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TOXICITY OF ACID ALUMINIUM-RICH WATER TO SEVEN FRESHWATER FISH SPECIES: A COMPARATIVE LABORATORY STUDY

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Abstract

The present study focuses on the relative sensitivity among freshwater fish species to aqueous aluminium. Seven common Scandinavian fish species were exposed to acidic Al-rich water, acidic Al-poor water, and approximately neutral water as a control. The relative sensitivity among the species to an acute aluminium challenge was documented, and was in the following order: Atlantic salmon, Salmo salar, *as the most sensitive; then roach,* Rutilus rutilus; *minnow,* Phoxinus phoxinus; *perch,* Perca fluviatilis; *grayling,* Thymallus thymallus; *brown trout,* Salmo trutta; *and Arctic char,* Salvelinus alpinus. *Substantial mortality was observed in all species when exposed to the Al-rich medium. Some mortality was also observed in minnow, roach, and brown trout exposed to the acidic Al-poor medium and the control medium. A high resistance to aluminium was observed in Arctic char, while perch was found to be more sensitive to aluminium than expected and, for the first time, a toxic response to aqueous aluminium in grayling was documented. Through controlled experimental studies, the results confirm that aluminium is an important factor in the toxicity of acidified waters to freshwater fish species.* © *1997 Elsevier Science Ltd*

Keywords: Acidification, Al-toxicity, Al-chemistry, relative sensitivity, mortality.

INTRODUCTION

The most serious effect of the acidification of surface waters in Scandinavia seems to be the decline or elimination of natural fish populations (Schofield, 1976; Muniz and Leivestad, 1980; Muniz, 1984). According to recent estimates, Norwegian lakes and rivers within an area of 86 000 km^2 are severely influenced by acidification (Henriksen and Hesthagen, 1993). Fish populations are extinct or significantly reduced in many of these lakes and rivers. The effect of acid deposition upon lake and surface water pH was probably first described by Mackereth (1957) and Gorham (1957, 1958). It is now more or less evident that aluminium, and not the H^+-concentration, is the principal toxicant killing the fish in acidified waters (Burrows, 1977; Howells *et al.*, 1990; Poléo, 1995). Low-molecular weight inorganic forms of aluminium, often referred to as inorganic monomeric aluminium, are believed to be the most important Al-species causing Al-toxicity in fish (Driscoll *et al.*, 1980; Fivelstad and Leivestad, 1984; Exley *et al.*, 1991; Poléo, 1995).

The literature includes substantial knowledge about the effects of aluminium on salmonids, especially Atlantic salmon, *Salmo salar*; brown trout, *Salmo trutta*; rainbow trout, *Oncorhynchus mykiss*; and brook trout, *Salvelinus fontinalis* (see Howells *et al.*, 1990). Among these, only Atlantic salmon and brown trout have a natural distribution in Scandinavia. The Atlantic salmon is reported to be more sensitive to acidic water (Grande *et al.*, 1978), and particularly to acidic Al-rich water (Rosseland and Skogheim, 1984), compared to the brown trout. The relative sensitivity of several other freshwater fish species to acidified waters in Scandinavia has also been studied (Almer, 1974; Bergquist, 1991). The problem with these studies, however, is that they are based upon field survey investigations in which the sensitivity is correlated to water pH, and not to the concentration of the principal toxicant, aluminium. By combining the results of a fish status survey and a water chemistry survey, Rask *et al.* (1995) showed that the concentration of labile aluminium was essential in determining whether or not populations of different species were affected by acid precipitation. This illustrates the importance of considering the aqueous Al-chemistry when interpreting the effects of acidification on fish. Because most field survey investigations cover great numbers of lakes with large variation in water chemistry, more specific knowledge on the sensitivity of freshwater fish species to aqueous aluminium is needed. A significant part of the area affected by acidification in Scandinavia corresponds with the area of highest fish species diversity. Thus, it is of major importance, for fishery management and the restoration of acidified

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lakes and streams, to know more about the significance of aqueous aluminium to the survival of various species living in these areas.

We have performed a comparative laboratory study on the acute toxicity of aqueous aluminium on seven common Scandinavian freshwater fish species (Atlantic salmon, *S. salar*; brown trout, *S. trutta*; roach, *Rutilus rutilus*; minnow, *Phoxinus phoxinus*; perch, *Perca fluviatilis*; Arctic char, *Salvelinus alpinus*; and grayling, *Thymallus thymallus*). Among these, little is known about the toxicity of aqueous aluminium in roach, minnow, perch, Arctic char and grayling. In the experiments, we have exposed the fish to three different media: acidic Al-rich and acidic Al-poor water (both with pH about 5.0), and 'neutral' Al-poor water (pH 6.5) as a control.

MATERIALS AND METHODS

Experimental animals

All except one of the experimental fish species (Table 1) were obtained from either lakes, streams, or hatcheries near Oslo. Roach (two sizes, 0+ fry and >1+ mature fish), minnow, and perch were caught by electrofishing. Parr of Atlantic salmon, brown trout, and Arctic char were obtained from local hatcheries. The fish were brought into the fish holding department at the University of Oslo, and acclimatised for at least three weeks before experimental use. In the present study, we also used grayling parr reared in the fish holding department. All salmonids were first generation hatchery fish. The fish holding department receives dechlorinated Oslo tapwater (see Table 2). The water systems from which the fish were obtained are all low in total aluminium, and have a circumneutral pH. Therefore, there is no reason to believe that prior exposure to aluminium may have resulted in acclimation and reduced toxicity in the fish obtained from the wild.

Analytical techniques

Size and charge fractionation of aqueous aluminium was performed using the Barnes–Driscoll extraction–cation exchange method combined with hollow-fiber ultrafiltration according to Lydersen *et al.* (1992).

