Transactions of the American Fisheries Society 137:1363–1377, 2008

DOI: 10.1577/T07-069.1

[Article]

Thresholds for Survival of Brown Trout during the Spring Flood Acid Pulse in Streams High in Dissolved Organic Carbon

IGNACIO SERRANO*[1] AND ISHI BUFFAM[2]
Department of Forest Ecology, Swedish University of Agricultural Sciences, Umeå 90183, Sweden

DANIEL PALM AND EVA BRÄNNÄS
Department of Aquaculture, Swedish University of Agricultural Sciences, Umeå 90183, Sweden

HJALMAR LAUDON
Department of Forest Ecology and Management,
Swedish University of Agricultural Sciences, Umeå 90183, Sweden

Abstract.—The survival of brown trout *Salmo trutta* embryos and first-year juveniles was studied using in situ bioassays during the snowmelt-driven spring flood in 12 streams in northern Sweden. Unlike in most previous studies on the impact of acidity on brown trout, the streams in this study were high in dissolved organic carbon (DOC), and as a result organic acids play a primary role in controlling pH. During the spring flood period DOC concentrations increased strongly in most streams and, in combination with dilution of acid-neutralizing capacity, resulted in a decrease in pH and an increase in total and inorganic monomeric aluminum. High mortality of brown trout juveniles occurred during the spring flood and was best correlated to the high H^+ concentration. No toxic effect could be directly attributed to measured inorganic aluminum concentrations. An empirical model to predict juvenile brown trout mortality in DOC-rich streams was developed, and based on these predictions a critical chemical threshold of pH 4.8–5.4 is proposed for first-year juveniles. There was high embryo and yolk sac fry survival during the spring flood, even at sites with pH as low as 4.0, suggesting that the pH threshold in DOC-rich waters is lower than the previously established threshold for low-DOC systems. We discuss the complex role that DOC has in humic-rich surface waters, where it both causes a pH decrease and protects against aluminum toxicity in fish. The results suggest that first-year juveniles are likely to be the stage most vulnerable to the effects of episodic pH depression associated with the snowmelt period in northern boreal systems. This results from asynchrony in the seasonality of the spring flood acid pulse and the seasonality of trout embryo development, which is slow in cold northern waters.

Streams and rivers in poorly buffered watersheds are vulnerable to episodes of acidic runoff associated with rainstorms and snowmelt events that result in a transitory decrease in pH (Jeffries et al. 1979; Sharpe et al. 1984; Wigington et al. 1992). Toxicity to fish populations caused by surface water acidification is well documented and is mainly due to the effects of increased concentrations of H^+ (i.e., reduced pH) and aluminum (Wood and McDonald 1987; Parkhurst et al. 1990; Havas and Rosseland 1995; Gensemer and Playle 1999). Increased H^+ levels impede the active uptake of sodium and stimulate efflux (Leivestad and Muniz 1976), which bring about net losses of electrolytes (especially Na^+ and Cl^-) across the gills (Booth et at. 1988; Weatherley et al. 1989). Ionoregulatory disturbances also occur due to intracellular accumulation of Al, which alters the barrier properties of the gill epithelium and affects transcellular processes (Exley et al. 1991; Havas and Rosseland 1995). In addition, Al accumulation in the gills reduces gill diffusion capacity causing respiratory distress (Havas and Rosseland 1995).

Toxicity is modified by water chemistry variables such as dissolved organic carbon (DOC), calcium, fluoride, citrate, and silicon (Driscoll et al. 1980; Howells et al. 1983; Birchall et al. 1989; Wilkinson et al. 1990). High levels of DOC are reported to reduce the effect of aluminum on fish (Simonin et al. 1993; Witters et al. 1990; Baldigo and Murdoch 1997). However, high DOC levels also promote an increase in the H^+ concentration (Eshleman and Hemond 1985; Köhler et al. 2000). In the humic-rich waters of northern Sweden an increase in DOC concentration coupled with dilution of acid-neutralizing capacity

* Corresponding author: ignacio.serrano@emg.umu.se
[1] Present address: Department of Ecology and Environmental Sciences, Umeå University, Umeå 90187, Sweden.
[2] Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706, USA.

Received March 24, 2007; accepted February 12, 2008
Published online September 11, 2008

(ANC) results in a natural pH decline of up to two units during the spring snowmelt period (Bishop et al. 2000; Laudon et al. 2000). Although few studies have evaluated the impact of acidity on fish in high-DOC watercourses, the results suggest that fish in these systems show higher tolerance to acidic Al-rich waters than fish in low-DOC systems (Laudon et al. 2005).

Sensitivity to acidity varies with species and life stage (McCormick and Leino 1999). Under equivalent exposure conditions, fish embryos and alevins are generally regarded as the most sensitive stages to acidification (Fivelstad and Leivestad 1984; Skogheim and Rosseland 1984; Reader et al. 1991; McCormick and Leino 1999). However, several factors may combine to make other life stages more susceptible to the effects of low pH. To investigate the impact of acidification on fish populations, it is necessary to consider the sensitivity of the life stage present at each point in the seasonal pH cycle (McCormick and Leino 1999). In northern latitudes, water temperature causes slow embryonic development in fish species such as brown trout *Salmo trutta* (Crisp 1981; Elliott et al. 1987). Prolonged development time decreases the probability that newly hatched alevins are exposed to the snowmelt runoff, which normally occurs in late April and early May in northern Sweden. Consequently, for brown trout, eggs and first year juveniles are the young stages with the most likelihood of being exposed to the most acidic conditions during spring.

The brown trout is among the most common native fish species in Scandinavian streams and lakes, but even within its potential range its distribution is patchy, especially in smaller streams. Surface water acidity is one of the commonly cited reasons for the lack of brown trout in some Scandinavian streams and lakes (Andersson and Andersson 1984; Degerman and Lingdell 1993; Hesthagen et al. 1999). However, few studies have explicitly considered the implications of high DOC concentrations on acid toxicity (Lacroix 1989; Laudon et al. 2005). The aim of our study was to shed some light on the role of acid stress in the scattered distribution of brown trout populations in DOC-rich waters such as those in northern Sweden. In this context, the purpose of this study was to (1) investigate the survival of eggs and first year juveniles of brown trout during the spring snowmelt period in DOC-rich streams, (2) identify chemical variables that best explain differences in egg and juvenile survival among streams, and (3) define pH and Al toxicity thresholds for early life stages of brown trout in DOC-rich waters.

Methods

Study sites.—The fieldwork was conducted in 12 streams situated within the 68-km^2 Krycklan catchment in the County of Västerbotten, northern Sweden, 235–310 m above sea level (Figure 1). Catchment characteristics and mean stream water chemistry characteristics vary among the study streams (Table 1), which range from first to fourth order. For streams in this region, the spring flood constitutes the major hydrological event of the year, with close to half of the annual runoff (Laudon et al. 2004) and the lowest pH values (Bishop et al. 2000). Poorly weathering gneissic metasediment is the predominant bedrock in the catchment and the forest soils are mainly iron podzols that have developed on a till overburden several meters thick. In low-lying areas paludification has led to the development of bogs and wetlands, characterized by spongy peat deposits, acidic waters, and a floor covered by a thick carpet of sphagnum moss. Strips of agricultural land over fine sorted sediments are found at lower altitudes within the catchment. The climate in the area is characterized by long winters and short summers. The 20-year mean annual precipitation (1980–1999) is 600 mm, of which one-third falls as snow (Ottosson Löfvenius et al. 2003). The snow cover is typically maintained from the end of October until the beginning of May. Many of the streams in the catchment are inhabited by brown trout or brook trout *Salvelinus fontinalis*, or both; Eurasian perch *Perca fluviatilis* and northern pike *Esox lucius* dwell in some of the ponds and small lakes within the area.

Experimental fish.—Eggs as eyed ova (spring 2003) and age-1 juveniles (spring 2004) of brown trout were obtained from a local hatchery (Norrfors fiskodling, Vattenfall AB). Eggs originated from a mixture of five males and five females of wild anadromous brown trout. The juveniles were first generation of wild anadromous brown trout. Fish mean weight was 6.4 $\pm$ 2.0 g (mean $\pm$ SE; range, 3.4–12.7 g). Hatchery water was untreated water from the nearby Vindel River. Thus, water chemistry at the hatchery varies throughout the year along with the variation in natural waters of the area (total Al = 30–160 μg/L, inorganic monomeric Al = 0–8 μg/L, and pH = 6.6–7.1 during spring 2003 and 2004).

Exposure conditions.—In situ bioassays measure the direct effect of stream water chemistry on fish mortality. Bioassays were carried out in the spring of 2003 and 2004. The objective was to test embryo and fry brown trout in 2003, and yearling brown trout in 2004. For more information regarding bioassays see Baker et al. (1996) and Van Sickle et al. (1996).

