The effects of ionic strength on the toxicity of aluminium to Atlantic salmon (*Salmo salar*) under non-steady state chemical conditions

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ABSTRACT

We have tested the influence of water ionic strength on the toxicity of aluminium in fish by comparing the mortality of Atlantic salmon (Salmo salar) *parr exposed to Al-rich water with additions of* Ca^{2+} *or* Na^+. *The fish were exposed in parallel to Al-rich water (Al 500 µg* l^{-1}, *pH 5.8) under non-steady state conditions, with and without the addition of one of the two base cations. The amount of* Na^+ *and* Ca^{2+} *added to the water was calculated in order to obtain an identical increase in water ionic strength. Fish mortality was dependent on water residence time and whether or not base cations were added to the Al-rich water. In all Al-exposures, the highest mortality was always observed in fish exposed to water with the shortest residence time. Mortality decreased systematically with increasing water residence time through the exposure set-up. The addition of a base cation,* Ca^{2+} *or* Na^+, *to the Al-rich water reduced fish mortality significantly compared to the Al-only exposures. Furthermore, increasing ionic strength with* Na^+ *reduced mortality to a larger extent than the corresponding increase in ionic strength by the addition of* Ca^{2+}. *The variation in mortality between the various aluminium and base cation treatments is discussed in terms of aluminium chemistry, specific mitigating effects of* Ca^{2+} *and* Na^+, *and the general importance of water ionic strength. This study clearly demonstrates that* Ca^{2+} *does not play an unique role as an ameliorating cation for Al-toxicity in fish under non-steady state chemical conditions. Thus, ionic strength seems to be important, probably for the interaction between aluminium and the gill surface, reducing the possibility for positively charged aluminium species to bind to negatively charged sites.*

Key words: acidification, aluminium toxicity, fish, Atlantic salmon, ionic strength

1. INTRODUCTION

One of the most important consequences of the acidification of soil water systems is the mobilisation of aluminium from the edaphic to the aquatic environment (Cronan & Schofield 1979; Seip *et al*. 1989). Aqueous aluminium is recognised by many authors as the principal toxicant killing fish in acidified waters (Burrows 1977; Driscoll *et al*. 1980; Howells *et al*. 1994; Gensemer & Playle 1999). Aluminium primarily leaches out from soils with low pH-buffering capacity, i.e. low dissolution rates of base cations. Such acid sensitive catchments are characterised by slowly weatherable soils and rocks, often with only a thin soil cover. Within these catchments, the soil water and runoff concentration of terrestrial derived base cations, like Ca^{2+} and Mg^{2+}, are normally low. Furthermore, acidification is responsible for the depletion of base cations from the soil complex and runoff (Kirchner & Lydersen 1995, Likens *et al*. 1996). Accordingly, it has repeatedly been observed that the negative impacts of acidification on freshwater fish are most severe in low conductivity (i.e. low ionic strength) waters (Bua & Snekvik 1972; Leivestad *et al*. 1980; Muniz 1984; Hutchinson *et al*. 1989; Bulger *et al*.1993).

Ca^{2+} is the predominant cation in most natural waters, and the concentration of Ca^{2+} is positively cor-related with ionic strength. It is well documented that high concentrations of Ca^{2+} can reduce Al-toxicity in fish (Brown 1981, 1983; Playle *et al*. 1989). This mitigating effect has most often been attributed to Ca^{2+} per se, through its effect on fish gill permeability. This is not surprising since Ca^{2+} is important in stabilising biological membranes, maintaining the integrity of cell to cell junctions, and controlling ion and water permeability across epithelial tissues (Schoffeniels 1967; Oduleye 1975; Steen & Stray-Pedersen 1975; McDonald 1983). In accordance to this, early field survey investigations of acidic lakes in southernmost Norway indicated that the concentration of Ca^{2+} in the water was of the same importance for the fish population status as pH (Wright & Snekvik 1978). Later, however, correspondent surveys indicate no correlation between water Ca^{2+} and fish population status (Muniz & Walløe 1990; Baker *et al*. 1993). It is therefore possible that the mitigating effects of Ca^{2+}, and base cations in general, to some extent can be explained by the water ionic strength (Lydersen *et al*. 2002). The importance of ionic strength was not tested in any of the field surveys mentioned above.

