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Relationships between stream acidity and bacteria, macroinvertebrates, and fish: a comparison of north temperate and south temperate mountain streams, USA

Patrick J. Mulholland,[1] Charles T. Driscoll,[2] Jerry W. Elwood,[1] Marcy P. Osgood,[3] Anthony V. Palumbo,[1] Amy D. Rosemond,[4] Michael E. Smith,[5] & Carl Schofield[6]
[1] *Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6036, USA;* [2] *Department of Civil Engineering, Syracuse University, Syracuse, NY 13244-1190, USA;* [3] *Department of Biology, Clarkson University, Potsdam, NY 13699, USA;* [4] *Department of General Biology, Vanderbilt University, Nashville, TN 37235, USA;* [5] *Department of Biology, Valdosta State College, Valdosta, GA 31698, USA;* [6] *Department of Natural Resources, Cornell University, Ithaca, NY 14850, USA*

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Abstract

A comparative study of relationships between stream acidity and bacteria, macroinvertebrates, and fish in the Adirondack Mountains of upper New York state and in the Southern Blue Ridge Mountains of eastern Tennessee, USA, was conducted. Although the study sites in both regions spanned a pH range from approximately 4.5 to 6.4, considerably greater seasonal variability in pH and higher monomeric Al concentrations characterized the Adirondack sites. Relationships between several biological characteristics and stream water acidity were similar in both regions, including lower production of epilithic bacteria and bacteria on decomposing leaves, lower leaf decomposition rates, lower density and generic richness of scraper/grazer macroinvertebrates, particularly Ephemeroptera, and lower fish abundance and survival in more acidic streams. Densities of total macroinvertebrates and densities of macroinvertebrates and bacteria inhabiting or closely associated with stream sediments were generally not related to stream water acidity.

Regional differences occur in some of the relationships between biological characteristics and stream water acidity. Negative correlations between bacterial production on rocks and pH, between bacterial production on decomposing leaves and pH, and between densities of Ephemeroptera and scrapers and pH were stronger in the Adirondacks than in the Southern Blue Ridge. Higher Al concentrations in the Adirondacks may be responsible for the stronger relationships with pH there. The steeper slopes of the relationships between Ephemeroptera density and all forms of Al in the Adirondacks compared with the Southern Blue Ridge suggests that there may be some adaptation among a few acid/aluminum-tolerant species in the seasonally more constant acidic Southern Blue Ridge streams. Fish bioassays indicated longer survival times in acidic streams in the Adirondacks compared with the Southern Blue Ridge, but these results may be an artifact associated with the use in the Southern Blue Ridge of rainbow trout as the test species which is known to be more acid sensitive compared with brook trout, the test species used in the Adirondacks.

Introduction

A substantial amount of evidence has accumulated on the relationships between stream water acidity and stream organisms and processes (see reviews by Burton *et al.*, 1982; Elwood & Mulholland, 1989). Fish are particularly sensitive to acidification, with few streams with pH < 5.5 having reproducing fish populations (Townsend *et al.*, 1983; Sadler & Turnpenney, 1986; Haines, 1986). Numerous studies of effects of acidic stream water on macroinvertebrates have shown that species richness and diversity, functional feeding group diversity, and in some cases, the density and biomass of invertebrates are positively related to stream water pH, particularly at pH < 6.0 (Sutcliffe & Carrick, 1973; Ziemann, 1975; Townsend *et al.*, 1983; Otto & Svensson, 1983; Stoner *et al.*, 1984; Burton *et al.*, 1985; Kimmel *et al.*, 1985; Mackay & Kersey, 1985; Smith *et al.*, 1990; Rosemond *et al.*, in press). These studies have indicated that several species of macroinvertebrates, particularly those belonging to the Ephemeroptera and to the grazer/scraper functional feeding group, are particularly acid-sensitive.

Low pH alone may not account for all of the negative effects of acidic stream water on fish and macroinvertebrates. Fish mortality at low pH has been shown to be primarily the result of high concentrations of Al (Zischke *et al.*, 1983; Gagen & Sharpe, 1987; Ormerod *et al.*, 1987). Experimental studies have demonstrated additive negative effects of low pH and high Al concentrations on stream macroinvertebrates (Burton & Allan, 1986; Hall *et al.*, 1987; Ormerod *et al.*, 1987). In one study Al additions had no effect on macroinvertebrates beyond that of H^+ alone, but this may have been the result of complexation of the Al by moderately high concentrations of dissolved organic carbon (5.5 to 6 mg/l) in stream water (Allard & Moreau, 1987).

Considerably less is known about the relationship between stream microbial communities and stream water acidity. Productivity of epilithic bacteria and bacteria associated with decomposing leaf material has been shown to be positively correlated with stream water pH (Palumbo *et al.* 1987a, b, 1988; Osgood & Boylen, 1990). Rates of leaf decomposition are also reduced at pH < 6, probably because of reduced microbial activity rather than reduced consumption by macroinvertebrate shredders (Minshall & Minshall, 1978; Otto & Svensson, 1883; Kimmel *et al.*, 1985; Mackay & Kersey, 1985; Chamier, 1987; Mulholland *et al.*, 1987; Osgood, 1987). Bacterial biomass and productivity in fine sediments, however, are unrelated to the pH of stream water (Palumbo *et al.*, 1987a; Osgood & Boylen, 1990).

Important questions still remain concerning the effect of acidity on stream organisms and processes. The effect of stream episodes (seasonal snowmelt, storm events) on stream communities is unclear as are the relative effects of H and Al concentrations. Although in most streams, pH and Al covary, the predominant form of Al can differ due to speciation and complexation with other solutes, particularly dissolved organic carbon (DOC). The toxicity of Al has been shown to vary with its chemical form and chelation of Al by DOC reduces Al toxicity for fish and macroinvertebrates (Baker & Schofield, 1982; Burton & Allan, 1986; Omerod *et al.*, 1987; McCahon & Pascoe, 1989; Peterson *et al.*, 1989).

The purpose of this paper is to compare the relationships between stream biological communities and stream water acidity in two regions of the United States, the Adirondack Mountains of New York and the Southern Blue Ridge of eastern Tennessee. Streams of these regions differ in the importance of seasonal variations in acidity (acidification episodes due to seasonal snowmelt) and the concentration and chemical form of Al. We have conducted studies in several streams encompassing a similar range of stream water pH in both regions. Results of the studies on bacterial abundance and metabolic activity (Palumbo *et al.*, 1987a, b, 1988; Osgood & Boylen, 1990), leaf decomposition (Mulholland *et al.*, 1987; Osgood & Boylen, in press), and macroinvertebrate abundance (Smith *et al.*, 1990; Rosemond *et al.*, in press) have been published separately for each region. This paper compares the results from both regions to determine if there are differences in the communities of bacteria, macroinvertebrates, and fish between regions that can be attributed to differences in temporal variability in acidity or differences in Al concentrations.

Study sites

Several first- to third-order, high elevation streams in the western Adirondack Mountains, New York, USA, and in the Southern Blue Ridge Province of eastern Tennessee, USA (Fig. 1) were selected on the basis of having low acid neutralizing capacity (ANC $< 100\ \mu eq\ l^{-1}$), and a range of pH and Al concentrations. In the Adirondack Mountains, Pancake-Hall Creek (PA) generally had lowest pH and ANC, Moss Lake Inlet (MO) had intermediate pH, and Beaver Brook (BE) had the highest pH and ANC, and lowest Al concentrations. Catchment areas for PA, MO, and BE were 74, 220, and 770 ha, respectively. Two or three sampling sites were selected over longitudinal gradients in acidity on each stream.

