Survival of Atlantic Salmon (*Salmo salar* L.) and Brown Trout (*Salmo trutta* L.) Exposed to Episodic Acidification during Spring Flood

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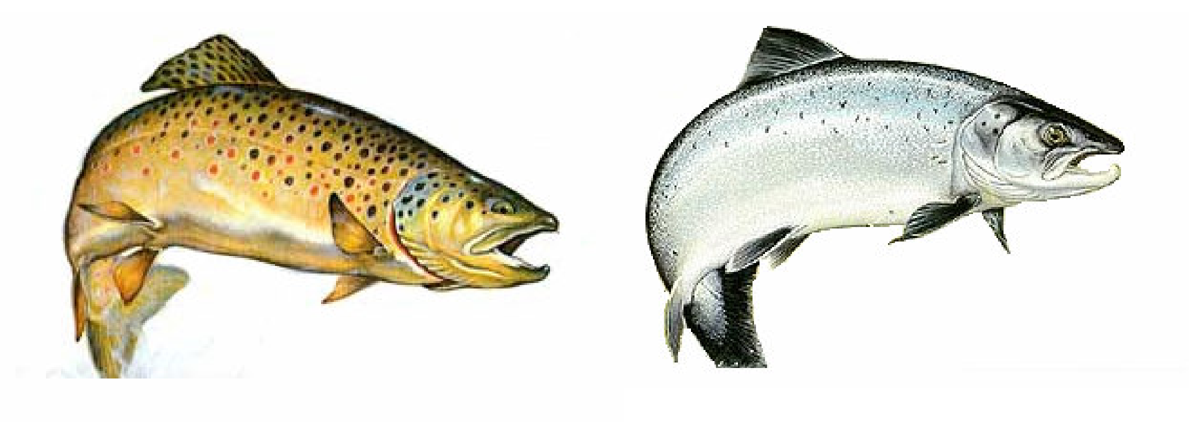

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ABSTRACT

This study was designed to evaluate the survival of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) parr (1+) during the spring snow melt period in order to identify variables that best explain differences in fish survival among streams, and to define critical toxicity thresholds in organic rich waters. The experiments were carried out in 10 streams in northern Sweden. Different hydrological parameters were monitored continuously, and water samples for chemical analysis were collected throughout the experimental period. During the spring snow melt, DOC concentration increased strongly, causing a natural depletion of pH and an increase in total aluminium, which resulted in high fish mortality. Atlantic salmon was more sensitive than brown trout to acidic water in all streams during the experiments. Also, the mortality data confirmed that pH and ANC/H^+ ratio constitute good predictors of Atlantic salmon and brown trout survival. The results also suggest that fish in DOC-rich streams can tolerate higher acidity and inorganic aluminium levels than fish in low DOC systems. Accordingly, a critical chemical threshold of pH (5.9 and 5.1) can be defined for Atlantic salmon and brown trout populations, respectively, in humic rich waters (DOC > 10 mg/l), about 0.3 pH units lower than for humic poor waters.

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SAMMANFATTNING

Överlevnaden av lax (*Salmo salar*) och öring (*Salmo trutta*) under tillfälliga pH-sänkningar under snösmältningen studerades i tio vattendrag i norra Sverige. Olika hydrologiska parametrar mättes kontinuerligt och vattenprover för laboratorieanalys insamlades under försöksperioden. Undersökningens syfte var att utvärdera dödligheten hos öring- och laxyngel (1+) under snösmältningen genom fältstudier för att identifiera vilka variabler som bäst förklarar skillnader i överlevnad i vattendrag. Syftet var också att bestämma kritiska toxicitets gränser i vattendrag med naturligt höga DOC-halter. Under vårens smältperiod ökade koncentrationen av DOC kraftigt vilket orsakade en naturlig pH-sänkning och en ökning av aluminiumhalter. Detta resulterade i hög dödlighet hos den studerade fisken. Undersökningen visar att lax var mindre motståndskraftig mot låga pH-värden i vattnet jämfört med öring. Det insamlade materialet bekräftar även att pH och ANC/H^+ kvoten utgör goda indikatorer på överlevnaden hos lax och öring. Studien indikerar vidare att fisk i vattendrag med höga DOC halter har högre överkevbad låga pH värden och oorganiska aluminiumhalter än fisk i vattendrag med låga DOC halter. Resultaten visar också att en kritisk kemisk pH-nivå kan fastställas för lax och öring i humusrika vattendrag (DOC >10mg/l). Enligt de kemiska observationerna av vattendragen indikerar detta att ca 40 % respektive 10 % av Krycklans 68 km^2 stora avrinningsområde inte är lämpliga för lax och öring.

INTRODUCTION

Episodic acidification, the short term decrease of pH during hydrological events associated with rainstorms and snowmelt periods (Wigington et al., 1992) represents, in small catchments in northern Sweden, one of the most important natural transient processes affecting the biodiversity of aquatic ecosystems (Laudon et al., 2005). The toxicity to aquatic organisms 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 aluminium on gill function or structure (reviewed by Havas and Rosseland, 1995; Gensemer and Playle, 1999). Aqueous aluminium (Al) occurs in many different forms with varying toxicity but inorganic monomeric aluminium species (Al_i) are generally considered the most toxic forms to fish (Poléo, 1995; Gensemer and Playle, 1999). At lethal concentrations, H^+ acts principally on the permeability of the cell membrane causing ion regulatory disorders, whereas aluminium accumulates on and in the gill tissue, disrupting ion regulation and impairing respiration (Kroglund and Finstad, 2003). Respiratory dysfunction seems to dominate at pH above 5.5 (Neville, 1985) and ion regulatory disturbances predominate at pH below 4.5 (Gensemer and Playle, 1999). At sublethal concentrations, different responses have been described, such as impairing chemoreception, reproduction failure, etc. (Havas and Rosseland, 1995). Toxicity is modified by physico-chemical parameters such as DOC, calcium, fluoride, citrate, and silicon (Driscoll et al., 1980, Lydersen et al., 2002a), but is also related to fish species and life stages (MacCormik and Leino, 1999). A species specific tolerance to low pH may explain the habitat distribution of different species of fish. Atlantic salmon represents the most sensitive species to acidic water (Grande et al., 1978), and particularly to acidic Al-rich water amongst the naturally occurring salmonids in Europe (Rosseland and Skogheim, 1986; Poléo et al., 1997). A critical chemical limit of pH (6.2) and Al_i (15-20 µg/l) has been defined for Atlantic salmon in soft waters (0.7-2.3 mg Ca/l) (Staurnes et al., 1993; Kroglund and Staurnes, 1999; Rosseland et al., 2001). Atlantic salmon generally inhabit the main stream of rivers and the larger tributaries, where the pH fluctuations are small. Brown trout is one of the most tolerant salmonids to acidification (Poléo, 1997) and inhabit smaller tributaries, with higher pH fluctuations. At pH below 5.0 and Al_i concentrations above the range 100 µg/l have been shown to cause significant mortality (Fivelstad and Leivestad, 1984; Barlaup and Åtland, 1996), although it is difficult to set critical chemical limits for this species, since there is no agreement between the observed toxic response to acidic Al-rich water among different studies (Howells et al., 1990, Dalziel et al. 1995), due probably to high genetic variability within and between populations of brown trout for tolerance to acid water (Rosseland et al., 2001).

The aim of the investigation was to assess fish response during episodic acidification through several objectives: (1) To monitor the water chemistry of streams that undergo episodic acidification, (2) to determine the survival of brown trout and Atlantic salmon parr (1+) during the spring snow melt by conducting in situ bioassays, and (3) to identify variables that best explain differences in fish survival among streams, defining pH and Al_i toxicity thresholds in naturally high DOC waters.