Embryo and fry study.—The egg experiments were conducted at 9 of the 12 sites from April to June 2003 (Table 1). On April 17 the eggs were put into 40 mesh bags (mesh size, 3 mm; volume, 250 cm^3) each containing 100 eggs, 20 from each family. Thereafter,

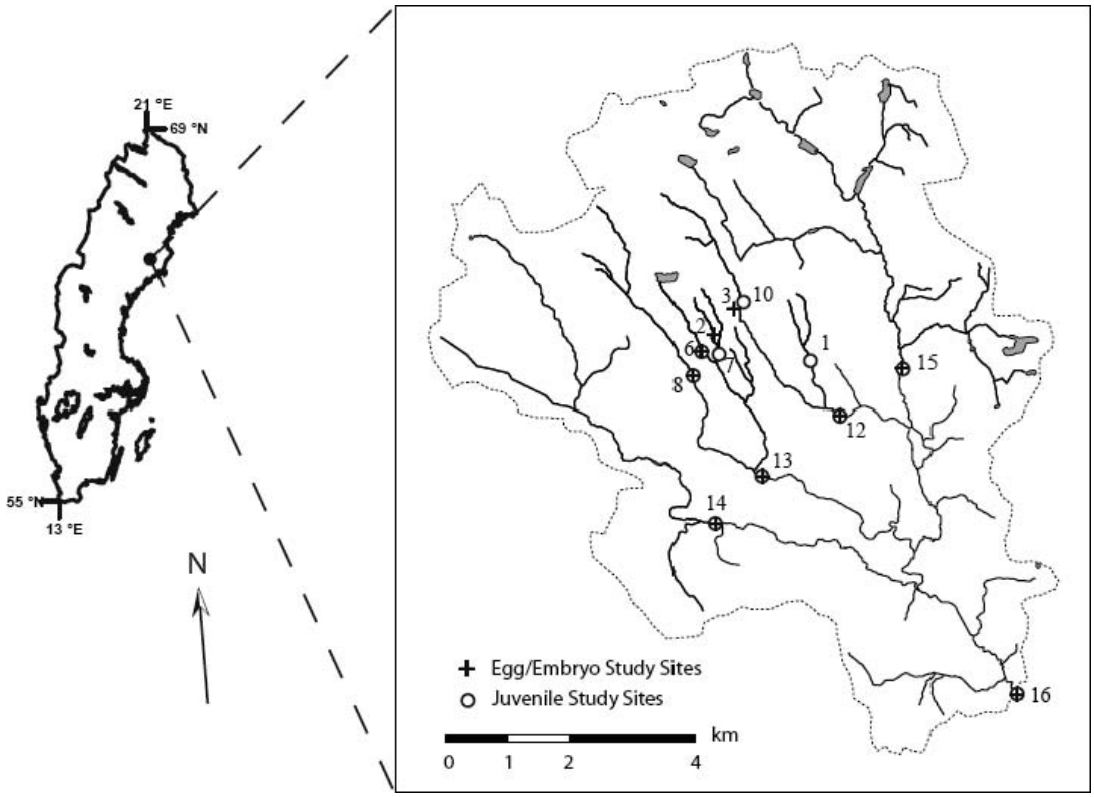

FIGURE 1.—Map of the Krycklan catchment in Sweden indicating the locations of the experimental sites (see Table 1). Streams (black lines) and lakes (gray areas) within the catchment and catchment boundary (dotted line) are also shown.

the eggs were placed in each of the nine experimental streams (Figure 1). Eggs were transported in tanks with oxygenated freshwater. At each site four bags were placed into a perforated plastic frame cage to ensure protection and a continuous flow of water. Finally the plastic frame cages were covered with pebbles. The eggs were kept in the stream until June 27 when the total number of surviving fry and eggs was counted. Another set of four mesh bags, containing the same egg mixture, was kept as control in a tank at the hatchery.

Juvenile study.—The juvenile study was carried out as three separate exposures of fish to the stream water at 10 of the 12 sites during the spring of 2004 (Table 1). During each trial fish were exposed for

TABLE 1.—Catchment descriptions and annual mean stream pH at sites where experiments with brown trout eggs and juveniles were conducted in 2003 and 2004. Sites 4, 5, 9, and 11 have been described in previous studies (Ågren et al. 2007, Buffam et al. 2007). We have kept site numeration for consistency.

Site	Site name (stream order)	Egg experiment 2003	Juvenile experiment 2004	Area (km^2)	% Forest	% Wetland	pH (mean ± SD)
1	Risbäcken (1)		x	0.66	98.7	1.3	5.6 ± 0.3
2	Västrabäcken (1)	x		0.14	100.0	0.0	5.2 ± 0.3
3	Lillmyrbäcken (1)	x		0.03	24.0	76.0	4.0 ± 0.1
6	Stortjärnbäcken (1)	x	x	1.4	72.8	24.1	5.8 ± 0.4
7	Kallkällsbäcken (2)		x	0.50	85.1	14.9	5.0 ± 0.6
8	Fulbäcken (2)	x	x	2.5	88.7	11.3	5.7 ± 0.6
10	Stormyrbäcken (2)		x	2.9	74.2	25.8	5.6 ± 0.6
12	Nymyrbäcken (3)	x	x	5.4	84.1	15.5	5.9 ± 0.5
13	Långbäcken (3)	x	x	7.2	89.1	9.9	5.9 ± 0.3
14	Åhedbäcken (3)	x	x	13.6	90.4	5.1	6.4 ± 0.2
15	Övre Krycklan (4)	x	x	19.9	83.2	14.0	6.6 ± 0.2
16	Krycklan (4)	x	x	67.8	88.0	8.3	6.7 ± 0.3

approximately 2 weeks (13–16 d). Trial 1 was started on April 21, trial 2 on May 4, and trial 3 on May 27. The third trial was carried out in only half of the streams (sites 1, 8, 10, 12, and 13). Depending upon the trial, between 8 and 12 fish were placed in Whitlock–Vibert (WV) hatching boxes in every stream, one cage for each individual. The boxes were placed at the middle depth of the stream. The midwater location was chosen to avoid disturbances of ice and debris, and to avoid desiccation of the fish due to changes in water level during the initial snowmelt. Fish were transported to the experimental sites in tanks with oxygenated freshwater (transport time about 2 h) and then transferred to the WV boxes. The boxes were placed inside a metal frame cage for physical protection. The test fish were not fed during the bioassays. Mortality was recorded every 24–48 h. Fish were considered to be dead when no swimming response could be elicited through stimulation of the lateral line organ. At one site, one of the bioassays was terminated before the end of the 2-week period (site 16, trial 2) owing to the burial of the cages into the sediment by a branch. The control fish were placed individually in WV boxes in tanks with a water current mimicking field conditions (0.2–0.5 m/s).

Water sampling and analysis.—Hourly stream discharge was calculated for all sites using measurements of stream height as determined by WT-HR water level sensors (TruTrack, Christchurch, New Zealand) and established water level discharge rating curves. For illustrative purposes we present discharge data from site 7 (Kallkälsbäcken), which has been monitored since 1980. Measurements of discharge at the other 11 sites confirmed that changes in flow were generally synchronous with, for example, maximum spring discharge at all sites occurring during a single 72-h period (Ågren et al. 2007).

The water chemistry sampling program was based on weekly sampling of the base flow before the onset of the spring flood, followed by sampling every 2–3 d until the discharge returned to base flow levels. Water samples for laboratory analysis were collected in acid-washed 250-mL polyethylene bottles close to the same location and depth as the fish were kept. The samples were stored cold and in the dark until analysis. The pH was measured using a Ross 8102 combination electrode (ThermoOrion). Samples for DOC analysis were either frozen unfiltered (in 2003) or frozen after filtration with 0.45-μm mixed cellulose ester (MCE) membrane filters (in 2004). Organic carbon is greater than 95% in the dissolved form in the surface waters of this region (Ivarsson and Jansson 1994; Köhler et al. 1999); thus, we use the term DOC for both years. Dissolved organic carbon was measured using a Shimadzu TOC-V_{CPH} analyser. Samples for major cation analyses (K^+, Mg^{2+}, Na^+, Ca^{2+}) were preserved with ultrapure HNO_3 (1% v/v) and stored cool until analysis by inductively-coupled plasma optical emission spectroscopy (ICP-OES) on a Varian Vista Ax Pro instrument. In 2003, samples for cation analyses were not filtered. In subsequent years including 2004, both unfiltered and filtered (0.45-μm MCE membrane filters) samples were analyzed. The values reported herein for 2004 are from the filtered samples, since the dissolved fraction is of primary importance for acid chemistry in the streams. The use of unfiltered samples during 2003 is not believed to have substantially affected the analysis. Based on data from subsequent years, calcium (Ca) concentration for all studied streams averages >90% filterable Ca, expect on one site (site 16) with the highest Ca and pH, in which filterable Ca averages 84% of the total Ca. Samples for strong acid anions (SO_4^{2-} and Cl^-) were stored at 4°C until analysis, which was carried out with a Dionex DX-300 or DX-320 ion chromatograph system. The typical precision in anions and cations analyses based on measurements of certified standards was better than 2%. Nitrate (NO_3^-) concentrations are so low in these streams during the spring flood period that their contribution to charge balance is insignificant (Petrone et al. 2007). Base cation (BC) concentration was calculated as the sum of K^+, Mg^{2+}, Na^+ and Ca^{2+} concentrations expressed as μeq/L of charge. Acid-neutralization capacity was calculated as the difference between BC and the sum of SO_4^{2-} and Cl^- expressed as μeq/L of charge (Laudon et al. 2000). Total and organic fractions of aluminum were measured using ICP-OES. Organic monomeric aluminum (Al_o) was separated using an ion exchange method (Driscoll 1984), with an exchange column directly coupled to an ICP-OES (Varian Vista AX), without acid addition. Inorganic monomeric aluminum (Al_i), which includes all cationic forms (Al^{3+}, $Al[OH]^{2+}$, $Al[OH]_2^+$, AlF^{2+}, AlF_2^+, $Al[SO_4]^+$), was calculated as the difference between total monomeric aluminum (Al_{tot}) and Al_o. Analytical uncertainty at a 95% confidence level is 6.4% for the determination of Al_{tot} and 8.4% for Al_o. The error associated with Al_i (10.6%) was calculated with equation (1):

$$\delta Al_i = [(\delta Al_{tot})^2 + (\delta Al_o)^2]^{1/2}. \quad (1)$$

For a more detailed description of the method see Cory et al. (2006). In 2003 only a subset of all samples was analyzed for Al_i.