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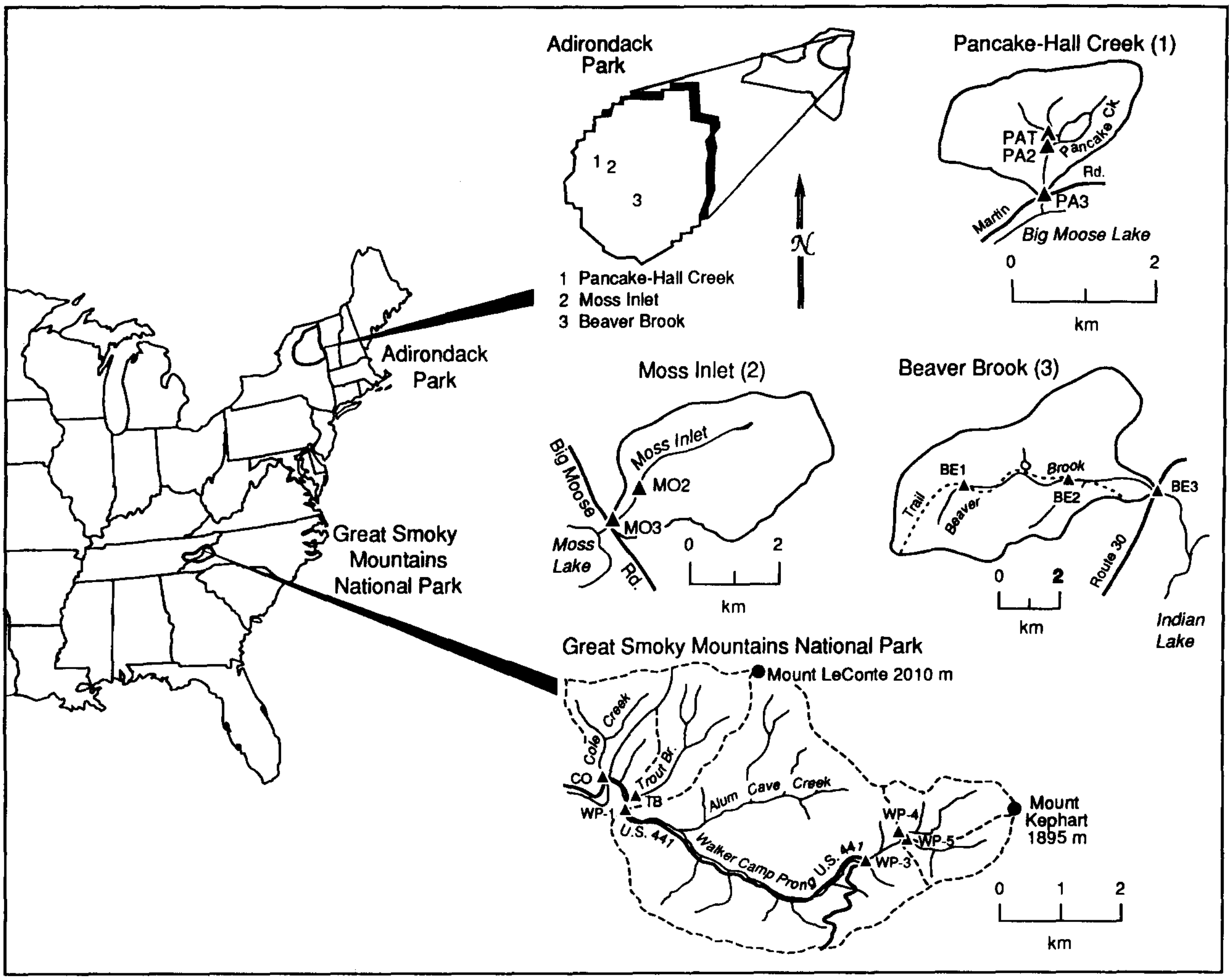

Fig. 1. Location of Adirondack Mountains and Southern Blue Ridge study sites located in the Great Smoky Mountains National Park. The Southern Blue Ridge sites in the Cherokee National Forest (not shown) are located approximately 60 km to the southwest of the Great Smoky Mountains National Park sites.

In the Southern Blue Ridge, several sampling sites were selected along Walker Camp Prong (WP) and two of its tributaries, Trout Branch (TB) and Cole Creek (CO), all in the Great Smoky Mountains National Park (GSMNP). One of the upper WP sites (WP4), a middle WP site (WP3), and TB were the most acidic sites and CO was the least acidic site. Two streams in the Cherokee National Forest, Sugar Cove (SC) and Hemlock Creek (HL) were also used in some of the bacterial studies to provide a greater range of acidity. The Cherokee National Forest streams (not shown in Fig. 1) are located approximately 60 km southwest of the GSMNP streams and are in the same physiographic province. The upper station on Hemlock Creek (HL2) is highly acidic (pH 4.6–4.9), whereas the Sugar Cove stations are both circumneutral (pH 6.2–6.7). Catchment areas ranged from 120 ha at WP5 to 380 ha at WP3.

Both regions consist of both coniferous and deciduous forests. In the Adirondacks, catchments for all three study streams contain primarily second-growth deciduous stands of sugar maple, beech, and yellow birch with small, interspersed coniferous stands (red spruce, balsam fir, hemlock). Beaver activity and/or bog conditions are present in portions of each catchment. Annual precipitation is about 160 cm. In the Southern Blue Ridge, GSMNP catchments are dominated by mature stands of red spruce and Frazer fir at high elevation, with beech, yellow birch, and hemlock becoming important in the lower elevations and along streams. Rhododendron is abundant, forming a dense subcanopy throughout most catchments. Cherokee National Forest catchments are dominated by second-growth stands of beech, yellow birch, sugar maple, red maple, and oak, with hemlock becoming important in coves and along the stream. Annual pre-

Table 1. Chemical characteristics of the study sites. Values are means of monthly samples collected during the period January 1985 to January 1986 in the Adirondacks and January 1984 to January 1986 in the GSMNP. All values are mg l^{-1} except ANC (μeq /l).

	pH mean (range)	ANC	Ca	Mg	SO_4	NO_3	Total monomeric Al	DOC	F
Adirondacks sites:									
PAT	4.9 (4.5–5.8)	−6	1.6	0.3	7.3	0.50	0.52	3.6	0.06
PA2	5.3 (4.7–5.9)	25	2.0	0.4	5.6	0.28	0.27	6.6	0.07
PA3	5.4 (4.7–6.4)	18	1.6	0.3	5.9	0.40	0.27	5.9	0.07
MO2	5.4 (4.9–6.1)	11	2.2	0.4	6.9	0.38	0.20	4.4	0.08
MO3	5.8 (5.1–6.6)	39	2.5	0.5	6.8	0.45	0.12	3.2	0.11
BE1	5.8 (5.0–6.6)	32	2.2	0.3	6.4	0.39	0.11	2.0	0.05
BE2	6.2 (5.4–6.9)	47	2.3	0.4	6.0	0.44	0.06	2.3	0.04
BE3	6.2 (5.4–7.0)	57	2.5	0.5	6.2	0.40	0.04	2.1	0.05
Southern Appalachian Sites:									
GSMNP Sites:									
WP4	4.5 (4.5–4.6)	−31	1.2	0.5	4.0	4.0	0.24	2.1	0.01
WP3	4.8 (4.7–4.9)	−17	1.4	0.6	3.6	4.1	0.14	1.3	0.02
TB	5.0 (4.9–5.1)	−15	1.2	0.7	4.2	3.4	0.13	0.7	0.01
WP5	5.7 (5.6–5.8)	0	1.7	0.6	2.8	4.6	0.03	0.5	0.01
CO	6.4 (6.0–6.6)	20	1.5	0.6	2.0	3.8	0.02	1.1	0.01
Cherokee National Forest Sites:									
HL2	4.8 4.6–4.9)	−9	4.3	2.0	23.5	0.3	0.32	0.9	0.07
HL1	5.8 (5.1–6.1)	10	2.9	1.3	12.8	0.1	0.09	0.7	0.02
SC2	6.5 6.2–6.7)	44	1.0	0.2	1.6	0.4	<0.01	0.8	0.01
SC1	6.6 (6.5–6.7)	50	1.1	0.2	1.6	0.5	<0.01	0.9	0.00

cipitation for the Southern Blue Ridge is about 220 cm.

