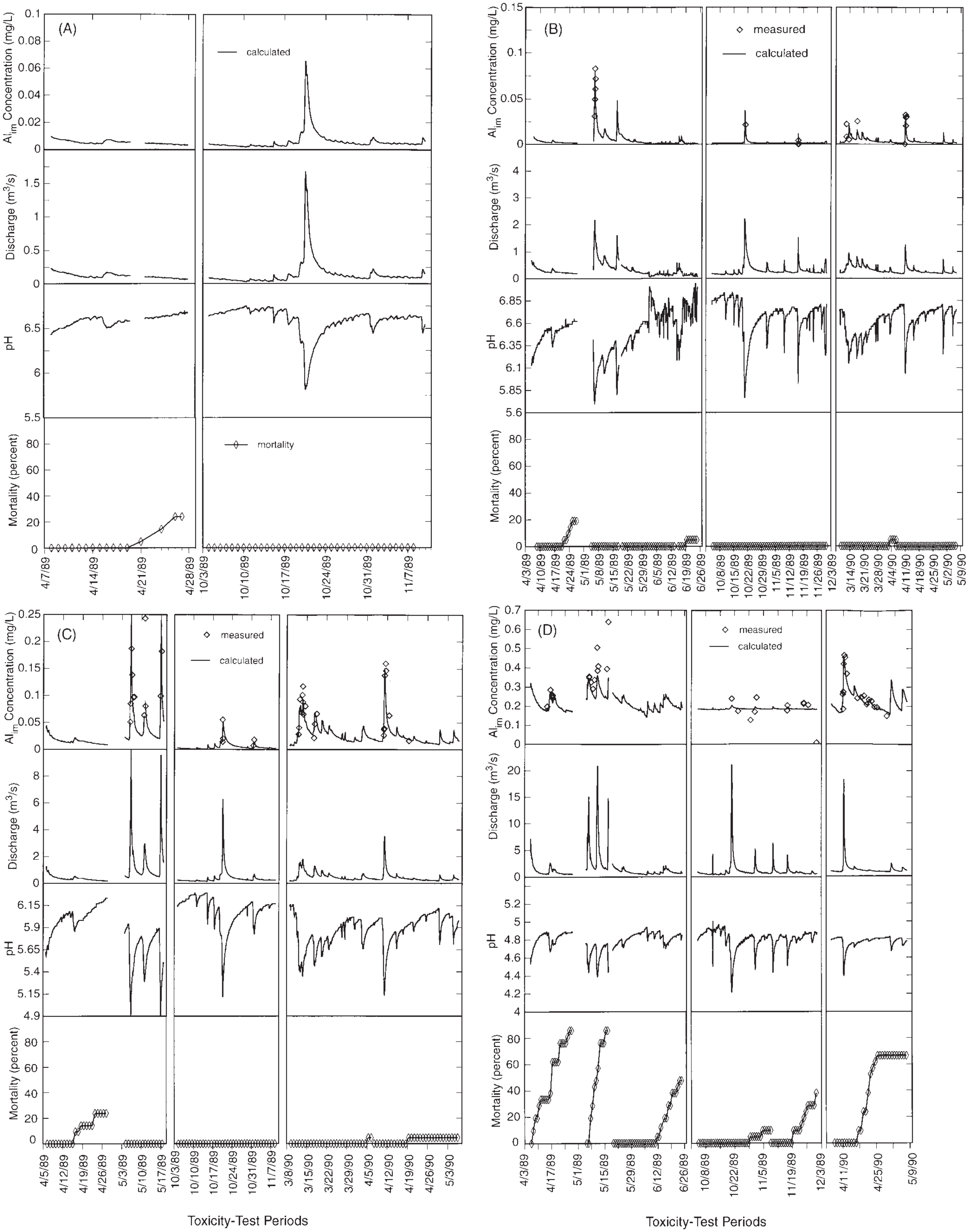


Table 2. Test periods, percent mortality, and statistics for selected constituents in samples obtained from the four study streams during test periods.

Test dates		Percent	Calculated Al_{im}		Measured Al_{im}		DOC	pH	Mean	Mean
Start	End	mortality	Mean	Median	Mean	Median	Mean	Mean	Ca^{2+}	Cl^-
East Branch Neversink River										
4/6/89	4/27/89	81	0.208	0.193	0.248	0.253	3.19	4.83	1.5	0.638
5/5/89	5/16/89	86	0.279	0.266	0.398	0.369	3.84	4.57	1.23	0.54
5/19/89	6/24/89	48	0.204	0.199	na	na	3.63	4.7	1.71	0.469
10/4/89	11/7/89	10	0.184	0.183	0.189	0.172	4.92	4.68	1.26	0.599
11/8/89	11/30/89	38	0.184	0.183	0.201	0.204	3.15	4.77	1.4	0.594
4/6/90	5/6/90	67	0.227	0.211	0.272	0.243	2.78	4.69	1.4	0.544
Biscuit Brook										
4/6/89	4/27/89	24	0.015	0.014	na	na	1.87	5.99	2.87	0.683
5/4/89	5/17/89	0	0.048	0.031	0.099	0.096	3.16	5.35	2.44	0.573
10/4/89	11/8/89	0	0.005	0.003	0.023	0.018	3.20	5.97	2.66	0.686
3/9/90	4/6/90	5	0.030	0.027	0.071	0.074	2.32	5.64	2.91	0.542
4/6/90	5/6/90	5	0.022	0.018	0.083	0.063	2.42	5.64	2.79	0.55
High Falls Brook										
4/6/89	4/27/89	19	0.003	0.002	na	na	2.03	6.28	3.97	0.673
5/4/89	5/17/89	0	0.013	0.009	0.059	0.06	3.20	5.99	3.24	0.601
5/19/89	6/24/89	5	0.002	0.001	na	na	2.12	6.54	3.85	0.547
10/4/89	11/8/89	0	0.002	0.001	0.021	0.021	4.12	6.34	3.72	0.688
11/8/89	11/30/89	0	0.002	0.001	0.001	0	3.20	6.4	3.79	0.64
3/9/90	4/6/90	5	0.005	0.004	0.015	0.015	2.11	6.42	3.79	0.536
4/6/90	5/6/90	0	0.003	0.002	0.022	0.029	2.65	6.32	3.67	0.534
Black Brook										
4/8/89	4/27/89	24	0.005	0.004	na	na	1.57	6.51	4.53	0.661
10/4/89	11/9/89	0	0.006	0.004	0.072	0.072	3.48	6.35	3.99	0.621