Statistical analysis.—Mean, minimum, and maximum values for water chemistry variables were obtained for each site for the periods corresponding

to in situ incubations using linear daily interpolation of measured water chemistry. Analyses of the mortality and water chemistry data were done with the statistical packages SPSS for Windows version 11.0 and MINITAB release 14. All water chemistry variables, except pH, were $\log_{10}$ transformed before regression analysis with mortality to improve normality. Exploratory multiple regression analysis was conducted using the Best Subsets Regression Maximum R^2 criterion procedure (SPSS version 11.0) and included the following predictor variables: mean pH, minimum pH, mean Al_i, maximum Al, mean DOC, mean Ca, mean ANC, and the ANC:H^+ ratio. The ratio of ANC to H^+, an index introduced to integrate measures of acidity and buffering capacity, correlated well with the mortality of age-2+ brown trout in a previous study in DOC-rich waters (Laudon et al. 2005).

Probit analysis (Prentice 1976) was used (Minitab 14) to test the influence of the various chemical features on fish mortality. Probit regression is a log-linear approach proved to be useful to analyze dose–response data in experimental studies on acid toxicology in fish (Simonin et al. 1993). Output is determined by a logistic equation relating the probability of fish mortality (P) to the value of an environmental predictor variable (X):

$$P = \frac{1}{1 + e^{-(\beta_0 + \beta_1 X)}}. \quad (2)$$

The models were evaluated using the Hosmer–Lemeshow goodness-of-fit test. Lower χ^2 values and higher P-values indicate a stronger relationship between predictor and response variables.

Complementarily, lifetime regression analysis (Minitab 14) was used to analyze the survival data. This analytical technique handles data in which the variable of interest is the time taken for a certain event to occur (Lawless 1982); in this case the time from when the fish were placed in the streams until death. In this analysis, data from individual fish are used. Survival analysis has been used previously for studies of Al toxicology in fish (Lydersen et al. 2002). In the lifetime regression analysis, the function $Y(t)$ denotes the probability that an individual survives until time $t + 1$, given that it has survived to time t. The regression model estimates the percentiles of the failure time distribution (equation 3):

$$Y_p = \beta_0 + \beta_1 X + \sigma\varepsilon_p; \quad (3)$$

Y_p = the pth percentile of the failure time distribution;
β_0 = the y-intercept (constant);
β_1 = the regression coefficient;
X = the predictor variable;
σ = the scale parameter;
ε_p = the pth percentile of the error distribution.

The models were evaluated by probability plots for the standardized and Cox–Snell residuals and by the Anderson–Darling goodness-of-fit statistic. A smaller Anderson–Darling statistic indicates that the distribution provides a better fit. For all statistical tests, a critical significance level of $\alpha = 0.05$ was chosen.

Results

Discharge and Water Chemistry

The snowmelt spring flood at Krycklan in 2003 had two distinct phases. The first was a small rise in flow at the end of March, resulting from a short warm spell. After an intervening period of cold weather, the main spring flood occurred in mid to late April, peaking on April 21 with flow remaining elevated above base flow levels until the start of June (Figure 2). The pH declined at eight of the nine sites during the spring flood. Site 3, draining a wetland-dominated catchment, behaved differently from the others, with consistently low pH occurring throughout the study period (Figure 2).

The first trial of 2004 began on the rising limb of the spring flood and ended just after the peak discharge, flow generally decreasing thereafter at all sites. The water temperature remained low during the first two bioassays (ranges, 0–1°C and 2–5°C, respectively) and was slightly higher at all sites (range, 5–10°C) during the last bioassay period.

The onset of the snowmelt period in 2004 coincided in time with that from the previous year and comprised a large episode lasting about 5 weeks. The chemical conditions of the water varied greatly during the experimental period and between streams. The pH dropped up to 1.5 units in some of the streams during the snowmelt period (Figure 3), with an average pH decline of approximately 1 pH unit from base flow to peak flow (detailed data in Buffam et al. [2007]). In 2004 the beginning of the first trial occurred during a period of rapid pH decrease for most of the sites, and minimum pH was reached during the first trial or within the first few days of the second trial, depending upon the site. There was also a clear drop in ANC during snowmelt, and an increase in DOC matching the pH decline. The decrease in ANC during the spring flood resulted largely from the dilution of base cation concentrations, including Ca^{2+}, which was over 2 mg/L in all streams during base flow, but decreased to about 1 mg/L in some streams during the spring thaw. Concentrations of SO_4^{2-} also became diluted at all study sites during the spring flood, decreasing on average by 35% to reach means ranging from 30 to 150

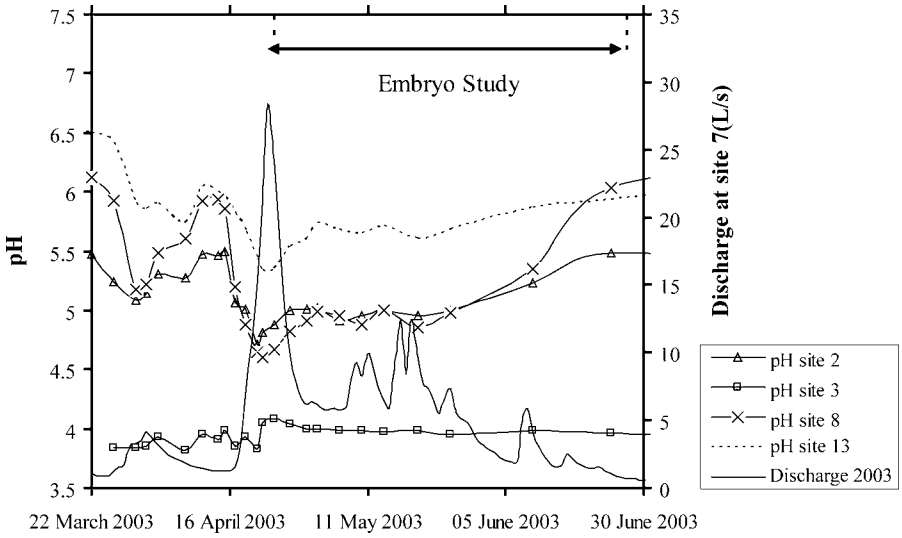

FIGURE 2.—Stream pH and discharge during the experimental period in 2003. For clarity, the time courses for pH are shown only for selected streams; refer to Table 3 for more detailed information.

μeq/L for the different sites. The concentration of Al_i varied between 0 and 180 μg/L during the spring snowmelt period. The concentration of Al_o varied between 110 and 590 μg/L. Organic monomeric aluminum constituted generally more than 70% of the amount of total aluminum (detailed data in Cory et al. [2006]).

Fish Responses and Acidity Thresholds

Embryo experiment.—The results from the experiments with brown trout embryos and yolk sac fry during the spring flood of 2003 are presented in Table 2 and Figure 4. Almost no mortality occurred in the acid waters during the 10 weeks of exposure, with greater than 80% survival at the site with lowest pH (Lillmyrbäcken site 3, pH = 4.0). In two of the high pH sites (sites 12 and 14) some of the bags were covered with sediments and embryos and fry exhibited high mortality, apparently due to suffocation. No mortality was observed in the control. No correlation between embryo or yolk sac fry mortality and the various water quality indices were found.

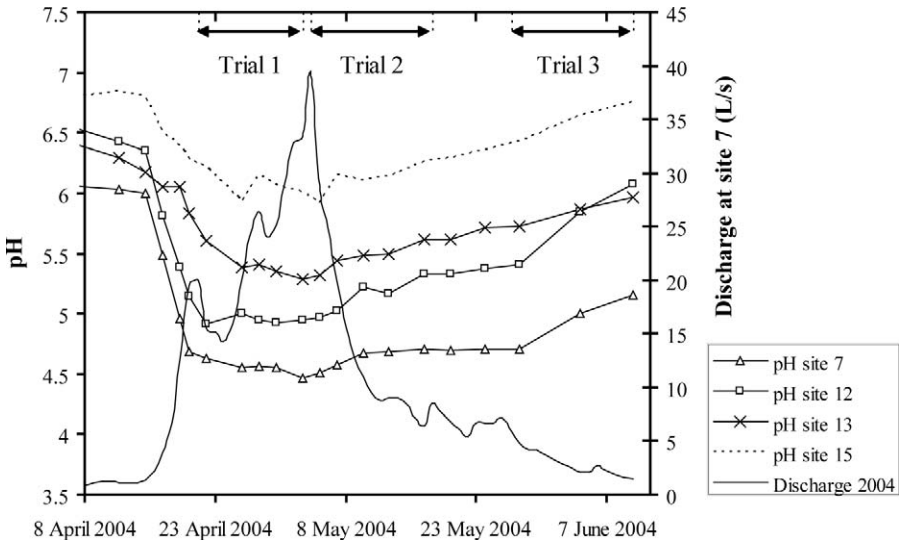

FIGURE 3.—Stream pH and discharge during the experimental period in 2004. For clarity, the time courses for pH are shown only for selected streams; refer to Table 3 for more detailed information.