The reason to believe that ionic strength is important for the Al-toxicity in fish, is that the interaction between aqueous aluminium and the gill surface is central for the mechanism of acute Al-toxicity in fish (Exley *et al*. 1991, 1996; Poléo 1995; Poléo & Bjerkely 2000). The ionic strength (I) is defined as:

$$I = 0.5 \Sigma c_i z_i^2$$

(c_i) is the molar concentration and (z_i) the charge of an ion (i), and the sum is taken over all ions in the solution. Ionic binding is probably of major importance for the interaction between aluminium and the gill surface, and there are two main reasons to anticipate that the ionic strength could affect the interaction between them (Lydersen *et al.* 2002). The first reason is that cations may compete for the negatively charged sites on the gill surface. The second reason is that increased ionic strength will reduce the rate of ionic binding between opposite charged molecules (Atkins 1982), because of enhanced competition between anions and the negatively charged gill surface for the cationic Al-species. In other words, the layer above a surface in which dissolved ions can be influenced by its charge is dependent on ionic strength (Chu 1967). In a medium of low ionic strength, this layer will extend further into the solution than in a medium of higher ionic strength. Due to this, the availability of monomeric inorganic Al-species that can bind to the gill surface by ionic bonds is higher in low ionic strength water than in high ionic strength water (Lydersen *et al.* 2002).

Thus, increased ionic strength may reduce Al-toxicity both by reducing 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.* 2002). According to this, it has been indicated that not only calcium, but also high water Na$^+$-concentrations may reduce Al-toxicity in fish (Brown 1981; Dietrich *et al.* 1989).

Present understanding of the effect of aluminium in natural waters and its interactions with aquatic biota is based largely upon chemical equilibrium constants. The environment, however, and in particular the biological environment, is not in steady state. Accordingly, true chemical equilibrium is seldom approached in natural systems like surface waters, and some ecotoxicological studies have indicated that a non-steady state Al-chemistry can predominate in these systems (Weatherley *et al.* 1991; Rosseland *et al.* 1992; Lydersen *et al.* 1994; Poléo *et al.* 1994). The same studies also indicate that a non-steady state transient Al-chemistry may dictate the Al-toxicity to fish in acidified freshwater systems. In the present study we have therefore investigated the possible effect of ionic strength on the toxicity of aluminium under non-steady state chemical conditions. We test the prediction that an equal increase in ionic strength, by the addition of Ca^{2+} and Na$^+$ respectively, will reduce the Al-toxicity to the same extent.

2. METHODS

2.1. Experimental animals

One year old Atlantic salmon (*Salmo salar*) parr, 5.4 ± 0.6 cm and 1.7 ± 0.1 g, were obtained from a local hatchery near Oslo. The experimental fish were brought into the fish holding department at the University of Oslo, where they were kept and acclimatised for two weeks prior to the experiments. The fish holding department receives dechlorinated Oslo tap water (Tab. 1).

Tab. 1. Major water quality parameters of the water in the fish holding department at University of Oslo measured during the experimental period. *Conductivity was measured at ambient temperature (4.8 – 6.1 °C).

Parameter		mean ± s.d.	N
pH		6.5 ± 0.1	28
Conductivity*	µS cm^{-1}	19.7 ± 0.8	28
Ca^{2+}	mg l^{-1}	3.11 ± 0.07	6
Na$^+$	mg l^{-1}	2.14 ± 0.05	6
Mg^{2+}	mg l^{-1}	0.54 ± 0.01	8
K$^+$	mg l^{-1}	0.36 ± 0.01	8
Cl$^-$	mg l^{-1}	2.63 ± 0.15	4
NO$_3^-$	µg l^{-1}	275	2
SO$_4^{2-}$	mg l^{-1}	6.00 ± 0.49	8
TOC	mg l^{-1}	3.19 ± 0.31	8

2.2. Test conditions

The experiments were performed in the laboratory of the fish holding department at the University. We used a flow-through exposure system for the experiments (Fig. 1), consisting of three channels (218 cm long, 42 cm wide, and 16 cm deep). Each channel was divided into 5 chambers in which the fish were exposed. The water flow rate into the channels was approximately 3.0 l min^{-1}, and the water residence time through each channel was about 20 min. The water was well aerated on its way through the channels, and the water flow of 3.0 l min^{-1} provided at least 5.0 l of water per gram of fish per day. This is well above 2.0 l g^{-1} day^{-1} which is recommended for this kind of experiments (Sprague 1973). The fish were sheltered by covers over the channels.