Aqueous aluminium was analysed by the HQ–MIBK extraction method described by Barnes (1975), with an extraction time of 20 s. The extracts were stored at 4°C for at least 24 h, and thereafter at room temperature (20°C) for 2 h before absorbance measurements were carried out on a Shimadzu UV-1201 spectrophotometer at 395 nm (Tikhonov, 1973; Bloom *et al.*, 1979). Absorbance was also measured at 600 nm in order to correct for iron interference (Sullivan *et al.*, 1986).

The amount of total monomeric Al-species (Al_a) was determined by direct extraction of water samples (Driscoll, 1984). Water samples were also run through a column of Amberlite IR-120 as cation exchange resin prior to extraction. As recommended by Driscoll, the cation exchange flow-rate was 3.8 ml min^{-1} ml^{-1} bed-volume. The resin was prepared by displacing some of the exchangeable hydrogen ions with sodium ions. When an eluant of comparable ionic strength to that of the water samples being analysed was passed through the exchanger, the pH of the effluent should have been similar to the pH of the sample. A volume of 60 ml 10^{-4} M NaCl was always used for conditioning the resin between runs of samples. For preconditioning purposes, 60 ml of water were eluted before another 60 ml of eluate were collected for extraction.

The aluminium present in the eluate was defined as non-labile Al. In natural fresh water, this fraction is often defined as organic monomeric Al, termed Al_o (Driscoll, 1984). The concentration of labile or inorganic monomeric Al (Al_i) was calculated as the difference between Al_a and Al_o. Total aluminium (Al_r) was also analysed by HQ–MIBK extraction after acidifying water samples with HNO_3 to pH 1.0 for 24 h. The standard deviation of the Barnes–Driscoll method is assumed to approximate 1% of the mean (Sullivan *et al.*, 1986).

Ultrafiltration was performed using an Amicon H1 P10-8 hollow-fiber cartridge with a nominal molecular weight cut-off level of 10 kD. Thus, it is possible to divide water samples into two molecular weight fractions, one high (Mw > 10 kD) and one low (Mw < 10 kD). The filtering flow-rate was about 300 ml min^{-1} with a transmembrane pressure of about 10 psi (69 kPa). The pore-size distribution of the membrane has been shown to be relatively narrow (Salbu *et al.*, 1985). Sorption on the fibers (by mass balance calculations) is minimal, providing the fibers are preconditioned with 250 ml of the sample water before the ultrafiltrate is collected for analysis (Lydersen *et al.*, 1987).

Table 1. The fish species used in the experiments

Species	Exposure period	Weight (g)	Total length (mm)	
Roach (*Rutilus rutilus*) >1+	26.06–07.07	14.9 ± 6.5	118 ± 18	(n = 136)
Atlantic salmon (*Salmo salar*)	09.07–19.07	4.0 ± 1.2	74 ± 12	(n = 230)
Arctic char (*Salvelinus alpinus*)	15.09–05.10	9.1 ± 2.9	103 ± 11	(n = 222)
Minnow (*Phoxinus phoxinus*)	10.10–11.11	2.4 ± 1.4	65 ± 10	(n = 188)
Perch (*Perca fluviatilis*)	15.11–21.12	9.7 ± 5.5	100 ± 14	(n = 115)
Roach (*Rutilus rutilus*) 0+	15.11–21.12	1.1 ± 0.6	53 ± 10	(n = 170)
Grayling (*Thymallus thymallus*)	10.01–20.02	9.4 ± 3.4	121 ± 14	(n = 61)
Brown trout (*Salmo trutta*	10.01–20.02	2.7 ± 0.9	64 ± 7	(n = 89)

The exposure periods of each experiment are indicated, and listed chronologically. Weight and total length are also given (mean ± s.d.). The same exposure period for two species means that they were exposed simultaneously in the channels.

Table 2. Major water quality parameters of the water in the fish holding department at University of Oslo

pH		6.5
Temperature	°C	9.5
Conductivity	$\mu s\,cm^{-1}$	23.0
Na^+	$mg\,litre^{-1}$	1.89
K^+	$mg\,litre^{-1}$	0.38
Ca^{2+}	$mg\,litre^{-1}$	2.98
Mg^{2+}	$mg\,litre^{-1}$	0.47
SO_4^{2-}	$mg\,litre^{-1}$	5.6
Cl^-	$mg\,litre^{-1}$	2.80
F^-	$\mu g\,litre^{-1}$	70
NO_3	$\mu g\,litre^{-1}$	260
SiO_2	$mg\,litre^{-1}$	3.3
Fe	$\mu g\,litre^{-1}$	10.0
TOC	$mg\,C\,litre^{-1}$	2.7

ALCHEMI (Version 4.0) was used to calculate the theoretical distribution of inorganic Al-species from the Al_i-fraction (Schecher and Driscoll, 1987, 1988), assuming equilibrium of dissolved species and a carbonate buffering system open to the atmosphere. In the ALCHEMI program, the effects of temperature and ionic strength on the Al-chemistry are accounted for. The uncertainties in the concentrations of all species obtained from these calculations are considered to be low (Schecher and Driscoll, 1987, 1988).

pH was measured using a Radiometer PHM-80 with a Radiometer GK-2401C combined glass-electrode. The pH readings were taken when the pH-meter drifted less than 0.01 pH unit per min. The standard deviation of the measured pH was ±0.01 pH unit. The conductivity was measured with a Radiometer CDM-80. The conductivity was read when three consecutive measurements were identical within one tenth of a unit ($\mu S\,cm^{-1}$). The pH and conductivity measurements as well as Al-extractions were performed immediately after the water samples were taken.

Total fluoride (F^-) was analysed according to Harwood (1969) using an Orion Model 94-09 ion selective electrode, connected to an Orion Research Microprocessor Ion Analyser-901 with the Orion Model 90-01-00 as reference electrode. The precision of the ion selective fluoride electrode is expected to be ±2% (Harwood). Na^+, K^+, Ca^{2+} and Mg^{2+} were analysed by Atomic Absorption Spectroscopy (AAS), SO_4^{2-} and Cl^- by ion chromatography (IC), NO_3 by the indophenolblue method, SiO_2 with the standard molybdate-complexing method, and total organic carbon (TOC) by a combined photochemical (UV) wet-chemical ($S_2O_8^{2-}$) oxidation method.

