

INCREASED MORTALITY OF FISH DUE TO CHANGING AL-CHEMISTRY OF MIXING ZONES BETWEEN LIMED STREAMS AND ACIDIC TRIBUTARIES

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Abstract. The present study is mainly focusing on mortality variations of fish due to changing Al-chemistry of mixing zones. An artificial mixing zone was made by pumping water from a limed stream and an acidic tributary into a mixing channel. Atlantic salmon (*Salmo salar* L.) parr were exposed to the mixed water, limed stream water, and acidic tributary water. Mortality, blood haematocrit and plasma Cl^- -concentration were recorded. Neither mortality, nor changes in haematocrit and plasma Cl^- were observed when fish were exposed to limed water, while in both acidic and mixed water, mortalities and loss of plasma Cl^- were observed. The highest mortality rates were found within the initial part (0 to 20 s) of the mixing zone. Blood haematocrit increased only in fish exposed to acidic tributary water. Our results shows that changes in Al-chemistry and subsequent Al-polymerization occur when acidic tributary water is mixed with limed stream water. We have also demonstrated that the toxicity which can arise in mixing zones are greater than in the original acidic water before mixing. The variations in mortality observed are associated with the quality and quantity of Al-polymerization as well as ageing of the polymers.

1. Introduction

Liming is extensively used in the restoration of acidified lakes and streams, not only to increase the water pH and alkalinity, but also to reduce the amount of toxic inorganic Al species. Subsequent survival of sensitive fish-species is often greatly enhanced. Immediately after liming, however, fish mortality has been reported (Dickson, 1983). Recently, field studies in the mixing zones between limed streams and acidic tributaries, have documented Al-toxicity to fish (Weatherly *et al.*, 1991; Rosseland *et al.*, 1992).

Aluminium is found to be highly toxic at a pH level of 5.0 to 5.4 (Schofield, 1977; Baker and Schofield, 1982; Cleveland *et al.*, 1986; Leivestad *et al.*, 1987). The toxicity is particularly high in oversaturated solutions with ongoing Al-precipitation (e.g. Dickson, 1983; Baker and Schofield, 1980; Poléo *et al.*, 1991). Within pH 5.0 to 5.4, the hydrolysis products, $\text{Al}(\text{OH})(\text{H}_2\text{O})_5^{2+}$ and $\text{Al}(\text{OH})_2(\text{H}_2\text{O})_4^+$, may

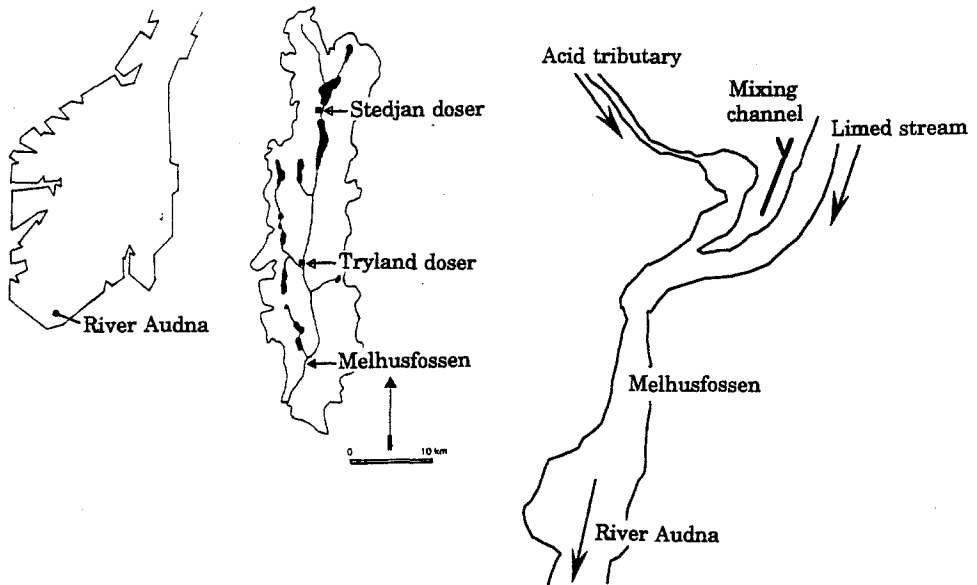


Fig. 1. Location of the experimental field. Left; map of southern Norway with the site of River Audna marked out. Center; The River Audna catchment area. Right; The position of the mixing channel between the acid tributary and the limed stream just upstream of Melhusfossen.