In two of the channels, dynamic Al-chemistry conditions were prepared by addition of an acidic Al-stock solution to the department water at the inlet of the channels (Fig. 1). The Al-stock solution was prepared by dissolving Al(NO$_3$)$_3$ × 9H$_2$O, and HNO$_3$ in distilled water. The pH of this stock solution was kept low (pH 2.0) in order to ensure that the total amount of Al was present as Al^{3+} before it was added to the department water. The fish were exposed to a dynamic Al-chemistry because the pH (2.0) of the Al-stock solution was rapidly risen to 5.8, as this solution was mixed with the approximately neutral department water (pH 6.5). The nominal Al-concentration was about 500 µg l^{-1} (18.5 µM). Depending on the experiment, we also added base cation solutions, either Ca^{2+} or Na$^+$, to the experimental water (Fig. 1). We used chloride salts of the base cations to prepare the base cation solutions. The amount of Na$^+$ added to the experimental water was calculated in order to obtain an identical ionic strength increase corre-
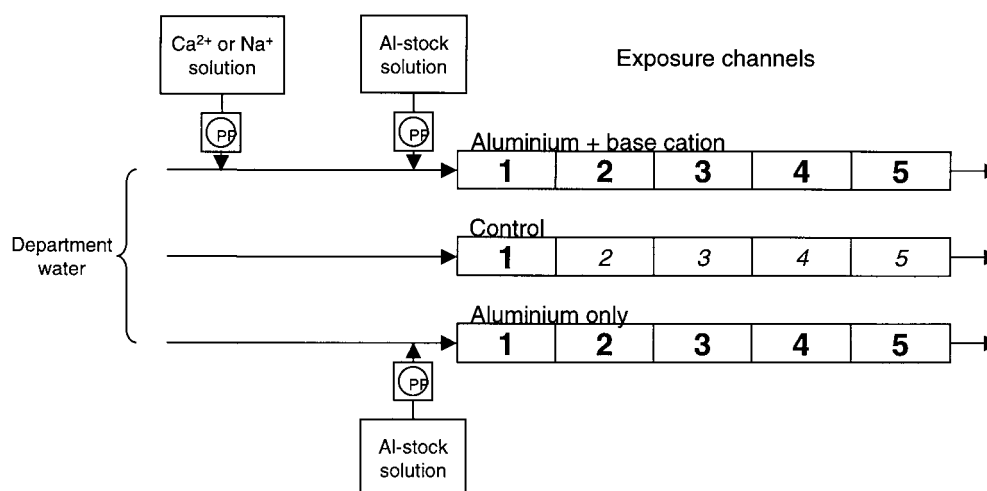
Fig. 1. A schematic presentation of the flow-through exposure system used in the experiments. Three exposure channels were divided into five chambers (1-5) in which the fish were exposed. Aluminium and base cation solutions were added to the department water by means of peristaltic pumps (PP). The water residence time after the addition of aluminium to the department water was 1, 5, 10, 15 and 20 min in chamber 1-5 respectively.

sponding to an addition of 2 mg Ca^{2+} l^{-1}. The third experimental channel always acted as control, receiving only untreated department water (Tab. 1). Water temperature varied between 4.8 and 6.1 °C during the experimental period.

2.3. Experimental protocol

The present study was performed as two experiments, each carried out for 140 h. The fish were exposed in parallel to; dynamic Al-chemistry medium with addition of a base cation (Ca^{2+} in the first experiment and Na^+ in the second), dynamic Al-chemistry medium only, and untreated department water. We used approximately 25 fish in each of the 5 exposure chambers within each of the two Al-chemistry channels. In the control channel, however, we used only 10 fish in the first chamber. The fish were always introduced into the channels one day before the start of the experiments.

Each exposure chamber was examined for dead fish at least once a day. 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 experiments. The chemical dosages and water flow into each channel were also controlled every day. Three or four times during each experiment, in situ aluminium fractionations and analyses were conducted. Water samples for later analyses of all major ions were collected.