Water chemistry for all study streams is provided and discussed in detail elsewhere (Mulholland *et al.*, 1986; Smith *et al.*, 1990) and is only briefly summarized here (Table 1). All sites are low in ionic strength and ANC. Mean annual pH for individual sites ranged from 4.9 to 6.2 in the Adirondacks and 4.5 to 6.4 in the Southern Blue Ridge.

The primary differences in water chemistry between the two regions were greater seasonal variability in pH (as indicated by the pH ranges for individual sites), higher concentrations of F, dissolved organic carbon (DOC), and monomeric Al, and lower concentrations of NO_3 at the Adirondack sites compared with the Southern Blue Ridge sites, particularly those in the GSMNP (Table 1). The large seasonal changes in pH at the Adirondack sites are largely the result of considerably lower pH values during the spring snowmelt period than during other times of the year. Seasonal variation in pH at the Southern Blue Ridge sites is small, although short-term (1–2 d) depressions in pH and increases in Al during large storms have been observed for several GSMNP streams with pH values normally >6 (Herrman & Baron, 1980). The low pH values of some of the GSMNP streams and Hemlock Creek are thought to be primarily the result of the presence within their catchments of a pyritic phyllite (Anakeesta formation), which is exposed to oxidation at outcrops and by natural landslides (Huckabee *et al.*, 1975). Despite the known geologic source of SO_4 in the Southern Blue Ridge catchments, SO_4 concentrations in the study streams in the Southern Blue Ridge (with the exception of Hemlock Creek) were generally less than those in the Adirondack study streams. However, SO_4 concentrations in our study streams in the Southern Blue Ridge were somewhat higher than most other streams in this region probably because of higher rates of atmospheric deposition, lower retention within the catchment, and leaching of geologic SO_4 (Elwood *et al.*, 1991). The high NO_3 concentrations in the GSMNP streams are probably the result of relatively high atmospheric deposition and low retention of N by the mature forests of these high elevation catchments .

Methods

Bacteria

To determine the abundance and activity of bacteria the following measurements were made: bacterial numbers in stream water; bacterial numbers, ATP levels, and bacterial production in fine sediments; bacterial production on rock surfaces; and rates of mass loss and bacterial production on decomposing leaves.

Bacteria numbers in the water column (water AODC) were measured on two or three replicate 20 ml samples preserved with 1 ml of filtered (2 μm pore size) 37% formaldehyde. Bacteria were stained with acridine orange, filtered (black Nuclepore filters, 0.2 μm pore size) and bacteria counted at 1000 $\times$ magnification (Hobbie *et al.*, 1977).

Fine sediments were collected from surficial deposits (top 5 cm) at each site. Sediment subsamples of 1 or 2 cm^3 were preserved for bacteria enumeration (sediment AODC) by adding 10 ml of filtered 37% formaldehyde in the field. In the laboratory (within 2 weeks) preserved subsamples were diluted with sterile-filtered distilled water and mixed in a Waring blender at high speed for 3 min. Subsamples (100 μl) of the diluted samples were stained, filtered, and bacteria counted as described above. ATP in 1 or 2 cm^3 subsamples of fine sediments was extracted in 50–70 ml of either DMSO or phosphoric acid (1 M). Subsamples of the extract were diluted 10 $\times$ –40 $\times$ with Tris buffer and analyzed for ATP using Lumac reagents and a Lumac integrating photometer with internal standardization. Bacterial production in fine sediments was determined by measuring the incorporation of tritiated thymidine into DNA. Tritiated thymidine (0.064 nmol, specific activity = 1.716×10^{14} Bq $mmol^{-1}$) was added to 1 or 2 cm^3 of sediment diluted to a volume of 5–10 ml. The sediment slurries were incubated in plastic tubes for 0.5–2 h in the field at ambient stream temperatures. At the end of the incubation, thymidine uptake was halted by adding 0.5–1 ml of 37% formaldehyde. Sediments were then centrifuged, the supernatant discarded, and the tritium-labelled DNA was extracted according to the methods of Findlay *et al.* (1984).

To determine bacterial production on rock surfaces, 3–5 rocks were collected and placed in plastic jars with 50–100 ml of stream water and tritiated thymidine (0.064 nmol, specific activity = 1.716×10^{14} Bq mmol^{-1}). Rocks were incubated for 0.5 h in the field at ambient stream temperatures. After terminating the incubations with the addition of 5–10 ml of 37% formaldehyde, rocks were transferred to separate jars and the tritium-labelled DNA extracted and measured.

Mass loss rates of decomposing leaves were measured by placing pre-weighed 5 g leaf packs (in 4 mm mesh nylon bags) at selected stream stations and measuring dry mass remaining over time. Fifty leaf packs were placed in pools at each station and 5 bags were retrieved at 1, 2, 4, 7, 10, and 15 (or 16) weeks. The Adirondack study was begun on September 21, 1985 and the Southern Blue Ridge study on August 27, 1985. Leaves were dried at 80 °C for determinations of dry mass and combusted at 500 °C for 16 h to compute ash free dry mass (AFDM). Mass loss rates (k) were determined using an exponential decay model for leaf dry mass (Adirondack sites) or leaf AFDM (GSMNP sites) over time (Petersen & Cummins, 1974). Bacterial production on decomposing leaves was measured in the laboratory within 1 d of field collections (leaves were kept on ice until measurements were made). Five leaf discs (1 cm diameter) cut from five different leaves from each bag were incubated in plastic tubes with 5 ml of stream water at ambient stream water temperatures. Tritiated thymidine (0.16 nmol, specific activity = 2.886×10^{12} Bq mmol^{-1}) was added to each tube and the incubation was stopped after 20 min by addition of 0.5 ml of 37% formaldehyde. The liquid in each tube was discarded, the leaf discs were rinsed three times with distilled water, and tritium-labelled DNA was extracted from the leaves following the procedure of Findlay *et al.* (1984) and Palumbo *et al.* (1987a).

Macroinvertebrates

Benthic macroinvertebrates were collected quarterly for one year (Adirondacks: January to October 1985; Southern Blue Ridge: April 1985 to February 1986) using a modified Hess sampler (0.1 m^2; 250 μm mesh size) in riffle areas of comparable substrate type. Five replicate samples were collected at each site and immediately preserved in 95% ethanol. Invertebrates were removed by hand in the laboratory, identified (usually to genus) and counted. Chironomids were sorted and counted but were not identified as to specific taxa and therefore were not included in the computations of total taxa richness. Organisms, except chironomids, were assigned to appropriate functional feeding groups using Merritt and Cummins (1984) or by analysis of gut contents. Benthic organic matter (BOM; > 250 μm in size) was measured by loss on ignition (450 °C for 24 h) after invertebrates were removed and the samples dried (60 °C).