constitute the predominant part of the inorganic dissolved Al within the actual temperature regime of most natural freshwaters, i.e. 0 to 25 °C (Lydersen, 1990), providing that the F^- -concentration is low. Because the Al-polymerization may start as soon as the $Al(OH)_2(H_2O)_4^+$ starts to form (Hem and Roberson, 1967), polymerization might be the main cause of the increased Al-toxicity observed within this pH region (pH 5.0 to 5.4) (Poléo *et al.*, 1991; Rosseland *et al.*, 1992; Poléo and Muniz, 1992). The gills may act as nucleating surfaces upon which Al can polymerize (Zaug, 1982; Playle and Wood, 1989, 1990), and it has recently been demonstrated that Al on fish gills arises from polymerization of low molecular weight Al-species (Oughton *et al.*, 1992). This deposition of Al onto the gill surfaces may result in clogging of the interlamellar spaces.

In this study, limed river water was mixed together with acidic water containing relatively high amounts of low molecular weight (LMW) inorganic monomeric Al (Al_i), and led through a 35 m long channel with a residence time of 6 min and 3 s. Atlantic salmon (*Salmo salar* L.) parr were exposed to the mixed water at different sites along the channel. Thus, it was possible to study the initial Al-polymerization processes and test if there were any changes in the Al-toxicity as the polymerization proceeded with time, i.e. along the channel.

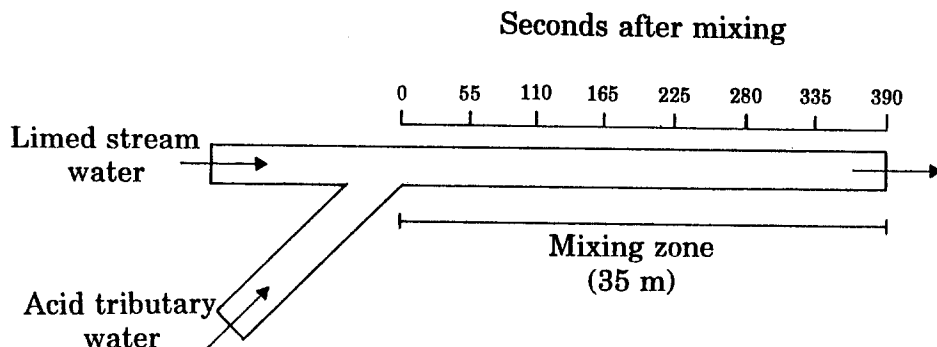


Fig. 2. Schematic representation of the experimental setup, indicating the relationship between residence time and length of the mixing zone.

2. Material and Methods

2.1. EXPERIMENTAL DESIGN

Atlantic salmon parr, ranging 12 to 14 cm in total length and 17 to 24 g in weight, were obtained from a research hatchery in Dirdal, Rogaland in Southwestern Norway. The experiment was performed in the River Audna, Vest Agder, Southern Norway, in november 1990. The river has been limed with automatic dosers since 1985 (Rosseland and Hindar, 1988). The fish were acclimatized to the limed water for 1 mo prior to the experiment. A complete laboratory was set up in the field just upstream of Melhusfossen, where an acidic tributary entered the limed river (Figure 1). An artificial mixing zone was made by pumping water from the river Audna and from the acidic tributary into separate channels (Figure 2). The mixing zone consisted of 33% limed stream water and 67% acidic tributary water.

The channel was 35 m long, 20 cm wide and 15 cm deep. During the experiment, the fish were kept in rectangular cages, 20 cm wide and 30 cm long. 14 cages were distributed along the channel; 6 cages containing between 7 and 18 fish within the first 2 m, and 8 cages containing between 5 to 11 fish along the remaining 33 m. A cage was also placed in each of the two branches in front of the mixing zone. Thus, the fish were exposed to both limed ($n=14$) and acidic water ($n=16$) as well as mixed water. The fish were sheltered by covers over the cages.

2.2. ANALYTICAL TECHNIQUES

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

Aqueous Al was analyzed 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 hr, and thereafter at room temperature (20 °C) for 2 hr before absorbance measurements were carried out on a Shimadzu UV-120-02 spectrophotometer at

395 nm (Tikhonov, 1973; Bloom *et al.*, 1969). Absorbance was also measured at 600 nm in order to correct for iron interference (Sullivan *et al.*, 1986).