2.4. Water chemistry analyses

Water pH was measured using a Radiometer PHM-80 with a Radiometer GK-2401C combined glass-elec-

trode. 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 (μS cm^{-1}). The pH and conductivity measurements, as well as Al-fractionations were performed immediately after the water samples were taken. Aqueous aluminium was fractionated by the extraction technique described by Barnes (1975), combined with the cation exchange procedure described by Driscoll (1984), following Lydersen *et al*. (1990). The aluminium concentration in the various fractions was measured by a Shimadzu UV-1201 spectrophotometer at 395 nm. Absorbance was also measured at 600 nm in order to correct for iron interference (Sullivan *et al*. 1986).

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 and total organic carbon (TOC) by a combined photochemical (UV) wet-chemical ($S_2O_8^{2-}$) oxidation method.

2.5. Survival analyses

Survival Analysis (StatView 4.5, see Abacus Concepts 1994) was used to analyse the survival data. Survival Analysis handles data in which the variable of interest is the time taken for a certain event to occur (Fox 1993); in this case the time from the fish were placed in the experimental channels until death. Surviving fish were censored. This was the particular reason for using this analytical technique since the experiments were not driven until 100% mortality in all groups.

Tab. 2. Mean (± s.d.) pH, ionic strength (10^{-4} M), and concentration of various Al-fractions (µg l^{-1}) in the experimental waters. N = 3 for each chamber, while N = 15 for the control exposures. Al_r = total amount of aluminium, Al_c = colloidal aluminium, Al_o = organic monomeric aluminium, and Al_i = inorganic monomeric aluminium.

Exposure	Chamber	pH	Ionic strength	Al_r	Al_c	Al_o	Al_i
Experiment 1							
Al-only	1	5.76 ± 0.06	4.77 ± 0.01	570 ± 46	111 ± 38	137 ±15	321 ± 43
	2	5.75 ± 0.07	4.77 ± 0.02	608 ± 47	158 ± 28	163 ± 7	287 ± 44
	3	5.75 ± 0.07	4.76 ± 0.01	587 ± 80	160 ± 55	160 ± 8	266 ± 28
	4	5.74 ± 0.07	4.76 ± 0.01	609 ± 39	202 ± 23	165 ± 12	242 ± 9
	5	5.74 ± 0.07	4.76 ± 0.01	592 ± 10	195 ± 38	168 ± 14	230 ± 15
Al + Ca	1	5.83 ± 0.09	6.20 ± 0.20	497 ± 47	120 ± 13	131 ± 20	246 ± 60
	2	5.83 ± 0.05	6.28 ± 0.05	510 ± 29	157 ± 16	141 ± 15	212 ± 42
	3	5.83 ± 0.05	6.28 ± 0.05	561 ± 59	221 ± 13	146 ± 9	193 ± 54
	4	5.84 ± 0.05	6.28 ± 0.05	526 ± 29	199 ± 17	147 ± 10	180 ± 27
	5	5.84 ± 0.05	6.28 ± 0.05	512 ± 49	180 ± 30	158 ± 16	174 ± 23
Control		6.51 ±0.02	4.71 ± 0.01	57 ± 4	39 ± 4	23 ± 7	0
Experiment 2							
Al-only	1	5.79 ± 0.15	4.49 ± 0.01	520 ± 43	132 ± 21	130 ± 20	258 ± 14
	2	5.78 ± 0.16	4.49 ± 0.01	604 ± 75	225 ± 85	150 ± 15	229 ± 21
	3	5.76 ± 0.15	4.49 ± 0.01	614 ± 111	240 ± 114	158 ± 16	216 ± 23
	4	5.79 ± 0.06	4.49 ± 0.01	564 ± 32	215 ± 44	157 ± 7	192 ± 12
	5	5.78 ± 0.16	4.49 ± 0.01	562 ± 16	213 ± 29	160 ± 14	190 ± 12
Al + Na	1	5.71 ± 0.06	6.23 ± 0.13	537 ± 20	137 ± 24	134 ± 7	267 ± 46
	2	5.72 ±0.02	6.23 ± 0.13	614 ± 62	222 ± 68	149 ± 14	244 ± 20
	3	5.73 ± 0.02	6.24 ± 0.13	622 ± 101	248 ± 74	155 ± 12	259 ± 34
	4	5.73 ± 0.02	6.23 ± 0.13	632 ± 92	231 ± 84	157 ± 12	244 ± 19
	5	5.73 ± 0.02	6.23 ± 0.13	595 ± 70	225 ± 72	158 ± 14	212 ± 15
Control		6.46 ± 0.03	4.39 ± 0.01	96 ± 2	36 ± 22	31 ± 16	28 ± 13