TABLE 2.—Summary of the major chemical variables during the brown trout egg–embryo incubation period in 2003. Mean values (ranges in parentheses) are shown for pH, inorganic monomeric aluminum (Al_i), dissolved organic carbon (DOC), Ca, and acid-neutralizing capacity (ANC) during the incubation period together with the mean egg–embryo mortality at each site at the end of each experimental period. For Al_i, 4–6 samples were analyzed for each site; for all other analyses, 13–15 samples were analyzed for each site.

Site	Embryo mortality (%)	pH	Al_i (μg/L)	DOC (mg/L)	Ca (mg/L)	ANC (μeq/L)
2	12	5.0 (4.7–5.5)	67 (42–85)	15 (10–24)	2.1 (1.8–2.9)	56 (32–87)
3	18	4.0 (3.8–4.1)	9 (−1–20)	35 (28–47)	0.6 (0.5–1.0)	34 (21–61)
6	9	5.2 (4.8–6.1)	11 (−1–20)	16 (11–20)	1.5 (1.2–2.0)	84 (47–122)
8	7	5.0 (4.6–6.0)	32 (9–46)	24 (14–30)	2.1 (1.9–2.5)	107 (26–149)
12	56	5.3 (4.8–6.4)	21 (7–33)	17 (12–22)	2.0 (1.7–2.6)	97 (73–141)
13	12	5.6 (5.3–5.9)	23 (9–33)	17 (14–21)	2.1 (1.9–2.3)	119 (100–134)
14	75	6.2 (5.9–6.7)	13 (6–20)	12 (7–19)	2.5 (2.1–3.0)	140 (110–194)
15	45	6.4 (6.2–6.9)	3 (0–5)	12 (8–16)	2.4 (2.1–2.9)	138 (113–178)
16	17	6.5 (6.1–7.1)	50 (6–122)	12 (6–17)	2.8 (2.5–4.0)	189 (148–264)

First-year juvenile experiment.—The mortalities of the brown trout juveniles exposed to the 10 different streams during the spring 2004 flood are illustrated in Table 3. In general, the highest mortality was registered for all streams during the first trial, coinciding with maximum runoff and minimum pH. Survival was somewhat prolonged in the second trial and no mortality was observed during the third exposure. All fish survived the acid episode in the large well-buffered streams, Åhedbäcken (site 14) and Övre Krycklan (site15), and in Krycklan River (site16) only one individual was found dead. No mortality occurred in the fish kept as controls.

Mean fish weight did not vary significantly between treatments (one-way analysis of variance [ANOVA]: $P < 0.001$). Mean pH, ANC:H^+ ratio, and DOC were the indices with the best univariate linear correlations to brown trout mortality ($P < 0.001$; Table 4). All indices were correlated with each other in pair-wise comparisons ($P < 0.05$) with the exception of Al_i, which was not significantly correlated with Ca nor ANC. A strong correlation was found between pH and several other chemical variables, namely DOC ($r = -0.88$, $P < 0.001$), ANC ($r = 0.82$, $P < 0.001$), and ANC:H^+ ($r = 0.996$, $P < 0.001$). Exploratory multiple regression analysis of key variables did not provide significant multivariate models.

The use of probit analysis allowed us to create nonlinear models to predict mortality after 2 weeks of exposure (Table 5). The ANC:H^+ ratio, pH, and DOC were the only variables that showed significant levels of predicted mortality according to the probit models. As these three variables are highly co-correlated, we have chosen to focus the development of threshold values on pH. The relationship between mean pH and mortality is shown in Figure 5.

Lifetime regression analyses allowed the construction of empirical models to evaluate the effect of different variables on the time to fish mortality (Table 6). Mean pH during the experiment was the best estimator of the time to death (Figure 6).

Discussion

The results of this investigation reveal a high rate of survival of brown trout embryos and yolk sac fry during exposure to low pH snowmelt runoff in DOC-rich waters. These results support earlier investigations suggesting that salmonid eggs and yolk sac fry are less vulnerable to the combination of low pH and aluminum than other early life stages (Brown and Lynam 1981; Baker and Schofield 1982; Fivelstad and Leivestad 1984; Weatherley et al. 1990; Reader et al. 1991; Sayer et al. 1991). It must be emphasized that the eggs used in this study were fertilized in circumneutral hatchery water, and remained in water of that quality for

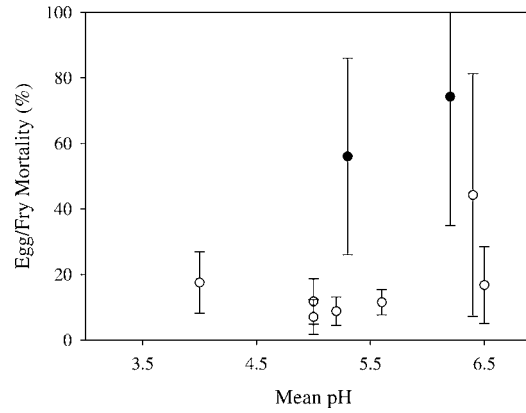

FIGURE 4.—Mean pH during the experimental period versus survival of brown trout eggs and fry (means ± SDs of four field replicates) at the end of the study. Fine sediment accumulated at sites 12 and 14 (solid symbols), causing suffocation of the eggs.

TABLE 3.—Summary of the major chemical variables during the spring 2004 juvenile brown trout experiment. For each time period, the mean, minimum, and maximum values were calculated by means of linear daily interpolation of measured values ($n = 3$–6 per run and site); see Table 2 for more details.

Run	Site	Fish mortality (%)	pH	Al_i (μg/L)	DOC (mg/L)	Ca (mg/L)	ANC (μeq/L)
1	1	12.5	5.0 (4.8–5.2)	91 (28–184)	20 (18–21)	1.9 (1.7–2.8)	116 (100–186)
	6	100	4.8 (4.7–5.2)	21 (4–44)	21 (17–24)	1.3 (1.0–1.9)	102 (68–147)
	7	100	4.6 (4.5–4.7)	39 (18–54)	22 (20–24)	1.5 (1.2–1.9)	82 (63–117)
	8	87.5	4.8 (4.7–4.9)	36 (18–56)	25 (21–29)	1.8 (1.3–2.9)	124 (86–219)
	10	100	4.7 (4.6–4.7)	18 (3–35)	20 (17–23)	1.4 (1.1–1.6)	83 (53–105)
	12	100	5.0 (4.9–5.1)	53 (11–115)	19 (17–23)	1.6 (1.3–2.1)	99 (74–151)
	13	75	5.5 (5.3–5.8)	42 (−6–130)	19 (18–20)	1.8 (1.5–2.1)	129 (92–174)
	14	0	6.0 (5.8–6.0)	8 (−9–33)	15 (12–17)	1.8 (1.7–2.0)	141 (114–180)
	15	0	6.1 (5.9–6.3)	1 (−13–17)	14 (11–14)	2.1 (1.7–2.5)	146 (119–184)
	16	12.5	6.1 (6.0–6.2)	51 (−16–167)	14 (10–16)	2.0 (1.7–2.5)	153 (117–204)
2	1	20	5.1 (4.9–5.3)	75 (49–116)	18 (16–20)	1.8 (1.6–2.0)	107 (99–119)
	6	70	5.0 (4.7–5.3)	15 (10–26)	18 (17–20)	1.1 (0.9–1.3)	82 (70–93)
	7	90	4.6 (4.5–4.7)	42 (27–56)	19 (18–19)	1.3 (1.2–1.4)	71 (65–77)
	8	40	4.9 (4.7–5.1)	32 (19–49)	20 (18–21)	1.4 (1.3–1.6)	99 (90–104)
	10	70	4.8 (4.7–5.0)	13 (10–18)	16 (16–17)	1.2 (1.1–1.4)	71 (60–77)
	12	40	5.2 (5.0–5.3)	23 (12–57)	16 (14–17)	1.4 (1.3–1.6)	85 (75–88)
	13	0	5.5 (5.3–5.6)	14 (1–44)	16 (14–18)	1.7 (1.5–1.9)	118 (101–141)
	14	0	6.1 (6.0–6.2)	−3 (−9–7)	11 (9–12)	2.4 (1.8–3.1)	146 (121–184)
	15	0	6.1 (5.9–6.3)	−3 (−6–2)	11 (11–13)	1.8 (1.6–2.0)	122 (112–129)
	16	0	6.2 (6.1–6.4)	2 (−14–49)	11 (10–13)	2.1 (1.6–2.7)	139 (101–176)
3	1	0	5.5 (5.4–5.7)	40 (9–75)	14 (12–17)	1.9 (1.8–2.0)	101 (74–119)
	8	0	5.6 (5.1–5.9)	19 (8–37)	16 (13–20)	1.7 (1.7–1.7)	121 (119–123)
	10	0	5.4 (5.0–5.7)	10 (−1–23)	16 (14–18)	1.6 (1.5–1.6)	103 (98–112)
	12	0	5.8 (5.4–6.1)	6 (−6–29)	14 (13–16)	1.7 (1.6–1.8)	105 (87–114)
	13	0	5.8 (5.7–6.0)	3 (−12–29)	13 (12–15)	1.8 (1.8–1.9)	120 (116–124)

approximately 350 degree-days (°C) before introduction to the stream water. It is possible that this delay in transfer may have resulted in greater survival than if fertilization had occurred within treatment. For several species, earlier exposure to Al–acid water resulted in greater embryonic mortality than when exposures started later (Parker and McKeown 1987; Hurley et al. 1989; Duis and Oberemm 2000). However, the change from circumneutral water during base flow to acidic water during the spring flood closely mimics the transient change in chemistry that many of the study streams experience.