Fish

Surveys of fish populations were conducted by repeated (3 passes) electrofishing of stream reaches (50 to 30 m depending on fish density) that had been isolated by seines. Total length, weight, sex, and scale samples were obtained from all fish collected before release.

In situ bioassays, utilizing 0+ age class trout fingerlings (Adirondacks: brook trout, *Salvelinus fontinalis*, Temiscamie strain; Southern Blue Ridge: rainbow trout, *Oncorhynchus mykiss*, Arlee strain), were conducted at several sites in each region to determine the acute toxicity of stream water to trout. The bioassays were conducted in April 1985 and October 1985 in the Adirondacks and in February 1986 in the Southern Blue Ridge. Fish were placed in plastic minnow cages (10 to

15 fish per cage) at control sites with circumneutral pH (Adirondacks: Eagle Creek, adjacent to Moss Lake Inlet drainage but which does not experience marked increases in acidity during high-flow periods; Southern Blue Ridge: Cole Creek) and sites with lower pH (Adirondacks: MO3 and PA3; Southern Blue Ridge: TB and WP3). The cages were placed in pools at each site, and fish mortality checked several times over periods of 7 d (Southern Blue Ridge sites) or 15 d (Adirondack sites). Water samples for pH and chemical analysis were collected several times during the bioassays at each site. Survivorship comparisons between sites and periods were made by the Mantel-Haenzel test (Mantel & Haenzel, 1959).

Water chemistry

Water samples for chemical analysis were collected from each site monthly. Stream water was collected from mid-water column in acid washed polyethylene bottles. Water temperature was measured in the field. Measurements of pH and extractions of monomeric Al were conducted either in the field (Southern Blue Ridge sites) or in the laboratory on refrigerated samples within 12 h of collection. Two fractions of Al, total monomeric Al and organic Al, were measured by atomic absorption spectrophotometry (AAS) in accordance with the fractionation procedures of Driscoll (1984). Other forms of monomeric Al (aquo, and hydroxy, flouride, and sulfate complexes) were estimated from application of an equilibrium water chemistry model (ALCHEMI, Schecher and Driscoll 1987) to the measured chemistry data at each site. Major cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) were determined on filtered samples (0.2 μm Nuclepore filters) by AAS, and major anions (SO_4^{2-}, NO_3^-, Cl^-) were measured by ion chromatography. Fluoride was measured potentiometrically with an ion specific electrode. Dissolved organic carbon was measured by persulfate oxidation and infrared absorption (OI Model 700 Carbon Analyzer) on samples filtered (0.4 μm Nuclepore filters) in the field, preserved with the addition of 0.1 ml of 6 N H_2SO_4, and placed on ice until analysis.

Statistical analysis

All statistical analyses were conducted using SAS (SAS Institute, 1985). Regression and correlation analyses between either macroinvertebrate or bacteria characteristics and chemistry variables were performed using a linear model (least squares) with untransformed (e.g., number of species, metabolic activity) or log-transformed (x + 1) (e.g., densities, due to nonhomogeneity of variances) data. Tests of equality of regression slopes were performed to determine differences in the biological characteristic-chemistry relationships between regions (Zar, 1984). Relationships are reported as significant if $P < 0.05$, and marginal if $0.05 < P < 0.15$.

Results

Aluminum chemistry

Concentrations of all forms of Al were higher with lower pH in both regions; however, at a given pH, Al concentrations were higher at the Adirondack sites than at Southern Blue Ridge sites (Fig. 2). The higher total monomeric Al concentrations in the Adirondacks were partially the result of higher DOC- and F-complexed Al because of higher DOC and F concentrations. Concentrations of the aquo (Al^{+3}) plus hydroxy [$AlOH^{+2}$, $Al(OH)_2^+$] forms were similar between regions, with the exception of slightly higher values at pH < 5 in the Adirondacks compared with the Southern Blue Ridge (Fig. 2c).

Bacteria

Very few bacteria-related characteristics were significantly correlated with stream water pH in both regions (Table 2). Only epilithic thymidine uptake was consistently related to stream water pH, al-

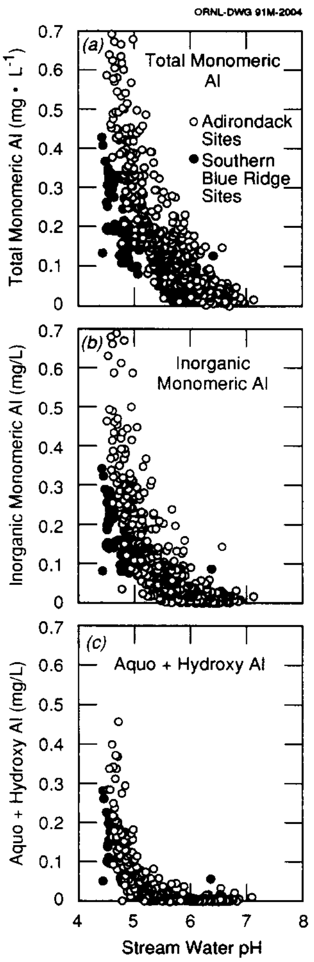

Fig. 2. Relationship between (a) monomeric Al concentration and pH, and (b) aquo + hydroxy Al concentration and pH for the Adirondack and Southern Blue Ridge sites.

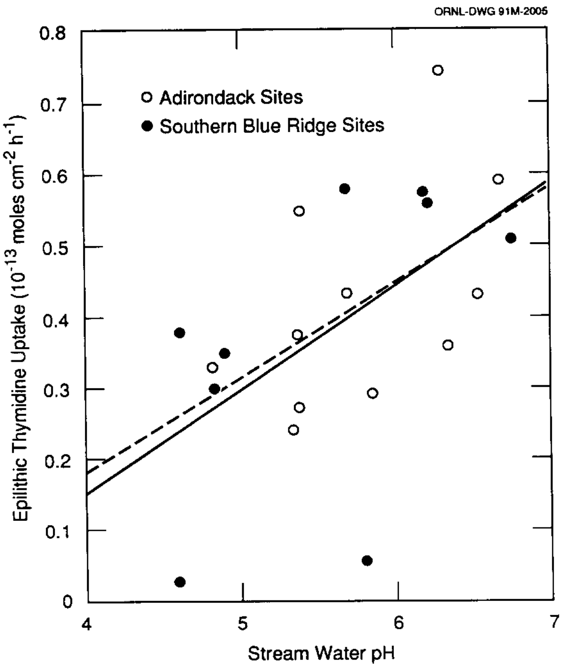

Fig. 3. Relationship between thymidine uptake by epilithic bacterial communities and stream water pH for Adirondack sites (open circles, dashed line) and Southern Blue Ridge sites (closed circles, solid line). The regression lines for the Adirondack sites ($F = 3.396$, $P = 0.098$, $df = 1{,}9$) and the Southern Blue Ridge sites ($F = 3.096$, $P = 0.122$, $df = 1{,}7$) are only marginally significant. The slopes of the regression lines are not significantly different ($F = 0.01$, $P = 0.9112$, $df = 1{,}16$).

though the correlations were only marginally significant in two of the four periods in which it was measured. Considering only the October data, the regressions between epilithic thymidine uptake and stream water pH are not significantly different between regions (Fig. 3). Some of the variability in epilithic thymidine uptake within each region in October may have been due to variability in water temperature among sites (7–13 °C at Adirondack sites, 9–16 °C at Southern Blue Ridge sites). Correlations between epilithic thymidine uptake and temperature, however, were not significant. Correlations between epilithic thymidine uptake and total monomeric, inorganic monomeric, or aquo + hydroxy Al were marginally significant for some periods, but were always lower than the correlation with stream water pH.

