

In situ Bioassays of Brook Trout (*Salvelinus fontinalis*) and Blacknose Dace (*Rhinichthys atratulus*) in Adirondack Streams Affected by Episodic Acidification

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In situ bioassays were conducted using native Adirondack brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in four headwater streams. Conductivity, pH, temperature, and stage height were monitored continuously, and water samples for laboratory analysis were collected during hydrologic episodes. Fish survived well during baseflow conditions, but during periods of spring snowmelt or large precipitation events, survival was poor. Blacknose dace were more sensitive than brook trout, and mortality was best correlated with the log of median inorganic monomeric aluminum (Al_{im}) concentration. Brook trout mortality was best correlated with a two-variable model that included dissolved organic carbon (DOC) and a concentration–duration variable (median Al_{im} during the episode times the duration of the episode). Brook trout mortality was inversely correlated with DOC. Bioassay fish that had been in the streams 15–24 d survived episodes better than fish that had either not become acclimatized or recovered from handling. Duration of exposure to acidic episodes was critical. Extended periods of poor water quality resulted in fish mortality and may be more important to native populations than short acidic episodes.

Des dosages biologiques in situ ont été réalisés avec des ombles de fontaine (*Salvelinus fontinalis*) et des naseux noirs (*Rhinichthys atratulus*) indigènes d'Adirondack dans quatre ruisseaux d'amont. La conductivité, le pH, la température et le niveau de l'eau ont fait l'objet d'une surveillance continue et des échantillons d'eau ont été prélevés pour analyse de laboratoire au cours des épisodes hydrologiques. Les poissons ont eu une bonne survie durant les conditions de débit de base, mais durant la fonte des neiges printanière et les grandes précipitations, la survie a été médiocre. Le naseux noir s'est révélé plus sensible que l'omble de fontaine et la mortalité présentait la meilleure corrélation avec le log de la concentration médiane d'aluminium monomère inorganique (Al_{im}). Chez l'omble de fontaine, la mortalité présentait la meilleure corrélation avec un modèle à deux variables, à savoir la concentration de carbone organique dissous (COD) et une variable concentration–durée (Al_{im} médiane au cours de l'épisode multiplié par la durée de l'épisode). La mortalité de l'omble de fontaine présentait une corrélation inverse avec la COD. Les poissons ayant séjourné dans les ruisseaux pendant 15–24 jours ont mieux survécu aux épisodes que les poissons qui n'avaient pas eu le temps de s'acclimater ou qui ne s'étaient pas rétablis de la manipulation. La durée de l'exposition à des épisodes où l'eau est plus acide était critique. Des périodes prolongées où la qualité de l'eau était médiocre ont entraîné de la mortalité et pourraient avoir plus d'importance pour les populations indigènes que les courtes périodes où l'acidité est élevée.

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Both laboratory and field bioassays have been used to demonstrate that fish are sensitive to acid and high aluminum concentrations (Baker and Schofield 1982; Sharpe et al. 1983; Johnson et al. 1987; Parkhurst 1987; Ingersoll et al. 1990). Relatively few studies have been conducted to evaluate the effects of episodic acidification that occurs during periods of snowmelt or heavy precipitation. Such episodes occur commonly in streams and rivers receiving acidic deposition and may vary in both intensity and duration. Following these episodes,

water quality typically returns to conditions acceptable to most fish life in all but chronically acidified streams. Detailed reviews of the effects of acidic episodes have been prepared by Marmorek et al. (1986) and Wigington et al. (1990).

Episodic acidification in the Adirondack region of New York State has been documented in lake outlets (Driscoll and Newton 1985; Galloway et al. 1987), large rivers (Colquhoun et al. 1981, 1984), and headwater streams (Colquhoun et al. 1982, 1984; Colquhoun and Aylesworth 1983). The most acidic epi-

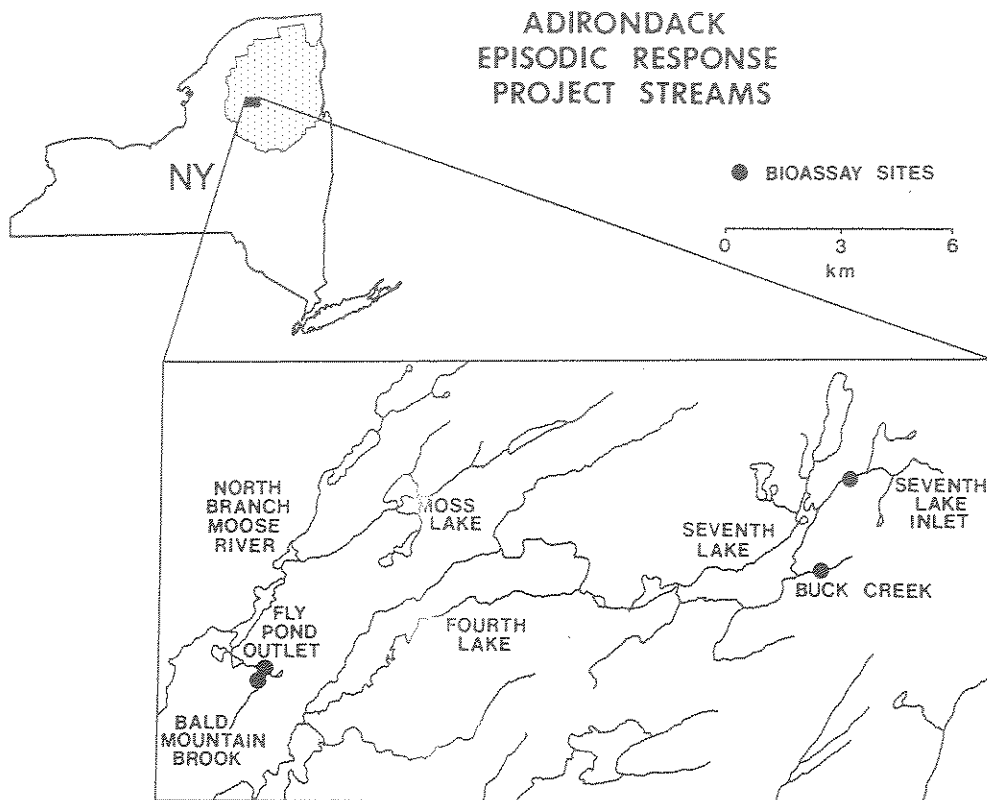


FIG. 1. Location of Adirondack stream bioassay sites.

sodes of longest duration generally occur during spring snowmelt, although episodes equal in acidity and duration may also occur at other times of the year, depending on the amount of precipitation.

Fisheries survey data have shown that fish are absent from many Adirondack streams that become acidic during the spring snowmelt period (Colquhoun et al. 1983). Schofield and Driscoll (1987) found that in the North Branch of the Moose River, acidity gradients in the watershed and the acid tolerance of the fish species were the key determinants in the species distributions they observed. Johnson et al. (1987) further suggested that the extent of the watershed that is acidic, and therefore toxic to fish, increases during snowmelt and major runoff events.

Studies in Pennsylvania (Gagen and Sharpe 1987; Sharpe et al. 1987) and in Norway (Hesthagen 1989) demonstrated fish mortalities in response to acidic episodes. During baseflow conditions, streams offered suitable fish habitat and water quality, but during spring snowmelt, poor water quality resulted in fish mortalities. Hesthagen (1989) counted dead fish daily in a Norwegian river and associated the cause of death with environmental acidification during spring snowmelt. He also observed that fish mortality was usually delayed relative to the onset of an acidic episode. Gunn (1986) concluded that acidic episodes may impact several species of salmonids at different periods in their life history. He also noted that few field studies had been conducted on the impacts of acidification on brook trout (*Salvelinus fontinalis*) populations.