The buffering capacity of the yolk sac with perivitelline fluids in eyed eggs and yolk sac fry (Peterson et al. 1980; Kugel and Peterson 1989), together with the protection conferred by the chorion, may explain why these stages are less vulnerable to high H^+ concentrations. No significant effect of Al_i on embryo mortality was found in our experiments. Some studies report that Al at high concentrations increases embryonic mortality (Baker and Schofield 1982), while other investigations have shown that Al has no effect on embryo mortality (Holtze and Hutchinson 1989; Weatherley et al. 1990; Buckler et al. 1995). Moreover,

TABLE 4.—Summary of results from linear regression analyses with juvenile brown trout mortality as the response variable ($n = 25$). All water chemistry variables except pH were log transformed before analysis. See Table 2 for variable descriptions; NS = not significant.

Predictor	r	Adjusted R^2	Sum of squares	P
pH	−0.81	0.65	2.75	<0.001
pH (minimum)	−0.73	0.53	2.26	<0.001
Al_i				NS
Al_i (maximum)				NS
DOC	0.77	0.59	2.52	<0.001
Ca	−0.66	0.44	1.92	<0.001
ANC	−0.62	0.39	1.70	0.001
ANC:H^+	−0.81	0.65	2.75	<0.001

TABLE 5.—Summary of results from probit analyses with juvenile brown trout mortality as the response variable ($n = 25$). All water chemistry variables (see Table 2) except pH were log transformed before analysis. Lower χ^2 and higher P-values indicate a stronger relationship between predictor and response variables. Parameters β_0 and β_1 are only included for those models considered significant ($P > 0.05$ denotes a significant relationship).

Predictor	β_0	β_1	χ^2	P
pH	25.9	−5.13	13.9	0.052
pH (minimum)			22.8	0.001
Al_i			52.3	<0.001
Al_i (maximum)			25.5	<0.001
DOC	−34.2	27.18	11.1	0.137
Ca			22.4	0.002
ANC			31.2	<0.001
ANC:H^+	4.55	−4.26	14.1	0.050

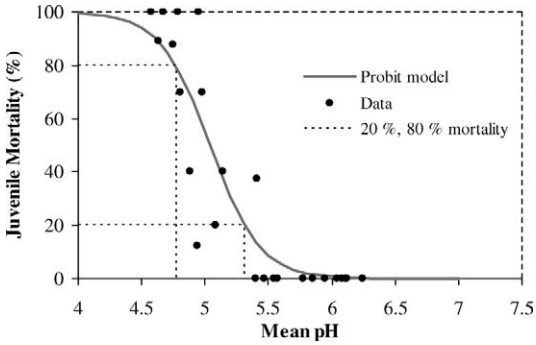

FIGURE 5.—Results of a probit model of brown trout mortality and mean pH during acid pulse episodes. The dashed lines show the threshold limits for the 20% and 80% probabilities of mortality.

low to moderate Al concentrations conferred defense against acid exposure in some salmonids at pH 4.0–4.5 (Baker and Schofield 1982; Holtze and Hutchinson 1989; Ingersoll et al. 1990; Sayer et al. 1991).

The lack of correlation between embryo and yolk sac fry mortality and pH or Al_i makes it difficult to define chemical critical thresholds for survival. Based on our experiments and the results of earlier studies (Table 7), it appears that the eyed egg and yolk sac fry stages of brown trout can survive acidic episodes of pH values of below 4.5 and that Al does not induce mortality in the concentration range observed (0–70 μg/L). We therefore suggest that in DOC-rich waters the pH threshold for survival of the embryo and yolk sac fry stages of brown trout is lower than the previously established clear water thresholds of 4.5–5.2.

The mortality of eggs at several high-pH sites due to oversilting and probable suffocation suggests that some of the sites are unsuitable for spawning because of too much fine sediment. Five of the sites (12, 13, 14, 15, and 16) are dominated by silt sediments according to the Quaternary sediment deposit map of the area. Among these five were the only three sites in the study experiencing substantial egg mortality, two of which resulted directly from visible silting over. Fine sediments are commonly implicated in salmonid egg mortality due to suffocation and result in poor spawning habitat (e.g., Soulsby et al. 2001; Julien and Bergeron 2006).

An objective of this investigation was to identify the variables that best explain fish mortality in these streams and identify acidity thresholds for brown trout survival in DOC-rich waters. Our results suggest that pH is the factor best correlated to brown trout mortality in these streams. The use of probit and lifetime regression analyses permitted the development of a model to estimate the effect of episodes of low pH on brown trout populations in high-DOC waters. For episodes lasting several weeks, a pH threshold of 4.8–5.4 is proposed (Figure 6). For shorter episodes pH thresholds would be lower.

We emphasize, however, that the extrapolation of these values to wild fish populations should be done cautiously. Free-ranging fish, unlike the caged fish we used in the experiments, can sometimes avoid acid and Al pulses (Carline et al. 1992; Vanoffelen et al. 1994). Laboratory studies on juvenile brown trout have shown that sense and avoidance of Al are only present when

TABLE 6.—Summary of results from the lifetime regression analyses for juvenile brown trout. Results from different predictors are shown, together with the estimated coefficients for the regression model and their *P*-values (*Z*-test) (NS = not significant). The term "residual" refers to the Andersson–Darling goodness-of-fit statistic for standardized residuals. A smaller value indicates a better fit. See Table 2 for model variables.

Model	Predictor	Coefficient	*P*
Mean pH	Intercept	−1746.99	<0.001
	Mean pH	411.63	<0.001
	Residual	2.61	
Minimum pH	Intercept	−1679.36	<0.001
	Minimum pH	412.25	<0.001
	Residual	4.59	
Al_i	Intercept	602.18	NS
	Mean Al_i	−1.24	NS
	Residual	147.07	
Ca	Intercept	−71.17	NS
	Mean Ca	0.32	NS
	Residual	139.00	
ANC	Intercept	137.27	NS
	Mean ANC	3.55	NS
	Residual	125.43	

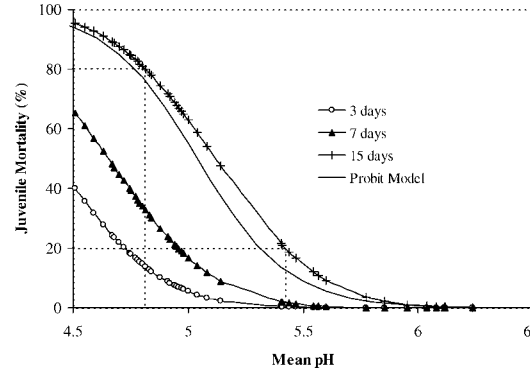

FIGURE 6.—Results of lifetime regression and probit models. The values shown on the lifetime regression curves are predicted mortalities based on the mean pH experienced by individual brown trout in our experiments. The dashed lines show the suggested thresholds for the 20% and 80% probabilities of mortality for episodes lasting 15 d.

TABLE 7.—Summary of studies on the effects of pH and Al on young stages of brown trout; Al_{tot} = total aluminum, Al_i = inorganic monomeric aluminum.

Stage	Values for mortality >40%	Experiment	Reference
Eggs	pH < 4.8–5.6 and Al_i > 20–310 μg/L	Lab	Skogheim and Rosseland (1984)
	Al_{tot} > 397 μg/L	Field	Weatherley et al. (1990)
Eyed eggs	pH < 4.5	Lab	Brown and Lynam (1981)
	pH = 4.5 and Al_i = 162 μg/L reduced embryonic mortality (75% hatching); pH = 4.5, Al_i = 162 μg/L, and Ca = 0.8–8 mg/L increased egg survival (77.6% hatching)	Lab	Sayer et al. (1991)
Yolk sac fry	Al_{tot} = 15 μg/L	Field	Weatherley et al. (1990)
	pH = 4.5 and Al = 323 μg/L	Lab	Reader et al. (1991)
	pH = 4.5–5.5 and Al_i = 162 μg/L	Lab	Sayer et al. (1991)
	pH = 4.5 and Al_i = 324 μg/L	Lab	Çalta (2002)
Eyed eggs, yolk sac fry	pH < 4.0; no Al_i correlation observed	Field	This study
Fry	pH < 4.5–5.1 and Al_{tot} > 200–350 μg/L	Field survey	Barlaup and Åtland (1996)
Alevins	pH = 4.8–5.6 and Al_i > 20–310 μg/L	Lab	Skogheim and Rosseland (1984)
1-year-old fry	pH = 5.2–5.6; no Al_i correlation observed	Field	This study

high inorganic Al concentrations (Al_i > 70 μg/L) are accompanied by very low pH (pH < 5.0) (Åtland 1998), conditions present in some of the study streams.