By direct extraction of water samples, the amount of total monomeric Al-species (Al_a) was determined (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 (1984), the cation-exchange flow-rate was 3.8 mL min^{-1} per mL bed-volume. The resin was prepared by displacing some of the exchangeable H^+ ions with Na^+ . When an eluant of comparable ionic strength to that of the water samples being analyzed was passed through the exchanger, the pH of the effluent should be similar to the pH of the sample. A volume of $60 \text{ mL } 10^{-4} \text{ M}$ 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 Al 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 analyzed by HQ/MIBK-extraction after acidifying water samples with HCl to pH 1.0 for 24 hr. 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 H1P10-8 hollowfiber cartridge with a nominal molecular weight cutoff level of 10 kD. Thus, it is possible to divide water samples into two molecular weight fractions, a high molecular weight fraction ($M_w > 10 \text{ kD}$) and a low molecular weight fraction ($M_w < 10 \text{ kD}$). A hollow fiber cartridge consists of several fine cylindrical tubes, each with a lumen diameter of 0.2 or 0.5 mm. The matrix is inert, non-ionic polymers. The high axial flow through hollowfiber lumens produces high shear forces at the membrane surface, minimizing concentration polarization, clogging and sorbtion by rejected solutes. As water flow through the fibers, it causes micro-solutes and salts to penetrate the membrane, while rejected solutes are discharged as waste. 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) are shown to be minimal, providing the fibers are preconditioned with 250 ml of the sample water before the ultrafiltrate is collected for analysis (Lydersen *et al.*, 1987).

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 minute. 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 ($\mu\text{S cm}^{-1}$). The pH and conductivity measurements as well as Al-extractions were performed immediately after the water samples were taken.

Total fluoride (F^-) was analyzed 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 ionselective fluoride electrode is expected to be $\pm 2\%$ (Harwood, 1969). Na^+ , K^+ , Ca^{2+} and Mg^{2+} were analysed by Atomic Absorption Spectroscopy (AAS), SO_4^- and Cl^- by ion chromatography (IC), NO_3^- by the indophenolblue method, reactive Si with the standard molybdate-complexing method, and total organic carbon (TOC) by a combined photochemical (UV) wet-chemical ($\text{S}_2\text{O}_8^{2-}$) oxidation method.

Blood samples were collected from the caudal artery by cutting the caudal fin, and centrifuged for five min. Haematocrit was measured, and the plasma samples were immediately analysed for chloride (Cl^-). The plasma Cl^- was determined colourmetrically using a Radiometer CMT-10 Chloride Titrator, with an expected precision of $\pm 0.5\%$.

3. Results

3.1. WATER CHEMISTRY

Water quality parameters of the limed river, the acidic tributary and the mixing zone are presented in Tables I and II. The water temperature was approximately the same in all three media (between 6.5 and 7.2 °C). During the experiment, the mean pH of the limed river was 6.41 ($[\text{H}^+] = 3.89 \times 10^{-7} \pm 0.54 \times 10^{-7}$ (n=7)) and the total concentration of Al was $226 \pm 16 \mu\text{g Al L}^{-1}$ (n=3). Despite the high concentration of total Al, the concentration of inorganic monomeric Al (Al_i) was very low, $14 \pm 6 \mu\text{g Al L}^{-1}$ (n=3) (Table II). In the acidic tributary, the mean pH was 4.89 ($[\text{H}^+] = 1.29 \times 10^{-5} \pm 0.13 \times 10^{-5}$ (n=7)) and the total concentration of Al was $258 \pm 10 \mu\text{g Al L}^{-1}$ (n=3), where $139 \pm 16 \mu\text{g Al L}^{-1}$ (n=3) was present as Al_i . In the mixed water (33% limed river water, 67% acidic tributary water), the mean pH in the 35 m long channel was 5.64 ($[\text{H}^+] = 2.29 \times 10^{-6} \pm 0.48 \times 10^{-6}$ (n=28)), and the total concentration of Al was $242 \pm 8 \mu\text{g Al L}^{-1}$ (n=36). The Al_i was $70 \pm 4 \mu\text{g Al L}^{-1}$, approximately half the concentration of Al_i in the acidic tributary water. No chemical changes were found analytically within the mixing zone, i.e. from the mixing site (t=0) to the end of the channel (t=6 min and 30 s).