The Adirondack Episodic Response Project was designed to address fish response during episodic acidification. This project was part of a larger effort coordinated by the U.S. Environmental Protection Agency with similar research conducted in Pennsylvania and in the Catskill Mountains of New York State. Several objectives of the Adirondack project were to (1) con-

tinuously monitor the water chemistry of several streams that experience episodic acidification, (2) determine the effects of acidic episodes on the survival of brook trout and blacknose dace (*Rhinichthys atratulus*) by conducting in situ bioassays, and (3) relate the bioassay results to fish population responses observed in the study streams. Additional components of the Adirondack Episodic Response Project are discussed in Kretser et al. (1991).

Methods

Four headwater streams were selected as study sites for the Adirondack Episodic Response Project (Fig. 1). The streams are located in the southwestern Adirondack region which receives over 120 cm of precipitation per year. Fly Pond Outlet and Bald Mountain Brook are in the North Branch of the Moose River drainage. Seventh Lake Inlet and Buck Creek are tributaries of Seventh Lake and are in the Middle Branch of the Moose River drainage. The watersheds are covered by state forest consisting of a northern hardwood/conifer mixture.

Conductivity, pH, temperature, and stage height of the streams were measured continuously. An instrument shelter was constructed at each stream to house a U.S. Geological Survey minimonitor, a Campbell (model CR10/WP) data logger/controller, an ISCO (model 2700) automated water sampler, and 12-V batteries. The ISCO sampler was programmed to collect 1-L samples at specified intervals based on changes in stream stage. Weekly grab samples were also collected from the streams near the instrument shelters. Water samples were analyzed for the following parameters: pH, acid-neutralizing capacity (ANC), specific conductance, Cl, NO₃, SO₄, Na, K, Ca, Mg, SiO₂, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), NH₄, total dissolved aluminum (Al_{tot}), total

monomeric aluminum (Al_{im}), and organic monomeric aluminum (Al_{om}). Inorganic monomeric aluminum (Al_{im}) was calculated by subtracting Al_{om} from Al_{tm} . Kretser et al. (1991) presented a more complete discussion of water chemistry methods and quality control procedures.

Bioassays were conducted four times during the study: fall 1988 (November 2 – November 19), spring 1989 (May 8 – June 15), fall 1989 (September 27 – October 31), and spring 1990 (May 1 – May 29). They were scheduled to coincide with times when acidic episodes generally occur and to coincide with concurrent intensive studies on fish movement in response to episodes (Kretser et al. 1991).

Native brook trout young-of-the-year and native blacknose dace adults were used for the fall bioassay experiments. Native yearling brook trout and blacknose dace adults were used for the spring bioassays. In addition, hatchery-reared (New York Department of Environmental Conservation, Rome Fish Hatchery) brook trout fry were also tested during the spring bioassays. The native brook trout and blacknose dace were collected from over 20 Adirondack streams with water chemistry comparable with that of the study streams. The fish were collected by electrofishing and held for several days in a large cage in Fly Pond Outlet prior to being used for the bioassays. At least 20 fish of each species were tested at each site during each bioassay.

The methods used for fish exposure in the study streams were those used by Johnson et al. (1987). The experimental fish were placed in 3.8-L Nalgene containers with two 7×12 cm openings screened with fiberglass (1.4-mm mesh) to ensure adequate water exchange. Three or four containers were used at each site for each species to ensure the fish were not crowded. The containers were then placed inside wood frame cages covered with 6.4-mm mesh plastic screen. The cages served to protect the bioassay containers during periods of high stream flow. The cages were placed in pool areas either adjacent to or near the instrument shelters. The test fish were not fed during the bioassays.

The survival of the bioassay fish was determined by daily checks at each stream. Fish were considered dead if there was no response to prodding and no observed movement. Bioassay experiments were restarted in all streams if greater than 10% mortality occurred in the reference stream (Fly Pond Outlet) or if more than 90% mortality occurred within 1 wk in any stream. As a result of these conditions, three yearling brook trout bioassays were conducted during spring 1989, two blacknose dace bioassays during spring 1989, and two brook trout bioassays during fall 1989.

Analysis of the bioassay and water chemistry data was done primarily with the Statistical Analysis System (SAS). Mortality data were also analyzed as probits (Fisher and Yates 1957) because they often show a sigmoidal relationship to chemical data. The aluminum concentrations were converted to log values prior to regression analysis with mortality in order to improve linearity. Exploratory multiple regression analysis was conducted using the SAS Stepwise Maximum R^2 procedure and the following variables: median field pH, minimum field pH, $\log(\text{median } Al_{td})$, $\log(\text{median } Al_{im})$, $\log(\text{peak } Al_{im})$, median DOC, $\log(\text{median DOC})$, median Ca, $\log(\text{median Ca})$, and a concentration-duration factor (CONCDUR) defined as the median Al_{im} concentration during an episode (period of daily median $Al_{im} > 100 \mu\text{g/L}$) multiplied by the duration of the episode in days. Daily Al_{im} values were predicted for each stream from the minimonitor pH records and the relationship

between minimonitor pH and the measured Al_{im} values. The water chemistry values used were those obtained while each specific bioassay was in progress.

Results

In order to better compare the hydrology of the four study streams, flow data were based on stream watershed areas. Stream baseflow conditions were identified as a daily average flow of $40 \text{ L} \cdot \text{s}^{-1} \cdot \text{km}^{-2}$ or less. Significant hydrologic episodes (over 2.5 times baseflow) occurred during three of the four bioassay periods. The spring 1989 bioassay occurred just following an episode and during a period of gradually decreasing stream flow. During periods of baseflow conditions, water quality in all streams usually allowed for the survival of the bioassay fish. With the exception of Fly Pond Outlet, episodes were generally associated with acidic conditions, higher concentrations of Al_{im} , and higher mortality in the bioassays.

Fly Pond Outlet (the reference stream) exhibited the best water quality of the four study streams. Baseflow pH values averaged 7.1 and during episodes were rarely below 6.0. The hydrologic episodes that occurred in Fly Pond Outlet were not usually associated with water chemistry changes severe enough to cause mortality in the bioassays. Fish mortality and median water chemistry values for selected parameters during the bioassays are presented in Table 1.

Daily Al_{im} concentrations were derived from the continuous record of minimonitor pH using stream-specific predictive equations. This allowed for an evaluation of gradual or small changes in Al_{im} which could be related to the daily observations of fish survival. An average of 80 measured Al_{im} concentrations for each stream were regressed with the corresponding minimonitor pH value for that day to develop the relationship. The following stream specific equations were developed:

Bald Mountain Brook:

$$\log(Al_{im}) = 4.84 - 0.55(\text{pH}) \quad (R^2 = 0.58)$$

Buck Creek:

$$\log(Al_{im}) = 5.27 - 0.65(\text{pH}) \quad (R^2 = 0.66)$$

Seventh Lake Inlet:

$$\log(Al_{im}) = 4.77 - 0.54(\text{pH}) \quad (R^2 = 0.48).$$

Each of these equations was significant at $P < 0.001$. The relationship for Fly Pond Outlet, however, was poor ($R^2 = 0.04$) because pH values were all greater than 5.6 and Al_{im} concentrations were low. The predicted daily Al_{im} concentrations for each stream were then plotted against percent occurrence (Fig. 2). Buck Creek exhibited the highest Al_{im} levels for the greatest percent of the time and Fly Pond Outlet had the lowest Al_{im} levels.