In contrast to the lack of relationships between sediment bacterial characteristics and stream water pH, sediment bacterial characteristics were consistently correlated with sediment organic content (Table 2). The correlations between the bacterial biomass-related characteristics (AODC, ATP) and sediment organic content were particularly strong. Bacterial production in sediments, as measured by thymidine uptake, was not as consistently correlated with sediment organic content as the biomass-related characteristics.

Mass loss rates of decomposing leaves were significantly correlated with stream water pH at the Southern Blue Ridge sites, but although the relationship was also positive at the Adirondack

Table 2. Significant correlations (r) between bacterial characteristics and stream water pH and sediment organic matter content for Adirondack and southern Blue Ridge sites. Data were analyzed by month in order to minimize temperature differences. NM = not measured.

Characteristic	Adirondacks				Southern Blue Ridge		
	Jan	Apr – May	Jul	Oct	Apr	Jul	Oct
Correlations with stream water pH:							
Water AODC	–	–	– 0.419*	–	–	–	NM
Sediment AODC	–	–	–	–	–	–	NM
Sediment ATP	–	–	–	–	0.975*	–	NM
Sediment Thymidine Uptake	– 0.824⁺	–	–	–	NM	–	NM
Epilithic Thymidine Uptake	0.808**	–	NM	0.523⁺	NM	NM	0.554
Correlations with Sediment organic matter content:							
Water AODC	–	–	–	–	NM	–	NM
Sediment AODC	0.869⁺ ⁺	0.996***	0.758***	0.979***	NM	0.928**	NM
Sediment ATP	0.998***	0.999***	0.726***	0.859***	NM	–	NM
Sediment Thymidine Uptake	–	–	–	0.739**	NM	0.970**	NM
Epilithic ATP	–	0.668*	–	–	NM	–	NM
Epilithic Thymidine Uptake	–	–	NM	–	NM	NM	–

⁺ $p < 0.10$
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

sites, it was not significant due to high variability resulting from one very high rate (Fig. 4a). The relationships between mass loss and pH were not significantly different between regions. Maximum thymidine uptake rate by bacteria on decomposing leaves was also positively related to stream water pH in both regions (Fig. 4b). The linear regression between maximum thymidine uptake rate and stream water pH was significant for the Adirondack sites, but only marginal for the Southern Blue Ridge sites. The slope of the relationship between thymidine uptake and stream water pH was significantly greater for the Adirondack sites than for the Southern Blue Ridge sites.

Macroinvertebrates

The macroinvertebrate communities at the Adirondack and Southern Blue Ridge sites were dominated by representatives of the orders Ephemeroptera, Plecoptera, Trichoptera, and Diptera. Multiple regression analyses revealed that stream water pH and benthic organic matter (BOM) were the two most important predictors of macroinvertebrate densities and taxa richness in both regions (Table 3). Stream water pH and BOM were uncorrelated in both regions ($r = 0.042$, $P = 0.818$ for the Adirondack sites; $r = 0.004$, $P = 0.987$ for the Southern Blue Ridge sites), indicating that correlations with each of these characteristics were independent.

For the Adirondack sites, macroinvertebrate densities were more often correlated with BOM than with stream water pH, whereas number of taxa was more highly correlated with stream water pH than BOM (Table 3). Most of the macroinvertebrate characteristics that were positively correlated with stream water pH were also negatively correlated with total monomeric, inorganic monomeric, and aquo + hydroxy Al, but the correlations with Al were weaker (lower r) than correlations with stream water pH. Logarithmic transformations of Al concentrations did not im-

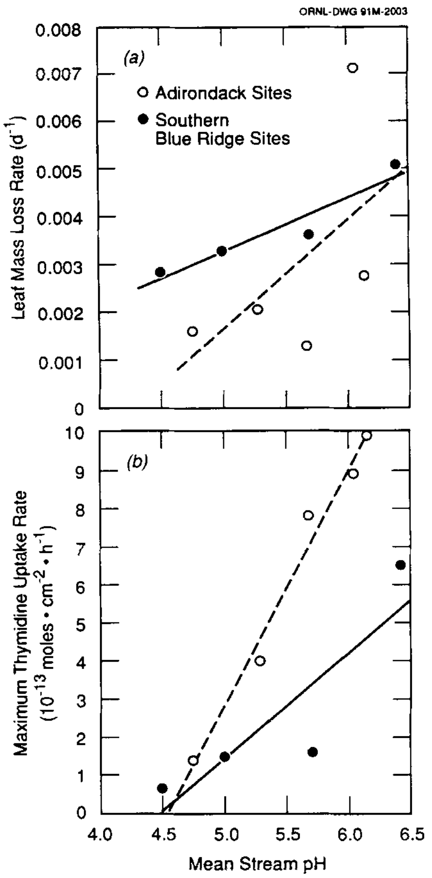

Fig. 4. Relationship between (a) mass loss rate of decomposing leaves, and (b) maximum uptake rate of thymidine by bacteria on decomposing leaves and stream water pH. The mass loss rate regressions are significant for the Southern Blue Ridge sites ($F = 19.72$, $P = 0.0471$, $df = 1,2$), but not for the Adirondack sites ($F = 1.303$, $P = 0.3365$, $df = 1,3$); slopes of the mass loss rate regressions are not significantly different between regions ($F = 0.34$, $P = 0.586$, $df = 1,5$). The thymidine uptake regressions in (b) are significant for the Adirondack sites ($F = 136.7$, $P = 0.0013$, $df = 1,3$), but only marginal for the Southern Blue Ridge sites ($F = 6.634$, $P = 0.123$, $df = 1,2$). The slope of the Adirondack relationship was significantly greater than that for the Southern Blue Ridge ($F = 7.62$, $P = 0.040$, $df = 1,5$).

prove the correlations above those for untransformed Al. All forms of Al were highly negatively correlated with stream water pH ($r = -0.808$, -0.750, and -0.640, for total monomeric, inorganic monomeric, and aquo + hydroxy respectively, $P < 0.001$).

At the Southern Blue Ridge sites, positive correlations between macroinvertebrate characteristics and stream water pH were more numerous than at the Adirondack sites (Table 3). Several of the macroinvertebrate characteristics at the Southern Blue Ridge sites were also correlated with BOM, but most of these correlations were weaker than they were at the Adirondack sites (Table 3). Stream water pH and BOM were also uncorrelated at the Southern Blue Ridge sites ($r = 0.004$, $P = 0.987$). The densities of Plecoptera and Trichoptera were more highly correlated (negatively) with concentrations of each of the three forms of Al than they were with pH. As expected, negative correlations between these forms of Al and stream water pH were highly significant ($r = -0.808$, -0.829, and -0.811, $P < 0.001$).