The toxicity of inorganic monomeric aluminum to fish is well documented (Driscoll et al. 1980; Sparling and Lowe 1996; Gensemer and Playle 1999). Significant mortality of brown trout in laboratory and in situ exposure experiments has been reported at Al_i concentrations >100 μg/L (Brown 1983; Fivelstad and Leivestad 1984; Rosseland and Skogheim 1984; Sadler and Lynam 1988; McCahon et al. 1989). A reason for the lack of correlation between trout mortality and Al_i concentration in this study could be due to the low levels of Al_i detected. Of the streams sampled for the juvenile study, only the headwater site (site 1) showed high levels (>90 μg/L Al_i) during both of the first two trials. Two other sites (sites 12 and 16) showed elevated levels (>50 μg/L Al_i) during the second trial. The rest of the streams remained well below the toxicity limits for brown trout. Despite pH values between 4.5 and 5.5 and total aluminum (Al_{tot}) concentrations well above 100 μg/L in all sites, only a few showed toxic levels of Al_i (Cory et al. 2006). This suggest that while Al_{tot} levels increase during the spring flood, the degree of organic complexation is high enough to maintain the concentration of Al_i below the toxic threshold for brown trout.

Another reason for the poor agreement between the observed mortality and the inorganic aluminum concentration could be the analytical method used. In high-DOC streams with high levels of organic aluminum (Al_o) such as those studied here, the calculation of Al_i as the difference between Al_{tot} and Al_o means that the analytical error for Al_i is relatively large (10.6%). It is possible that the error on Al_i determination could mask any patterns linking the Al_i and fish mortality. This was found in a similar study by Laudon et al. (2005), where physiological evidence from the fish gills supported the hypothesis that aluminum caused the observed mortality despite no correlation between stream water Al_i and fish mortality. Furthermore, previous studies suggest that Al_i cannot be determined precisely in surface waters with DOC concentrations greater than 10 mg/L (Driscoll 1984; Simonin et al. 1993; Lawrence et al. 2007). The majority of the samples from this study fall within this category. Also, aluminum speciation in water samples may change even during short storage times as a result of rapid transformation processes (Andrén 1995), indicating the importance of in situ measurements of Al_i (Teien et al. 2007). Thus, as noted by Driscoll (1984), the classical methods to determine aluminum speciation following the Barnes–Driscoll laboratory fractionation principles might be inappropriate for the assessment of potentially toxic aluminum species in water with high DOC concentrations.

In our study, Ca concentrations were negatively correlated with the mortality of juvenile brown trout, but this correlation disappears once the effect of pH on mortality is accounted for. No difference in fish survival among streams could be directly attributed to Ca (see also Table 6). High concentrations of Ca ameliorate the toxic effects of acid–Al in fish through the action of Ca on fish gill permeability (Brown 1983; Playle and Wood 1989); the threshold at which Ca reduces gill membrane permeability is often quoted as 1.0 mg/L for brown trout (McWilliams 1982). Mean Ca concentrations exceeded this threshold for all trials in our juvenile trout study, and for 24 of 25 trials Ca remained above that threshold throughout the incubation period (Table 3). This may explain the lack of a strong response to variation in Ca concentrations, since all trials in this study experienced sufficiently high levels to mitigate gill membrane permeability.

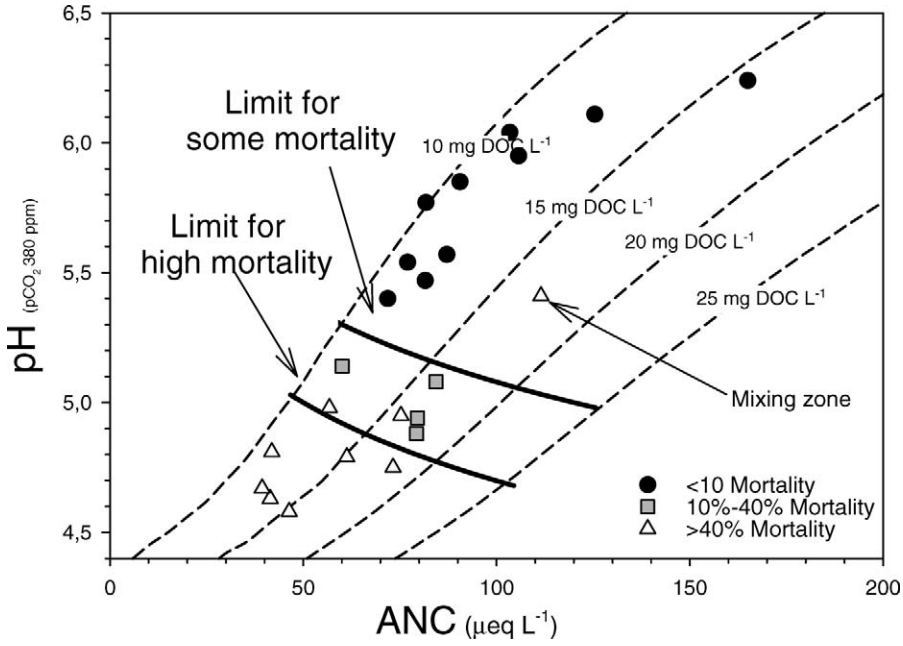

FIGURE 7.—Established relationships between acid-neutralizing capacity (ANC) and pH at different levels of dissolved organic carbon (DOC; dashed lines; Laudon et al. 2001. The response of brown trout was divided into (1) no mortality (<10%), (2) some mortality (10–40%), and (3) high mortality (>40%). The ANC:H^+ thresholds proposed by Laudon et al. (2005) for some mortality and high mortality of age-2 brown trout are shown as dark lines. The responses observed in our study of age-1 brown trout are shown as points, each representing one trial, broken down into the same three categories. Note that the calculation of ANC does not include the concentration of strong organic anions (Lawrence et al. 2007.)

A major goal of this investigation was to assess the role of DOC on the survival of brown trout during episodic acidification. Dissolved organic carbon is reported to reduce the effects of Al on fish due to the organic binding of Al (Wilkinson et al. 1993) and it has been hypothesized that DOC could prevent the precipitation or polymerization of inorganic Al in the gill microenvironment (Bertsch and Parker 1996; Roy and Campbell 1997; Gensemer and Playle 1999). In one of the few field experiments studying salmonid mortality during acid episodes in DOC-rich streams, acute toxicity to Atlantic salmon *Salmo salar* was attributed to ionoregulatory effects caused only by H^+ concentration, not to accumulation of Al on the gills (Lacroix and Townsend 1987). Even at high concentrations, DOC is not regarded to have any direct deleterious effect on fish (Richards et al. 1999). High levels of DOC, however, promote an increase in the H^+ concentration (Eshleman and Hemond 1985; Hruška et al. 2001), which in this study contributed to a pH decline of up to 1.5 units in some of the streams (Buffam et al. 2007). Moreover, Lawrence et al. (2007) recently proposed that organic acidity contributes to the mobilization of inorganic aluminum. The biological impact of these conflicting effects is still unclear. However, Laudon et al. (2005) proposed a method of expressing acidity thresholds in a way that acknowledges the dual effects of DOC in humic-rich streams that also contain Al. They exposed age-2+ brown trout to snowmelt in six streams in northern Sweden, obtaining similar responses to our study, and found that the ANC:H^+ ratio worked very well (better than pH) in defining mortality thresholds (Laudon et al. 2005). This empirical relationship gives rise to a pH threshold which decreases as DOC increases, and, thus, could be a useful tool to describe acidity thresholds in DOC rich streams that also contain Al. From this model fit to bioassays with age-2+ brown trout, Laudon et al. (2005) suggested that the critical limit for high mortality (>40%) in surface waters with 10 mg/L DOC will be reached at pH 5.0, while the same threshold in water with 25 mg/L DOC will occur at pH 4.7 (Figure 7).

Analogous responses were observed in this study of age-1 juvenile brown trout and in the field experiments carried out by Laudon et al. (2005), which used age-2 and older brown trout. It does not appear from this comparison that age-1 brown trout are substantially more sensitive than older trout. Only in site 13 during the first trial was mortality higher than expected based

on Laudon et al.'s (2005) thresholds. A possible explanation could be that the cages at site 13 were placed downstream (15 m) of the confluence of an acidic, Al-containing stream (pH = 5.7 ± 0.1, Al_{tot} = 360 ± 220 μg/L, $n = 4$) with a more neutral stream (pH = 6.1 ± 0.5, $n = 4$). In mixing zone areas such as this, high mortality related to Al speciation has been reported even though the mixed medium has a pH above that known to cause toxic effects (Muniz and Leivestad 1980; Poléo et al. 1994; Verbost et al. 1995).

In conclusion, our study shows that high concentrations of DOC and low pH during the spring flood can provide serious constraints for brown trout. The impact of reduced juvenile survival due to acidic events (see Figure 6) in brown trout populations should be taken into consideration for effective management. The effect of acidic episodes on abundance and the related effect on population structuring are still not well understood and constitute an obvious subject for study.

Similar studies in systems with lower DOC concentrations than the ones observed in our streams clearly indicate that Al_i is the primary source of stress and death for wild salmonids (Johnson et al. 1987; Baldigo et al. 2007). In DOC-rich waters it is possible that pH, not Al_i, places a primary constraint on the survival of brown trout, even if the uncertainty in the determination of Al_i makes it impossible to discard it as a potential toxic factor for fish in the region. It appears that in organic-rich boreal streams the concentrations of Al_i are either too low to exert a toxic effect on brown trout or the analytical uncertainty makes it difficult to establish a link with fish mortality. We suggest that pH is a biologically relevant factor that can be used to establish thresholds for the survival of brown trout during the spring flood in high-DOC streams.