In the survival analysis the hazard function $l(t)$: denotes the probability that an individual survives until time $t+1$, given that it has survived to time t. For any covariate Z, it is assumed that the hazard can be expressed as the baseline hazard (Z = 0) multiplied by an exponential function of Z; $l(t;Z) = l_0(t) \times e^{\beta Z}$. In the parametric Weibull model, it is assumed that the hazard function is either strictly increasing, strictly decreasing, or constant (i.e. the assumption of any specific form of the hazard function is relaxed). We used the Weibull model to compare survival times in fish exposed to Al-rich medium with increased ionic strength and in fish exposed to Al-rich medium only. Chamber number (i.e. the age of the mixed acidic water) influences mortality, and we therefore used chamber number as another factor in the analysis. Differences between exposures, and between chambers, were evaluated using a Wald χ^2-test, and differences in hazard are quantified using e^β. If the survival probabilities are not different between groups, the e^β will not be significantly different from 1. Low e^β, however, implies a low survival probability relative to the group with the highest survival in the experiment.

3. RESULTS

3.1. Water chemistry

The addition of chemicals to the department water altered the chemical composition of the experimental water. pH was lowered from 6.5 to between 5.7 and 5.9 in the Al-exposure channels (Tab. 2). In the calcium exposure, Ca^{2+} increased from 3.11 to 5.07 mg l^{-1}, while Na^+ increased from 2.14 to 5.44 mg l^{-1} in the sodium exposure. These increases, together with a corresponding increase in Cl^- concentration, resulted in an increase in ionic strength from between 4.5 and 4.8 10^{-4} M to between 6.2 and 6.3 10^{-4} M.

The total amount of aluminium (Al_r) in the Al-solutions increased from between 57 and 96 µg l^{-1}, to between 497 and 632 µg l^{-1} (Tab. 2). The Al-analyses revealed that the various Al-fractions changed as the water passed through the experimental channels. The concentration of colloidal aluminium (Al_c) and organic monomeric aluminium (Al_o) increased systematically through the channels, while the concentration of inorganic monomeric aluminium (Al_i) showed a correspondent systematic decrease. Even though the concentration of Al_r varied between the exposure chambers, we found no systematic changes in this fraction through the channels (Tab. 2).

We observed that the Al_o-fraction in the Al-enriched waters was much higher than in the untreated control water. The total organic pool in the natural water may have a proportion of non-complexed organic molecules that represent a sink for binding of additional aluminium. A non-complexed organic pool like this could therefore, to some extent, contribute to the increase in
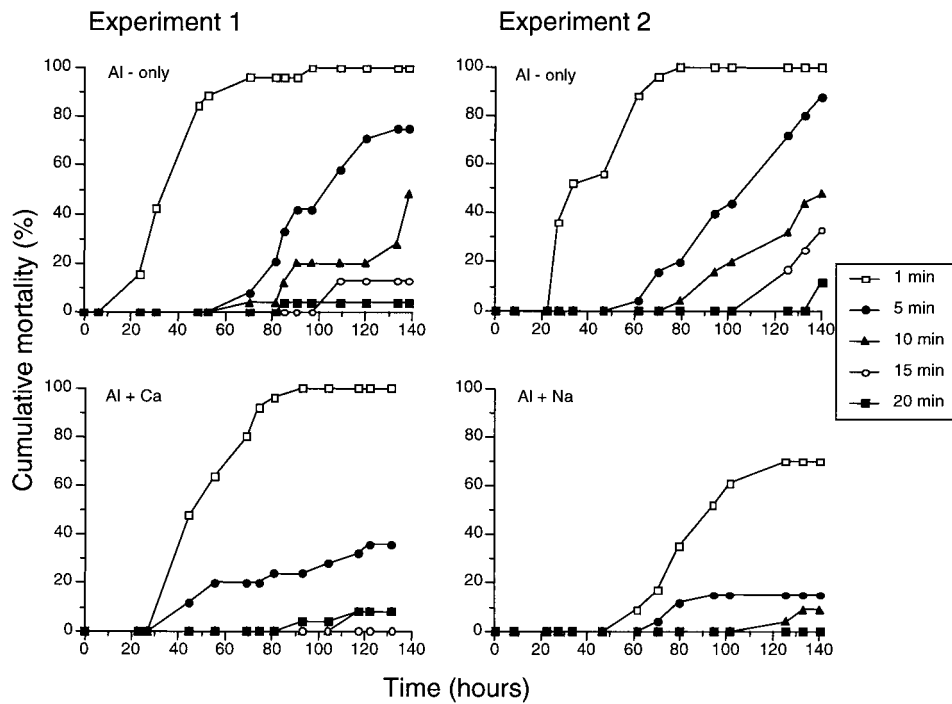
Fig. 2. Cumulative mortality of Atlantic salmon exposed to various combinations of aluminium and base cations. For each combination, fish were exposed to the water at 5 different residence times after the addition of aluminium to the department water: 1, 5, 10, 15 and 20 min.