In the limed river, only 24% ($55 \pm 8 \mu\text{g Al L}^{-1}$) of the total amount of Al (Al_r) was present as low molecular weight (LMW) species (Mw < 10 kD), and only $14 \pm 6 \mu\text{g Al L}^{-1}$ was present as Al_i . In the acidic tributary, 79% of total Al ($204 \pm$

TABLE I

Major water quality parameters of the limed river, the acid tributary, and the mixing zone. The values from the mixing zone corresponds to an ageing time of about 3 min and 10 sec after mixing

		Limed river	Acid trib.	Mixing zone
pH		6.41	4.89	5.64
Temp.	°C	6.5	7.2	7.0
TOC	mg C L ⁻¹	3.13	2.33	2.30
Na ⁺	mg L ⁻¹	3.29	4.23	3.93
K ⁺	mg L ⁻¹	0.40	0.28	0.32
Ca ²⁺	mg L ⁻¹	2.72	1.35	1.81
Mg ²⁺	mg L ⁻¹	0.48	0.56	0.54
SO ₄ ²⁻	mg L ⁻¹	4.00	4.00	4.00
Cl ⁻	mg L ⁻¹	5.60	7.59	6.95
Si	mg L ⁻¹	2.10	1.70	1.83
HCO ₃ ⁻	μeq L ⁻¹	45	0	7
NO ₃ -N	μg L ⁻¹	220	210	213
F ⁻	μg L ⁻¹	50	46	46

TABLE II

The pH and concentration of different Al-species in total and hollowfiber ultrafiltered water from the limed river, the acid tributary, and the mixing zone. Hollowfiber cutoff: 10 kDalton

	pH	Al _a	Al _o	Al _i	Al _r -Al _a
		μg Al L ⁻¹			
Limed river	6.41	74 ± 8 (n=3)	60 ± 6 (n=3)	14 ± 6 (n=3)	152 ± 16 (n=3)
Ultrafiltered	6.44	24 ± 7 (n=3)	10 ± 8 (n=3)	14 ± 6 (n=3)	31 ± 8 (n=3)
Acid Tributary	4.89	179 ± 14 (n=3)	40 ± 4 (n=3)	139 ± 16 (n=3)	79 ± 9 (n=3)
Ultrafiltered	4.96	149 ± 8 (n=3)	23 ± 3 (n=3)	126 ± 12 (n=3)	55 ± 10 (n=3)
Mixing zone	5.64	131 ± 4 (n=36)	61 ± 2 (n=36)	70 ± 4 (n=36)	111 ± 8 (n=36)
Ultrafiltered	5.66	71 ± 4 (n=36)	17 ± 3 (n=36)	54 ± 5 (n=36)	50 ± 3 (n=36)

TABLE III

Distribution of monomeric Al species present in total and hollowfiber ultrafiltered samples, from the limed river, the acid tributary, and the mixing zone. The amounts of inorganic monomeric Al species are calculated by means of ALCHEMI (Schecher and Driscoll 1987, 1988), while the organic monomeric Al-fraction (Al_o) is measured

	Total			Filtered (Mw < 10 kDalton)		
	Limed river	Acid trib.	Mixing zone	Limed river	Acid trib.	Mixing zone
pH	6.41	4.89	5.64	6.44	4.96	5.66
Temp.	6.5	7.2	7.0	6.5	7.2	7.0
	$\mu\text{g Al L}^{-1}$					
Al^{3+}	0	41	3	0	3	2
$AlOH^{2+}$	0	8	3	0	7	2
$AlOH_2^+$	2	2	5	2	2	3
AlF^{2+}	1	44	15	1	43	13
AlF_2^+	1	3	4	1	3	5
$AlOHF^+$	8	13	26	8	13	21
$AlOHF_2$	1	0	0	1	0	0
$AlH_3SiO_4^{2+}$	1	28	14	1	24	8
Sum Al_i	14	139	70	14	127	54
Al_o	60	40	61	95	49	81

$10 \mu\text{g Al L}^{-1}$) was present as LMW species, and 91% ($126 \pm 12 \mu\text{g Al L}^{-1}$) of Al_i was on LMW forms. In the mixing zone, 50% of total Al ($121 \pm 4 \mu\text{g Al L}^{-1}$) was present as LMW species, and about 80% of the Al_i ($54 \pm 5 \mu\text{g Al L}^{-1}$) was present in LMW forms.