Mechanisms of H^+ and Al-toxicity to fish

Freshwater fishes exchange sodium for hydrogen ions and chloride for bicarbonate, which allow the animal to make acid–base adjustments and to maintain ion and osmoregulatory homeostasis by extracting NaCl from the dilute environment (Claiborne et al, 2002). Increased hydrogen ion activity in the surrounding medium impedes the active uptake of sodium and stimulates 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).

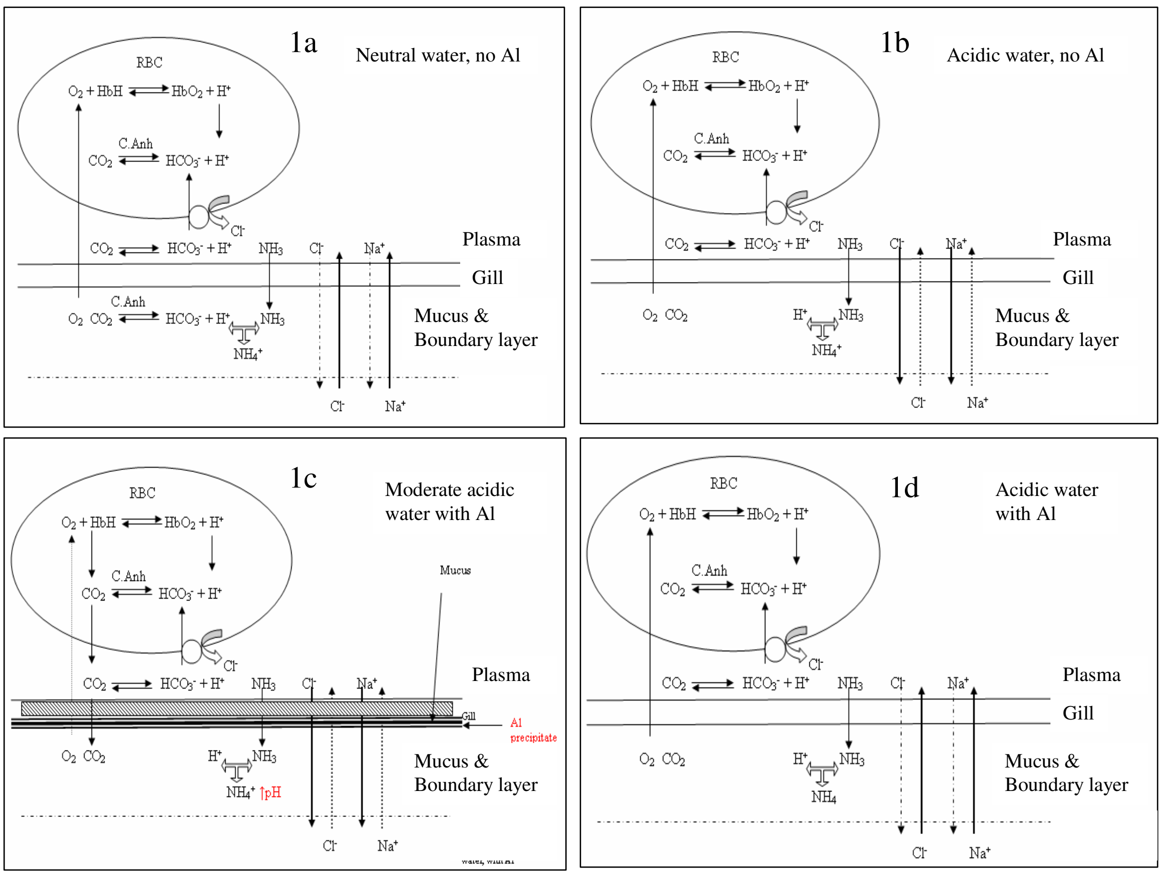


Figure 1. Model of Al interactions at fish gills, modified from Gensemer & Playle (1999). RBC= red blood cell. 1. A Neutral conditions in the absence of Al. 1.b Moderate acidic conditions (pH 5-6) in the absence of Al. 1.c Moderate acidic conditions (pH 5-6) in the presence of Al (1.d). Very acidic conditions (pH< 5) in the presence of Al. In acidic water (b) H^+ ions impede the active uptake of sodium and stimulate efflux, which bring about net losses of electrolytes (especially Na+ and Cl-) across the gills. Moderate acidity (pH 5 to 6) in the presence of Al (c) is when toxicity is most severe for fish in soft waters. pH increase in the gill microenvironment (due to NH3 excretion) causes Al-hydroxides (the main form of dissolved Al at this pH range) to accumulate on the gill surface. In very acid soft waters (d), the accumulation of Al is slow due to competition with H^+ for binding sites (d). In such case, Al can reduce the toxic effects caused by H^+ concentration alone (Neville, 1985).

Aluminium exposure in fish affects gill function, causing respiratory and ion regulatory disorders. Whereas the consequences of Al are characterized to some extent, the mechanisms underlying Al-toxicity are not completely understood. There are two major theories to explain the mechanisms of Al-toxicity to fish. According to Exley et al. (1991), positively charged Al-hydroxides bind to the external surface of fish gills (mucus and epithelia), rich in negatively charged sites, increasing gill permeability, with a consequent alternation in transmembranal ion fluxes, which in turn allows Al^{3+} to accumulate intracellularly in epithelial cells, causing a disruption in cytosolic calcium homeostasis leading to cell sloughing and death (Exley et al. 1991). Poléo (1995) suggests that the process of Al polymerization is the mechanism that explains acute toxicity of aluminium to fish, especially above pH 5.0 or after a rise in pH. According to his theory, Al-hydroxides bind to functional groups at the gill surface and then these polymerize (grow) and age on the gill surface. This, together with the associated increased mucus secretion cause severe clogging of the interlamellar spaces (Poléo, 1995). The primary consequences are a reduced water flow over the respiratory surfaces, and suffocation due to the increased thickness of the diffusion barrier for gases and ions. Hypoxia and acidosis will in turn cause increased ventilation and enhance O_2 consumption. Figure 1 describes Al-gill interaction trying to incorporate both theories.

Parameters conditioning the toxicity of pH and Aluminium to fish

Aluminium toxicity is mainly dependent on factors that influence Al chemistry, such as water pH, temperature, calcium, dissolved organic carbon (DOC), fluoride, citrate, and silicon (Driscoll et al. 1980, Poléo et al. 1991).