The bioassay that began on May 18, 1989, demonstrated that during periods of low flow, water quality in the study streams is suitable for the survival of blacknose dace. When the 1989 Bald Mountain Brook and Seventh Lake Inlet bioassays are compared with the spring 1990 bioassays for these streams, the effect of the spring 1990 episode is evident (Fig. 3 and 4). Blacknose dace exposed to 50–110 $\mu\text{g } Al_{im}/\text{L}$ experienced no mortality, but when an episode began on May 11, 1990, and continued for over 10 d the Al_{im} concentrations increased to over 250 $\mu\text{g/L}$ and resulted in 100% mortality. In Fly Pond Outlet the Al_{im} concentrations remained below 50 $\mu\text{g/L}$, and no blacknose dace mortality was observed during either 1989 or 1990.

1. Selected water chemistry and brook trout and blacknose dace mortality during in situ bioassays.

Stream	Median pH	Median Al _{lim} µg/L	Median DO mg/L	Median Cl ⁻ mg/L	Median Al _{lim} calculated µg/L	Dissolved Al _{lim} µg/L	Mortality %
<i>Brook trout (size range 50–123 mm)</i>							
Mountain Brook	5.75	42	3.4	1.96	100	12	45
	5.87	41	3.5	2.21	156	5	30
	5.70	51	4.5	2.25	177	19	10
	4.91	138	4.3	1.83	287	25	65
k Creek	4.67	340	7.5	2.40	340	17	80
	5.28	70	5.1	2.33	111	3	45
	4.72	158	5.4	2.40	158	12	50
	4.89	124	7.4	2.27	199	10	10
	4.37	283	6.7	2.05	287	25	70
Pond Outlet	6.70	21	7.8	3.80	21	0	5
	6.42	24	4.0	4.78	24	0	0
	6.95	18	2.9	5.75	18	0	15
	6.65	21	6.4	5.10	21	0	10
	6.58	22	5.1	4.11	22	0	5
Seventh Lake Inlet	5.12	210	9.3	3.01	210	17	20
	5.27	86	6.3	2.51	104	3	0
	5.47	63	6.7	2.76	108	1	10
	5.48	62	8.3	2.82	134	5	5
	4.57	191	7.7	2.32	242	21	25
<i>Brook trout fry (size range 25–39 mm)</i>							
Bald Mountain Brook	5.31	85	3.4	1.98	124	8	74
	4.91	134	4.3	1.83	177	19	27
Buck Creek	4.79	146	5.4	2.26	146	8	89
	4.47	233	7.0	2.12	233	17	100
Fly Pond Outlet	6.38	25	4.1	4.63	25	0	0
	6.58	22	5.1	4.11	22	0	7
Seventh Lake Inlet	5.12	210	9.3	3.01	210	17	20
	5.27	86	6.3	2.51	104	3	0
	5.47	63	6.7	2.76	108	1	10
	5.48	62	8.3	2.82	134	5	5
	4.57	191	7.7	2.32	242	21	25
<i>Blacknose dace (size range 27–80 mm)</i>							
Bald Mountain Brook	5.09	111	6.4	2.24	133	12	85
	5.00	125	3.5	1.93	125	9	100
	5.74	49	3.3	1.97	69	0	0
	5.86	42	4.0	2.25	146	8	0
	5.05	121	4.5	1.83	205	13	100
Buck Creek	4.66	350	7.7	2.42	350	11	95
	4.77	149	5.6	2.07	149	6	100
	5.01	106	5.0	2.31	106	7	48
	4.84	132	5.6	2.48	139	20	90
	4.56	201	5.0	2.28	201	9	100
Fly Pond Outlet	6.70	21	7.8	3.80	21	0	5
	6.28	25	4.3	3.74	25	0	0
	6.39	25	4.0	4.80	25	0	0
	6.70	21	6.4	5.51	21	0	0
	6.80	20	5.1	4.11	20	0	0
Seventh Lake Inlet	5.05	220	9.3	3.01	220	15	95
	4.72	163	6.7	2.44	163	8	95
	5.18	95	6.0	2.55	105	6	0
	5.47	64	8.3	2.82	118	6	5
	4.83	130	7.8	2.36	255	13	95

*Daily Al_{lim} values were calculated from minimonitor pH measurements using the stream-specific relationship between pH and periodic measurements of Al_{lim}.

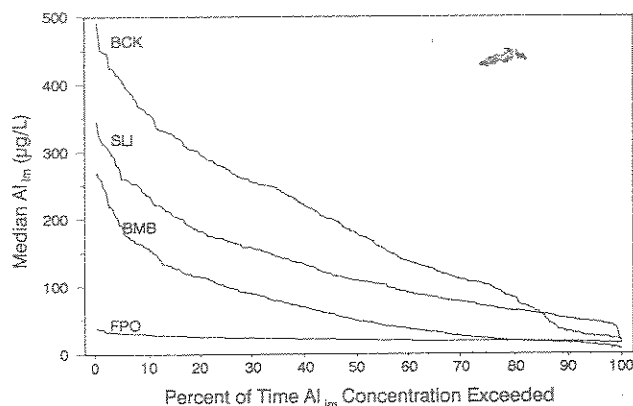


FIG. 2. Percent occurrence of daily median Al_{im} concentrations at the four study streams during the period November 1988 to June 1990. Daily median Al_{im} values were calculated from continuous records of minimonitor pH using stream specific predictive equations. BCK, Buck Creek; SLI, Seventh Lake Inlet; BMB, Bald Mountain Brook; FPO, Fly Pond Outlet.

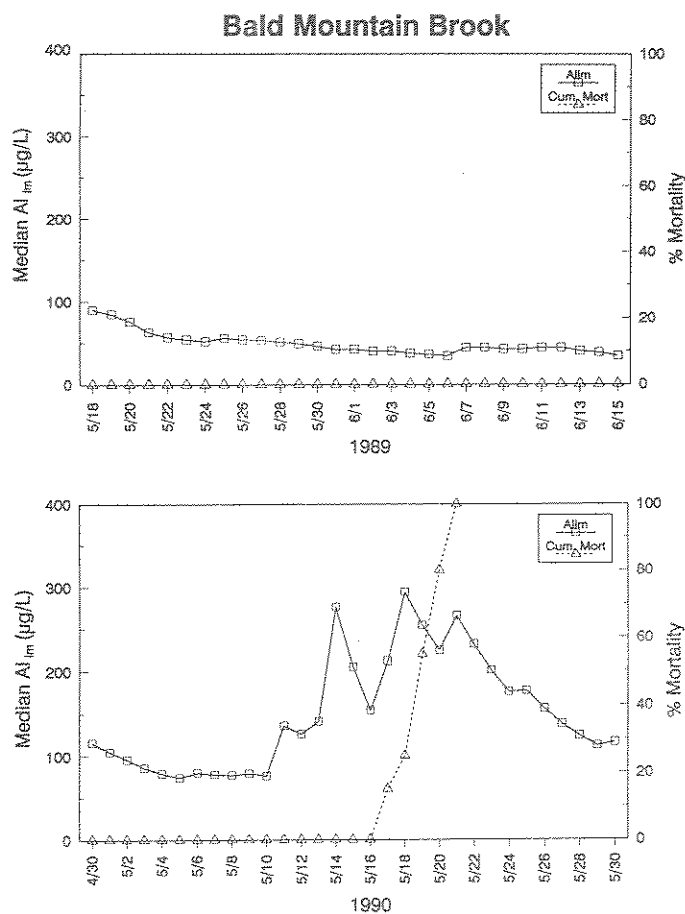


FIG. 3. Cumulative mortality of blacknose dace and calculated median daily Al_{im} during the spring 1989 and 1990 bioassays in Bald Mountain Brook.