The distribution of inorganic monomeric Al-species is shown in Table III. Based on chemical modelling (ALCHEMI), most of the inorganic monomeric Al are assumed to be present as Al-fluorides in the three media. In the acidic water, there was also a substantial amount of Al^{3+} -ions present.

3.2. FISH MORTALITY AND PHYSIOLOGY

There was no mortality of salmon parr exposed to limed water within the 68 hr experimental period. In the acidic tributary and in the mixing zone, however, mortality was observed (Figure 3). In the first 0.5 to 2.0 m of the mixing channel, i.e. within 20 s after mixing, the mortality was substantially higher ($74.8 \pm 12.0\%$ ($n=6$ cages)) than in the remaining 30 m (>1 min after mixing) of the channel ($16.0 \pm 10.3\%$ ($n=8$ cages)), and in the acidic tributary (27% ($n=1$ cage)). From

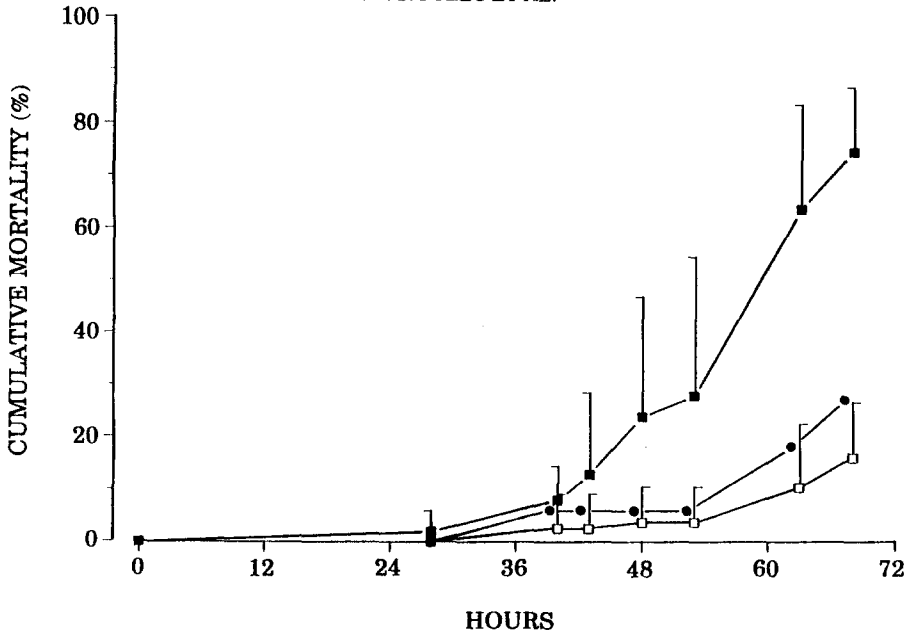


Fig. 3. Cumulative mortality of Atlantic salmon *Salmo salar* L. parrin; (●) the acid tributary ($n=1$ cage), (□) the first 2.0 m of the mixing zone (0–20 s after mixing, $n=6$ cages), and (□) the last 30 m of the mixing zone (> 1 min after mixing, $n=8$ cages). Mean with SD. No mortality occurred in the limed stream.

5.0 m (1 min after mixing) and down along the channel, the mortality-rate was approximately similar to the mortality-rate in the acidic tributary.

Fish exposed to acidic and mixed water lost plasma Cl^- throughout the experiment (Figure 4). The plasma Cl^- -concentrations decreased from $119 \pm 10 \text{ mmol L}^{-1}$ ($n=5$), to $53 \pm 2 \text{ mmol L}^{-1}$ ($n=4$) in the acidic tributary, and to 73 ± 18 ($n=5$) mmol L^{-1} and $71 \pm 15 \text{ mmol L}^{-1}$ ($n=15$) in the first (0.5 to 2 m) and second (5 to 35 m) part of the channel, respectively. There was no decrease in plasma Cl^- -concentration of fish exposed to limed water within the first 48 hr of the experiment. During the period 48 to 68 hr, the plasma Cl^- showed a decrease from $113 \pm 10 \text{ mmol L}^{-1}$ ($n=4$) to $94 \pm 8 \text{ mmol L}^{-1}$ ($n=4$).