Relationships between density (log-transformed) and taxa richness of some macroinvertebrate groups and stream water pH varied between regions (Fig. 5). Although the total density/pH relationships were significant only for the Southern Blue Ridge sites (Table 3), there was no significant difference in the slopes between regions ($F = 0.43$, $P = 0.516$, $df = 1,44$) (Fig. 5a). The relationships between Ephemeroptera and scraper density and stream water pH were significantly steeper (greater slopes) for the Adirondack sites compared with the Southern Blue Ridge sites ($F = 8.02$, $P = 0.007$, $df = 1,44$; $F = 9.50$, $P = 0.004$, $df = 1,44$, for the Ephemeroptera and scraper comparisons, respectively) (Fig. 5b,c). In contrast, the relationships between total and Ephemeroptera taxa richness and stream water pH were steeper for the Southern Blue Ridge sites than for the Adirondack sites ($F = 4.60$, $P = 0.0375$, $df = 1,44$; $F = 11.48$, $P = 0.0015$, $df = 1,44$, for the total and Ephemeroptera richness relationships, respectively) (Fig. 5d,e). The relationship between Trichoptera richness and stream water pH was also steeper for the Southern Blue Ridge sites than the Adirondack sites, but the difference was only marginally significant ($F = 2.38$, $P = 0.130$, $df = 1,44$) (Fig. 5f).

Because the relationships with stream water

Table 3. Macroinvertebrate characteristics significantly correlated with pH. Values presented are correlation coefficients (r).

Characteristic	Adirondack sites		Southern Blue Ridge sites	
	pH	BOM	pH	BOM
Density by order:				
Total	–	0.761***	0.527*	–
Ephemeroptera	0.703***	–	0.669**	– 0.502*
Thrichoptera	–	–	0.512*[1]	0.490^{+}
Plecoptera	–	0.624***	0.456^{+}[1]	0.582*
Diptera	–	0.705***	–	–
Density by feeding group:				
Scrapers	0.751***	–	0.783***	–
Shredders	–	0.617***	0.498*[1]	0.574*
Collectors–gatherers	–	0.673***	–	0.497^{+}
Predators	–	0.752***	–[2]	–
Number of taxa:				
Total	0.486**	0.338^{+}	0.847***	–
Ephemeroptera	0.750***	–	0.907***	–
Trichoptera	0.487**	–	0.729**	0.457^{+}
Plecoptera	–	–	–	–

$^{+}$ $p<0.10$
* $p<0.05$
** $p<0.01$
*** $p<0.001$
[1] Characteristic is more highly correlated with one or more forms of Al than with pH.
[2] Significant correlation with total monomeric, inorganic monomeric, and aquo + hydroxy Al ($r = -0.659$, -0.618, -0.627, respectively, $p <= 0.01$).

pH were strongest for the Ephemeroptera in both regions and because these relationships were significantly different between regions, we compared the relationships between Al concentrations and Ephemeroptera density and richness between regions. There was considerable scatter in Ephemeroptera densities and richness at low concentrations of Al in both regions (Fig. 6). At higher Al concentrations (total monomeric Al > 0.10 mg l^{-1}, inorganic monomeric Al > 0.08 mg l^{-1}, aquo + hydroxy Al > 0.03 mg l^{-1}) there were significant negative relationships between all forms of Al and Ephemeroptera density and Ephemeroptera richness for the Adirondack sites, but not for the Southern Blue Ridge sites. Also, the relationships with Al concentration were steepest (slopes most negative) for aquo + hydroxy Al, and least steep for total monomeric Al for the Adirondack sites.

Fish

Salmonids dominated the fish communities in both regions. In the Adirondacks, brook trout, creek chub, mud minnow, and yellow perch were present at the MO sites, but only brook trout were present at the BE sites. Brook trout collected in BE ranged in age from 0 + to 3 + years; however, only 0 + and 1 + age fish were collected from MO sites. Fish were absent at the PA sites. In the Southern Blue Ridge only brook trout were found at most sites. These species were present at sites with pH > 5.7 (WP5, CO, SC1, SC2) and absent

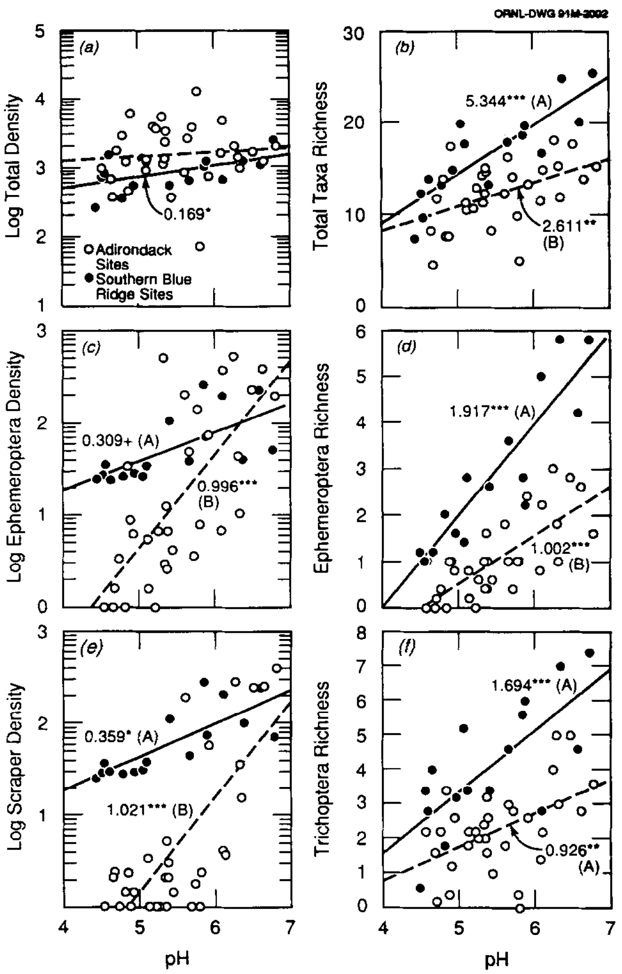

Fig. 5. Relationships between stream water pH and (a) logarithm of total macroinvertebrate density, (b) logarithm of Ephemeroptera density, (c) logarithm of scraper density, (d) total taxa richness, (e) Ephemeroptera richness, and (f) Trichoptera richness at Adirondack sites (open circles) and Southern Blue Ridge sites (closed circles). Slopes of the least squares regression lines are given if significant ($+P<0.10$, $*P<0.05$, $**P<0.01$, $***P<0.05$). Slopes with different letters in parentheses are significantly different between regions ($P<0.05$). Data are means of 5 replicate samples collected from each site each quarter.