The in situ bioassays consistently demonstrated that the blacknose dace were more sensitive than the brook trout to the episodes of acidic water. Cumulative mortality was greater and occurred sooner in blacknose dace than in brook trout (Fig. 5 and 6). In many cases, all of the blacknose dace died over a

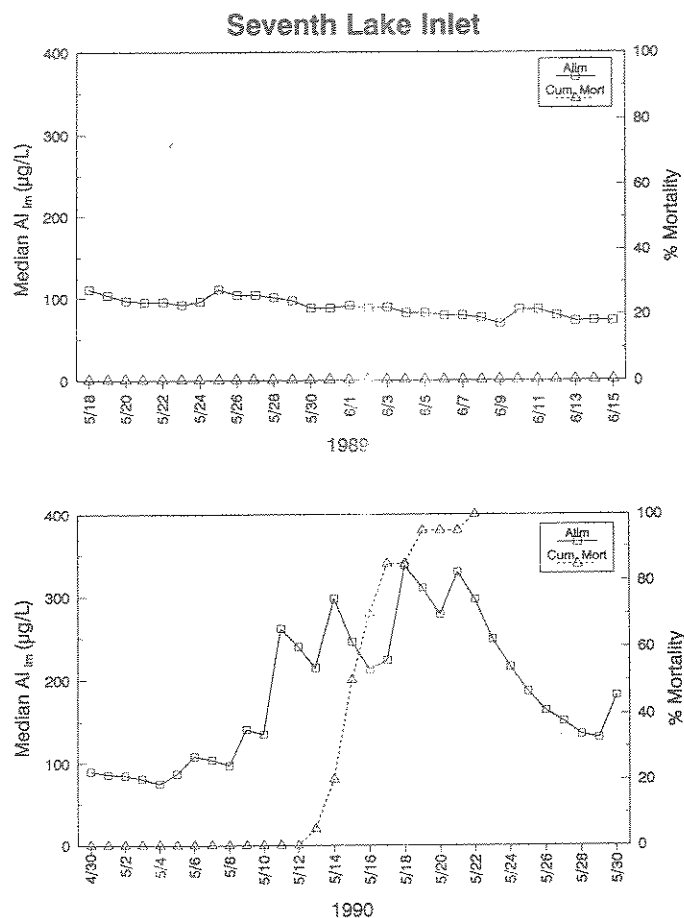


FIG. 4. Cumulative mortality of blacknose dace and calculated median daily Al_{im} during the spring 1989 and 1990 bioassays in Seventh Lake Inlet.

short length of time. Brook trout mortality occurred slower and over a longer time period.

Early unexplained mortality of brook trout in the reference stream, Fly Pond Outlet, during the spring 1989 and fall 1989 bioassays may have been caused by handling stress. These bioassays were restarted with new fish in all four study streams. Bioassays were generally considered to be acceptable until the mortality in Fly Pond Outlet exceeded 10%. Because of the variability in water quality among streams, the duration of bioassays varied. Buck Creek exhibited the most toxic water chemistry and therefore the shortest bioassays.

Seventh Lake Inlet was generally more acidic and had higher Al_{im} levels than Bald Mountain Brook both during baseflow and in response to episodes. The bioassay results (Fig. 5 and 6) showed that Bald Mountain Brook was consistently less toxic to blacknose dace than Seventh Lake Inlet. However, Bald Mountain Brook was more toxic to both brook trout and brook trout fry than Seventh Lake Inlet. Although a major episode occurred during the spring 1990 bioassay the brook trout fry in Seventh Lake Inlet suffered no mortality whereas the Bald Mountain Brook fish experienced 30% mortality. Seventh Lake Inlet had consistently higher DOC and higher Ca concentrations than Bald Mountain Brook, and this difference may explain the observed bioassay results.

Differences in water chemistry were observed among the four bioassay periods (Table 1), and combining the data allowed for full evaluation of the factors causing fish mortality. DOC, for

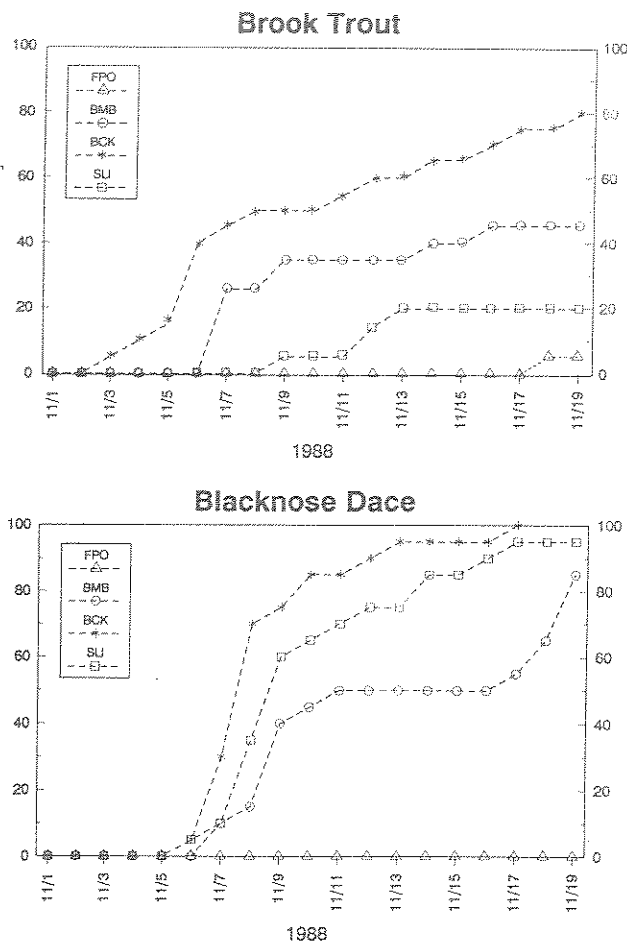


Fig. 5. Cumulative mortality of brook trout and blacknose dace observed during the fall 1988 bioassays. FPO, Fly Pond Outlet; BMB, Bald Mountain Brook; BCK, Buck Creek; SLI, Seventh Lake Inlet.

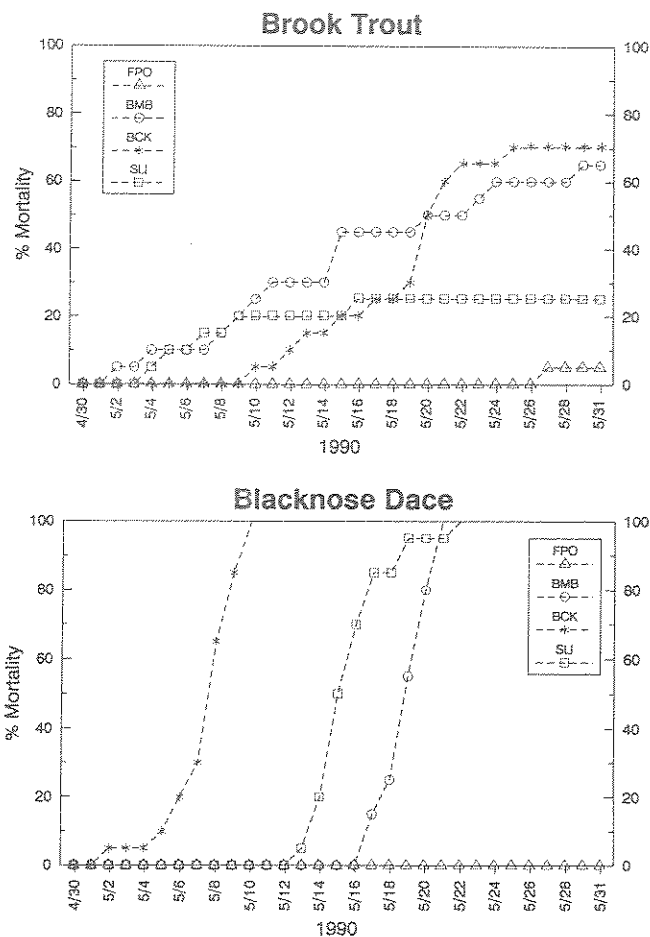


Fig. 6. Cumulative mortality of brook trout and blacknose dace observed during the spring 1990 bioassays. FPO, Fly Pond Outlet; BMB, Bald Mountain Brook; BCK, Buck Creek; SLI, Seventh Lake Inlet.

ample, appeared to be somewhat higher in Fly Pond Outlet and Seventh Lake Inlet during the fall bioassays than during spring bioassays. Two years of data, however, were inadequate to analyze seasonal variation.