Blood haematocrit in fish exposed to acidic tributary water increased from $47 \pm 8\%$ ($n=5$) to $67 \pm 8\%$ ($n=4$) during the experiment. The haematocrit in fish exposed to limed water was unchanged throughout the experiment. Even in the mixed water, haematocrit did not change significantly during the experiment, but the values were somewhat higher than in the limed water (Figure 5).

4. Discussion

In the present study it is documented for the first time that rapid changes in Al-chemistry after mixing of limed stream water and acidic tributary water can create highly toxic mixing zones with enhanced mortality of fish. Due to mixing of the

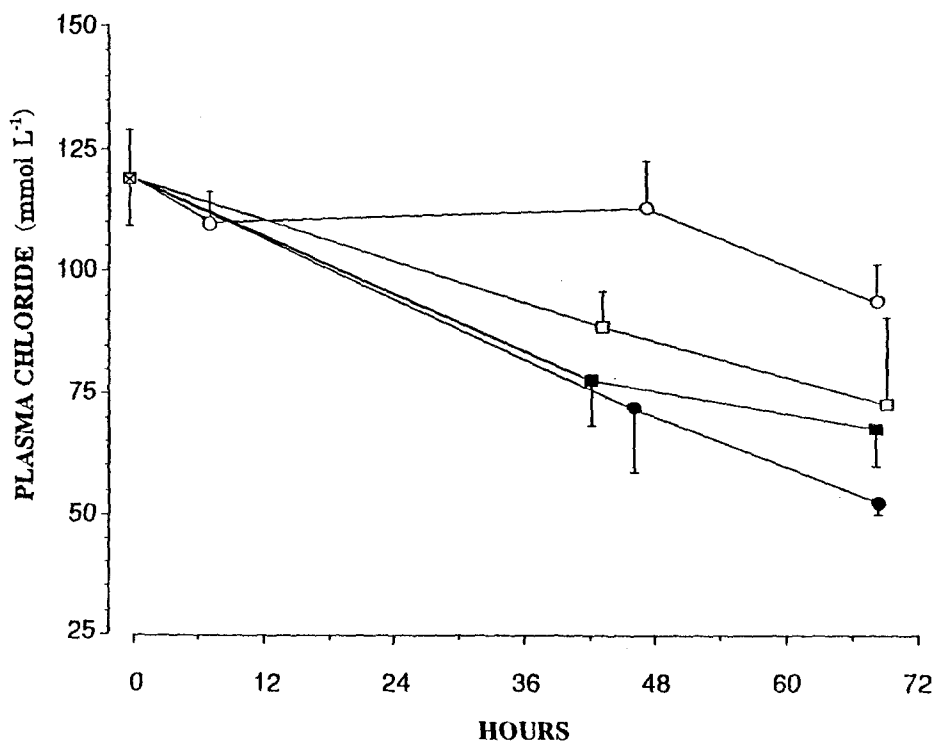


Fig. 4. Mean concentration of plasma chloride (Cl^-) among Atlantic salmon parrin in; (○) the limed stream, (●) the acid tributary, (□) the first 2.0 m of the mixing zone (0–20 s after mixing), and (□) the last 30 m of the mixing zone (> 1 min after mixing). Mean with SD. (□) control fish.

media, pH increased and caused better conditions for Al-polymerization which is believed to be the major factor for the acute toxicity of Al to fish.

No mortality was observed when salmon were exposed to limed water, while in both acidic and mixed water, mortalities were observed (Figure 3). The highest mortality-rates were found within the first 20 s after mixing (0 to 2 m) in the mixing channel, and not in the acidic tributary as might have been expected. The aluminate ion, $\text{Al}(\text{OH})_4^-$, Al-fluorides and polymeric hydrolysis products are all possible causes of the observed variations in mortality (Leivestad *et al.*, 1987; Lydersen *et al.*, 1990a; Wilkinson *et al.*, 1990; Poléo *et al.*, 1991; Weatherly *et al.*, 1991; Rosseland *et al.*, 1992).