Note: Number of values, *n*, used for each statistic ranged from 5 to 40. All chemical concentrations (except pH) are in mg/L. na, not available.

and High Falls Brook, 0.077 mg/L at Biscuit Brook, and 0.211 mg/L at the East Branch Neversink.

Most high flows were accompanied by pronounced fluctuations in pH, ANC, and concentrations of Al_{td}, Al_{tm}, Al_{im}, DOC, NO_3^-, SO_4^{2-}, Cl^-, and Ca^{2+} in the two acidified streams (East Branch Neversink and Biscuit Brook) and by smaller fluctuations in the two reference streams (High Falls Brook and Black Brook). Hourly discharge, calculated pH, and measured and calculated Al_{im} concentrations for each stream during toxicity-test periods (Figs. 2A–2D) suggest that pH and Al_{im} concentrations are directly related to discharge in the four streams, but the relations differ among streams and change seasonally within streams. For example, discharge in the East Branch Neversink increased about 20-fold during the middle of October 1989 and both measured and estimated concentrations of Al_{im} did not change significantly; however, similar increases in discharge during the spring of 1989 and 1990 were accompanied by large increases in Al_{im} concentration of as much as 0.500 mg/L (Fig. 2D). Seasonal changes in the relation between stream discharge and Al_{im} concentrations at Biscuit Brook (Fig. 2C) and at High Falls Brook (Fig. 2B) are also evident, although to a lesser degree. Conversely, fluctuations in stream pH consistently reflected changes in discharge at all sites; therefore, the relation between stream pH and discharge did not change greatly from season to season within any of the four streams.

Brook trout mortality

Brook trout mortality in the four streams ranged from 0 to 86% at the end of each test, and differed by stream and season (Table 2). Generally, fewer than 25% of brook trout died in spring tests (1989, 1990) in the three least acidified streams (Black Brook, High Falls Brook, and Biscuit Brook), whereas 48–86% of fish died in spring tests in the chronically acidified stream (East Branch Neversink) (Table 2). Mortality levels were lower in fall tests than spring tests at all sites (Figs. 2A–2D); during the fall of 1989, mortality was 0 at Black Brook, High Falls Brook, and Biscuit Brook, and 10–38% at the East Branch Neversink River. Mortality levels at the end of test periods typically correlated closely to mean and median pH and Al_{im} concentrations that fish were exposed to during the test period (Table 2).

Relations between brook trout mortality and chemical constituents

Results of simple regression analyses show that either median or mean Al_{im} concentrations alone could account for 76–85% of the variability in brook trout mortality (Table 3). The largest r^2 values and smallest SDRs generally resulted when mean or median Al_{im} concentration was used as the single independent variable; SDRs were 13–16% (eqs. 1–3 in Table 3). The statistics for other constituents typically had weaker relations with mortality levels (Table 3). Mean and median estimates of Al_{td}, Ca^{2+}, and pH could account for 40–65% of the variability in mortality (eqs. 6–11 in Table 3). Chloride and DOC were not significantly related to mortality (Table 3) but were included in stepwise multiple-regression analyses because they can alter either water toxicity or the sensitivity of fish to toxic condition (Schofield and Trojnar 1980; Baker and Schofield 1982; Driscoll 1985; Parkhurst 1987).

Table 3. Slope, intercept, standard deviation of residuals (SDR), r^2 or adjusted r^2 value, p value, and degrees of freedom (df) for simple, logistic, and multiple regression equations defining relations between percent brook trout mortality (y) and statistics from measured constituents (x) during tests in the four study streams, 1989–1990.