During the fall 1989 bioassay, a major episode occurred after a change of baseflow conditions (Fig. 7). The episode resulted in significant mortality in both blacknose dace and brook trout in Bald Mountain Brook and Seventh Lake Inlet, even though high levels of Al_{im} were reached. Other bioassay tests with both species indicated that had this episode occurred at the beginning of the bioassay, significant mortality would have occurred. An early spring 1989 bioassay with blacknose dace (Fig. 7) began with Al_{im} concentrations greater than $100 \mu\text{g/L}$ and resulted in significant mortalities after 6 d. The fall 1989 episode occurred after fish may have acclimated to the stream or recovered from handling stress. The water quality conditions during this test were typical of those under which fish in a stream would survive. The data from these fall 1989 bioassays showed that blacknose dace in Bald Mountain Brook were able to survive a 10-d episode that resulted in a median Al_{im} concentration of $199 \mu\text{g/L}$ and median pH of 4.9. Brook trout in Buck Creek experienced only 10% mortality during a 10-d episode that resulted in a median Al_{im} concentration of $199 \mu\text{g/L}$, median pH of 4.6, and median DOC of 8.5 mg/L .

A stepwise regression analysis of key variables was conducted to determine which were the best predictors of mortality

in the bioassay fish. For blacknose dace the log of median Al_{im} was the best predictor of mortality ($R^2 = 0.75$). Converting the mortality data to probits did not result in better correlations. Figure 8 demonstrates that for blacknose dace, a median Al_{im} concentration of $100 \mu\text{g/L}$ or less over the entire bioassay period resulted in good survival. The median Al_{im} for the fall 1989 bioassay discussed above was $42 \mu\text{g/L}$ (Table 1).

The length of time blacknose dace were exposed to elevated Al_{im} concentrations was important and was demonstrated during several episodes. Mortality of blacknose dace during the spring 1990 bioassay did not occur until several days after the start of the episode (Fig. 3 and 4). If an episode occurred for over 6 d, high mortality occurred in the blacknose dace bioassays (Fig. 8). CONCDUR was also identified as a key variable in the stepwise analysis. The data indicate that median Al_{im} concentrations greater than $100 \mu\text{g/L}$ and episodic peaks of over 6 d resulted in mortality to blacknose dace. When the predicted Al_{im} concentrations for each stream (Fig. 2) are examined, it is evident why blacknose dace do not survive well in Buck Creek, Seventh Lake Inlet, and Bald Mountain Brook. The percentage of time the predicted Al_{im} was greater than $100 \mu\text{g/L}$ was approximately 76% for Buck Creek, 57% for Seventh Lake Inlet, and 25% for Bald Mountain Brook.

When a stepwise regression analysis of key variables was conducted on the brook trout bioassay data, CONCDUR was the best predictor of mortality and resulted in the best one-

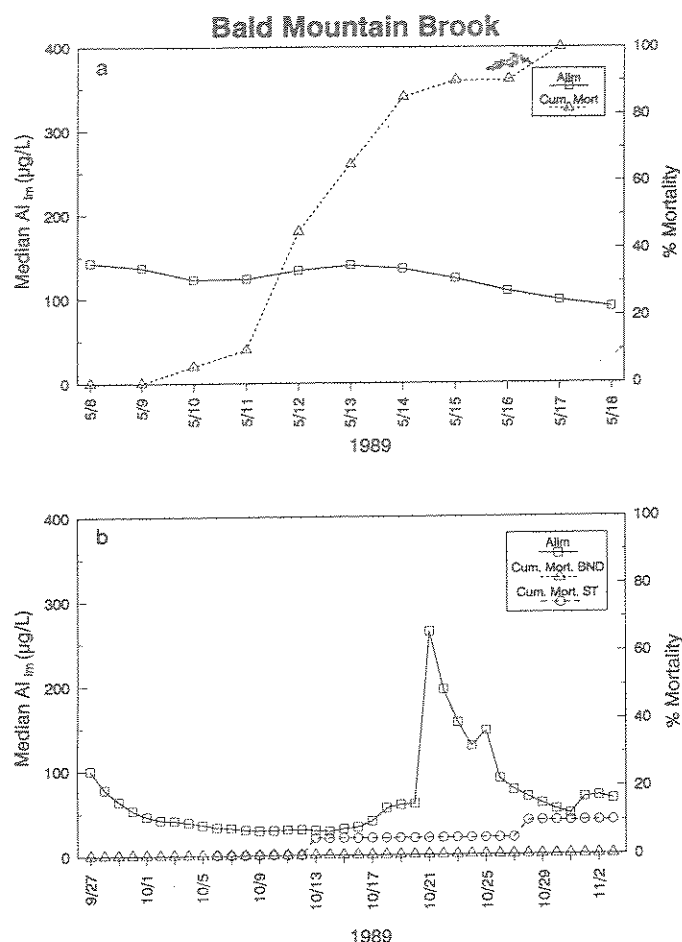


FIG. 7. Cumulative mortality and calculated median daily Al_{im} of (a) blacknose dace during the spring 1989 bioassays and (b) blacknose dace (BND) and brook trout (ST) during the fall 1989 bioassays in Bald Mountain Brook.

variable model ($R^2 = 0.44$). As with the blacknose dace, converting the mortality data to probits did not improve the R^2 term. The second most important variable of those tested proved to be DOC, and when both CONCDUR and DOC were used to predict mortality the R^2 term increased to 0.61. Figure 9 shows the predicted response surface if these two variables are used to predict brook trout mortality. Increases in DOC result in decreased predicted mortality to brook trout. The best two-variable predictive equation for brook trout was therefore as follows:

$$\text{Percent mortality} = 49.7 + 0.01(\text{CONCDUR}) - 6.46(\text{DOC}).$$

The brook trout bioassay data were more variable than the blacknose dace data with mortalities occurring over a greater range of water chemistry. Although cumulative mortality figures similar to Fig. 8 were developed for brook trout, they included a number of unexplainable outliers. However, it is evident that brook trout can withstand a slightly higher median Al_{im} concentration for a longer duration than blacknose dace.