The limed water (pH 6.41, 6.5 °C) was the only medium having conditions making the presence of the monomeric aluminate ions possible. The limed water, however, was not observed to be toxic despite high amounts of Al ($226 \pm 16 \mu\text{g Al L}^{-1}$). Most of the Al was present as high molecular weight (HMW) colloidal Al-polymers ($152 \pm 16 \mu\text{g Al L}^{-1}$) and as organic monomeric Al-species ($60 \pm 6 \mu\text{g Al L}^{-1}$), which is both shown to be non-toxic to fish (Baker and Schofield, 1980,

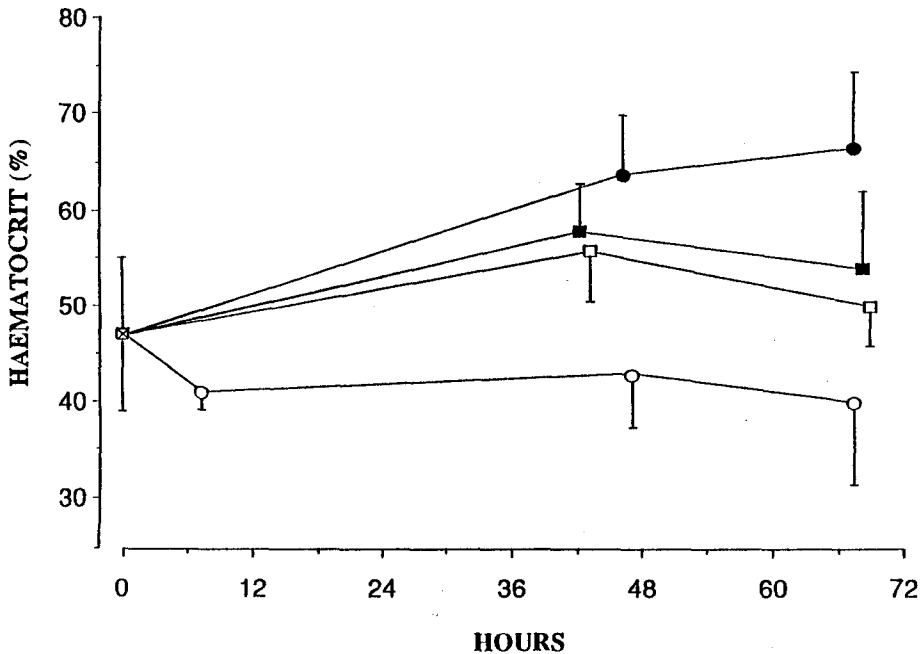


Fig. 5. Mean haematocrit among Atlantic salmon parr in; (○) the limed stream, (●) the acid tributary, (■) the first 2.0 m of the mixing zone (0–20 s after mixing), and (□) the last 30 m of the mixing zone (> 1 min after mixing). Mean with SD. (□) control fish.

1982; Driscoll *et al.*, 1980; Skogheim *et al.*, 1986; Witters *et al.*, 1990; Lydersen *et al.*, 1990a; Poléo *et al.*, 1991). Thus, when the pH of natural acidic, Al-rich water is raised to a pH between 6.0 and 7.0, the aluminate ion actually does not exist in significant amounts, probably because it is incorporated into polymerized Al-species (Bertsch, 1990).

Besides pH and temperature, the most important parameters for the Al-speciation and consequently the Al-toxicity of dilute fresh waters, are the concentrations of F^- , H_4SiO_4 , Ca^{2+} and TOC. Presence of one or several of these chemical constituents will reduce the Al-toxicity to fish compared with solutions containing Al-hydrolysis products only (Baker and Schofield, 1980; Driscoll *et al.*, 1980; Baker, 1982; Cleveland *et al.*, 1986; Skogheim *et al.*, 1986; Wood *et al.*, 1988, 1990; Birchall *et al.*, 1989; Lydersen *et al.*, 1990a; Wilkinson *et al.*, 1990; Witters *et al.*, 1990; Poléo *et al.*, 1991). The concentrations of these components, however, are almost the same in the limed water, the acidic water, and the mixed water (Table I and III). Therefore, these components cannot explain the toxicity variations observed, i.e. from non-toxic limed river water to the highly toxic mixed water.