Acknowledgments

The authors gratefully acknowledge Peder Blomkvist, Elon Manfredsson, and Martin Wiss for field and laboratory assistance. We extend our appreciation to Barry P. Baldigo and two anonymous reviewers for their helpful comments. This research was funded by the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning (FORMAS) and the Knut and Alicia Wallenberg Foundation. Researchers and staff at the Vindeln Experimental Forest Research Station provided logistical support and research infrastructure. All experiments were approved by the Swedish Animal Ethical Board.

References

Ågren, A., I. Buffam, M. Jansson, and H. Laudon. 2007. Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. Journal of Geophysical Research [online serial] 112: G03003.

Andersson, B., and P. Andersson. 1984. The distribution of trout (*Salmo trutta* L.) in relation to pH: an inventory of small streams in Delsbo, central Sweden. Institute of Freshwater Research Drottningholm Report 61:28–33.

Andrén, C. 1995. Aluminium speciation: effects of sample storage. Water, Air, and Soil Pollution 85:811–816.

Åtland, Å. 1998. Behavioural responses of brown trout, *Salmo trutta*, juveniles in concentration gradients of pH and Al: a laboratory study. Environmental Biology of Fishes 53:331–345.

Baker, J. P., and C. L. Schofield. 1982. Aluminium toxicity to fish in acidic waters. Water, Air, and Soil Pollution 18:289–309.

Baker, J. P., J. Van Sickle, C. J. Gagen, D. R. DeWalle, W. E. Sharpe, R. F. Carline, B. P. Baldigo, P. S. Murdoch, D. W. Bath, W. A. Kretser, H. A. Simonin, and P. J. Wigington, Jr. 1996. Episodic acidification of small streams in the northeastern United States: effects on fish populations. Ecological Applications 6:422–437.

Baldigo, B. P., G. Lawrence, and H. A. Simonin. 2007. Persistent mortality of brook trout in episodically acidified streams of the southwestern Adirondack Mountains, New York. Transactions of the American Fisheries Society 136:121–134.

Baldigo, B. P., and P. S. Murdoch. 1997. Effect of stream acidification and inorganic aluminum on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York. Canadian Journal of Fisheries and Aquatic Sciences 54:603–615.

Barlaup, B. T., and Å. Åtland. 1996. Episodic mortality of brown trout (*Salmo trutta* L.) caused by sea-salt-induced acidification in western Norway: effects on different life stages within three populations. Canadian Journal of Fisheries and Aquatic Sciences 53:1835–1843.

Bertsch, P. M., and D. R. Parker. 1996. Aqueous polynuclear aluminum species. Pages 117–168 *in* G. Sposito, editor. The environmental chemistry of aluminum. Lewis Publishers, New York.

Birchall, J. D., C. Exley, J. S. Chappell, and M. J. Phillips. 1989. Acute toxicity of aluminum to fish eliminated in silicon-rich acid waters. Nature (London) 338:146–148.

Bishop, K. H., H. Laudon, and S. Köhler. 2000. Separating the natural and anthropogenic components of spring flood pH decline: a method for areas that are not chronically acidified. Water Resources Research 36:1873–1884.

Brown, D. J. A. 1983. Effect of calcium and aluminium concentrations on survival of brown trout (*Salmo trutta*) at low pH. Bulletin of Environmental Contamination and Toxicology 30:582–587.

Brown, D. J. A., and S. Lynam. 1981. The effect of sodium and calcium concentrations on the hatching of eggs and the survival of the yolk sac fry of brown trout, *Salmo trutta* L. at low pH. Journal of Fish Biology 19:205–211.

Buckler, D. R., L. Cleveland, E. E. Little, and W. G. Brumbaugh. 1995. Survival, sublethal responses, and tissue residues of Atlantic salmon exposed to acidic pH and aluminum. Aquatic Toxicology 31:203–216.

Buffam, I., H. Laudon, J. Temnerud, C.-M. Mörth, and K. Bishop. 2007. Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal

stream network. Journal of Geophysical Research [online serial] 112:G01022.

Çalta, M. 2002. Does brown trout (*Salmo trutta* L.) larval development retardation caused by short-term exposure to low pH and elevated aluminum concentration affect a second episode of toxicity? Journal of Applied Ichthyology 18:210–215.

Carline, R. F., D. R. De Walle, W. E. Sharpe, B. A. Dempsey, C. J. Gagen, and B. Swistock. 1992. Water chemistry and fish community responses to episodic stream acidification in Pennsylvania, USA. Environmental Pollution 78:45–48.

Cory, N., I. Buffam, H. Laudon, S. Köhler, and K. Bishop. 2006. Landscape control of stream water aluminum in a boreal catchment during spring flood. Environmental Science and Technology 40:3494–3500.

Crisp, D. T. 1981. A desk study of the relationship between temperature and hatching time for the eggs of five species of salmonid fishes. Freshwater Biology 11:361–368.

Degerman, E., and P.-E. Lingdell. 1993. pHisces: fisk som indikator på lågt pH. [pHisces: the fish fuana as an indicator of low ph.] Information från Sötvattenslaboratoriet, Drottningholm 3:37–54.

Driscoll, C. T. 1984. A procedure for the fractionation of aqueous aluminium in dilute acidic waters. Internal Journal of Environmental Analytical Chemistry 16:267–283.

Driscoll, C. T., J. P. Baker, J. J. Bisogni, and C. I. Schofield. 1980. Effect of aluminium speciation on fish in dilute acidified waters. Nature (London) 284:161–164.

Duis, K., and A. Oberemm. 2000. Sensitivity of early life stages of vendace, *Coregonus albula*, to acid pH in postmining lakes: an experimental approach. Environmental Toxicology 15:214–224.

Elliott, J. M., U. H. Humpesch, and M. A. Hurley. 1987. A comparative study of 8 mathematical models for the relationship between water temperature and hatching time of eggs of freshwater fish. Archiv für Hydrobiologie 109:257–277.

Eshleman, K. N., and H. F. Hemond. 1985. The role of organic acids in the acid–base status of surface waters at Bickford watershed, Massachusetts. Water Resources Research 21:1503–1510.

Exley, C. A., J. S. Chappell, and J. D. Birchall. 1991. A mechanism for acute aluminium toxicty in fish. Journal of Theoretical Biology 151:417–427.

Fivelstad, S., and H. Leivestad. 1984. Aluminium toxicity to Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.): mortality and physiological response. Institute of Freshwater Research Drottningholm Report 61:69–77.

Gensemer, R. W., and R. C. Playle. 1999. The bioavailability and toxicity of aluminum in aquatic environments. Critical Reviews in Environmental Science and Technology 29:315–450.

Havas, M., and B. O. Rosseland. 1995. Response of zooplankton, benthos, and fish to acidification: an overview. Water, Air, and Soil Pollution 85:51–62.

Hesthagen, T., J. Heggenes, B. M. Larsen, H. M. Berger, and T. Forseth. 1999. Effects of water chemistry and habitat on the density of young brown trout *Salmo trutta* in acidic streams. Water, Air, and Soil Pollution 112:85–106.

Holtze, K. E., and N. J. Hutchinson. 1989. Lethality of low pH and Al to early life stages of 6 fish species inhabiting Precambrian shield waters in Ontario. Canadian Journal of Fisheries and Aquatic Sciences 46:1188–1202.

Howells, G. D., D. J. A. Brown, and K. Sadler. 1983. Effects of acidity, calcium, and aluminium on fish survival and productivity: a review. Journal of the Sciences of Food and Agriculture 34:559–570.

Hruška, J., H. Laudon, C. E. Johnson, S. Köhler, and K. Bishop. 2001. Acid–base character of organic acids in a boreal stream during snowmelt. Water Resources Research 37:1043–1056.

Hurley, G. V., T. P. Foyle, and W. J. White. 1989. Differences in acid tolerance during the early life stages of 3 strains of brook trout, *Salvelinus fontinalis*. Water, Air, and Soil Pollution 46:387–398.

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*). Canadian Journal of Fisheries and Aquatic Sciences 47:1580–1592.

Ivarsson, H., and M. Jansson. 1994. Regional variation of dissolved organic matter in running waters in central northern Sweden. Hydrobiologia 286:37–51.

Jeffries, D. S., C. M. Cox, and P. J. Dillon. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. Journal of the Fisheries Research Board of Canada 36:640–646.

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.

Julien, H. P., and N. E. Bergeron. 2006. Effect of fine sediment infiltration during the incubation period on Atlantic salmon (*Salmo salar*) embryo survival. Hydrobiologia 563:61–71.

Köhler, S., J. Hruška, and K. Bishop. 1999. Influence of organic acid site density on pH modelling of Swedish lakes. Canadian Journal of Fisheries and Aquatic Sciences 56:1461–1470.

Köhler, S., H. Laudon, A. Wilander, and K. Bishop. 2000. Estimating organic acid dissociation in natural surface waters using total alkalinity and TOC. Water Research 34:1425–1434.

Kugel, B., and R. H. Peterson. 1989. Perivitelline fluid pH of rainbow trout (*Oncorhynchus mykiss*) eggs in relation to ambient pH. Canadian Journal of Fisheries and Aquatic Sciences 46:2070–2073.

Lacroix, G. L. 1989. Ecological and physiological responses of Atlantic salmon in acidic organic rivers of Nova Scotia, Canada. Water, Air, and Soil Pollution 46:375–386.