The hatchery-reared brook trout fry were treated as a separate group of fish, and the data from these bioassays were also analyzed using the maximum R^2 procedure. The log of the median calcium concentration proved to be the best single variable, but only produced an R^2 term of 0.33. The best two-variable model

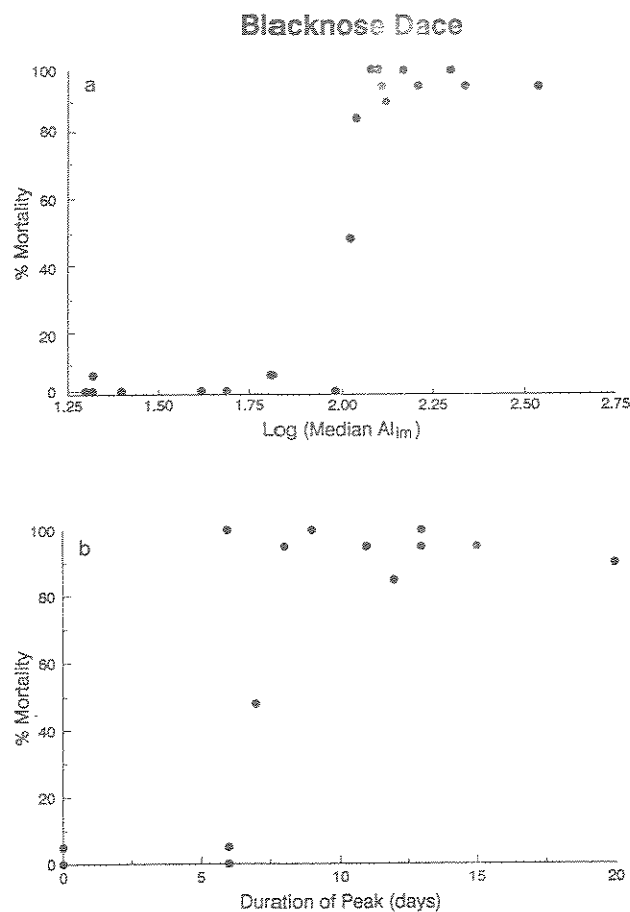


FIG. 8. Cumulative mortality of blacknose dace in relation to (a) the log of median Al_{im} during the bioassay and (b) the duration of the episode. Each data point represents a separate bioassay in one of the four streams.

of the brook trout fry data included CONCDUR and the log of median Al_{im} and resulted in an R^2 of 0.71. The brook trout fry data therefore appear to be consistent with the findings from the wild brook trout and the blacknose dace bioassays.

Discussion

The results of the bioassay tests from this study confirm previous findings that blacknose dace are more sensitive to acidic, high- Al_{im} waters than are brook trout. Johnson et al. (1987) presented similar bioassay results, and Kretser et al. (1989) presented survey data of 1469 Adirondack waters which showed that blacknose dace occurred at a minimum pH of 5.59 and brook trout at a minimum of 4.64. Schofield and Driscoll (1987) listed blacknose dace as a species sensitive to acidification and brook trout as indeterminate in its sensitivity.

Fish population studies were also a part of this project (Kretser et al. 1991). The results of those studies are in general agreement with the bioassay data and show Buck Creek to be the most toxic of the four study streams. Blacknose dace stocked in the three most acidic streams suffered poor survival, while greater numbers of brook trout were able to survive. In some cases, brook trout and a few blacknose dace in the stream were able to survive, while in situ bioassay fish died. This may be due to several factors including stress on caged fish resulting from handling and confinement. As discussed below, the bioas-

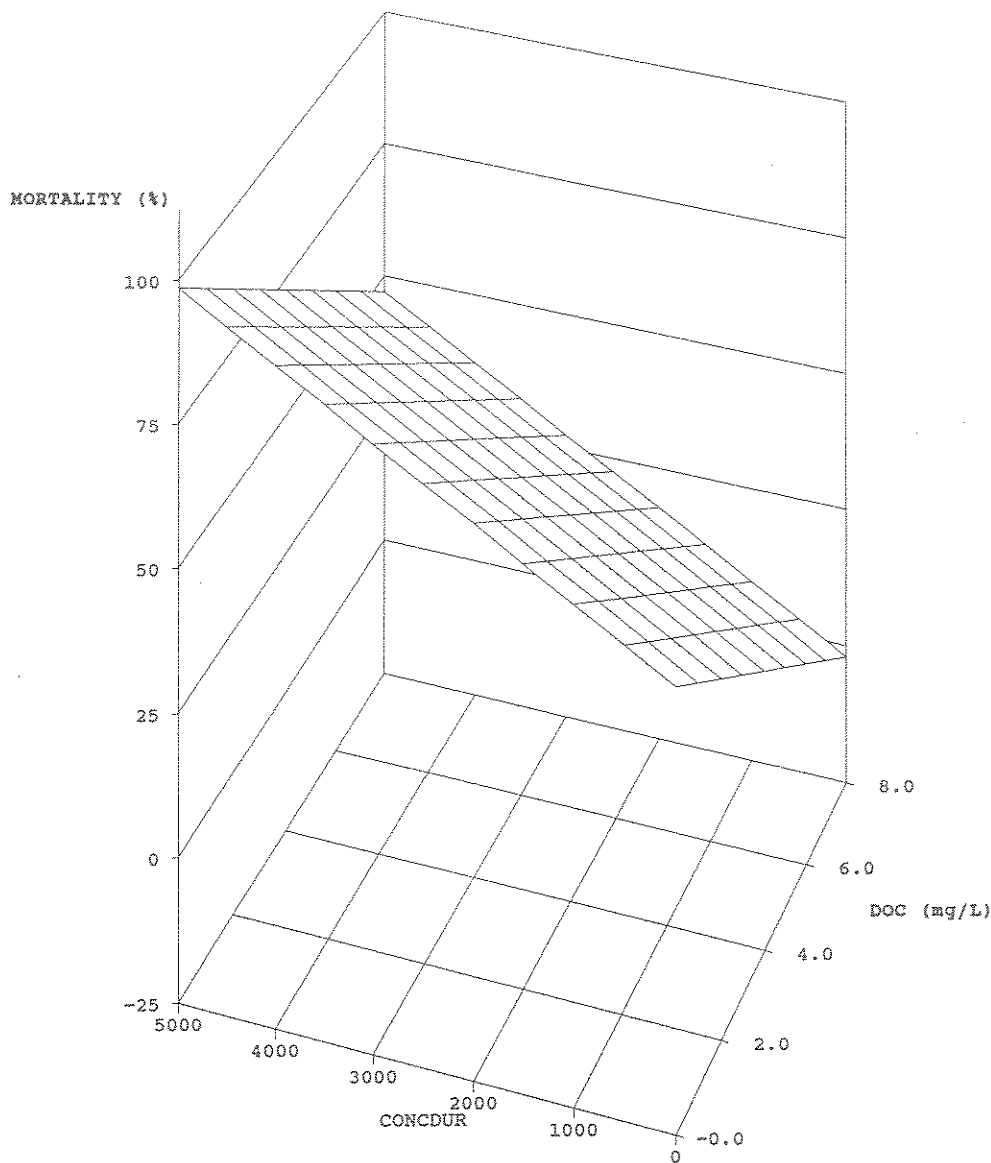


FIG. 9. Predicted mortality of brook trout in terms of DOC and CONCDUR. CONCDUR was defined as the median Al_{im} concentration during an episode (period of daily median $Al_{im} > 100 \mu g/L$) multiplied by the duration of the episode in days. The equation for the response surface is Percent mortality = $49.7 + 0.01(CONCDUR) - 6.46(DOC)$.

fish may not have been acclimated to baseflow conditions as long a period as the stream fish, resulting in higher mortality in the bioassay fish. There is also the possibility that wild fish may encounter small areas under banks with slightly better water quality.

The bioassay results showed that after the test fish were in the stream for 15–24 d, they could better withstand acidic episodes. They were able to survive several days of water quality fluctuations that would have caused fish mortality if they had been exposed early in the bioassay period. Although the bioassay fish may not have survived a major episode of long duration, they appeared to become at least partially acclimated to the poor water quality and/or to recover from the initial stress of being captured and caged. This acclimation was observed in both brook trout and blacknose dace. Mount et al. (1990) also observed similar acclimation to baseflow conditions in laboratory experiments with brook trout. Fish that they exposed for

18 d to baseline conditions (pH 5.21 and Al_{im} concentration of $21 \mu g/L$) survived a 6-d acidic episode (pH 4.59 and Al_{im} of $244 \mu g/L$), while fish without prior acclimation that had been reared at pH 6.5 suffered over 50% mortality when exposed to the same acidic, high-aluminum episode.