Test conditions

The experiments were performed in the laboratory of the fish holding department at the University of Oslo. Two test media, A and B, were prepared by chemical addition of HNO_3 and $Al(NO_3)_3$ to the water of the department. Medium A = acidic Al-rich water (pH about 5.0) was prepared by addition of an acidic $Al(NO_3)_3$-solution. Medium B = acidic Al-poor water (pH about 5.0) was prepared by addition of HNO_3 only. Untreated water (pH 6.5) acted as the control medium.

Three flow-through channels were used for the exposures (Fig. 1). The channels were 218 cm long, 42 cm wide, and 16 cm deep. The water flow-rate into each channel was approximately 3 liters min^{-1}, and the residence time was about 30 min. The water was well aerated on its way through the channels, and the water flow of 3 liters min^{-1} provided at least 4.7 liters of water per gram of fish per day. This is well above 2.0 liters $g^{-1}\,day^{-1}$ which is recommended for this kind of experiment (Sprague, 1973). The fish were sheltered by covers over the channels.

Experimental protocol

The present study was performed as six separate experiments, carried out within periods of between 11 and 42 days (Table 1). In each of these experiments, one or two of the fish species were exposed to all three media in parallel. An experiment was not carried further if all fish in medium A had died. We stopped feeding the fish two days prior to the exposures, and fish were not fed during the experiments. The fish were introduced into the channels one day before the exposures were started. Cumulative mortality was used as a measure of Al-toxicity, and when mortality was higher than 50% it was quantified in terms of LT_{50}, i.e. the time until 50% of the fish were dead. LT_{50} (with 95% confidence limits) was estimated by logistic regression when mortality was 100% or close to 100%. When mortality was significantly lower than 100%, we read the LT_{50} values directly from the mortality curves, i.e. the point where the mortality curve crossed the 50% line. Fish were judged to be dead when opercular movements had ceased and no swimming response could be elicited by stimulation of the caudal peduncle.

Water temperature, conductivity, and pH were measured daily throughout the experimental period. The chemical dosage and water flow into the channels were also controlled each day. Once, before the experiments with the fish were carried out, the Al-chemistry of the three channels was characterised. In addition to this, Al_r, Al_a, Al_o and Al_i were determined between one and six times during each exposure.

RESULTS

Water chemistry

Water quality parameters and Al-fractionation data are presented in Tables 2 and 3. The results from the chemical characterisation of the channels, which was performed prior to the experiment with the fish, are presented in Tables 4 and 5.

The water temperature was approximately the same in the three channels, and varied by less than 1°C during each exposure (Table 3). Throughout the experimental period, the mean water temperature was between 4.4 and 9.9°C. During the experiments, the mean pH in medium A was 5.07 ($[H+] = 8.61 \times 10^{-6} \pm 2.18 \times 10^{-6}$ (mean ± s.d., $n = 308$)), in medium B, 5.08 ([H+]

$= 8.38 \times 10^{-6} \pm 3.72 \times 10^{-6}$ ($n = 308$)); and in the control, 6.50 ([H+] $= 3.19 \times 10^{-7} \pm 6.06 \times 10^{-8}$ ($n = 308$)). The electrical conductivity in the media was stable and varied with less than 2.0 $\mu S\,cm^{-1}$ throughout the experimental period (Table 3). The conductivity in medium A (27.0 $\mu S\,cm^{-1}$) and B (25.0 $\mu S\,cm^{-1}$) was somewhat higher than in the control water (22.0 $\mu S\,cm^{-1}$) due to the addition of the chemicals.

The results from the chemical characterisation of the channels (Table 4) showed that, in medium A, the total concentration of Al was 402 ± 6 $\mu g\,liters^{-1}$ (mean ± s.d., $n = 16$), where 294 ± 7 $\mu g\,liters^{-1}$ ($n = 16$) was present as Al_i. During the experiments, however, the variabilities in the Al measurements were larger than during the initial characterisation of the channels (Table 3 and Table 4). The reason for this is probably that the Al measurements during the chemical characterisation were carried out within a period of one day. The measurements during the experiments, however, were done within periods of several days, with small fluctuations in water flow, dosage flow, water temperature, and pH, thus giving rise to somewhat larger variabilities.

Ultrafiltration revealed that about 25% of the total amount of aluminium (Al_r) in our test medium was high-molecular weight species, and that the major part of the inorganic Al was present as low-molecular weight species (about 82%). Based on the chemical modelling (ALCHEMI, Table 5), a large part of the inorganic monomeric Al (42%) was present as Al^{3+} (81 μg $liters^{-1}$) and Al-hydroxides (42 $\mu g\,liters^{-1}$). There were also substantial amounts of Al-fluorides (95 $\mu g\,liters^{-1}$, e.g. 32%) and $AlH_3SiO_4^{2+}$ (74 $\mu g\,liters^{-1}$, e.g. 25%) present.

We calculated the mean concentration of total aluminium in medium A for each exposure, and the means varied between 381 ± 32 $\mu g\,liters^{-1}$ ($n = 4$) and 492 ± 11 $\mu g\,liters^{-1}$ ($n = 4$). Within each experiment, however, the total Al-concentration varied less than throughout the whole experimental period (see Table 3). The Al-chemistry was approximately the same in medium B and the control water (Table 3 and Table 4). The total concentration of Al was similar in medium B and in the control, around 75 $\mu g\,liters^{-1}$. Of this, very little was present as Al_i (between 0 and 25 $\mu g\,liters^{-1}$).