Equation	Variables (x)[a]	Slope	Intercept	SDR	r^2	p	df
1	Median Al_{im}	267.2	–10.3	0.013	0.85	≤0.0001	13
2	Mean Al_{im}	248.5	–9.6	0.013	0.84	≤0.0001	13
3	Maximum Al_{im}	154.8	–7.0	0.016	0.76	≤0.0001	13
4	LCR mean Al_{im}	na	na	0.005	0.98	<0.001	14
5	LCR median Al_{im}	na	na	0.003	0.99	<0.001	14
6	Mean Al_{td}	206.7	–7.7	0.019	0.65	≤0.0001	13
7	Mean pH	–27.7	178.6	0.020	0.52	<0.001	18
8	Minimum pH	–29.5	174.8	0.022	0.40	0.003	18
9	Mean Ca^{2+}	–17.6	70.7	0.021	0.46	0.001	18
10	Mean DOC	4.1	8.7	0.029	0.02	0.609	18
11	Mean Cl^-	–91.5	75.4	0.028	0.04	0.387	18
12	Mean Al_{im}	264.9	16.0	0.012	0.85	≤0.0001	12
	Mean DOC	–8.6				0.094	
13	Median Al_{im}	284.1	14.9	0.012	0.87	≤0.0001	12
	Mean DOC	–8.5				0.080	
14	Median Al_{im}	386.6	–273.8	0.012	0.87	0.0001	
	Mean pH	57.2				0.088	11
	Mean Ca^{2+}	–26.4				0.178	
15	Median Al_{im}	325.0	–70.3	0.009	0.92	≤0.0001	11
	Mean DOC	–16.9				0.011	
	Mean Cl^-	181.3				0.003	
16	Mean Al_{im}	377.2	–156.5	0.008	0.93	≤0.0001	10
	Mean DOC	–17.7				0.002	
	Mean Cl^-	201.5				0.006	
	Mean pH	12.3				0.127	
17	Median Al_{im}	393.2	–143.0	0.008	0.93	≤0.0001	10
	Mean DOC	–16.1				0.003	
	Mean pH	12.0				0.013	
	Mean Cl^-	173.0				0.011	
18	Mean Al_{im}	372.9	–246.9	0.007	0.95	≤0.0001	9
	Mean DOC	–16.1				0.002	
	Minimum pH	30.4				0.029	
	Mean Cl^-	237.6				0.001	
	Mean Ca^{2+}	–8.5				0.246	
19	Median Al_{im}	377.3	–195.6	0.008	0.94	≤0.0001	9
	Mean DOC	–14.9				0.005	
	Mean Cl^-	201.1				0.005	
	Minimum pH	23.0				0.118	
	Mean Ca^{2+}	–5.8				0.481	

Note: na, not applicable.

[a] LCR, logistic concentration response (equation factors are provided in text); DOC, dissolved organic carbon; Al_{im}, inorganic monomeric aluminum; Al_{td}, total dissolved aluminum.

The sigmoid-shaped Gaussian or logistic concentration response (LCR) function (Fig. 3),

$$y = a + b/(1 + (x/c)^d),$$

generally describes mortality and toxic-constituent associations more accurately than linear equations (Rand and Petrocelli 1985). LCR equations that use mean or median measured Al_{im} data (x) account for 98–99% of the variability in mortality levels (y), and SDR values are as low as 3% (eqs. 4 and 5 in Table 3). Concentrations of other constituents did not generate practical LCR functions when related to our brook trout mortality data. Results indicate that percent mortality of brook trout (y) in Catskill tests is most accurately described when x is mean Al_{im} concentration, in milligrams per litre, and factors a, b, c, and d are equal to 78.03, 76.52, 0.20, and 32.54, respectively (Fig. 3A).

The two-, three-, four-, and five-variable regression equations that most accurately define brook trout mortality include median or mean Al_{im} concentration as the primary variable (eqs. 12–19 in Table 3). The linear associations of one or two constituent coefficients (t values) are not statistically significant in several equations but are included because the F value remains significant, and inclusion of the constituent typically improves the SDR by at least 1%. The two most accurate two-variable equations (eqs. 12 and 13) consist of mean or median Al_{im} and mean DOC (SDR = 12%, r^2 = 0.85 and 0.87). The

Fig. 3. Logistic concentration response (LCR) curve (solid lines) and 95% confidence interval (broken lines) describing the relations between percent brook trout mortality and mean inorganic monomeric aluminum (Al_{im}) concentration. (A) Relations based on measured Al_{im} concentrations. (B) Relations based on calculated hourly Al_{im} concentrations.

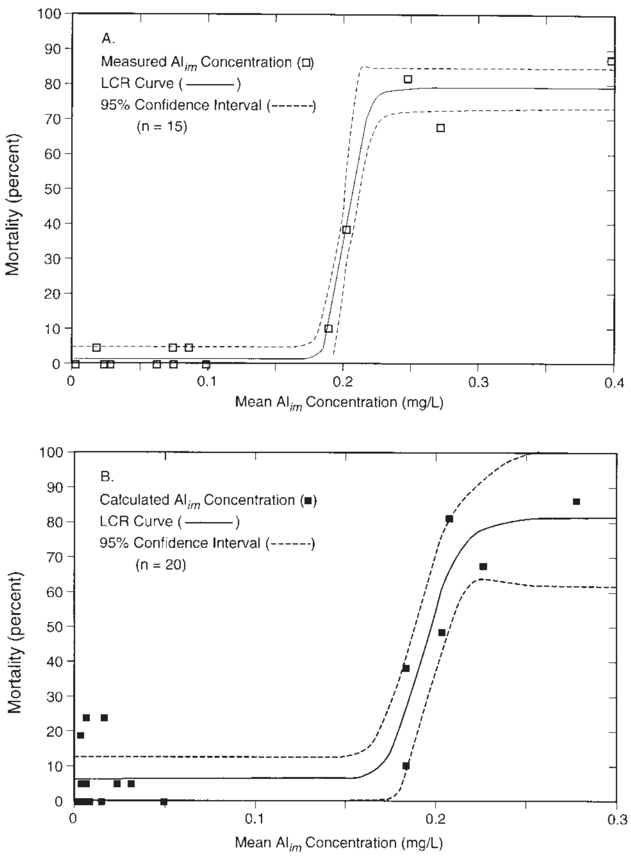
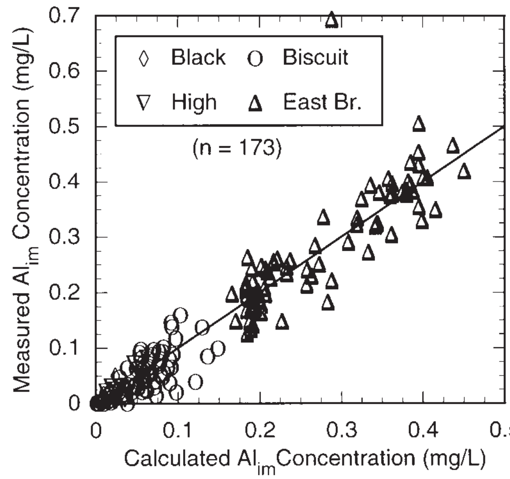