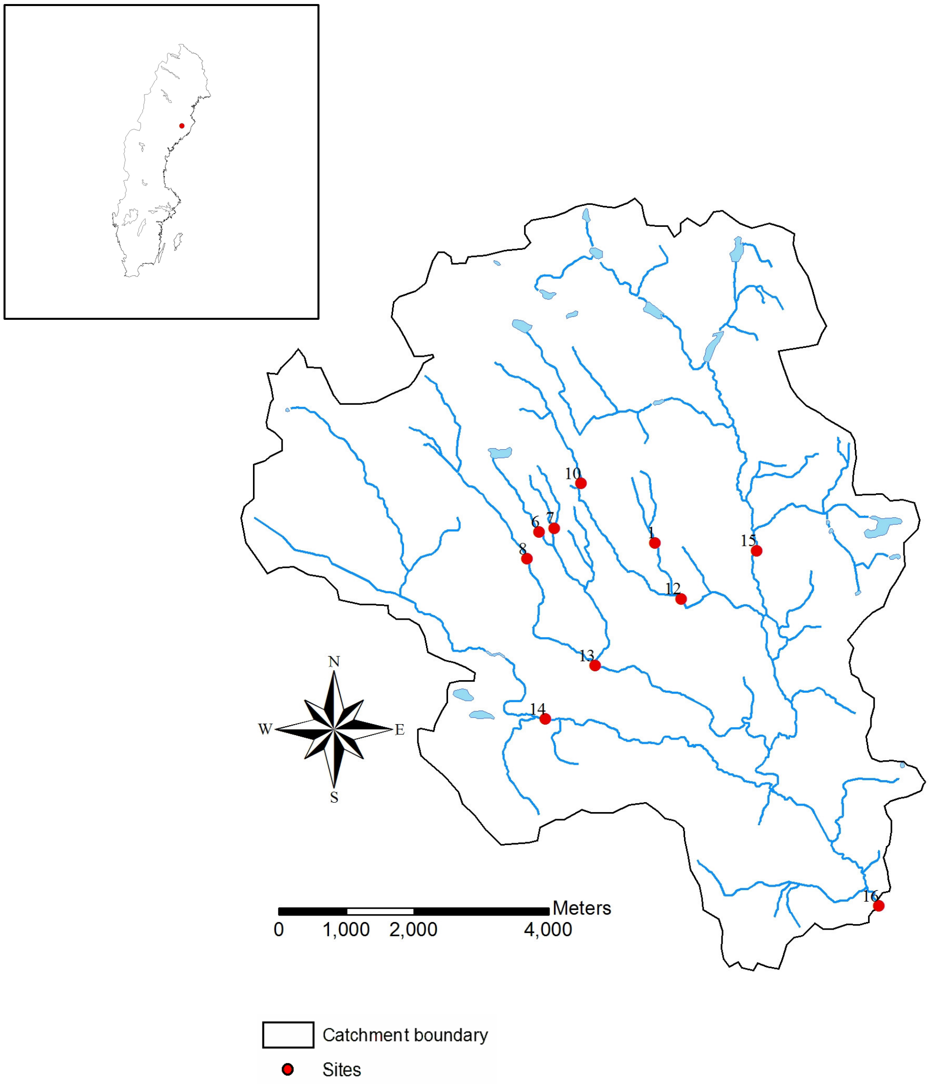
High concentrations of Ca^{2+} and, to a lesser extent, other cations, have been reported to reduce Al-toxicity in fish, through its effect on fish gill permeability (Brown, 1983; Playle and Wood, 1989), and it has been proposed that increased ionic strength may reduce Al-toxicity both by impairing the ability of aluminium to bind to the gill by ionic bonds, and by competition between base cations and positively charged aluminium for negatively charged sites on the gill surface (Lydersen et al., 2002b). It is well documented that DOC generally reduces the effects of aluminium on fish due to the organic binding of aluminum (Poléo et al. 1991) and prevents the precipitation or polymerization of inorganic Al in the more alkaline gill microenvironment (Roy and Campbell, 1997). These examples, however, come largely from low DOC systems. In the humic rich water of northern Sweden, high levels of DOC promote an increase in the H^+ concentration that may result in natural pH declines of up to 2 units during the spring snow melt period (Bishop et al., 2000). Few studies have tried to evaluate the chemical impact on fish in high DOC watercourses, and the results suggest that fish in these streams show higher tolerance to acidic Al-rich waters than fish low DOC systems (Laudon et al., 2005). As well as pH, temperature affects the solubility, the hydrolysis and molecular weight distribution of aqueous Al species (Lydersen et al., 1990). In concordance with this, it has been observed that Al toxicity is reduced at low temperatures (Poléo et al., 1991). Increased temperature, by increasing fish metabolism and decreasing O_2 solubility, can lead to hypoxia in fish already exposed to aqueous aluminium (Lydersen et al. 2002a). Fluoride and citrate are complexing agents that strongly inhibit Al polymerization and have been reported to reduce Al toxicity (Driscoll et al, 1980, Wilkinson et al. 1990), and silicic acid, through the formation of hydroxyl-aluminosilicate species in the alkaline gill environment, blocks the precipitation of Al-OH species on the gills (Birchall et al. 1989).

MATERIAL AND METHODS

Study Sites

This study was conducted on ten streams of the in the County of Västerbotten, northern Sweden (Figure 2). The elevation ranges from 235 m to 310 m above sea level, with slopes between 5% and 10% (Bishop, 1991) and an area of 68 ha. The climate is characterized by long winters and short summers. There is a continuous snow cover from the end of October until the beginning of May, which is followed by a growing season lasting from the end of May until the end of September. The mean annual precipitation is 600 mm, of which one-third falls as snow. During the period of study (from 22/4 to 16/6) the precipitation in the area was 64.5 mm (VFP, unpublished data). The annual mean temperature is 0 °C (20 year average). About a third of the annual runoff comes during the spring snowmelt period (Laudon, pers. comm.). Another period of increased runoff occurs in the autumn, associated to the rains which typically last from mid August till October.

The catchment is forested with Norway spruce (*Picea abies*) in the low elevation areas and Scots pine (*Pinus sylvestris*) at higher elevations (Anonymous, 1978). Cowberry, *Vaccinium vitis-idaea*, and bilberry, *Vaccinium myrtillus,* constitute the major components of the understory vegetation on the hillslopes, together with the lichen *Deschampsia flexuosa*. Deciduous trees (silver birch, *Betula pendula*, white birch, *Betula Pubescens*, grey alder, *Alnus incana* and willow, *Salix sp.*) are found along the streams, and mosses (*Sphagnum spp.* and *Polytrichum spp.*) dominate on the streambanks. Granite is the predominant bedrock in the catchment (Laudon & Bishop 2002) and the forest soils, mainly iron podzols, have developed on a till overburden that is several meters thick (Bishop 1994). These till soils have a high infiltration capacity, typical of many Swedish till catchments (Bishop, 1991). 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 glacier fluvial sediments are found further down in the valley (Eriksson, 2004).



Nr	Site Name	Stream Order	Area (km^2)	Water	Forest	Wetland	Agricultural
1	Risbäcken	1	0,66	0,0%	98,7%	1,3%	0,0%
6	Stortjärnbäcken	1	1,40	3,1%	72,8%	24,1%	0,0%
7	Kallkällsbäcken	2	0,50	0,0%	85,1%	14,9%	0,0%
8	Fulbäcken	2	2,48	0,0%	88,7%	11,3%	0,0%
10	Stormyrbäcken	2	2,94	0,0%	74,2%	25,8%	0,0%
12	Nymyrbäcken	3	5,40	0,0%	84,1%	15,5%	0,3%
13	Långbäcken	3	7,21	0,6%	89,1%	9,9%	0,4%
14	Åhedbäcken	3	13,60	0,6%	90,4%	5,1%	3,9%
15	Övre Krycklan	4	19,91	1,7%	83,2%	14,0%	1,0%
16	Krycklan	4	67,84	0,7%	88,0%	8,3%	3,0%

Figure 2. Map of the catchment of Krycklan and the stream study sites

Experimental Fish

1-year-old juveniles of Atlantic salmon, *Salmo salar* L., and Brown trout, *Salmo trutta* L., were obtained from a local hatchery. The hatchery water is untreated water from the Vindeln river. Thus, water chemistry at the hatchery varies throughout the year along with the variation in natural waters of the area. Water quality of the hatchery water is considered to be good, low in total aluminium, and with circumneutral pH (Table 4, appendices). Therefore, there is no reason to believe that prior exposure to aluminium may have resulted in acclimation and reduced toxicity in the fish. Fish kept at Umeå Marine Science Centre (UMF) acted as controls (Table 5, Appendices).