Al_o. In the present study, however, we used an acidic $Al(NO_3)_3$-stock solution (pH about 2.0) to prepare the dynamic Al-chemistry conditions. In this acidic solution the octahedral hexahydrate $Al(H_2O)_6^{3+}$ (often written as Al^{3+}) should be the only significant Al-species present (Hem & Roberson 1967; Lydersen 1990). According to Hem & Roberson (1967) and Lydersen *et al.* (1990), aluminium starts to polymerise as soon as pH is raised to above 4.0-4.5, depending on water temperature. 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.* 1994). The increase in Al_o is therefore a confirmation of reduced cationic charge of aluminium during the initial phase of the polymerisation process. The increase in Al_c, calculated as the difference between Al_r and total monomeric aluminium (Al_a), confirm that monomeric Al-species were converted to larger, less reactive polymeric species through the channels.

3.2. Fish mortality

While no mortality was observed in the control fish, mortality was recorded in all Al-exposures. Mortality rates differed, however, with water composition and residence time through the channels (Fig. 2). Survival analysis showed that there were no differences in mortality between the two Al-only exposures (Weibull $\chi^2 =$ 0.083, P = 0.77). However, there were significant differences between the chambers within the channels (Tab. 3), with the lowest mortality observed in fish exposed in the last chamber, i.e. the water with the longest residence time. Mortality increased systematically from chamber 5 to chamber 1. The estimated survival probability in chamber 1 was only 20% of that observed in chamber 5. There were significant differences in mortality between the Al-only exposures and the Al-exposures with base cations added (Tab. 4). Mortality was lowest in the Al+Na-exposure, and highest in the Al-only exposures. Also in the Al+Ca-exposure mortality was significantly lower than the corresponding Al-only exposure (Wald $\chi^2 = 304.89$, P <0.001), but significantly higher than in the Al+Na-exposure (Wald $\chi^2 = 58.80$, P <0.001).

4. DISCUSSION

Our results confirm previous observations that the acute toxicity of aluminium under non-steady state chemical conditions is reduced with increasing age of the water, and that additions of base cations to Al-rich water reduce the toxic effect of aluminium. In addition to this, we observed that an equal increase in ionic strength due to the addition of Ca^{2+} and Na^+, respectively, did not reduce the Al-toxicity to the same extent. Sodium seems to be the more effective base cation in reducing Al-toxicity.

Tab. 3. Model coefficients from the survival analysis of the Al-only exposures. The model coefficients (e^β) with 95% confidence intervals (c.i.) gives the survival probabilities of the Atlantic salmon in the exposure chambers within the experimental channels, compared to the survival in chamber 5 (the chamber with the lowest mortality). There were significant differences in survival probability for the fish between the chambers (Weibull χ^2 = 350.4, P <0.001).