We observed that the Al_o-fraction in medium A was higher than in medium B and the control water. The total organic pool in the natural water may have a proportion of non-complexed organic molecules which represents a sink for binding of additional aluminium. A non-complexed organic pool like this could, therefore, to some extent contribute to the increased Al_o values measured in medium A. In the present study, however, we used an acidic $Al(NO_3)_3$-solution (pH about 2.0) to prepare the acidic Al-rich medium A. In this acidic Al-solution, the octahedral hexahydrate $Al(H_2O)_6^{3+}$ (often written as Al^{3+}) should be the only significant Al-species present (Hem and Roberson, 1967; Lydersen, 1990). Ultrafiltration showed that inorganic colloidal Al-polymers were formed when the acidic Al-solution was added to the untreated water with a pH of about 6.5 (Table 4). According to Hem and Roberson, and Lydersen *et al.* (1990), Al starts to polymerise as soon as pH is raised to above 4.0. Because some of these Al-polymers are not cation-exchangeable (Driscoll, 1984), but are HQ–MIBK extractable (Barnes, 1975), they are determined as Al_o by the analytical method used (Lydersen *et al.*, 1990).

Fish reactions and mortality

In all experiments, the fish exposed to medium A showed a rapid decline in activity. After only a few hours, fish exposed to this medium were not moving at all. No such decline in activity was observed in fish exposed to medium B or C.

The Arctic char was the only species exposed to medium A in which less than 50% mortality was observed (Fig. 2). Atlantic salmon, minnow, and roach (both 0+ and >1+) showed almost 100% mortality

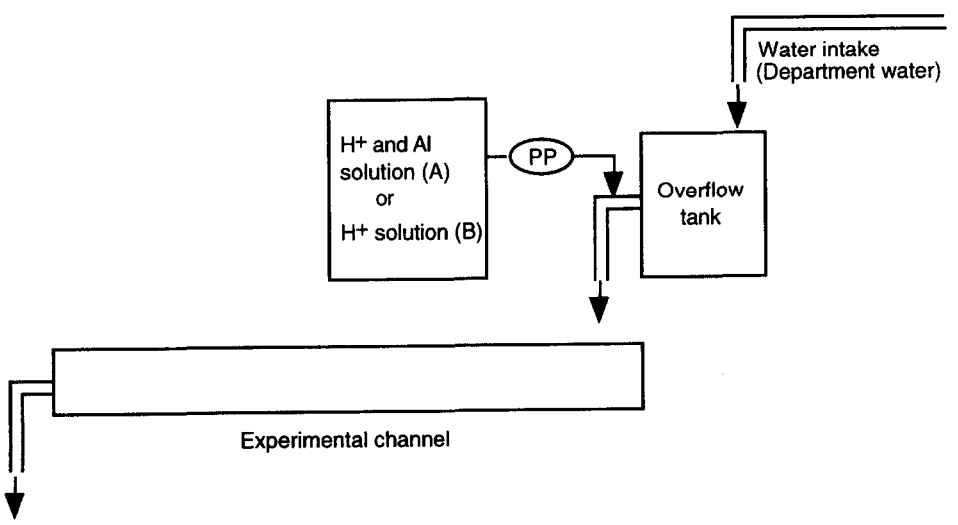

Fig. 1. A schematic presentation of the experimental flow-through channels which were used in the experiments. The H^+/Al-solution and the H^+-solution were added to the department water as it entered the channels. The pH in these solutions was about 2.0. PP = peristaltic pump.

Table 3. Water quality data of the two test media A and B, and the control water

Species	pH	Temp. (°C)	Cond. ($\mu S\,cm^{-1}$)	n_a	Al_r	Al_a ($\mu g\,litre^{-1}$)	Al_o ($\mu g\,litre^{-1}$)	Al_i	n_b
Medium A									
Roach >1+	5.32	8.0±0.2	26.1±0.7	24	390±12	332±9	53±3	279±7	6
Atlantic salmon	5.28	9.2±0.9	26.9±0.9	22	381±32	316±3	50±1	266±4	4
Arctic char	5.08	9.9±0.8	27.4±1.2	42	399±17	351±0	41±2	311±1	4
Minnow	5.07	8.5±1.1	27.5±0.6	64	464±44	378±65	40±22	338±44	4
Perch	5.06	5.5±0.6	26.2±0.8	72	492±11	442±13	65±11	377±22	4
Roach 0+	5.06	5.5±0.6	26.2±0.8	72	492±11	442±13	65±11	377±22	4
Grayling	4.97	4.5±0.1	26.7±1.2	84	477±39	433±34	64±5	369±33	4
Brown trout	4.97	4.5±0.1	26.7±1.2	84	477±39	433±34	64±5	369±33	4
Medium B:									
Roach >1+	5.42	8.0±0.2	24.5±0.6	24	81±8	40±6	19±5	21±2	6
Atlantic salmon	5.31	9.2±0.9	25.5±0.6	22	63±5	26±2	21±3	5±3	4
Arctic char	5.20	9.9±0.8	24.9±1.3	42	67±7	45±1	20±3	25±1	2
Minnow	5.17	8.5±1.1	24.9±0.9	64	72±2	38±5	27±1	10±7	4
Perch	4.95	5.6±0.6	24.8±1.4	72	86±1	49±8	35±6	14±3	4
Roach 0+	4.95	5.6±0.6	24.8±1.4	72	86±1	49±8	35±6	14±3	4
Grayling	4.99	4.5±0.1	24.2±1.3	84	83±8	46±5	37±9	9±3	4
Brown trout	4.99	4.5±0.1	24.2±1.3	84	83±8	46±5	37±9	9±3	4
Control water									
Roach >1+	6.41	7.9±0.3	21.5±0.4	24	81±4	40±7	22±5	18±2	3
Atlantic salmon	6.42	9.1±0.9	24.6±3.3	22	64±5	21±1	23±1	0	2
Arctic char	6.42	9.9±0.8	22.4±1.0	42	72	39	28	11	1
Minnow	6.54	8.5±1.1	22.2±0.9	64	74	44	28	16	1
Perch	6.55	5.5±0.7	20.9±0.5	72	87±6	50±6	41±4	9±2	2
Roach 0+	6.55	5.5±0.7	20.9±0.5	72	87±6	50±6	41±4	9±2	2
Grayling	6.51	4.4±0.1	20.5±0.7	84	81±4	46±5	37±9	9±3	2
Brown trout	6.51	4.4±0.1	20.5±0.7	84	81±4	46±5	37±9	9±3	2

Mean ± s.d. are shown, n_a = number of pH, temperature, and conductivity observations, while n_b = number of Al-analyses.

during their exposure periods to medium A. Medium A was most toxic to Atlantic salmon and roach >1+ (LT_{50} was estimated as 85 and 97 h, respectively; Table 6). Except for the low mortality in the Arctic char (37%), the highest LT_{50} (approximately 500 h) was observed in the brown trout.