Lacroix, G. L., and D. R. Townsend. 1987. Responses of juvenile Atlantic salmon (*Salmo salar*) to episodic increases in acidity of Nova Scotia rivers. Canadian Journal of Fisheries and Aquatic Sciences 44:1475–1484.

Laudon, H., S. Köhler, and I. Buffam. 2004. Seasonal TOC export from seven boreal catchments in northern Sweden. Aquatic Sciences 66:223–230.

Laudon, H., A. B. S. Poléo, L. A. Vollestad, and K. Bishop. 2005. Survival of brown trout during spring flood in DOC-rich streams in northern Sweden: the effect of

present acid deposition and modelled preindustrial water quality. Environmental Pollution 135:121–130.

Laudon, H., O. Westling, and K. Bishop. 2000. Cause of pH decline in stream water during spring melt runoff in northern Sweden. Canadian Journal of Fisheries and Aquatic Sciences 57:1888–1900.

Lawless, J. F. 1982. Statistical models and methods for lifetime data. Wiley, New York.

Lawrence, G. B., J. W. Sutherland, C. W Boylen, S. A. Nierzwicki-Bauer, B. Momen, K. M. Roy, B. P. Baldigo, H. A. Simonin, and S. B. Capone. 2007. Acid rain effects on aluminum mobilization clarified by inclusion of strong organic acids. Environmental Science and Technology 41:93–98.

Leivastad, H., and I. Muniz. Fish kills at low pH in a Norwegian river. Nature 259:391–392.

Lydersen, E., S. Löfgren, and R. T. Arnesen. 2002. Metals in Scandinavian surface waters: effects of acidification, liming, and potential reacidification. Critical Reviews in Environmental Science and Technology 32:73–295.

McCahon, C. P., A. F. Brown, M. J. Poulton, and D. Pascoe. 1989. Effects of acid, aluminum, and lime additions on fish and invertebrates in a chronically acidic Welsh stream. Water, Air, and Soil Pollution 45:345–359.

McCormick, J. H., and R. L. Leino. 1999. Factors contributing to first-year recruitment failure of fishes in acidified waters with some implications for environmental research. Transactions of the American Fisheries Society 128:265–277.

McWilliams, P. G. 1982. The effects of calcium on sodium fluxes in the brown trout, *Salmo trutta*, in neutral and acidic waters. Journal of Experimental Biology 96:439–442.

Muniz, I. P., and H. Leivestad. 1980. Toxic effects of aluminium on the brown trout, *Salmo trutta* L. Pages 320–321 *in* D. Drabløs and A. Tollan, editors. Proceedings of the international conference on the ecological impact of acid precipitation. SNFS project. Oslo-Ås, Norway.

Ottosson Löfvenius, M., M. Kluge, and T. Lundmark. 2003. Snow and soil frost depth in two types of shelterwood and a clear-cut area. Scandinavian Journal of Forest Research 18:54–63.

Parker, D. B., and B. A. McKeown. 1987. The effects of low pH on egg and alevin survival of kokanee and sockeye salmon, *Oncorhynchus nerka*. Comparative Biochemistry and Physiology 87C:259–268.

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 acidic water toxicity to brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 47:1631–1640.

Peterson, R. H., P. G. Daye, and J. L. Metcalfe. 1980. Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. Canadian Journal of Fisheries and Aquatic Sciences 37:770–774.

Petrone, K., I. Buffam, and H. Laudon. 2007. Hydrologic and biotic control of nitrogen export during snowmelt: a combined conservative and reactive tracer approach. Water Resources Research [online serial] 43:W06420.

Playle, R. C., and C. M. Wood. 1989. Water chemistry changes in the gill microenvironment of rainbow trout: experimental observations and theory. Journal of Comparative Physiology B 159(5):527–537.

Poléo, A. B. S., E. Lyderson, B. O. Rosseland, F. Kroglund, B. Salbu, R. D. Vogt, and A. Kvellestad. 1994. Increased mortality of fish due to changing Al-chemistry of mixing zones between limed streams and acidic tributaries. Water, Air, and Soil Pollution 75:339–351.

Prentice, R. L. 1976. A generalization of the probit and logit methods for dose response curves. Biometrics 32:761–768.

Reader, J. P., T. R. K. Dalziel, R. Morris, M. D. J. Sayer, and C. H. Dempsey. 1991. Episodic exposure to acid and aluminium in soft water: survival and recovery of brown trout, *Salmo trutta* L. Journal of Fish Biology 39:181–196.

Richards, J. G., B. K. Burnison, and R. C. Playle. 1999. Natural and commercial dissolved organic matter protects against the physiological effects of a combined cadmium and copper exposure on rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 56:407–418.

Rosseland, B. O., and O. K. Skogheim. 1984. A comparative study on salmonid fish species in acid aluminium-rich water, II. Physiological stress and mortality of one- and two-year-old fish. Institute of Freshwater Research Drottningholm Report 61:186–194.

Roy, R. L., and P. G. C. Campbell. 1997. Decreased toxicity of Al to juvenile Atlantic salmon (*Salmo salar*) in acidic soft water containing natural organic matter: a test of the free-ion model. Environmental Toxicology and Chemistry 16:1962–1969.

Sadler, K., and S. Lynam. 1988. The influence of calcium on aluminium-induced changes in the growth rate and mortality of brown trout, *Salmo trutta* L. Journal of Fish Biology 33:171–179.

Sayer, M. D. J., J. P. Reader, and R. Morris. 1991. Embryonic and larval development of brown trout, *Salmo trutta* L.: exposure to aluminium, copper, lead, or zinc in soft, acid water. Journal of Fish Biology 38:431–455.

Sharpe, W. E., D. R. DeWalle, R. T. Leibfried, R. S. Dinicola, W. G. Kimmel, and L. S. Sherwin. 1984. Causes of acidification of four streams on Laurel Hill in southwestern Pennsylvania. Journal of Environmental Quality 13:619–631.

Simonin, H. A., W. A. Kretser, D. W. Bath, M. Olson, and J. Gallagher. 1993. In situ bioassays of brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in Adirondack streams affected by episodic acidification. Canadian Journal of Fisheries and Aquatic Sciences 50:902–912.

Skogheim, O. K., and B. O. Rosseland. 1984. A comparative study on salmonid fish species in acid aluminium-rich water, I. Mortality of eggs and alevins. Institute of Freshwater Research Drottningholm Report 61:177–185.

Soulsby, C., A. F. Youngson, H. J. Moir, and I. A. Malcolm. 2001. Fine sediment influence on salmonid spawning habitat in a lowland agricultural stream: a preliminary assessment. Science of the Total Environment 265:295–307.

Sparling, D. W., and T. P. Lowe. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and

wildlife. Reviews of Environmental Contamination and Toxicology 145:1–127.

Teien, H. C., B. Salbu, F. Kroglung, L. S. Heier, and B. O. Rosseland. 2007. The influence of colloidal material on aluminium speciation and estimated acid neutralising capacity (ANC). Applied Geochemistry 22:1202–1208.

Vanoffelen, H. K., C. C. Krueger, C. L. Schofield, and C. Keleher. 1994. Survival, distribution, and ion composition in 2 strains of brook trout (*Salvelinus fontinalis*) fry after exposure to episodic pH depressions in an Adirondack lake. Canadian Journal of Fisheries and Aquatic Sciences 51:792–799.

Van Sickle, J., J. P. Baker, H. A. Simonin, B. P. Baldigo, W. A. Kretser, and W. E. Sharpe. 1996. Episodic acidification of small streams in the northeastern United States: fish mortality in field bioassays. Ecological Applications 6:408–421.

Verbost, P. M., M. H. G. Berntssen, F. Kroglund, E. Lyderson, H. E. Witters, B. O. Rosseland, B. Salbu, and S. E. Wendelaar Bonga. 1995. The toxic mixing zone of neutral and acidic river water: acute aluminium toxicity in brown trout (*Salmo trutta* L.). Water, Air, and Soil Pollution 85:341—346.

Weatherley, N. S., A. P. Rogers, X. Goenaga, and S. J. Ormerod. 1990. The survival of early life stages of brown trout (*Salmo trutta* L.) in relation to aluminum speciation in upland Welsh streams. Aquatic Toxicology 17:213–230.

Weatherley, N. S., S. P. Thomas, and S. J. Ormerod. 1989. Chemcal and biological effects of acid, aluminium, and lime additions to a Welsh hillstream. Environmental Pollution 56:283–297.

Wigington, P. J., T. D. Davies, M. Tranter, and K. N. Eshleman. 1992. Comparison of episodic acidification in Canada, Europe, and the United States. Environmental Pollution 78:29–35.

Wilkinson, K. J., P. M. Bertsch, C. H. Jagoe, and P. G. C. Campbell. 1993. Surface complexation of aluminum on isolated fish gill cells. Environmental Science and Technology 27:1132–1138.

Wilkinson, K. J., P. G. C. Campbell, and P. Couture. 1990. Effect of fluoride complexation on aluminum toxicity towards juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 47:1446–1452.

Witters, H. E., S. Van Puymbroeck, J. H. D. Vangenechten, and O. L. J. Vanderborght. 1990. The effect of humic substances on the toxicity of aluminium to adult rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 37:45–53.

Wood, C. M., and D. G. McDonald. 1987. The physiology of acid aluminum stress in trout. Annales de la Societe Royale Zoologique de Belgique 117:399–410.