Fig. 4. Calculated and measured inorganic monomeric aluminum (Al_{im}) concentrations from all toxicity-test periods in the four Catskill Mountain streams, 1989–1990.



best three-variable equation (eq. 15) includes median Al_{im}, mean DOC, and mean Cl^- (SDR = 9%, $r^2 = 0.92$). A second three-variable equation (eq. 14), nearly as accurate as the two-variable equations, includes median Al_{im}, mean pH, and mean Ca^{2+} (SDR = 12%, $r^2 = 0.87$). Two four-variable equations (eqs. 16 and 17) include median or mean Al_{im} and mean DOC, mean Cl^-, and mean pH (SDR = 8%, $r^2 = 0.93$). The best five-variable equation (eq. 18) includes median Al_{im}, mean DOC, mean Cl^-, mean Ca^{2+}, and minimum pH (SDR = 7%, $r^2 = 0.95$). The second five-variable equation (eq. 19) is almost as accurate as eq. 18; it differs only in its use of mean Al_{im} rather than median Al_{im} (SDR = 8%, $r^2 = 0.94$).

Relations between brook trout mortality and calculated Al_{im} data

Hourly calculated Al_{im} concentrations represent measured Al_{im} concentrations fairly accurately and also depict temporal fluctuations in Al_{im} concentration for each stream. Although some Al_{im} measurements differ from the calculated Al_{im} record, most of the differences are small (Figs. 2A–2D). The accuracy of equations (not shown) used to calculate hourly Al_{im} concentrations is demonstrated by the relatively small SDR (0.033 mg/L) of the relation between all measured and calculated Al_{im} values from the four streams (Fig. 4). The SDRs for relations between measured and calculated Al_{im} values are 0.018 mg/L ($r^2 = 0.53$) for Black Brook, 0.013 mg/L ($r^2 = 0.75$) for High Falls Brook, 0.031 mg/L ($r^2 = 0.49$) for Biscuit Brook, and 0.039 mg/L ($r^2 = 0.83$) for East Branch Neversink River. These SDRs are comparable to the total analytical error of approximately 0.024 mg/L for Al_{im} measurements in the laboratory.

Magnitude statistics (mean, median, and maximum values) for calculated Al_{im} concentrations account for less variability in percent mortality ($r^2 = 0.62$–0.74; eqs. 1–3 in Table 4) than do equivalent statistics for measured Al_{im} concentrations ($r^2 = 0.76$–0.80; eqs. 1–3 in Table 3); however, the SDR values of 15–18% are analogous. Even though statistics for measured Al_{im} data (Table 3, Fig. 3A) explain variability in mortality better than those for calculated Al_{im} data (Table 4, Fig. 3B), hourly calculated Al_{im} concentrations represent exposure conditions more completely than the former. Out of all the magnitude statistics for calculated Al_{im} data, the mean Al_{im} concentration explains the largest amount of variability in brook trout mortality (74%) and yields an SDR of 15% (eq. 1 in Table 4). The median calculated Al_{im} concentration (SDR = 15%, $r^2 = 0.73$) describes percent mortality nearly as well (eq. 2 in Table 4). The LCR equation that uses mean calculated Al_{im} concentrations (eq. 4 in Table 4) accounts for 83% of the variability in trout mortality (SDR = 11%).

Linear relations between brook trout mortality and the cumulative duration (hours) of exposure estimated above calculated-Al_{im} concentrations from 0.025 to 0.350 mg/L were significant; however, the slopes, r^2 values, and significance levels (p) decreased, and SDR values increased, in all equations in which Al_{im} concentrations were above and below the 0.225–0.250 mg/L range (Table 4). The relations between percent mortality and cumulative duration of exposure to Al_{im} concentrations of 0.225 and 0.250 mg/L (and higher) yielded the smallest SDR values (14 and 15%) and largest r^2 values (0.73 and 0.76) (eqs. 7 and 8 in Table 4). These results suggest that Al_{im} concentrations of 0.225–0.250 mg/L are pivotal to brook trout mortality.

Relations between percent mortality and the number (frequency) of acidic–Al_{im} episodes during which Al_{im} concentrations

Table 4. Slope, intercept, standard deviation of residuals (SDR), r^2 or adjusted r^2 value, p value, and degrees of freedom (df) for simple and logistic regression equations defining the relations between percent brook trout mortality (y) and statistics of magnitude, duration, and frequency (x) for calculated Al_{im} concentrations (in milligrams per litre) in tests at the four Catskill Mountain streams, New York, 1989–1990.

Equation	Variable (x)	Slope	Intercept	SDR	r^2	p	df
Magnitude statistics for calculated Al_{im} concentrations							
1	Mean concentration	249.8	2.8	0.015	0.74	≤0.0001	18
2	Median concentration	256.2	3.5	0.015	0.73	≤0.0001	18
3	Maximum concentration	149.7	–2.5	0.018	0.62	≤0.0001	18
4	LCR mean concentration	na	na	0.011	0.83	<0.001	18
Duration of exposure (number of hours) above Al_{im} concentration threshold in column 2							
5	0.175 mg/L	0.058	10.1	0.023	0.36	0.003	18
6	0.200 mg/L	0.117	11.2	0.019	0.56	≤0.0001	18
7	0.225 mg/L	0.236	9.2	0.015	0.73	≤0.0001	18
8	0.250 mg/L	0.388	9.9	0.014	0.76	≤0.0001	18
9	0.275 mg/L	0.626	12.3	0.017	0.64	≤0.0001	18
10	0.300 mg/L	0.784	13.9	0.019	0.53	<0.001	18
11	0.325 mg/L	1.048	14.8	0.021	0.46	<0.001	18
Frequency of exposure (number) to increases in Al_{im} concentration of at least the value in column 2							
12	0.050 mg/L	16.7	6.6	0.022	0.42	0.002	18
13	0.100 mg/L	20.6	7.5	0.021	0.46	0.001	18
14	0.200 mg/L	35.9	11.9	0.024	0.32	0.010	18