Exposure conditions

The study was carried out as 3 separate exposures of fish to the stream water during the spring of 2004. We exposed the two different species for two weeks at a time (11-16 days) during the period from April 2004 to June 2004 in 10 different streams. Trial one was started on April 20, trial two on May 4, and trial three on May 27. Last trial was carried out in only half of the streams (sites 1, 8, 10, 12, and 13). Approximately 10 fish from each species were placed in Whitlock-Vibert (W-V) hatching boxes in every stream (Figure 7, appendices), one cage for each individual. The boxes were placed in the middle depth of the stream. The midwater location was chosen to avoid disturbances of ice and debris, and to avoid desiccation of the fishes due to the extreme changes in water level during the initial snow melt. Fish were transported to the experimental sites in tanks with oxygenated freshwater (transport distance about 50 km) and thereafter transferred to the W-V boxes. The boxes were then placed inside a metal frame cage to protect them during periods of high stream flow. The test fish were not fed during the bioassays. Mortality was recorded every 24 or 48 h, and dead fish were removed from the cages and preserved by freezing (-20°C). Fish were considered to be dead when no swimming response could be elicited through stimulation of the lateral line organ. Fish surviving the two weeks exposure were killed by a blow to the head. In one site, due to the burial of the cages into the sediment by a branch, one of the bioassays had to be finished before the two week period. The control fish were placed individually in W-V boxes in a fish fence with a water current mimicking field conditions. All experiments were approved by the animal ethical board

Water Analysis

The sampling program was based on weekly sampling of base flow prior to the onset of spring flood, and then sampling every two days 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. pH was measured using a Ross 8102 combination electrode (ThermoOrion). Samples for dissolved organic carbon (DOC) analysis were filtered using 0.45 μm MCE membrane filters and then frozen until analysis. DOC was measured at Örebro University using a Shimadzu TOC-V_{PCH} analyser. Samples for major cation analyses (K, Mg, Na, Ca) were filtered (0.45 μm), preserved with ultrapure HNO_3 (1 % v/v) and stored cool until analysis by ICP-OES (inductively-coupled plasma optical emission spectroscopy) on a Varian instrument at Stockholm University. Samples for strong acid anions (SO_4^{2-} and Cl^-) were stored at 4 ºC until analysis, which utilized a Dionex DX-300 or DX-320 ion chromatograph system at Stockholm University. The typical precision in anions and cations analyses based on measurements of certified standards was better than 4%. 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 (ANC) was calculated as the difference between BC and the sum of SO_4^{2-} and Cl^- expressed as µeq/l of charge. Total and organic fractions of monomeric Aluminium were measured using ICP-OES at SLU, Uppsala (Department of Environmental Assessment). Organic monomeric Aluminium (Al_o) was separated using an ion exchange method (Driscoll 1984). Inorganic monomeric aluminium (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 Al and Al_o.

Statistical analysis

Analysis of the bioassay and water chemistry data was done with the statistical packages SPSS for windows 11.0 and MINITAB® Release 14.1. All water chemistry variables, except pH, were log transformed in order to improve linearity. Possible relationship among variables was examined using Pearson product moment correlation and evaluated by two-tailed tests. Exploratory multiple regression analysis was conducted using the Best Subsets Regression Maximum R^2 criterion procedure and including the following variables: mean pH, minimum pH, mean Ali, log (mean Al), maximum Al, log (peak Ali), mean DOC, log (mean DOC), mean Ca, log (mean Ca), mean ANC, and ANC/H^+. For the significant variables, probit analysis was used to test the influence of the various chemical parameters on fish mortality. This procedure measures the relationship between the strength of a stimulus and the proportion of cases exhibiting a certain response to the stimulus. Probit regression is a log-linear approach very useful to analyze dose-response data in experimental studies (Garson, 2001). Like in logit or logistic regression, the analysis determines which of the covariates are significant to explain the probability that Y, the dependent variable (probability of mortality in this case) equals 1. A logistic distribution function was used for probit analysis, since mortality showed a sigmoidal relationship to chemical data. The model can be written as in Finney (1971):

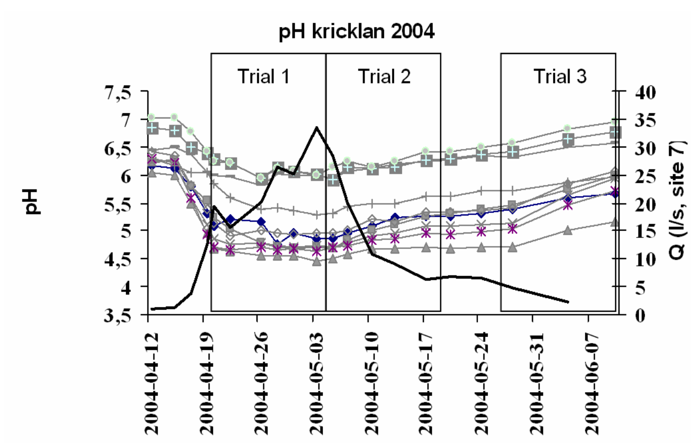
$\pi = g(\beta_0 + \beta_1 X_1 + ... + \beta_k X_k)$, where π is the probability of mortality=1, β are the coefficients of the probit model, and g (y) represents the logistic distribution function $g(y)= 1/(1+e^{-y})$.

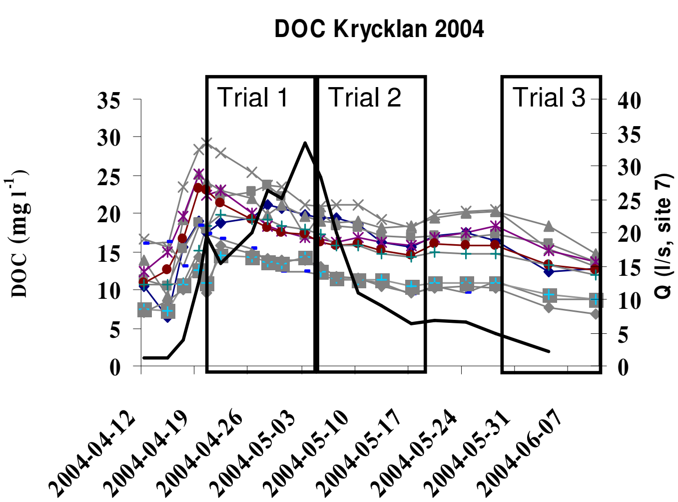
Finally, binary logistic regression was used to better define thresholds values for the significant parameters determining salmon and trout survival during short-time acidic episodes. The binary logistic equation is: $P= 1/(1+e^{-z})$, where $z= \beta_0 + \beta_1 X_1 + ... + \beta_k X_k$. $\mathbf{L_{T50}}$ (i.e. time to 50% mortality) below seven days qualified the mortality rate as high (class 0) and $\mathbf{L_{T50}}$ over 7 days were classified as class 1.

RESULTS

Water chemistry

The chemical parameters of the water varied greatly during the experimental period and within streams (Table 1). The water temperature remained low (≈ 0°C) although it increased slightly during the last bioassay period. The pH dropped up to 1.5 units in some of the streams during the experimental period (Figure 3.A), with an average pH decline of approximately 1 pH unit from baseflow to peak flow. There was also a clear drop in ANC during snowmelt, and a small increase in DOC matching the pH decline (Figure 3.B). The concentration of inorganic monomeric aluminium (Al_i) varied between 0 and 180 $\mu g/l^{-1}$ during the spring snowmelt period (Figure 3.C). The concentration of organic monomeric aluminium (Al_o) varied between 110 and 590 $\mu g\ l^{-1}$. Al_o constituted generally more than 70% of the amount of total Aluminum. Calcium concentration was high (over 2 mg/l) in all streams during baseflow, but it decreased substantially during the spring thaw in all streams (Figure 3.D).