In medium B, we observed 31% mortality in minnow, and somewhat less mortality in roach >1+ and 0+, and in brown trout (15, 9 and 10%, respectively). There was also some mortality in minnow (18%), roach >1+ and 0+ (31 and 2%, respectively), and brown trout (7%) in the control water (Fig. 2). We did not observe any mortality in the holding tanks, where fish were kept prior to the experiments.

DISCUSSION

The present study documents the relative sensitivity of seven common Scandinavian freshwater fish species to acidic Al-rich water. Our results, which are based on laboratory experiments, differ from reported relative sensitivities to acidified waters obtained from field studies.

Water chemistry

The composition and the water chemistry of medium A should represent water qualities found in acidified areas of Scandinavia (Henriksen *et al.*, 1988). In addition, the relatively high content of aluminium, especially Al_i, used in the present study corresponds with the content in natural waters in which fish populations are strongly affected or extinct (Henriksen *et al.*, 1988; Henriksen and Hesthagen, 1993), or to the content during acidic run-off episodes (Driscoll *et al.*, 1980; Gagen *et al.*, 1993), such as autumn and spring floods, which are known to cause severe fish kills (Leivestad and Muniz, 1976).

The chemical characterisation of the exposure channels used in our experiments shows that 25% of the total aluminium present in medium A was in high-molecular weight forms (Table 4). This indicates that the experiments in the present study have been conducted under relatively non-steady state aqueous Al-chemistry conditions. Under non-steady state conditions, especially after a rapid rise in pH, low-molecular Al-forms coalesce to form high-molecular weight Al-polymers (Hem and Roberson, 1967; Lydersen *et al.*, 1991). We believe that true chemical equilibrium is seldom approached in natural systems like surface waters. Our experimental conditions should therefore correspond more or less to the situation found in natural acidified waters.

Al-toxicity to individual fish species

The present study confirms earlier investigations that aluminium is acutely toxic to freshwater fish species, and also that aluminium seems to be the principal toxicant killing fish in acidified waters whatever the species concerned. Our results also confirm that acidic water at pH 5.0 is not toxic, or much less toxic to the fish species tested than acidic Al-containing water of the same pH.

Table 4. Mean pH and concentrations of different Al-fractions in total and hollow-fibre ultrafiltered water samples from the two test media, A and B, used in the exposure channels and the control channel

	pH	Al_r	Al_a ($\mu g\,litre^{-1}$)	Al_o ($\mu g\,litre^{-1}$)	Al_i	
Medium A	5.17	402 ± 6	336 ± 10	43 ± 6	294 ± 7	(n = 16)
Ultrafiltered	5.25	306 ± 7	263 ± 7	21 ± 4	242 ± 8	(n = 12)
Medium B	5.16	77 ± 6	31 ± 9	19 ± 5	12 ± 6	(n = 8)
Ultrafiltered	5.30	45 ± 10	19 ± 12	3 ± 2	17 ± 13	(n = 4)
Control	6.29	79 ± 5	32 ± 7	29 ± 3	4 ± 3	(n = 4)
Ultrafiltered	6.34	25 ± 2	0	4 ± 0	0	(n = 2)