Even though both pH and temperature of the acidic tributary water are low, Al-polymerization may occur, but probably to a small extent (Lydersen, 1990). Due to a higher pH in the mixing zone, Al-polymerization should be enhanced. Based on the

concentration of different Al-species in both the limed river and the acidic tributary, and on the mixing-ratio of river and tributary water, it is possible to some extent, to quantify the polymerization of Al in the mixing zone. In the mixed water, the concentration of Al_i decreased by $28 \pm 4 \mu\text{g Al L}^{-1}$ (29%), while the concentration of Al_o increased by $14 \pm 2 \mu\text{g Al L}^{-1}$ (23%). An increase in Al_o soon after a pH shift has earlier been demonstrated to be a result of physicochemical changes of inorganic Al (Lydersen *et al.*, 1991). Thus, both the decrease in Al_i and the increase in Al_o reflects Al-polymerization in the mixed water, Al-polymerization is also documented in the hollow-fiber ultrafiltered samples, where $35 \pm \mu\text{g Al}_i \text{ L}^{-1}$ was removed after mixing the two waters. This agrees with the decrease in total Al_i of $28 \pm 4 \mu\text{g L}^{-1}$ and the increase in total Al_o of $14 \pm 2 \mu\text{g L}^{-1}$, which together yield $42 \pm 4 \mu\text{g Al L}^{-1}$.

Several authors have documented high toxicity when fish were exposed to oversaturated Al-solutions with ongoing Al-precipitation (Dickson, 1978, 1983; Baker and Schofield, 1980, 1982; Grahn, 1980; Hutchinson *et al.*, 1989). Because ongoing Al-polymerization probably is of major importance for the Al-toxicity (Poléo *et al.*, 1991; Rosseland *et al.*, 1992; Poléo and Muniz, 1992), the decrease in toxicity observed along the channel (Figure 3) should be expected as the Al-chemistry is stabilized with time (aging). The variation in mortality observed along the channel reflects chemical changes which were not possible to detect analytically. However, we have previously demonstrated that such changes in the Al-chemistry can be determined analytically when total concentration of Al and temperature are high (Lydersen *et al.*, 1990b, 1991). Due to a longer aging time of the limed water (several hours) compared to the artificially mixed water (from 0 to 6 min and 30 s), the Al-polymerization is more advanced and the Al-toxicity therefore totally eliminated.

In addition to these differences in polymerization characteristics, the levels of Ca^{2+} is of importance for the toxicity. The Ca^{2+} -concentration was $2.72 \mu\text{g Ca}^{2+} \text{ L}^{-1}$ in the limed stream, compared to $1.81 \mu\text{g Ca}^{2+} \text{ L}^{-1}$ in the mixing zone. Although small differences, these levels have shown to be of critical importance for the survival of fish (Rosseland and Hindar, 1991). Because the Ca^{2+} -concentration was even lower in the acid tributary ($1.35 \mu\text{g Ca}^{2+} \text{ L}^{-1}$), having a lower mortality than the mixing zone, changing Al-chemistry must be the key factor for the toxicity variations in our system.

Only fish exposed to acidic tributary water had a significant increase in haematocrit (Figure 5). Despite a lower mortality rate, fish in the acidic water also showed a higher loss of plasma Cl^- , compared to the fish in the first part of the mixing zone (Figure 4). Generally, fish death in Al-rich water is believed primarily to be due to hypoxia and/or electrolyte loss (e.g. Neville, 1985; Wood and McDonald, 1987; Rosseland *et al.*, 1990). According to Neville (1985), fish death in Al-rich water was primarily due to hypoxia at pH 6.1 and to electrolyte loss at pH 4.5 and 4.0. Between pH 5.0 and 5.5, where fish mortality seems most abundant, Neville (1985) found a transition between the two mechanisms. In the acidic tributary

where Al-polymerization was low, the enhanced loss of electrolytes from plasma was probably the most important cause of the Al-toxicity. In the initial part of the mixing zone however, the mortality may be caused by hypoxia in addition to electrolyte loss. The hypoxia is probably due to Al-polymerization and subsequent accumulation onto the gill surface which contain numerous negatively charged organic sites (Clamp *et al.*, 1978). Because hypoxia and electrolyte loss separately are not as harmful as a combination of these two mechanisms (Wood and McDonald, 1987; Poléo and Muniz, 1992), fish survived for a longer time in the acidic tributary water than in the initial part of the mixing channel.

Our results show that changes in Al-chemistry and subsequent Al-polymerization occur when acidic tributary water is mixed with limed stream water. Under such circumstances, fish may suffer from a combination of hypoxia and electrolyte loss. Decreased toxicity along the mixing channel is explained by the stabilization of the Al-polymers with time, and not by any other chemical factors, for example pH and Ca^{2+} , in our system. Thus, the variations in mortality observed are associated with the quality and quantity of Al-polymerization as well as aging of the polymers.

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