Note: Al_{im}, inorganic monomeric aluminum; LCR, logistic concentration response (equation factors are provided in the text); na, not applicable.

increased by 0.050, 0.100, 0.150, and 0.200 mg/L were significant; however, the SDR values were high, and r^2 values were low (eqs. 12–14 in Table 4). The lowest SDR, 21%, and highest r^2 value, 0.46, were obtained from the regression between percent mortality and the number of episodes in which Al_{im} concentrations increased by at least 0.100 mg/L.

The 10, 20, 50, and 100% brook trout mortality estimates (Fig. 5), as a function of exposure duration and Al_{im} concentration (eqs. 5–11 in Table 4 and others), suggest that certain Al_{im} magnitude and duration characteristics are important to brook trout mortality. The sharp decrease in slope of the 20 and 50% mortality lines at and below 0.200 mg/L Al_{im} provides evidence that this threshold is critical to brook trout mortality. The minimum period of exposure needed to cause death of caged brook trout in infrequently toxic waters of the Neversink River can also be hypothesized by comparing significant (20%) predicted mortality levels with the duration of exposure to Al_{im} concentrations in excess of the threshold for survival (0.225–0.250 mg/L). The 20% mortality line in Fig. 5 indicates that (*i*) only background mortality levels (defined as less than 20%) are expected in waters with mean Al_{im} concentrations below 0.175 mg/L for extended periods, and (*ii*) significant mortality levels (defined as 20% or greater) are expected in waters with mean Al_{im} concentrations of 0.225 mg/L (and higher) only when exposure durations are 2 days or longer.

Discussion

Our results demonstrate that waters of three low-order Catskill Mountain streams, Biscuit Brook, High Falls Brook, and the East Branch, were episodically acidified during events of increased discharge; the East Branch Neversink River was also chronically acidified. Discharge events on the three streams typically produced concurrent episodes of elevated Al_{im} concentration; the frequency, duration, and magnitude of increases in Al_{im} concentration depended upon stream and season. Al_{im} concentrations were often toxic to caged young-of-the-year brook trout in the East Branch site and seldom toxic to caged fish at the other two acidified sites or at the primary reference site, Black Brook. With the single exception of Van Sickle et al. (1996), no other studies have documented fish mortality in the Catskills or associated it with acidification of local streams.

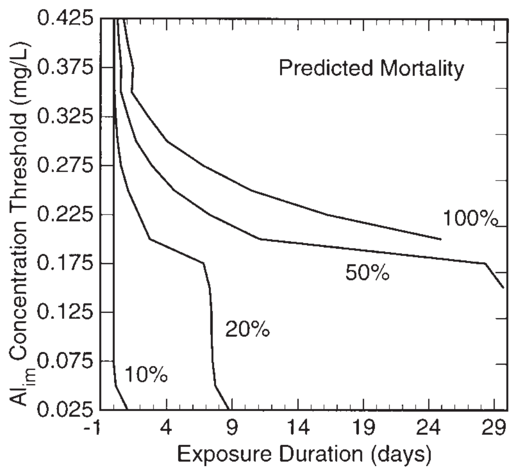
In our study, brook trout mortality was related to mean and median Al_{im} concentration more strongly than to other constituents. Mean pH and DOC, Ca^{2+}, and Cl^{-} concentrations were also significantly related to brook trout mortality. Acidic–Al_{im} episodes appeared to cause significant brook trout mortality when Al_{im} concentrations approached or exceeded 0.225 mg/L but only after fish were exposed to these conditions for 2 or more days. Except for the ERP (the present study; Gagen et al. 1993; Simonin et al. 1993; Van Sickle et al. 1996), few studies have assessed the relations between fish mortality and fluctuating concentrations of Al_{im} in natural systems. Many recent investigations, however, have found that temporal characteristics of fluctuating pH and Al_{im} concentrations (in the laboratory) and mean and median pH, Ca^{2+}, DOC, and Al_{im} concentrations (in both the laboratory and natural systems) strongly affect fish survival and are good predictors of brook trout mortality (see review by Baker et al. 1990).

Biologically relevant chemical constituents

Calcium

Our finding that mean Ca^{2+} concentrations can account for 46% of the variability in brook trout mortality is consistent with results of previous studies. Calcium has been shown to improve brook trout survival by increasing their tolerance to Al_{im} in (*i*) pH-neutral waters at Ca^{2+} concentrations above 0.5–1.0 mg/L, and (*ii*) acidic waters at Ca^{2+} concentrations of

Fig. 5. Estimated 10, 20, 50, and 100% brook trout mortality as a function of exposure duration (0–30 days) and inorganic monomeric aluminum (Al_{im}) concentration (0.025–0.425 mg/L).