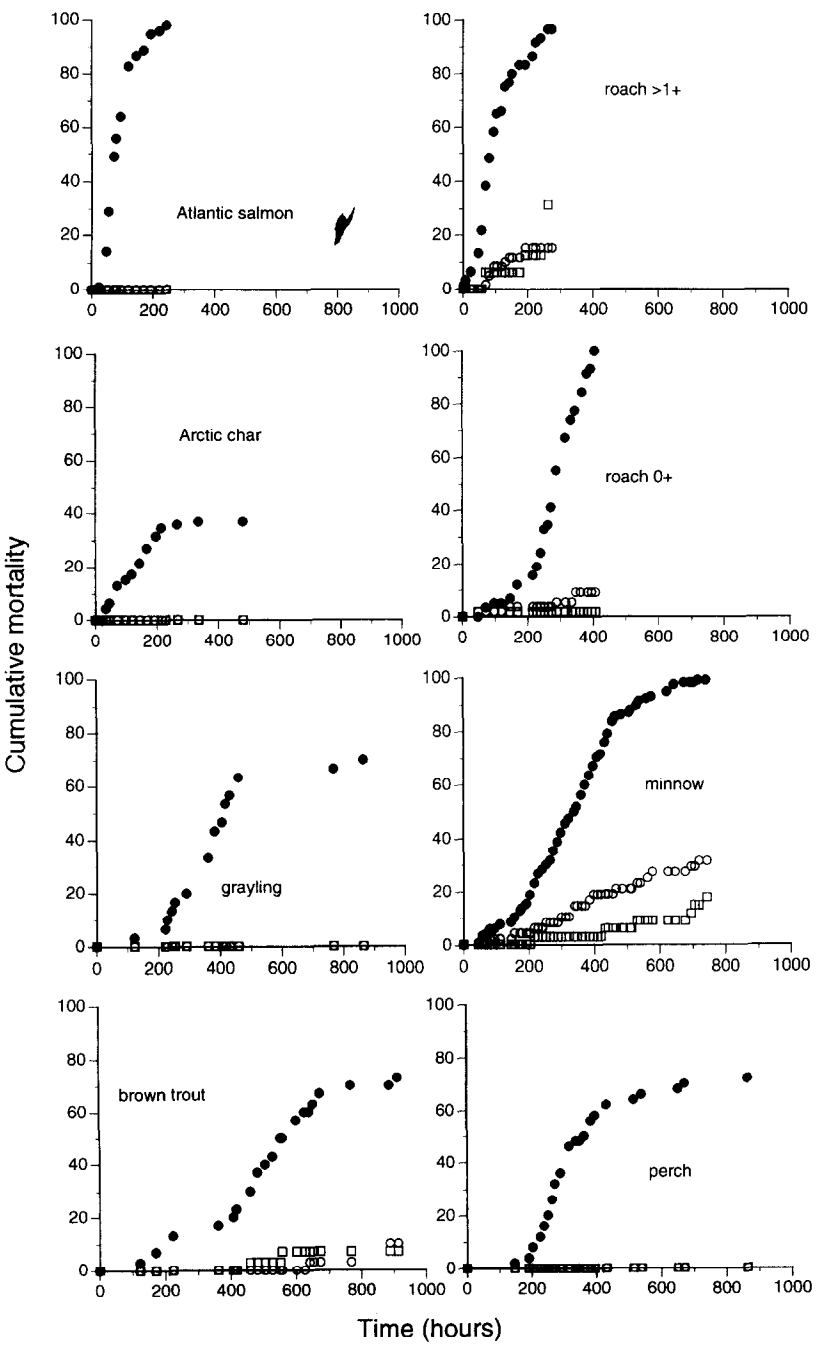

Fig. 2. Cumulative mortality of fish exposed to medium A (●), medium B (○), and control water (□). Salmonids are shown in the left column and non-salmonid species in the right column.

Table 5. Distribution of inorganic monomeric Al-species present in total (T) and hollow-fibre ultrafiltered (UF) water samples from the two test media, A and B, used in the exposure channels

	Medium A		Medium B	
	T	UF	T	UF
pH	5.17	5.25	5.16	5.30
Temp (°C)	9.5	9.5	9.5	9.5
Al^{3+}	81	52	0	0
$AlOH^{2+}$	29	23	0	0
$AlOH_2^+$	13	13	0	0
$AlOH_4^-$	0	0	0	0
AlF^{2+}	63	56	4	5
AlF_2^+	3	4	5	6
$AlOHF^+$	29	32	2	4
$AlSO_4$	3	2	0	0
$AlH_3SiO_4^{2+}$	74	60	0	0
Sum Al_i	295	242	11	15

The amounts of inorganic monomeric Al-species ($\mu g\,liter^{-1}$) are calculated by means of ALCHEMI (Schecher and Driscoll, 1987, 1988).

Roach

We observed a low tolerance to acidic Al-rich water in the roach compared to the other fish species in our study (Fig. 2). This observation corresponds well with a recent report on a toxicity study where different fish species have been exposed to acidic Al-containing water by Vuorinen *et al.* (1993) who found that roach larvae were more sensitive to different combinations of pH and Al-concentration than larvae of pike, *Esox lucius*; whitefish, *Coregonus lavaretus*; and pike perch, *Stizostedion lucioperca*. The high sensitivity in roach to aluminium observed by Vuorinen *et al.* (1993) and ourselves corresponds to the high sensitivity to acidification in this species, which is reported from various field survey investigations of acidified areas (Almer, 1974; Johansson and Milbrink, 1976; Bergquist, 1991; Degerman *et al.*, 1992).

It is well known that Al-sensitivity can differ among life history stages within a fish species (Howells *et al.*, 1990). In the present study, the roach was the only species in which different life stages were exposed, and larger mature >1+ roach appeared to be much more sensitive than the smaller 0+ fry (Fig. 2). However, we have to be careful concluding that roach >1+ are more sensitive than the 0+ fry, since the two experiments were run at slightly different water temperatures, and since we observed significant mortalities in the control media (Fig. 2). Poléo *et al.* (1991) and Poléo and Muniz (1993) have demonstrated that Al-toxicity in fish is dependent on water temperature, with higher toxicity at higher temperatures. Thus, the higher mortality in roach >1+, exposed to 8.0–9.9°C, compared to roach 0+ exposed to 4.5–5.0°C, may largely be explained by a temperature effect. The higher mortality in roach >1+ compared to 0+ can also to some extent be explained by an initial lower quality of the experimental fish. The fish could have been suffering from a disease or a parasite infection which we were not aware of. Therefore, more thorough research on Al-toxicity in roach and the influence of life history stages is needed before we can compare this species with other fish in which stage sensitivity has been studied.

Atlantic salmon

The Atlantic salmon is known to be extremely sensitive to acidification in general, and to aluminium in particular (Howells *et al.*, 1990), and comparative studies of various salmonid fish species have clearly demonstrated that Atlantic salmon, apart from rainbow trout, is the most sensitive salmonid (Grande *et al.*, 1978; Fivelstad and Leivestad, 1984; Rosseland and Skogheim, 1984). The high mortality of Atlantic salmon observed in the present study (Fig. 2) confirms that this species is extremely sensitive to aluminium, and that it is more vulnerable than other Scandinavian salmonids and non-salmonid fish species.