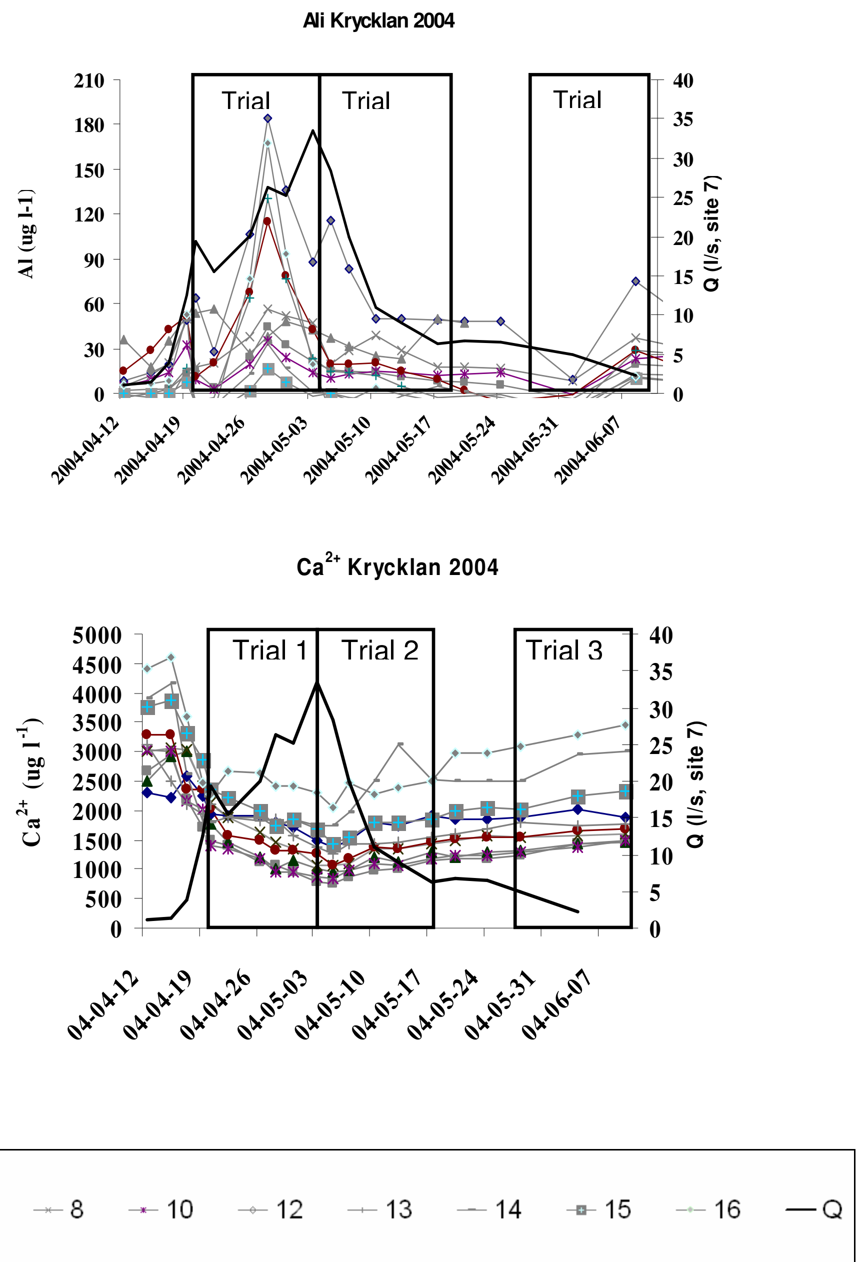


Figure 3. Variation in physico-chemical water parameters in Krycklan catchment during the spring snowmelt period: pH, DOC, Al_i and Ca^{2+}

Table 1. Values of the major chemical parameters measured in the stream water during the experimental period (Average ± SD)

Site	Run	Atlantic Salmon (Salmo salar) Initial N of fish	pH	Ali (µg/l)	DOC (mg/l)	Calcium (µg/l)	Brown Trout (Salmo trutta) Initial N of fish	pH	Ali (µg/l)	DOC (mg/l)	Calcium (µg/l)
1	1	8	4.94 ± 0.165	129 ± 42.0	20.303 ± 0.758	1728.88 ± 177.08	8	4.94 ± 0.165	129 ± 42	20.303 ± 0.758	1728.88 ± 177.08
6	1	7	4.94 ± 0.240	22 ± 16.9	21.636 ± 2.598	1270.94 ± 197.18	8	4.79 ± 0.148	25 ± 15	21.583 ± 2.622	1066.89 ± 227.32
7	1	8	4.55	35	21.890	1202.50	8	4.58 ± 0.044	35 ± 17	21.586 ± 1.638	1217.40 ± 226.97
8	1	7	4.77 ± 0.035	47 ± 12.7	24.535 ± 1.338	1542.31 ± 113.94	8	4.75 ± 0.033	48 ± 8	23.460 ± 1.754	1370.80 ± 236.41
10	1	8	4.68 ± 0.035	27 ± 11.0	19.154 ± 1.231	1061.93 ± 147.32	8	4.67 ± 0.025	26 ± 8	18.679 ± 1.198	1024.49 ± 122.71
12	1	8	4.96 ± 0.036	87 ± 25.0	18.269 ± 0.844	1381.70 ± 104.96	8	4.95 ± 0.038	70 ± 39	19.038 ± 1.685	1428.94 ± 127.57
13	1	8	5.48 ± 0.201	47 ± 53.0	18.833 ± 0.710	1768.33 ± 251.35	8	5.41 ± 0.117	58 ± 51	18.926 ± 0.752	1701.95 ± 214.29
14	1	8	5.96 ± 0.076	8 ± 16.0	14.641 ± 2.137	1813.98 ± 75.65	8	5.95 ± 0.082	11 ± 16	14.090 ± 1.853	1816.81 ± 84.23
15	1	8	6.04 ± 0.092	6 ± 8.2	13.912 ± 0.418	1819.75 ± 139.26	8	6.04 ± 0.092	6 ± 8	13.912 ± 0.418	1819.75 ± 139.26
16	1	8	6.13 ± 0.159	55 ± 52.8	13.335 ± 3.344	2528.85 ± 232.47	8	6.08 ± 0.184	32 ± 64	15.245 ± 0.683	2662.60 ± 27.53
1	2	10	5.14 ± 0.142	58 ± 16.8	17.434 ± 1.749	1739.63 ± 179.14	10	5.08 ± 0.177	70 ± 30	17.819 ± 1.742	1666.85 ± 224.84
6	2	9	4.92 ± 0.113	15 ± 0.7	18.242 ± 0.253	929.91 ± 89.41	10	4.98 ± 0.223	13 ± 3	18.120 ± 1.249	958.13 ± 145.73
7	2	10	4.62 ± 0.071	43 ± 7.4	18.840 ± 0.148	1095.47 ± 162.88	9	4.63 ± 0.084	38 ± 8	18.590 ± 0.400	1116.13 ± 144.71
8	2	10	4.93 ± 0.124	29 ± 8.6	19.901 ± 1.465	1306.60 ± 148.07	10	4.88 ± 0.145	27 ± 9	20.120 ± 1.360	1259.81 ± 165.51
10	2	10	4.80 ± 0.064	14 ± 1.3	16.476 ± 0.410	1042.80 ± 61.44	10	4.81 ± 0.104	13 ± 2	16.375 ± 0.462	1030.49 ± 130.72
12	2	10	5.12 ± 0.141	20 ± 0.4	15.949 ± 0.134	1271.03 ± 133.00	10	5.14 ± 0.147	16 ± 5	15.539 ± 0.722	1286.16 ± 158.10
13	2	10	5.51 ± 0.073	7 ± 7.6	15.200 ± 0.766	1467.60 ± 55.60	10	5.47 ± 0.104	8 ± 8	15.605 ± 1.123	1426.99 ± 102.78
14	2	9	6.16 ± 0.079	-3 ± 5.9	10.753 ± 0.873	2522.00 ± 475.72	10	6.11 ± 0.114	-2 ± 5	11.006 ± 0.944	2368.18 ± 536.69
15	2	10	6.17 ± 0.066	-5 ± 1.9	11.211 ± 0.484	1751.09 ± 135.02	10	6.12 ± 0.123	-4 ± 3	11.446 ± 0.671	1686.47 ± 185.89
16	2	10	6.27 ± 0.118	-2 ± 4.5	10.850 ± 0.911	2401.89 ± 94.35	9	6.24 ± 0.119	-4 ± 7	11.264 ± 1.217	2329.76 ± 180.80
1	3	10	5.54 ± 0.148	42 ± 46.7	13.887 ± 2.188	1927.75 ± 86.73	10	5.54 ± 0.148	42 ± 47	13.887 ± 2.188	1927.75 ± 86.73
8	3	10	5.51 ± 0.339	23 ± 20.5	16.289 ± 3.759	1572.61 ± 40.79	10	5.57 ± 0.339	23 ± 21	16.289 ± 3.759	1572.61 ± 40.79
10	3	10	5.25 ± 0.297	11	16.779 ± 2.345	1327.07 ± 54.98	10	5.40 ± 0.339	11 ± 17	15.772 ± 2.406	1380.32 ± 100.09
12	3	9	5.77 ± 0.335	14 ± 21.2	13.891± 1.742	1632.60 ± 66.09	11	5.77 ± 0.335	14 ± 21	13.891 ± 1.742	1632.60 ± 66.09
13	3	10	5.85 ± 0.126	9 ± 29.0	13.397 ± 1.392	1767.16 ± 22.90	9	5.85 ± 0.126	9 ± 29	13.397 ± 1.392	1767.16 ± 22.90