DOC was an important variable in the brook trout bioassays. Fish mortality was less in streams with higher DOC levels, even though Al_{im} levels measured were the same. The higher DOC and calcium levels in Seventh Lake Inlet may have allowed better brook trout survival there than in Bald Mountain Brook. This was the case in spite of the fact that Al_{im} concentrations appeared in most cases higher in Seventh Lake Inlet. The blacknose dace in these streams may either have been more sensitive to the Al_{im} concentrations than the brook trout or were not protected by the higher DOC and calcium levels.

The finding that in situ DOC concentrations were positively correlated with brook trout survival agrees with laboratory data.

TABLE 2. Number of in situ bioassays where observed mortality was correctly predicted (within $\pm 10\%$) by predictive models developed in other studies. Brook trout feeding fry bioassays were conducted during spring 1989 and spring 1990. Brook trout yearling and blacknose dace bioassays were conducted during spring and fall 1989 and spring 1990. The predictive models assume stable water chemistry conditions, and the field data used to test the models were 10-d periods of relatively stable conditions. The number of bioassays in which the model predicted more mortality than was observed is given in parentheses. NA = not applicable.

	Johnson et al. (1987)	Parkhurst (1987) 1	Parkhurst (1987) 2	Ingersoll et al. (1990)	Parkhurst et al. (1990)	Baker et al. (1990)
Brook trout feeding fry	2 of 8 (6)	0 of 8 (8)	2 of 8 (6)	5 of 8 (1)	3 of 5 (5)	5 of 8 (1)
Brook trout yearlings	7 of 12 (1)	NA	NA	NA	NA	NA
Blacknose dace adults	2 of 12 (10)	NA	NA	NA	NA	NA

Parkhurst et al. (1990) conducted laboratory bioassays that demonstrated that brook trout mortality was reduced at higher DOC concentrations. The predicted response surface based on our field studies (Fig. 9) is similar to the values they presented, showing the highest brook trout survival at low aluminum and high DOC concentrations. Parkhurst et al. (1990) also found that the best predictive equation of brook trout survival for their data included Al_{im} , DOC, and pH. In earlier field studies, Parkhurst (1987) had not observed a beneficial effect of higher DOC concentrations, possibly because in those studies, DOC was negatively correlated with pH.

It is interesting to note that DOC is generally thought to bind the toxic Al_{im} and convert it to Al_{om} . Therefore, measurements of Al_{im} should already include the benefit derived from DOC. The interpretation of the DOC and Al_{im} interactions may be complicated by the analytical method used for Al_{im} measurement. Recent research at the University of Wyoming has shown that using an ion exchange resin column (Driscoll 1984) to separate the organic and inorganic fractions of monomeric aluminum (as was done in our study) may lead to an overestimation of Al_{im} (Bergman 1992). Weak organic acids may be able to bind with Al_{im} and reduce its bioavailability, but may not be strong enough to remain bound to the Al_{im} in an ion exchange resin column. Consequently the measured Al_{im} value may include some of the Al_{om} . In higher DOC waters, such as Seventh Lake Inlet, much of what was measured as Al_{im} may in fact be Al_{om} and not be toxic to the fish. A better method of analyzing for the biologically available fraction of aluminum is needed.

Our bioassay and water chemistry monitoring results indicated that Al_{im} was the primary parameter that caused fish mortality during acidic episodes. Baker and Schofield (1982), Johnson et al. (1987), Parkhurst et al. (1990), and others also identified Al_{im} as a primary determinant in the survival of fish exposed to acid waters. These researchers also developed models to predict fish survival under various water quality conditions. These models, however, did not generally predict what we observed in our bioassays (Table 2). The observed mortality was correctly predicted in greater than 50% of the bioassays only when we used models developed by Ingersoll et al. (1990) or Baker et al. (1990) for brook trout feeding fry or the model developed by Johnson et al. (1987) for brook trout yearlings.

The reasons for the relatively poor agreement between the predicted mortality and our observed bioassay data may be attributed to a number of factors. These may include the use of

other methods to measure Al_{im} , variation in fish strain or age, variation in fish handling, the fact that test fish may not have been acclimated to low-conductivity water quality conditions, and/or the fact that the brook trout feeding fry used in the study described here were of hatchery origin. Each predictive model had a set of experimental conditions that may have been different from the bioassays we conducted. Although these models are useful in gaining a better understanding of how variations in water chemistry affect fish survival, they do not fully characterize the variables occurring in the stream environment. The occurrence of acidic episodes is the primary factor that is not taken into account in any of these predictive models.

The variable CONCDUR was developed to incorporate acidic episodes into predictive models. CONCDUR was found to be an important variable correlated with mortality of bioassay fish. Brook trout were able to survive episodes for a longer duration than blacknose dace, and both appeared to survive longer than expected based on research by Gagen and Sharpe (1987). They found that in Linn Run, Pennsylvania, brook trout exposed to over 200 $\mu\text{g Al/L}$ for durations greater than 1 d suffered mortality. In our streams, exposures of longer duration were necessary to cause brook trout mortality. DOC may not have been as important in Linn Run as in our study streams. As well, the unacclimated hatchery brook trout they used may have been more sensitive to the test conditions than were the wild brook trout used in this study.

The in situ bioassay tests reported here documented that our study streams were toxic to fish at certain times and that episodes of toxic water chemistry did occur. During these episodes, pH levels were frequently less than 5.0 and Al_{im} levels greater than 100 $\mu\text{g/L}$. The continuous water chemistry monitoring data showed that episodes often lasted for many days and in some cases several weeks (spring 1990 bioassays). Episodes of sufficient duration resulted in mortality in the bioassay fish.

Extended seasonal periods of poor water quality resulting in fish mortality may be more important in our study streams than short acidic episodes. These periods can result from a number of snowmelt or precipitation episodes occurring one after another before the stream returns to baseflow conditions. High flows and associated high Al_{im} levels for over 6 d resulted in blacknose dace mortality. Episodes of longer duration were necessary to cause significant brook trout mortality. Although duration of acidic episodes appears to be a critical characteristic, the term "chronically acidified" does not truly apply to

se streams. During periods of baseflow conditions, which in streams occur about half of the time, each stream provides acceptable water quality for fish survival. Critical conditions for fish survival occur when episodes of toxic water quality continue for many days. Fish are able to survive episodes of short duration.

It has been hypothesized that wild fish may move to refuge areas of better water quality during episodes whereas caged assay fish are confined to one location. Large refuge areas where fish can congregate to withstand acidic episodes did not appear to be an important characteristic of our Adirondack study streams. A small refuge site in Buck Creek was located, and Seventh Lake was a potential downstream refuge area for both Buck Creek and Seventh Lake Inlet. However, they did not play an important role in terms of fish survival during episodes (W. Bath, Adirondack Lakes Survey Corporation, Ray Brook, NY, unpublished data). The primary factor affecting fish populations in our study streams appeared to be the occurrence of episodes of poor water quality. Fish were not observed to actively seek refuge sites during episodes and then disperse throughout the stream following the episodes.

Carline et al. (1992) reported that in Pennsylvania, greater than 3000 km of trout streams have been adversely influenced by acidification. Water chemistry survey data for Adirondack headwater streams are limited, but studies by Colquhoun et al. (1983, 1984) showed that many streams are fishless, particularly those in the southwestern Adirondacks, and have spring pH levels less than 5.0. The periodic toxicity of certain Adirondack streams due to episodic acidification is likely the reason why they either are fishless or support limited fish populations. One month or less of acidic water quality could either reduce or eliminate fish populations. The impact may be evident for years in streams that have steep gradients or barriers that block fish from moving back upstream into otherwise suitable habitat. Steep gradients are common in Adirondack headwater streams and may explain the occurrence of some fishless waters.