Arctic char

To our knowledge, the present study is the first documentation of the toxicity of aqueous aluminium in Arctic char. We observed that Arctic char was the most tolerant species to medium A among the seven different species we tested (Fig. 2). Only 37% of the char died during the 480 h exposure (20 days). This resistance to acidic Al-rich water is substantially higher than what has been documented in any salmonid fish species in earlier studies (for an overview, see Howells *et al.*, 1990). Andersen *et al.* (1984) studied the effects of acidification on Arctic char and brown trout populations in three lakes in a coastal area in southern Norway. Based on catch per unit effort, age structure and recruitment, as well as growth and condition factors, it was suggested that Arctic char were more affected than brown trout. Furthermore, it was concluded that the observed differences in stock structure probably reflect species–specific differences in tolerance. Our results, however, indicate that this species–specific tolerance is not to aqueous aluminium. The lower resistance in the Arctic char to acidification observed by Andersen *et al.* may very well be an effect of changes in biotic factors favouring the brown trout in interspecific competition. In accordance with this, Almer (1974) found Arctic char to be one of the most sensitive species to acidification in a field survey investigation. Bergquist (1991), on the other hand, reported that Arctic char had an intermediate sensitivity to acidification among different fish species, but that it was less sensitive than brown trout. Because the previous reports referring to the sensitivity of Arctic char are based upon field data, and are not thoroughly correlated with the concentration of aqueous aluminium, we have to be careful comparing them with our experimental study.

An interesting observation concerning the Arctic char was that 35% mortality was reached after approximately 250 h of exposure in medium A, and that only two out of the remaining 54 fish died during the next 230 h of the experiment (Fig. 2). This observation

Table 6. Mortality, as LT_{50}, of the fish exposed to medium A. The species are grouped according to their sensitivity to medium A

Species	LT_{50}			B	C
Sensitive					
Atlantic salmon	85	(81–90)*	(n=100)	101	29
Roach >1+	97	(92–103)*	(n= 60)	60	16
Roach 0+	284	(276–291)*	(n= 58)	55	57
Intermediate					
Minnow	331	(326–338)*	(n=116)	48	24
Perch	360		(n= 50)	50	15
Grayling	410		(n= 30)	15	16
Brown trout	500		(n= 30)	30	29
Tolerant					
Arctic char	**		(n= 92)	90	30

*Expected mean time with upper and lower 95% confidence limit.
**Only 37% mortality was observed in Arctic char. Number (n) of Exposed fish to medium B (B) and the control water (C) are also indicated.

suggests that Arctic char either has an ability to adapt to acidic Al-rich water, or that the sensitivity to acidic Al-rich water differs between individuals in the population. Further investigations, however, are needed to point out a possible mechanism behind the observed resistance in the surviving individuals of Arctic char.

Minnow

Almer (1974) reported that the minnow was wiped out from acidic waters, and that it seemed to be as sensitive to acidification as the sensitive roach. In addition, Bergquist (1991) reported that the minnow had the highest frequency of extinction from 87 acidified Swedish lakes compared to a large number of other species. For example, the minnow had an extinction frequency of 70% compared to 40% in brown trout and 35% in roach (Bergquist). Our results correspond relatively well with these field surveys, since the minnow was observed to be the third most sensitive fish to our Al-exposure (Fig. 2). However, because a substantial number of minnow died in the control water and in medium B, the quality of the fish which we used was not optimal. We should, therefore, make the comment that the sensitivity of minnow to aluminium might be somewhat lower than what we observed in the present study.

The higher mortality of minnow observed in medium B than in the control (Fig. 2) indicates that the minnow is sensitive to acidification in general, and to acidic Al-containing water in particular. According to this, Norrgren *et al.* (1991) have documented mortality of minnow at pH 5.0 when no aluminium was present. They also found that the mortality at pH 5.0 was increased if aluminium was added to their experimental water, which supports our observations.

Perch

Through controlled experimental studies, our results demonstrate for the first time that aluminium is an important factor in the toxicity of acidic water to perch, and that the perch is more sensitive to acidic Al-rich water than previously pointed out. The perch is generally believed to be one of the most tolerant freshwater teleost species to acidification (Almer, 1974; Rask, 1983; Bergquist, 1991; Vuorinen *et al.*, 1992; Rask *et al.*, 1995). In our study, however, the perch was relatively sensitive to the Al-challenge used in the experiments. For example, the LT_{50} observed in perch was 350 h, compared to 330 h in minnow (Table 6). We also observed that the perch was more sensitive than the salmonids, grayling, brown trout, and Arctic char. Thus, our results are somewhat contradictory to the general opinion that perch is an acid-tolerant fish. We believe that this contradiction can be explained to some extent by the ability of perch to live in humic lakes and ponds. Within areas where such water systems are common, the perch is well distributed. In humic lakes, aluminium is readily complexed to the humic substances, and it is well known that organic bound aluminium is not toxic to fish (Baker and Schoefield, 1980; Driscoll *et al.*, 1980; Fivelstad and Leivestad, 1984; Witters *et al.*, 1990; Poléo *et al.*, 1991). Thus, within a large part of its distribution, the perch is seldom, or never, exposed to elevated concentrations of toxic inorganic aluminium, even when these areas are subject to acid precipitation and soil and water acidification. Indeed, recent estimates show that perch seems to be relatively tolerant to acidic water as long as the Al-concentration is low (Rask *et al.*, 1995). One interesting observation made by Rosseland *et al.* (1980) supports our argument. They observed that, in some cases, perch populations were lost before brown trout populations from acidified lakes in the southern part of Norway. This part of Norway is dominated by clearwater lakes with low contents of organic substances and high concentrations of inorganic aluminium. We therefore propose that the perch is an aluminium-sensitive, but H^+-tolerant species, and this corresponds well with the finding made by Rask *et al.* (1995), that the concentrations of labile aluminium are essential in determining whether or not perch populations in acidified lakes are affected.

Finally, the contradiction between the general opinion of a high resistance to acidification in perch, and our observation of a low resistance to aqueous aluminium, may also be explained partly by the fact that many studies have not paid enough attention to the presence of toxic aluminium in the waters.