Fish Responses

Mortalities of the different species exposed to the ten different streams are illustrated in Figure 4. Mortality rates were generally higher in low order streams with the exception of Risbäcken brook (#1), and Långbäcken (#13). The bioassays demonstrated that Atlantic salmon were more sensitive than brown trout to episodic acidification, showing higher and faster mortality in all streams during the three experiments. No mortality was observed in the fish kept as controls in Norrbyn during 5 weeks.

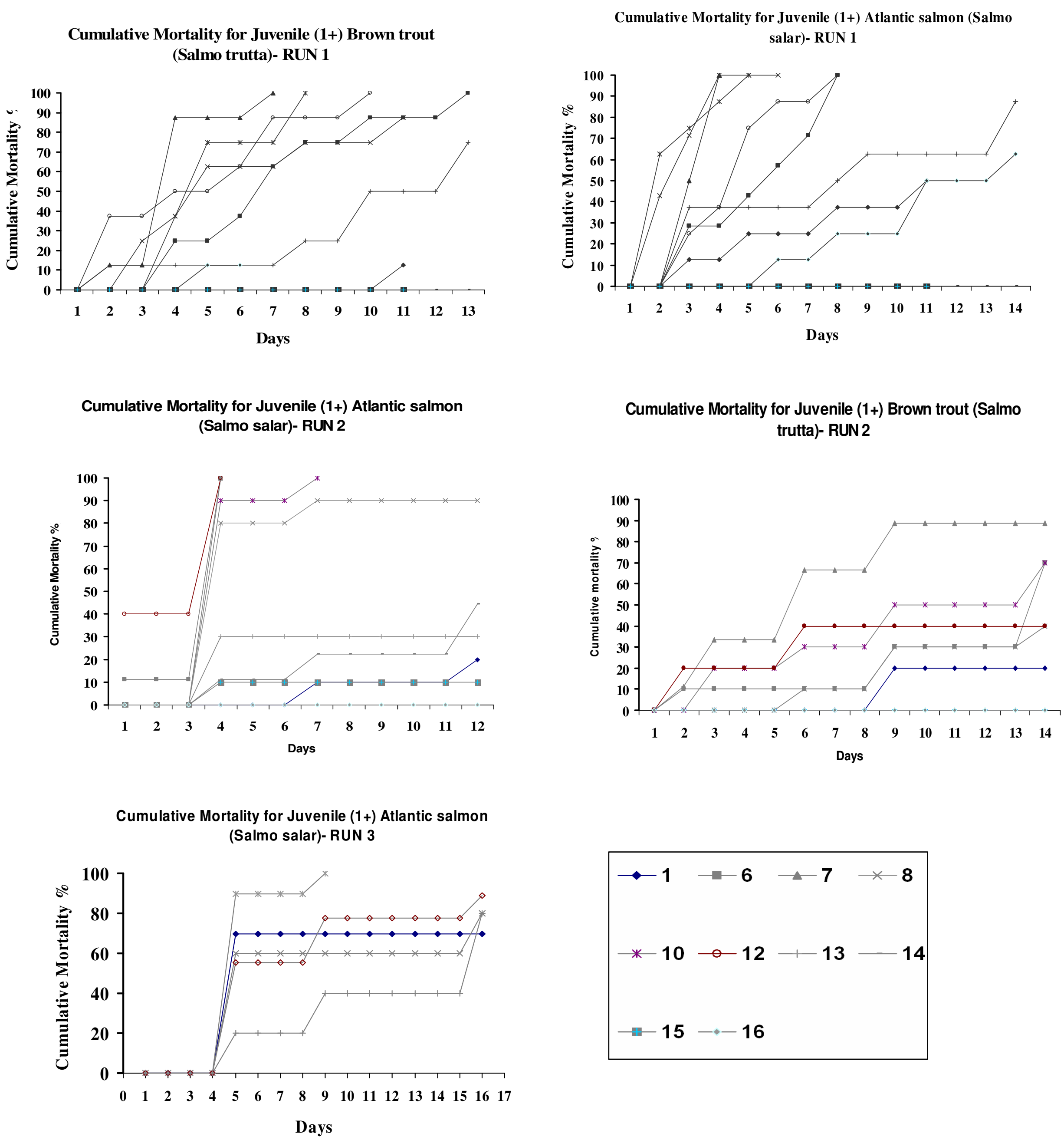


Figure 4. Cumulative mortalities of Atlantic salmon and brown trout in the bioassays. No mortality was observed for brown trout during the third experiment or in the control fish.

Statistical Analysis

Correlation analysis provided us with a first insight into the potential relevant parameters for fish survival. pH and ANC/H^+ were the indices that best correlated with salmon mortality ($p < 0.001$). For brown trout, pH and DOC were the factors best correlated with fish mortality ($p < 0.001$). As expected, all indices were correlated with each other in pair-wise comparisons ($p < 0.05$) with the exception of Al_i, not significantly correlated with Ca, ANC or ANC/H^+. A strong correlation was found between pH and DOC (illustrated in Figure 5), which exposes the importance of DOC as a key factor in controlling pH decline during the spring snow melt.