As was noted by Johnson et al. (1987), we found that the number of waters that are acidic is greatest during spring snowmelt and major runoff events. Toxic water conditions that occur during such episodes cause fish mortality and reductions in stream fish populations. Future research on this subject needs to incorporate at least three key factors: a better method for measuring the biologically relevant Al_{im} , consideration of the fact that fish do become acclimated to low-conductivity water quality, and consideration of the fact that episodes of acidic water quality play a key role in determining the survival of stream fish populations.

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not been subject to the USEPA review process and therefore does not necessarily reflect the views of the agency, and no official endorsement should be inferred. Likewise, the ALSC makes no warranty with respect to the accuracy, completeness, or usefulness of the information contained, nor assumes any liability with respect to the use of any information, apparatus, method, or process disclosed in this paper.

References

- BAKER, J., AND C. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic water. *Water Air Soil Pollut.* 18: 289-309.
- BAKER, J.P., D.P. BERNARD, M.J. SALE, AND S.W. CHRISTENSEN. 1990. Biological effects of changes in surface water acid-base chemistry, NAPAP SOS/T Rep. 13. In *Acidic deposition: state of science and technology*, National Acid Precipitation Assessment Program. Vol. II. Washington, DC.
- BERGMAN, H.L. 1992. Development of biologically relevant methods for determination of bioavailable aluminum in surface waters. Final Technical Report for the U.S. Geological Survey, Reston, VA. 32 p.
- CARLINE, R.F., W.E. SHARPE, AND C.J. GAGEN. 1992. Changes in fish communities and trout management in response to acidification of streams in Pennsylvania. *Fisheries* 17: 33-38.
- COLQUHOUN, J.R., AND G.D. AYLESWORTH. 1983. Stream water quality related to discharge of six Adirondack streams in 1983. NYS Department of Environmental Conservation, Albany, NY. 4 p.
- COLQUHOUN, J.R., H.J. DEAN, J. SYMULA, J.C. SKEA, AND S.J. JACKLING. 1983. Non-target effects of the Adirondack black fly (*Simuliidae*) treatment program. NYS Dep. Environ. Conserv. Tech. Rep. 83-1.
- COLQUHOUN, J.R., W. KRETZER, AND M. PFEIFFER. 1984. Acidity status update of lakes and streams in New York State. NYS Dep. Environ. Conserv. Tech. Rep. 140 p.
- COLQUHOUN, J.R., J. SYMULA, AND R.W. KARCHER, JR. 1982. Report of Adirondack sampling for stream acidification studies — 1981 supplement. NYS Dep. Environ. Conserv. Tech. Rep. 82-3: 46 p.
- COLQUHOUN, J.R., J. SYMULA, M. PFEIFFER, AND J. FEURER. 1981. Preliminary report of stream sampling for acidification studies. 1980. NYS Dep. Environ. Conserv. Tech. Rep. 81-2: 116 p.
- DRISCOLL, C.T. 1984. A procedure for the fractionation of aqueous aluminum in dilute acidic waters. *Int. J. Environ. Anal. Chem.* 16: 267-284.
- DRISCOLL, D.T., AND R.M. NEWTON. 1985. Chemical characteristics of Adirondack lakes. *Environ. Sci. Technol.* 19: 1018-1024.
- FISHER, R.A., AND F. YATES. 1957. Statistical tables for biological, agricultural, and medical research. 5th ed. Hafner Publishing Company, Inc., New York, NY. 138 p.
- GAGEN, C.J., AND W.E. SHARPE. 1987. Influence of acid runoff episodes on survival and net sodium balance of brook trout (*Salvelinus fontinalis*) confined in a mountain stream. *Ann. Soc. R. Zool. Belg.* 117(Suppl. 1): 219-230.
- GALLOWAY, J.N., G.R. HENDREY, C.L. SCHOFIELD, N.E. PETERS, AND A.H. JOHANNES. 1987. Processes and causes of lake acidification during spring snowmelt in the west-central Adirondack Mountains, New York. *Can. J. Fish. Aquat. Sci.* 44: 1595-1602.
- GUNN, J.M. 1986. Behaviour and ecology of salmonid fishes exposed to episodic pH depressions. *Environ. Biol. Fishes* 17: 241-252.
- HESTHAGEN, T. 1989. Episodic fish kills in an acidified salmon river in southwestern Norway. *Fisheries* 14(3): 10-17.
- INGERSOLL, C.G., D.R. MOUNT, D.D. GULLEY, T.W. LAPOINT, AND H.L. BERGMAN. 1990. Effects of pH, aluminum, and calcium on survival and growth of eggs and fry of brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* 47: 1580-1592.
- JOHNSON, D.W., H.A. SIMONIN, J.R. COLQUHOUN, AND F.M. FLACK. 1987. In situ toxicity tests of fishes in acid waters. *Biogeochemistry* 3: 181-208.
- KRETZER, W., J. GALLAGHER, AND J. NICOLETTE. 1989. Adirondack lakes study 1984-1987: an evaluation of fish communities and water chemistry. Adirondack Lakes Survey Corporation, Ray Brook, NY. 397 p.
- KRETZER, W.A., H.A. SIMONIN, D.W. BATH, B.P. BALDIGO, D. DiTOMMASO, J. GALLAGHER, AND M. OLSON. 1991. Episodic acidification and associated fish and benthic invertebrate responses of four Adirondack headwater streams: an interim report of the Episodic Response Project. EPA/600/3-91/036. U.S. Environmental Protection Agency, Corvallis, OR.
- MARMOREK, D.R., K.W. THORNTON, J.P. BAKER, D.P. BERNARD, AND B. REUBER. 1986. Acidic episodes in surface waters: the state of science. Final report for the U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR. 232 p.
- MOUNT, D.R., M.J. SWANSON, J.E. BRECK, A.M. FARAG, AND H.L. BERGMAN. 1990. Responses of brook trout (*Salvelinus fontinalis*) fry to fluctuating

1990. Responses of brook trout (*Salvelinus fontinalis*) fry to fluctuating acid, aluminum, and low calcium exposure. *Can. J. Fish. Aquat. Sci.* 47: 1623-1630.
- PARKHURST, B.R. 1987. A comparison of laboratory and in situ bioassays for evaluating the toxicity of acidic waters to brook trout. Ph.D. dissertation, University of Wyoming, Laramie, WY. 131 p.
- PARKHURST, B.R., H.L. BERGMAN, J. FERNANDEZ, D.D. GULLEY, J.R. HOCKETT, AND D.A. SANCHEZ. 1990. Inorganic monomeric aluminum and pH as predictors of acid water toxicity to brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* 47: 1631-1640.
- SCHOFIELD, C.L., AND C.T. DRISCOLL. 1987. Fish species distribution in relation to water quality gradients in the north branch of the Moose River basin. *Biogeochemistry* 3: 63-85.
- SHARPE, W.E., W.G. KIMMEL, E.S. YOUNG, JR., AND D.R. DEWALLE. 1983. In-situ bioassays of fish mortality in two Pennsylvania streams acidified by atmospheric deposition. *Northeast. Environ. Sci.* 2(3/4): 171-178.
- SHARPE, W.E., V.G. LEIBFRIED, W.G. KIMMEL, AND D.R. DEWALLE. 1987. The relationship of water quality and fish occurrence to soils and geology in an area of high hydrogen and sulfate ion deposition. *Water Resour. Bull.* 23: 37-46.
- WIGINGTON, P.J., R.D. DAVIES, M. TRANTER, AND K.N. ESHLEMAN. 1990. Episodic acidification of surface waters due to acidic deposition. NAPAP SOS/T Rep. 12. *In* Acidic deposition: state of science and technology. National Acid Precipitation Assessment Program. Vol. II. Washington, DC.