2.0–8.0 mg/L (see review by Baker et al. 1990). For example, during exposure of brook trout fry to toxic levels of Al and acidity, whole-body content of Na, Cl^-, Ca^{2+}, and K^+ in brook trout fry declined (susceptibility to ionoregulatory distress increased) except when Ca^{2+} concentrations exceeded 1.5–2.0 mg/L (Wood et al. 1990*a*, 1990*b*). The authors concluded that (*i*) a protective Ca^{2+} threshold exists for brook trout fry, (*ii*) it ranges from 2 to 8 mg/L, and (*iii*) the protective threshold varies with pH. Calcium increases the tolerance of fish to otherwise toxic levels of Al_{im} and acidity by increasing the efficiency of sodium ion regulation and oxygen exchange processes, which are impeded by low pH and high Al_{im} concentrations (Mount et al. 1988).

In our study, mean Ca^{2+} concentrations were less than 2.0 mg/L during tests in the East Branch Neversink River where pH averaged less than 5.0, and generally higher than 2.0 mg/L in the other three streams where pH averaged 5.4–6.5. Evidently, tolerance levels and survival rates of exposed fish were not positively affected by high Ca^{2+} concentrations at the East Branch Neversink site because of very low pH levels; high mean Ca^{2+} concentrations at the other sites may have provided some protection for brook trout exposed to high Al_{im} concentrations.

Chloride

In Catskill tests, mean Cl^- concentrations alone were not significantly related to percent mortality, but multiple regression analyses (eqs. 15–19 in Table 3) suggested that Cl^- was possibly the fourth or fifth most important constituent affecting brook trout mortality. These findings advocate a hypothesis that, during exposure to toxic acid and Al_{im} concentrations, elevated concentrations of Cl^- (and Ca^{2+}, Na^+, and K^+) in stream waters decrease the osmotic imbalance between fish and water (the NaCl gradient) and thereby reduce ionoregulatory stresses, sensitivity of fish, and mortality rates in a manner similar to that of Ca^{2+}. Circumstantial evidence from the literature suggests that high Cl^- concentrations may, in fact, decrease the sensitivity of brook trout to Al_{im} and pH toxicity. Several studies document a decrease in whole-body content of Cl^- (and Na^+, Ca^{2+}, and K^+) in salmonids during exposure to elevated acidity and Al concentrations (Wood et al. 1990*a*, 1990*b*; Cleveland et al. 1991; Gagen and Sharpe 1987; Gagen et al. 1993). None, however, have directly related increased fish survival with elevated Cl^- concentrations in acidified lakes and streams. Uniformly low concentrations of Cl^- in our study streams (0.5–0.7 mg/L; Tables 1 and 3) likely lessen the strength of any interrelations and, thus, the importance of Cl^- to brook trout mortality in our analyses.

Dissolved organic carbon

The higher levels of fish mortality and concentrations of Al_{tm} and Al_{im} during the spring than the fall (Fig. 2, Table 2), coupled with higher concentrations of DOC and Al_{om} during the fall than the spring (Table 2), suggest that seasonal differences in brook trout mortality in Catskill streams are due, in part, to seasonal differences in DOC concentrations. During the fall, high concentrations of DOC complex inorganic Al species, which in turn increases Al_{om} concentrations. During the spring, both DOC and Al_{om} concentrations are low. Although primarily controlled by pH, mean Al_{im} concentrations during the spring are much higher than they are during the fall at most study sites (Table 2); Al_{im} concentrations also increase more sharply during spring episodes of high flow and increased acidity than during comparable fall episodes (Fig. 2). In the three Catskill streams with measurable Al_{im} concentrations, shifts in the ratios of Al_{om} to Al_{tm} between seasons demonstrate a potential seasonal effect of DOC on Al_{im} concentration and on water toxicity. Mean ratios of Al_{om} to Al_{tm} at High Falls Brook, Biscuit Brook, and the East Branch Neversink were 1.00, 0.80, and 0.41 during the fall and 0.69, 0.46, and 0.18 during spring tests, respectively. The larger fraction of Al_{tm} that is Al_{im} during the spring as compared with the fall makes the waters potentially more toxic to aquatic life during the spring even at equivalent fall pH levels and Al_{tm} concentrations. The seasonal difference in Al_{im} concentration and water toxicity in these three streams may therefore be attributed indirectly to DOC concentration and the strong direct relation between Al_{om} and DOC concentrations ($r^2 = 0.50, p \leq 0.0001$). Driscoll et al. (1980) and Shultz et al. (1993) described similar relations between Al_{om} and DOC concentrations, and higher levels of both during fall than during spring in Adirondack and Catskill waters.

Given the strong association between DOC, Al_{om}, and Al_{im} and the seasonal differences in mortality levels, DOC, Al_{om}, and Al_{im} concentrations, it is surprising that DOC alone was not significantly related to brook trout mortality in the Catskill tests (Table 3). DOC was, however, found to be the second most important factor in all multiple-variable regressions (eqs. 12–19 in Table 3); thus, our findings were consistent with other studies that generally indicate that DOC is a strong secondary or tertiary determinant of brook trout mortality (Parkhurst et al. 1990; Simonin et al. 1993). Numerous studies confirm that DOC complexes with inorganic Al to create Al_{om} species that are less toxic than Al_{im} to fish (Driscoll et al. 1980; Driscoll 1985; Parkhurst 1987). Others have shown that concentrations of Al_{om}, like Al_{im}, are systematically dependent on pH (Browne and Driscoll 1993). Results from these and other investigations also suggest that, in moderately acidified waters, DOC concentrations greater than 2.0 or 3.0 mg/L may significantly decrease Al_{im} concentrations and, thus, decrease water toxicity and mortality of exposed brook trout. For example, Parkhurst (1987) and Parkhurst et al. (1990) showed that, for their studies, (*i*) the protective effects of DOC were most pronounced when the DOC concentration was greater than 3.1 mg/L and water pH was 5.2–5.6, (*ii*) very high Al_{im} concentrations (1.0 mg/L) inhibited the protective effects of DOC, (*iii*) DOC was the third most important factor related to brook trout mortality, and (*iv*) a logistic model that included Al_{im}, pH, and DOC best predicted brook trout survival in waters with DOC concentrations greater than 1.0 mg/L.