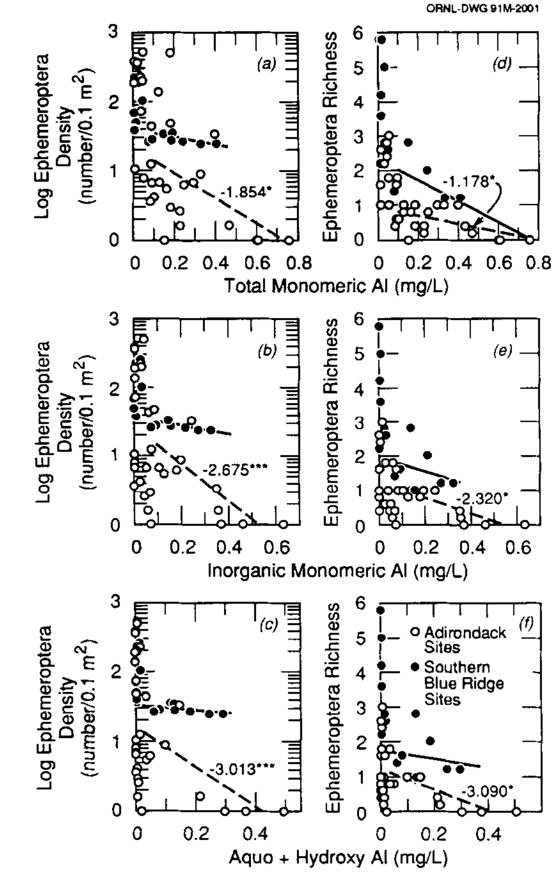

Fig. 6. Relationships between the concentrations of three different forms of Al in stream water and Ephemeroptera density and richness. Regressions were fit to the data for total monomeric Al > 0.10, inorganic monomeric Al > 0.08 mg l^{-1}, and aquo + hydroxy Al > 0.03 mg l^{-1}. Slopes of the regression lines are given if significant ($*P<0.05$, $**P<0.01$, $***P<0.001$). Data are means of 5 replicate samples collected from each site each quarter.

at sites with pH < 5.0 (TB, WP3, WP4, HL2). Rainbow trout were also found at WP1. At HL1 we found only 1 fish and this may have been a transient from a larger, higher pH stream whose confluence with HL is within 100 m of the sampling site. Although the mean pH of HL1 is 5.8, this site experiences seasonal pH depressions to 5.1 during higher flow periods in winter and spring. Age of fish collected in the Southern Blue Ridge could not be determined because scales were either absent from fish or scale resorption has been so extensive that annuli could not be identified and enumerated.

Results of *in situ* bioassays indicated significant mortality at sites with pH < 5.3. Studies conducted in the Adirondacks with brook trout indicated little mortality in Eagle Creek, a reference stream with pH 5.3–6.2, but almost complete mortality in MO3 and PA3, where the pH ranged from 4.6 to 5.4 during the 15 d study period

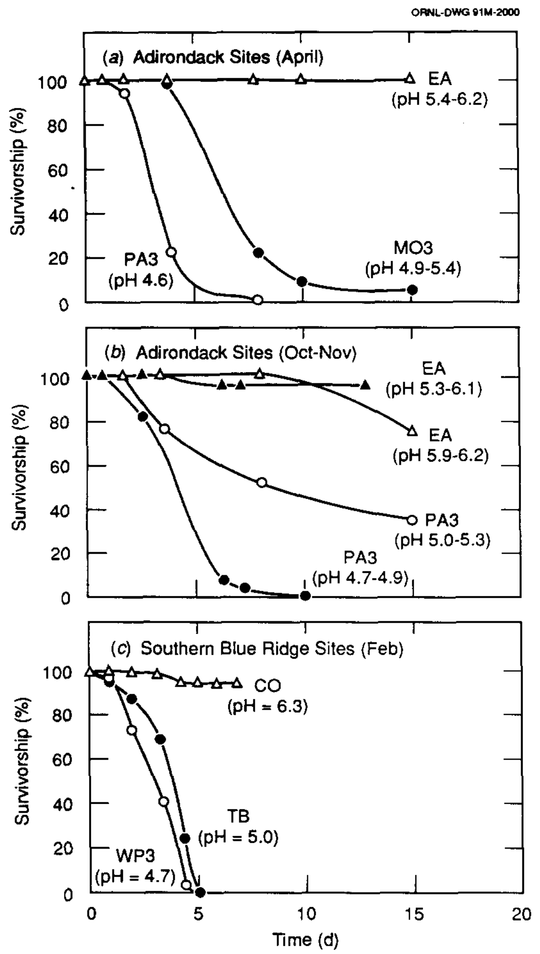

Fig. 7. Survivorship curves for *in situ* bioassays using brook trout at Adirondack sites in (a) April and (b) October–November, and using rainbow trout at sites in the Southern Blue Ridge in (c) February. The range in pH at each site during the bioassay is given in parentheses.

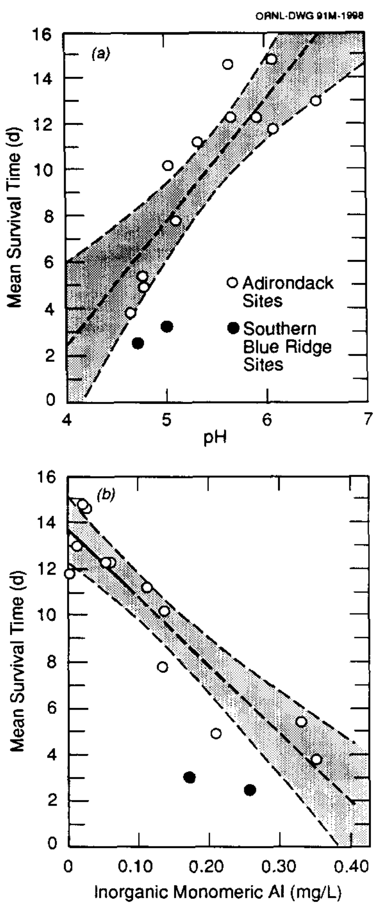

Fig. 8. Relationship between mean survival time and (a) mean stream water pH ($F = 27.64$, $P = 0.004$, $df = 1,10$) and (b) mean inorganic monomeric Al concentration ($F = 57.76$, $P = 0.0001$, $df = 1,10$) for the Adirondack sites (open circles, dashed line). The shaded area represents the 95% confidence interval for the regression lines. Two data points for the Southern Blue Ridge sites (closed circles) are also plotted.

(Fig. 7a,b). Mortality curves were steeper at lower pH in both studies. Bioassay studies at the Southern Blue Ridge sites using rainbow trout showed very rapid mortality at pH < 5.0 but little mortality at CO with a pH of 6.3 (Fig. 7c).

Mean survival times (time to 50% mortality) computed from bioassay survivorship curves were strongly related to mean pH ($r = 0.8567$) and mean inorganic monomeric Al concentration ($r = -0.923$) for the Adirondack sites (Fig. 8a,b). Mean survival times of rainbow trout at the Southern Blue Ridge sites plotted well outside the 95% confidence intervals for survival time/pH and survival time/Al relationships for brook trout at the Adirondack sites, indicating shorter survival time of rainbow trout for a given pH and Al concentration in the Southern Blue Ridge, a result consistent with the known greater sensitivity of this species to acidification (Grande *et al.*, 1978). Although it would appear that slopes of survival time vs pH or Al were less steep for the Southern Blue Ridge assays, we have only two data points and hesitate to make inferences from a line between them.

Discussion

Regional similarities in relationships with stream water acidity

The results suggested negative relationships between stream water acidity and some bacterial, macroinvertebrate, and fish characteristics. Most characteristics positively correlated with stream water pH were associated with organisms that tend to live in the water or on the surface of stream bottom substrata (the so-called epifauna and epiflora). These included production of epilithic bacteria and bacteria on decomposing leaves, leaf decomposition rates, the density and taxa richness of grazing and scraping macroinvertebrates (primarily Ephemeroptera), and fish survival. Characteristics associated with organisms that live in or closely associated with fine-grained sediments (the so-called infauna and inflora) generally were not related to stream water pH. These characteristics tended to be correlated with benthic organic matter, and included bacterial biomass and production in fine sediments, and density and richness of shredder and collector-gatherer macroinvertebrates. It has been suggested that the lack of a relationship with water acidity for infauna and inflora is the result of buffering of pH in sediments (Collins *et al.*, 1981; Palumbo *et al.*, 1987a; Elwood & Mulholland, 1989). Sharp increases in pH within the upper few centimeters of sediments of acidic lakes as a result of alkalinity generation within the sediments have been documented (Kelly *et al.*, 1984; Cook *et al.*, 1986). Although we are unaware of documented pH gradients in stream sediments, O_2 gradients in stream sediments have been reported (e.g., Grimm & Fisher, 1984), indicating that pore water chemistry in streams can be very different from that of the flowing water and alkalinity-generating anaerobic processes (e.g., sulfate reduction, denitrification) may be important in stream sediments.