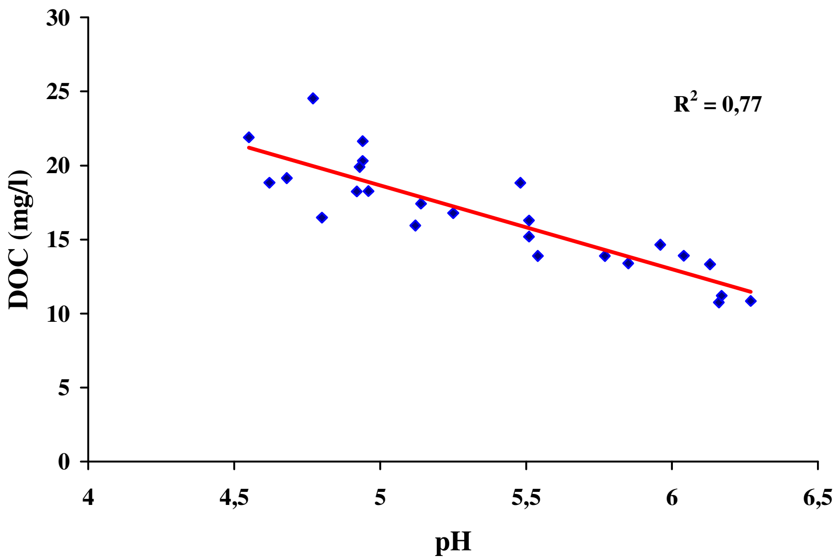
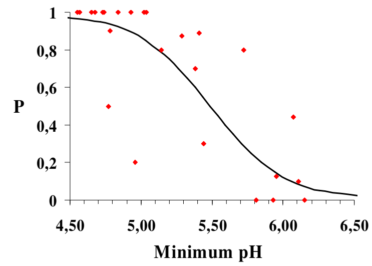
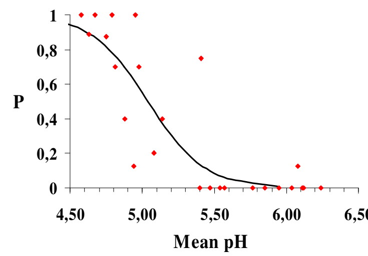
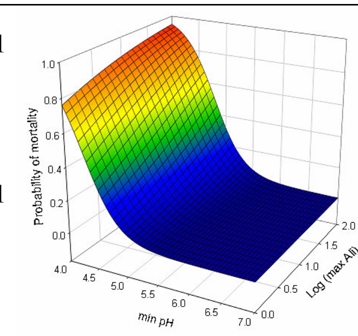


Figure 5. Correlation between pH and DOC

Exploratory multiple regression analysis of key variables was conducted to determine the best predictors of mortality in the bioassay fish. For Salmon, ANC/H^+ and pH were the best predictors (R^2= 0.61 and 0.52 respectively). pH and DOC were the best predictors for trout mortality (R^2=0.65, 0.51 and 0.59, respectively). Converting the mortality data to probits resulted, as expected, in better correlations, since mortality showed a sigmoidal relationship to chemical data. The analyses for salmon showed that "minimum pH" was the only significant variable predicting mortality, which varied also with stream order. For trout, "mean pH" proved to be the best single variable. The best model for brown trout was a two variable model that included minimum pH and log (max Al_i). As for salmon, the response varied also with stream order (Table 2).

Table 2. Model coefficients from the probit analysis for Atlantic salmon and brown trout. Hosmer-Lemershow $\chi2$ goodness of fit tests = 2.740, 13.837 and 7.271 (p-values = 0.908, 0.054 and 0.367) confirms that the logistic distribution fits the data adequately. Constant number refers to stream order. An average value was used in the graphs for simplicity.

Response Variable		Predictor	Coefficient	SE coefficient	Z	P	Graph
Atlantic Salmon (*Salmo salar*)		Min pH	-3.81990	1.72501	-6.29	<0.001	
		$Constant_1$	19.66627	3.12698	6.13	<0.001	
		$Constant_2$	21.72694	3.13673	2.97	0.003	
		$Constant_{\geq3}$	21.47815	3.54845	2.96	0.003	
Brown trout (*Salmo trutta*)	Model 1	Mean pH	-5.11148	0.692181	-7,38	<0.001	
		Constant	25.7636	3.48763	7,39	<0.001	
	Model 2	Min pH	-6.27326	0.978054	-6.41	<0.001	
		Log (max Ali)	1.38545	0.580448	2.39	0.017	
		$Constant_1$	27.28015	4.57041	5.97	<0.001	
		$Constant_2$	28.71374	4.71176	3.07	0.002	
		$Constant_{\geq3}$	32.28266	5.79840	3.11	0.002	

The results of the binary logistic regression analysis are summarized in Table 3. The quality of the models is expressed as goodness of fit (Nagelkerke R^2) and as percent correct classification (PC_{all}, PC_0 and PC_1). ANC/H^+ (R^2= 0.69) constitutes the best predictor of salmon mortality in the bioassays. Mean pH (R^2= 0.80) and ANC/H^+ (R^2=0.72) are the best predictors of trout mortality.

Table 3. Summary table of the binary logistic regression analysis. N_0 and N_1 are the number of experiments where $L_{T50} \leq 7$ days and where $L_{T50} > 7$ days. All models were significant ($p < 0.01$). PC_0, PC_1 and PC_{all} refer to the percentage of correctly predicted cases for the '0' group, the '1' group and the '0 and 1' group. Confidence limits refer to predictor values at P=0.5, 0.1 and 0.9.

Fish Species	Variable	N_0	N_1	R^2	PC_{all}	PC_0	PC_1	Confidence Limits
Atlantic Salmon (*Salmo Salar*)	Minimum pH	11	14	0.63	84.0	85.7	81.8	5.28 (4.78 - 5.78)
	Mean pH	11	14	0.58	80.0	78.6	81.8	5.45 (4.87 - 6.04)
	Mean ANC/H$^+$	11	14	**0.69**	88.0	92.9	81.8	**19.519 (3.243-35.794)**
Brown Trout (*Salmo trutta*)	Minimum pH	6	19	0.60	84.0	83.3	84.2	4.72 (4.46 - 4.97)
	Mean pH	6	19	**0.80**	92.0	83.3	94.7	**4.85 (4.71 - 4.99)**
	Mean ANC/H$^+$	6	19	**0.72**	92.0	83.3	94.7	**4.865 (1.555 - 8.174)**

DISCUSSION

The present study supports earlier investigations suggesting that low pH is acutely toxic to freshwater fish species and that episodic acidic events have a potentially large effect on the distribution of salmonid populations. It is well documented that during the spring snow melt, the strong increase in DOC concentration bring about a natural depletion of pH and ANC and an increase in total aluminium (Wigington et al., 1992; Laudon et al., 2004). Our data (Table 1) and the strong relationship found in this study between pH and DOC supports that theory. The experiments conducted in the streams reported that episodes of toxic water chemistry with pH levels less than 5.0 occur, which resulted in high fish mortality. Our mortality data confirm as well that pH is the main toxic factor to Atlantic salmon and brown trout in these streams. No correlation was found between Atlantic salmon mortality and Al_i or between fish mortality and Ca^{2+}. However, mortality decreased with increasing calcium concentration and a significant effect of ANC/H^+ ratio on fish survival was found.

The toxicity of inorganic monomeric labile aluminium (Al_i) to fish is well documented (Driscoll et al. 1980; Howells et al. 1994; Gensemer & Playle 1999), and Al_i concentrations of more than 50 $\mu g\ l^{-1}$ and 100 $\mu g\ l^{-1}$ are reported to be acutely toxic to Atlantic salmon and brown trout parr, respectively (Fivelstad & Leivestad 1984). Our investigation confirms earlier studies on aluminium toxicity to brown trout, although the toxic effects of Al_i were only significant in combination with pH (Table 2). No toxic effect of Al_i on salmon could be proved. The reason for the poor agreement between the observed mortality and the inorganic aluminium concentration can be due to the method to measure Al_i. Previous studies have reported that Al_i cannot be determined accurately in surface waters with high levels of organic acidity and relatively low levels of Al_i (even at toxic levels) (Laudon, 2000), and recently it has been shown that, due to rapid transformation processes, aluminum speciation in water samples may change even during short storage times (Andren, 1995), indicating the importance of in situ measurements of Al_i. Thus, the classical methods to determine aluminum speciation following the Barnes/Driscoll fractionation principles might be misleading for the assessment of potentially toxic aluminum species in the water (Royset et al., 2005).