In our analyses, the relation between brook trout mortality and DOC alone was only partially evident because any protective effect of high DOC concentrations (>2.0 mg/L) at the East Branch Neversink site was negated, as predicted by Parkhurst et al. (1990), by high Al_{im} concentrations (up to 0.639 mg/L) and very low pH values (4.6–4.8) (Table 1). Mean DOC concentrations at this site ranged from 2.8 to 3.8 mg/L during five tests where brook trout mortality exceeded 20% and averaged 4.9 mg/L in one test with mortality less than 20% (Table 2). Mean DOC concentrations in the well-buffered stream (Black Brook) and the two intermediate-pH streams (Biscuit Brook and High Falls Brook) were generally less than 2.0 mg/L when brook trout mortality was equal to or greater than 20% and were 2.0–4.1 mg/L when mortality was less than 20% (Table 2). Brook trout mortality and DOC concentrations were also not significantly related to each other during toxicity tests done in four Adirondack streams, yet median DOC was the second most important factor in multiple-variable analyses, accounting for an additional 17% of variability in mortality (Simonin et al. 1993). During all of their tests, median DOC ranged from 2.9 to 9.3 mg/L (Simonin et al. 1993). DOC concentrations in many Adirondack waters are typically high (>3.0 mg/L) and Al_{om} is usually the dominant Al form (Driscoll et al. 1980). Conversely, seasonal differences in brook trout mortality levels in several Pennsylvania streams were attributed to higher acidity and higher Al_{td} concentrations during the spring than in the fall; DOC was unimportant to brook trout survival because more than 90% of Al_{td} was found to be in a reactive inorganic form (Al_{im}) year round (Gagen et al. 1993). Except for Benner Run during the fall 1989, consistently low DOC concentrations, averaging from about 1 to 2 mg/L during spring tests and from 2 to 3 mg/L during fall tests, may have lessened the effect of DOC on Al speciation and fish mortality in their streams (Gagen et al. 1993). In a statistical analysis of chemistry and bioassay results from all of the ERP streams, Van Sickle et al. (1996) found that median DOC concentrations added explanatory power to higher order brook trout mortality models using time-weighted median Al_{im}, minimum pH, and median Ca^{2+}.

Findings of this study, Gagen et al. (1993), Simonin et al. (1993), and Van Sickle et al. (1996) suggest that the beneficial effect of DOC on brook trout mortality varies across the northeastern United States owing to differences in DOC concentrations encountered in streams of the regions. These differences may be enhanced, and the importance of DOC on fish mortality further altered, by complex geochemical interactions that underlie relations among pH, DOC, Al_{om}, and Al_{im}. Spatial (local and regional) and temporal differences in DOC concentrations and in the effect of DOC on Al_{im} concentration and fish mortality may be attributed to three factors: (*i*) Al_{im} and DOC can be important pH buffers in acidic waters (pH <5.3), where the buffering capacity of dissolved inorganic carbon is diminished, (*ii*) high DOC concentrations can contribute to water acidity and toxicity, and (*iii*) the surface-water concentration of Al_{im} is highly related to pH while Al_{om} concentration is highly related to DOC concentration (Driscoll 1985; Browne and Driscoll 1993).

pH

Our analyses indicate that pH is a secondary determinant of brook trout mortality in the Catskill study streams. Mean pH alone accounted for 52% of the variability in mortality, and including pH in equations with three or more predictor variables (Table 3) helped account for an additional 11% of the variability. These results corroborate findings of earlier studies that indicate that pH is often a primary or secondary determinant of fish mortality in lakes and streams of the northeastern United States that undergo acidification (Driscoll et al. 1980; Baker and Schofield 1982; Sharpe et al. 1987; Johnson et al. 1987; Parkhurst et al. 1990; Gagen et al. 1993; Van Sickle et al. 1996). Other investigations have also shown that acidity is acutely toxic to various life stages of brook trout in waters with a pH range of about 3.5–4.5 and that it is a nonlethal inhibitor of growth at moderate levels (pH 5.0–6.0) (see review by Baker et al. 1990). In our study streams, however, pH rarely approached acutely toxic levels for young-of-the-year brook trout, even in the chronically acidic East Branch Neversink site (Table 2, Fig. 2).

The effect of pH on brook trout mortality in the Catskill tests appears to be due primarily to its relation to mineral Al solubility. Analyses of Catskill stream-chemistry data through a simple chemistry equilibrium model, ALCHEMI (Schecher and Driscoll 1987), indicate that Al solubility is controlled by an aluminum hydroxide solid phase similar to gibbsite. Water pH controls dissolution of gibbsite and, therefore, also controls Al speciation and Al_{im} concentration (Driscoll 1985). Dissolution of gibbsite increases both above and below pH 6.0; thus, concentrations of inorganic Al species increase as pH decreases below pH 6.0 (Driscoll 1985). Because water pH strongly affects the concentration of Al_{im}, and because Al_{im} appears to govern brook trout mortality in the Catskill tests, pH is critical to brook trout mortality and to the distribution of native brook trout populations in Catskill streams that are affected by acidification.