Among the bacterial characteristics, the relationship with stream water pH was stronger for measures of metabolic activity than for measures of abundance in both regions. Previous reports have documented the lack of a relationship between pH and bacterial numbers in the water column and on decomposing leaves, despite significant relationships between pH and bacterial production (thymidine uptake) of epilithic and decomposing leaf communities at these sites (Palumbo *et al.*, 1987a, b, 1988; Osgood & Boylen, 1990). Further, leaf decomposition was slower at acidic sites in both regions, primarily as a result of lower microbial decomposition (Mulholland *et al.*, 1987; Osgood & Boylen, in press).

Other similarities in the relationships between biota and acidity in the Adirondack and Southern Blue Ridge sites were the macroinvertebrate community composition at acidic sites as defined by functional feeding groups, the strong positive relationships between pH and the density and taxa richness of the Ephemeroptera, and the general lack of a relationship between pH and total macroinvertebrate density. In both regions, scrapers made up a lower proportion of the macroinvertebrate communities at acidic sites, and both the density and taxa richness of Ephemeroptera were low (Smith *et al.*, 1990; Rosemond *et al.*, in press). Although total taxa richness of the macroinvertebrate community was positively related to stream water pH, total density of macroinvertebrates was not strongly related to stream water pH at both sites. Also, the densities of Plecoptera, and of shredders and collector-gatherers were more highly correlated with BOM than with pH or Al at both sites, perhaps indicating generally greater acid tolerance among these groups.

Regional differences in relationships with stream water acidity

Of most interest in this study were the differences in the relationships between stream biota and stream water acidity between the regions. Although the study sites in each region spanned approximately the same range in mean annual pH, considerably greater seasonal variability in pH and higher concentrations of total and inorganic monomeric Al characterized the Adirondack sites (Table 1, Fig. 2). These regional differences allow us to evaluate (1) effects on biological communities of seasonal variation in acid/base chemistry and (2) effects of Al compared with H^+ alone. By comparing the relationships between various biological characteristics and stream water pH between regions we can address the relative importance of these effects. For example, if the effects of seasonal acidic episodes on populations or biological processes are long-lasting (much longer than the episode itself), then we would expect to see a weakening of the relationship (lower or non-significant slope) between measured biological characteristics and stream water pH for the Adirondack sites compared with the Southern Blue Ridge sites. The lower slopes of relationships between biological characteristics and pH in the Adirondacks might be the result of low values of these characteristics at normally high pH sites due to pulses of acidity during the spring snowmelt season at these sites. Alternatively, if the effects of Al concentration are important, particularly total or inorganic monomeric forms, then we would expect to see a somewhat steeper relationship between biological characteristics and pH in the Adirondacks compared with the Southern Blue Ridge because of the higher Al concentrations in the Adirondacks. Because the impact of each of these effects on biological characteristic-pH relationships between regions would be opposite, we can only determine the net effect. Similar biological characteristic-pH relationships between regions could be a result of either (1) little effect of seasonal variability and Al concentrations or (2) effects that approximately cancel each other.

The relationships between thymidine uptake rate (epilithic and associated with decomposing leaves) and pH and between leaf mass loss rate and pH were either stronger (higher r, e.g., epilithic thymidine uptake) or steeper (greater slope, e.g., thymidine uptake associated with decomposing leaves) for the Adirondack sites compared with the Southern Blue Ridge sites. This is consistent with the hypothesized importance of monomeric Al in inhibiting bacterial production in acidic streams. These results are also consistent with past results indicating greater accumulations of Al on leaves in highly acidic streams and the maintenance of high levels of Al and low rates of bacterial production and respiration for relatively long periods of time following transplants of decomposing leaves from acidic to circumneutral sites (Mulholland *et al.*, 1987; Palumbo *et al.*, 1987a).

Among the macroinvertebrate characteristics measured, the relationships with pH between regions were mixed. The regressions between densities of Ephemeroptera and scrapers and pH were significantly steeper for the Adirondack sites than for the Southern Blue Ridge sites (Fig. 5b,c), but the regressions between total taxa richness and pH and between Ephemeroptera richness and pH were steeper for the Southern Blue Ridge sites (Fig. 5d,e). This would suggest that monomeric Al may be important in limiting Ephemeroptera abundance in acidic streams (because Al is higher at the acidic Adirondack sites compared to the Southern Blue Ridge sites for a given pH), but less so in controlling the number of taxa in these streams. Also, the relationships between Ephemeroptera density and all forms of Al were considerably steeper for the Adirondack sites compared with the Southern Blue Ridge sites (Fig. 6a,b,c), suggesting that the negative effect of Al is greater in the Adirondacks. This could be a result of adaptation of acid-tolerant species in the Southern Blue Ridge to the seasonally-constant high Al concentrations. Also the acidic sites in the Southern Blue Ridge are known to have been acidic for at least 50 years, a longer period than the acidic Adirondacks sites, thus increasing the potential for long-term adaptation to acidic environments.

Our results are generally consistent with other studies that have demonstrated additive negative effects of low pH and high Al concentrations on stream macroinvertebrates (Burton & Allan, 1986; Ormerod *et al.*, 1987). Further, the slopes of the regression lines relating Ephemeroptera density and Al concentrations for the Adirondack sites were lowest for total monomeric Al and greatest for aquo + hydroxy Al, suggesting that it is the latter form that is most toxic to invertebrates.

The relationships between mean survival times of fish and both pH and inorganic monomeric Al in the Adirondacks were strong. Mean survival times at acidic sites in the Southern Blue Ridge were substantially shorter than would be expected from the Adirondack relationships (Fig. 8). However, these regional differences may reflect different acid sensitivities of the fish used in the bioassays. Brook trout, which were used in the Adirondacks bioassays, are known to be more acid tolerant than rainbow trout which were used in the Southern Blue Ridge bioassays (Grande *et al.*, 1978).

In conclusion, there are considerable similarities in the relationships between bacteria, macroinvertebrates, and fish and stream water acidity in mountain streams in the Adirondack and Southern Blue Ridge regions. Bacterial activity in epilithic and decomposing leaf habitats, densities and generic richness of Ephemeroptera and grazer/scraper feeding groups of macroinvertebrates, and fish survival are all lower in more acidic streams. However, acidic streams in the Adirondacks generally have higher concentrations of monomeric Al than acidic streams in the Southern Blue Ridge. Therefore, differences in relationships between some biological characteristics (bacterial production on decomposing leaves, densities of Ephemeroptera and scrapers) and stream water pH between regions suggest that relatively high concentrations of monomeric Al, particularly the aquo + hydroxy forms, can increase the toxicity of acidic stream water at least to these organisms. Results from the Southern Blue Ridge where acidification is seasonally constant suggest that the negative effects of stream water acidity on stream biological communities over the long-term may be primarily on macroinvertebrate community composition and taxa richness and on bacterial metabolism rather than on total density or biomass of these organisms.

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