Even if high concentrations of Ca^{2+} has been extensively reported to ameliorate the toxic effects of acid/Al in fish, through its action on fish gill permeability (Brown 1983,

Playle & Wood 1989), no significant effect on fish survival was observed in the streams examined in this study. However, the ANC/H^+ ratio correlated well with fish mortality. In concordance with this, it has been recently proposed that the mitigating effects of Ca^{2+} and other base cations can be explained by the water ionic strength, instead of just by Ca^{2+} concentration per se. Increased ionic strength may reduce Al-toxicity by reducing the ability of aluminium to bind to the negatively charged sites on the gill surface (Lydersen et al. 2002b).
Dissolved organic carbon (DOC) is reported to reduce the effects of aluminium on fish due to the organic binding of aluminum (Poléo et al. 1991) and it has been hypothesized that it could prevent the precipitation or polymerization of inorganic Al in the more alkaline gill microenvironment (Roy and Campbell, 1997). These examples, however, comes largely from lower DOC systems. High levels of DOC promote an increase in the H^+ concentration (Bishop et al., 2000), which in this study resulted in a pH decline of up to 1.5 units in some of the streams (Figure 6). The outcome of this apparent contradiction is still to be solved, but a possible explanation was given by Laudon et al. (2005). They suggested that in humic rich waters the acid contribution of DOC will lead to a decrease in pH, but due to its ameliorating role on Al-toxicity, the critical toxic thresholds will be lower than in low DOC systems. That is, fish in high DOC-rich streams are able to tolerate higher acidity and Al_i than fish in low DOC streams.

In concordance with other studies of various salmonid fish species (Poléo, 1997), our results confirm that there are relatively large differences in toxic response between brown trout and Atlantic salmon (Figure 6), probably based on differences in metabolism and oxygen dependence (Andren, 2003). Atlantic salmon showed higher mortality than brown trout and therefore represents a more vulnerable species to low pH than other Scandinavian fish species.

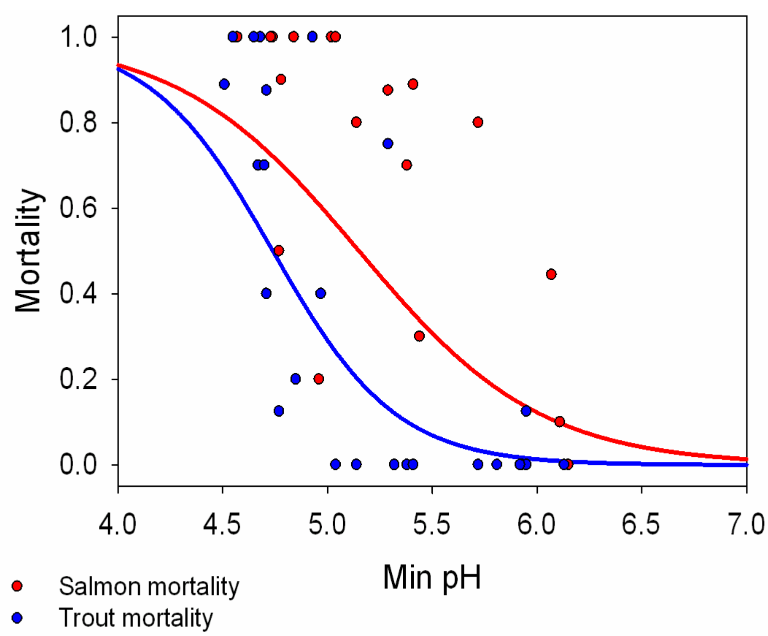


Figure 6. Predicted mortality of Atlantic salmon and brown trout in terms of minimum pH based on probit analysis

One of the main aims of this study was to define critical chemical levels for Atlantic salmon and brown trout in DOC-rich waters. Applying probit analysis to the data, the strength of the relationship between pH and mortality was determined, and therefore it is possible to define the pH level that will induce a certain proportion of mortality. Accordingly, 1+ Atlantic salmon and brown trout populations can significantly decrease (15 % mortality) during acid episodes (pH < 5.9 and pH< 5.1 respectively) in humic rich water (DOC > 10 mg/l) (Figure 6).

Binary logistic regression analysis helped to define threshold values for chemical acidity in a more useful and simplistic approach than probit analysis. Although the models worked better for trout than for salmon, they fit the data well and can be valuable for management purposes to predict fish responses in humic rich waters. pH and the ANC/H^+ ratio constitute good criteria. Stream waters where mean pH reach values below 5.75 and 4.96 or ANC/H^+ ratios fall below 32.4 and 7.5 can cause significant mortality (15%) of salmon and trout populations, respectively. These pH values are comparable to the ones provided by probit analysis.

In conclusion, we found out that toxic water conditions occur during the spring snow melt period, causing Atlantic salmon and brown trout mortality. We defined predictor variables and proposed chemical thresholds for management purposes of these species. Future research on this subject needs development of better methods to measure Al_i in high DOC system and consideration of the fact that acidic episodes exert large impact in determining the distribution of stream fish populations.

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APPENDICES

Table 4. Major water quality parameters in Vindelälven from May till June (5 year average). Data from Länsstyrelsen.

Parameter	Units	Mean	N
Temperature	°C	7,41	28
pH		6.97	28
Conductivity	µS/cm	4.193	28
N_{tot}	µg/l	207.57	28
TOC	mg/l	6.21	28

Table 5. Major water quality parameters in control tanks from May till June. Swedish environmental monitoring, Umeå Marine Sciences Center

Parameter[1]	Units	Mean	N
Temperature	°C	4.039 + 3.389	33
Oxygen	mg/l	13.21 + 0.366	4
Ntot	µg/l	256.56 + 42.201	18
N-NH4	µg/l	4.84 + 2.490	18
TOC	mg/l	4.00 + 0.455	14
Silicon	µS/cm	823 + 158.31	18

[1] Data for 5-15 m depth, best corresponding to water intake level. Johan Wikner (pers.comm.)

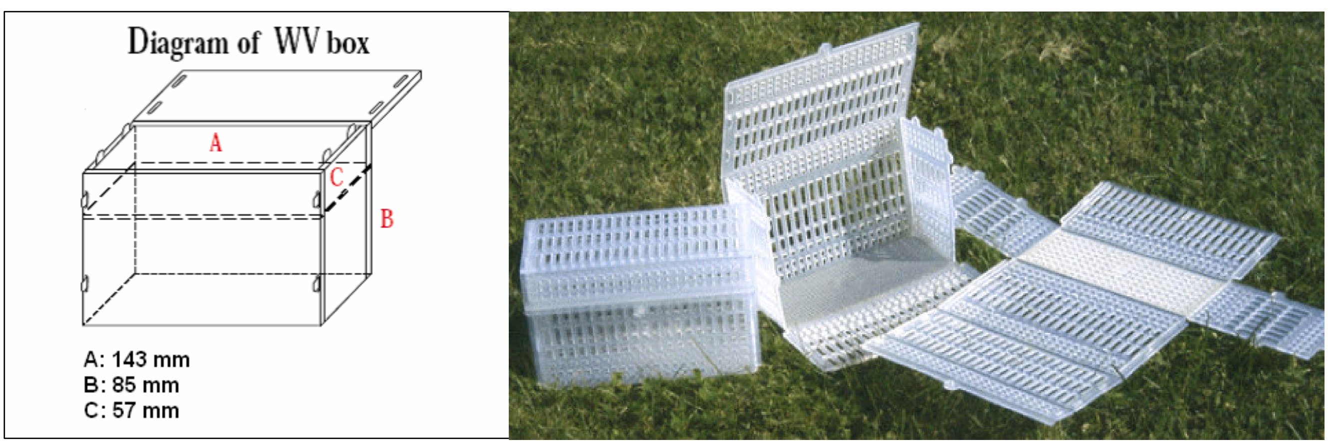

Figure 7. Whitlock Vibert box used in the experiments