Inorganic monomeric aluminum

Our results indicate that Al_{im} is the primary determinant of brook trout mortality in the study streams and that significant brook trout mortality occurs only after fish are exposed to Al_{im} concentrations in excess of a survival threshold for 2 days or longer. In fact, mean or median Al_{im} concentration accounted for 76–99% of the variability in brook trout mortality (Table 3). Recent investigations have also concluded that Al_{im} was the primary determinant (and that pH and DOC were significant secondary determinants) of mortality of brook trout in natural acidified waters (see review by Baker et al. 1990; Parkhurst et al. 1990). Mean or median Al_{im} (or Al_{td}) concentration or a combined duration and Al_{im} concentration factor consistently explained the greatest amount of variability in brook trout mortality in all three regions of the ERP (Gagen et al. 1993; Simonin et al. 1993; Van Sickle et al. 1996).

In our study streams, 0.200 mg/L Al_{im} could be designated as the concentration threshold for brook trout survival given that (*i*) 20% represents the maximum acceptable brook trout mortality level (based on the total error of mortality data; see Methods), (*ii*) the estimated 20% lethal Al_{im} concentration (LC_{20}) for brook trout is in the 0.181–0.195 mg/L range (LCR curves in Figs. 3A and 3B), and (*iii*) duration of exposure to Al_{im} concentrations in excess of 0.225 and 0.250 mg/L accounts for the greatest amount of variability in mortality (Table 4). This finding is consistent with recent field and laboratory studies that have documented significant brook trout mortality under low Ca^{2+} (<2.0 mg/L), DOC (<2.0 mg/L), and pH (4.4–5.2) conditions only when ambient concentrations of Al_{im} and (or) Al_{td} exceed the 0.200–0.300 mg/L range (see review by Baker et al. 1990; Van Sickle et al. 1996). For example, gill damage and mortality in brook trout increased at pH values of 4.2–5.2 only when total acid-soluble Al concentrations were equal to or greater than 0.200 mg/L (Schofield and Trojnar 1980; Baker and Schofield 1982); brook trout mortality occurred only when they were exposed (for some minimum duration) to Al_{td} concentrations greater than 0.200–0.300 mg/L (Gagen and Sharpe 1987).

Duration and frequency of acidic–aluminum episodes

Results from the Catskill study provide evidence that acidic–Al_{im} episodes strongly reduce survival of resident brook trout in parts of the Neversink River. Increased mortality was generally associated only with periods of high flow and episodes of elevated acidity and increased Al_{im} concentration (Figs. 2A–2D). Results also indicate that certain episode characteristics affect brook trout mortality more than others. In linear regression analyses, for example, episode frequency appears to have a minor effect on mortality, whereas the cumulative duration of exposure to Al_{im} concentrations in excess of 0.250 mg/L explained the greatest amount of variability (76%) in brook trout mortality (Table 4). Assuming that the Al_{im} concentration threshold for survival is about 0.200 mg/L (Figs. 3 and 5), the 20% mortality line in Fig. 5 shows that significant brook trout mortality is projected at 0.225 mg/L Al_{im} only when exposure durations are 2 days or longer. This critical minimum exposure is supported by physiological data from Gagen and Sharpe (1987), which show that ionoregulation fails and brook trout die at toxic Al_{td} concentrations (above the 0.200–0.300 mg/L range) only when exposure durations are 1.5 days or longer.

Mounting evidence from other field and laboratory investigations also suggests that the frequency of acidic–Al_{im} episodes and the duration of exposure to lethal and sublethal conditions strongly affect mortality levels. Death of brook trout occurs only after a minimum exposure period to toxic Al_{td} concentrations is surpassed, apparently when whole-body content and depletion rates of Na^{+} and other ions surpass a minimum physiological threshold for survival (Gagen and Sharpe 1987). A combined Al_{im} concentration and exposure-duration variable was the best single predictor of brook trout mortality in bioassays done in the Adirondack Mountains (Simonin et al. 1993). Repeated exposure to hydrogen ion pulses of varying durations (acid episodes) caused greater cumulative mortality of larval brook trout than continuous exposures to equivalent mean hydrogen ion concentrations (Curtis et al. 1989). Intermittently elevated Al_{td} concentrations caused greater cumulative mortality and decreased growth rates of juvenile brook trout than continuous exposures to similar concentrations (Siddens et al. 1986). These findings and the effect of episode number and duration of exposure to toxic Al_{im} concentration on brook trout mortality in Catskill tests suggest that (*i*) the frequency of toxic conditions can significantly affect brook trout survival, (*ii*) stresses and injury to brook trout exposed to toxic acidic–Al_{im} episodes may be additive, (*iii*) brook trout appear to undergo little recovery between frequent toxic acidic–Al_{im} episodes in headwater streams, and (*iv*) brook trout appear to assimilate effects of acidic–Al_{im} episodes through the cumulative damage–repair function described by Breck (1988).

The total exposure duration and the frequency of exposure to elevated concentrations of toxic constituents can be as important to fish survival as the ambient concentration of the toxicant. The duration of exposure affects mortality levels because stress and death of organisms are generally not provoked at low concentration of toxins during long exposures nor at high concentrations during extremely short exposures (Breck 1988); therefore, the duration of exposure can be assumed to determine the ultimate level of mortality for exposed fish once Al_{im} concentrations exceed the threshold for survival. The number or frequency of exposures to toxic conditions can also affect mortality rates if the resulting injury to an organism is additive and not repaired between successive sublethal exposures (Rand and Petrocelli 1985; Breck 1988). Thus, knowledge of the maximum duration that fish can survive acutely toxic Al_{im} concentrations (the minimum duration that causes mortality during exposure to Al_{im} concentrations at and above survival thresholds), and of the effects of frequent changes in Al_{im} concentration on fish mortality, is essential to assess the significance of acidic–Al_{im} episodes on individual fish. The effects of chronic acidification and acidic–Al_{im} episodes on mortality of one the most acid-tolerant native species (brook trout) suggest that endemic fish populations and fish communities throughout the Neversink River are also negatively affected by stream acidification.

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