Grayling

To our knowledge, the effect of acidified water and aluminium on grayling has never before been studied. The observed mortality in medium A is therefore the first documentation of a toxic response in grayling to acidic Al-rich water (Fig. 2). Our results also demonstrate that acidic Al-poor water with pH 5.0 is not acutely toxic to grayling which, according to the literature, corresponds well with what is found in other freshwater teleosts (Howells *et al.*, 1990). Compared to the other salmonids in the present study, the grayling is more sensitive to

aluminium than brown trout and Arctic char, but much less sensitive than Atlantic salmon (Table 6).

Brown trout

The sensitivity to medium A which we observed in the brown trout (Table 6) is substantially lower than what has been reported in many previous studies (Rosseland and Skogheim, 1984; Howells *et al.*, 1990; Reader *et al.*, 1991). Grande *et al.* (1978), however, reported a toxic response in brown trout similar to that of the present study. They exposed parr of different salmonids to acidic stream water and their results showed that the LT_{50} in brown trout was approximately 960 h, while the LT_{50} in Atlantic salmon was approximately 144 h. The brown trout/Atlantic salmon LT_{50} ratio in the study of Grande *et al.* (1978) and in the present study was exactly the same ($^{b.t.}LT_{50}/^{A.s.}LT_{50} = 6.6$). Fivelstad and Leivestad (1984), also reported relatively large differences in toxic response between brown trout and Atlantic salmon exposed to acidic Al-rich water. Apart from these reports, however, most studies of Al-toxicity in brown trout have demonstrated toxic responses more or less similar to other salmonids (Howells *et al.*, 1990). This disagreement between observed toxic response to acidic Al-rich water in brown trout among different studies might have many explanations. The most likely is probably the differences in bulk-water chemistry, especially Al-chemistry and speciation which varies greatly, in the large number of investigations. Genetic variation in tolerance among different trout populations, however, might in some cases also be an explanation (Gjedrem, 1980; Rosseland and Skogheim, 1987).

Relative tolerance to aluminium among species

In the present study, perch, roach 0+, grayling, and brown trout were exposed to slightly lower temperatures (4.5–5.5°C) than Atlantic salmon, roach >1+, Arctic char, and minnow (8.0–9.9°C). Experiments with Atlantic salmon have previously demonstrated that Al-toxicity to fish is dependent on water temperature (Poléo *et al.*, 1991; Poléo and Muniz, 1993). We believe, however, that the temperature variations between the exposures are not sufficient to explain the overall species differences we observed in Al-toxicity. In Table 6, we have grouped the seven species according to their sensitivity (sensitive, intermediate, and tolerant) to the acidic Al-rich medium A, based on LT_{50}.

Among the sensitive species are the Atlantic salmon and roach (both 0+ and >1+). Within this group, the mortality of roach 0+ was much lower compared to the older roach and Atlantic salmon. As already mentioned, however, this difference in mortality can to some extent be explained by the difference in test-water temperature between the exposures. Thus, a higher water temperature in the experiment with roach 0+ would probably have given a higher mortality, more like roach >1+ and Atlantic salmon which were exposed to temperatures between 8.0 and 9.9°C. The intermediate group consists of minnow, perch, grayling, and brown trout. The highest sensitivity within this group was recorded in the minnow. This species, however, was exposed to a higher test-water temperature (8.0–9.9°C) compared to the other three species (4.5–5.5°C). Finally, the tolerant group consists of only the Arctic char, which showed an unexpected low sensitivity to the aluminium challenge in the present study. The observed mortality in Arctic char would probably have been even lower if the char had been exposed when water temperature was between 4.5 and 5.5°C.

As already mentioned, it is generally accepted that salmonid fish species are the most sensitive freshwater teleosts to aqueous aluminium (Howells *et al.*, 1990). Except for the Atlantic salmon, which appeared to be the most sensitive species in our study, our results diverge from the general assumption made in the review of Al-toxicity in fish by Howells *et al.* (1990). We found that Arctic char, brown trout, and grayling were the most tolerant species to the high aluminium challenge in our experiment. In comparison, roach, minnow, and perch were more sensitive to aluminium than these salmonids. We believe that the disagreement between our toxicity study and previous field investigations can be explained partly by environmental factors which do not appear under laboratory conditions, and partly by the fact that many field surveys have not considered aqueous Al-chemistry when assessing the relative sensitivity among fish species. Among important environmental factors which do not appear under laboratory conditions are predator–prey interactions and interspecific competition for nursery and feeding areas. Apart from elevating the concentration of aqueous aluminium, acidification of the freshwater environment most certainly alters these other environmental factors. Both altered interactions between species within fish populations and elevated concentrations of toxic substances like aluminium are of major importance to understand the negative effects of freshwater acidification.

Among the non-salmonid species in the present study, roach was the most sensitive while minnow and perch were observed to be the more tolerant species to aluminium. This relative sensitivity among the non-salmonid species in our study, corresponds well with previous reports (Almer, 1974; Bergquist, 1991; Rask *et al.*, 1995). Almer found that the sensitivity among these three species for low pH was in the order: roach, minnow and perch, while Bergquist grouped them in the following order: minnow, roach and perch.

It has been hypothesized that hypoxia contributes significantly to the mortality caused by acutely toxic aqueous aluminium in fish (Neville 1985; Poléo, 1995; Poléo *et al.* 1995). Poléo (1995) suggested that the process of Al-polymerisation is the mechanism which best explains the hypoxia and acute toxicity of aqueous aluminium to fish, especially above pH 5.0 or after a rise in pH. It has been confirmed that mortality of Atlantic salmon and brown trout in Al-rich water increases several-fold when the fish are exposed to the initial phase of the Al-polymerisation process (Lydersen *et al.*, 1994; Poléo *et al.*, 1994). The lowest resistance to acidic

Al-rich water seems to be found in fish species which also have a very limited hypoxia tolerance, such as Atlantic salmon, rainbow trout, and roach (Almer, 1974; Grande *et al.*, 1978; Howells *et al.*, 1990; Rask *et al.*, 1995). Thus, the most sensitive species in our study were the Atlantic salmon and the roach (Table 6).

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