Chamber	e^β	95% c.i.
1	0.197	0.149 - 0.261
2	0.454	0.345 - 0.598
3	0.576	0.439 - 0.757
4	0.754	0.565 - 1.004
5	1.000	

Tab. 4. Model coefficients from the survival analysis of the four experiments (two Al-only additions, Na+Al addition, and Ca+Al addition). The model coefficients (e^β) with 95% confidence intervals (c.i.) gives the survival probabilities of the Atlantic salmon in the different experiments when controlling for different survival probabilities among chambers within the different experiments). There were significant differences between the experiments (Weibull χ^2 = 146.5, P <0.001).

Experiment	e^β	95% c.i.
Al only 1	0.396	0.339 - 0.464
Al only 2	0.403	0.344 - 0.472
Al + Ca addition	0.492	0.417 - 0.577
Al + Na addition	1.000	

True chemical equilibrium is seldom approached in natural systems like surface waters, and several toxicological studies have indicated that the acute Al-toxicity in fish may depend on the age of the Al-solution (Weatherley *et al*. 1991; Rosseland *et al*. 1992; Lydersen *et al*. 1994; Poléo *et al*. 1994; Poléo & Bjerkely 2000). In all these studies, Al-toxicity was highest during the initial phase of Al-polymerisation which takes place right after mixing of acidic Al-rich water with circumneutral river water. After this initial phase, Al-toxicity decreased significantly with water residence time, i.e. age of the Al-polymers present in the water. In the present study we observed exactly the same feature in our Al-exposures (Fig. 2). The addition of base cations did not influence the time dependent Al-toxicity, and therefore confirm that this time dependency is important for the present understanding of acute Al-toxicity in fish. According to Poléo (1995) and Poléo & Bjerkely (2000), the time dependent Al-toxicity under non-steady state conditions is due to the transformation of unstable monomeric aluminium to more stable polymeric Al-forms when pH in the Al-containing water is raised. By ageing, the Al-polymers increase in size, which also means that their cationic charge decreases (Hem & Roberson 1967). This is analytically revealed by increased concentrations of both Al_o and Al_c, and decreased concentration of Al_i under the initial Al-polymerisation phase. Accordingly, the ability of aluminium to bind to negatively charged sites at the gill surface is reduced.

In the present study we observed that both the addition of Ca^{2+} and Na^+ had an ameliorating effect on the acute Al-toxicity in salmon. This corresponds well with the literature. It has been accepted for a long time that aqueous Ca^{2+} mitigates Al-toxicity in fish (Brown 1981, 1983; Playle *et al*. 1989). It has also been demonstrated that high concentrations of Na^+ in the water reduces Al-toxicity (Brown 1981; Dietrich *et al*. 1989). The mitigating effect of aqueous Ca^{2+} has been attributed to its stabilising effect on the gill epithelium (reviewed by McDonald 1983). No such effect, however, is likely to be attributed to Na^+, which appeared to reduce Al-toxicity as efficiently as Ca^{2+} (Fig. 2). However, a passive loss of ions, mainly Na^+ and Cl^-, is to be expected in fish exposed to aluminium (reviewed by Howells *et al*. 1994; Gensemer & Playle 1999). Increasing the concentration of aqueous Na^+ might therefore reduce passive efflux and facilitate compensatory Na^+-uptake over the gill epithelium. In the present study, however, the salmon were exposed to aluminium under non-steady state conditions and at relatively high pH (between 5.7 and 5.9). Poléo *et al*. (1994) found that salmon exposed to aluminium under non-steady state conditions in the initial part of a mixing zone (pH 5.6) between limed and acidic water did not suffer from ion regulatory disturbances despite enhanced mortality (75% after 68 h). Accordingly, Neville (1985) demonstrated that Al-toxicity in rainbow trout (*Oncorhynchus mykiss*) at relatively high pH was predominately due to hypoxia rather than ion regulatory disturbances. We might therefore assume that the salmon in our experiment, at least to some extent, was suffering from hypoxia. If so, it is reasonable to attribute the mitigating effects of Ca^{2+} and Na^+ to their contribution to water ionic strength, reducing the possibility for aluminium to interact with the gill surface.

As already mentioned, our experiments demonstrate that increasing the water ionic strength by adding Na^+ or Ca^{2+} reduces the toxic effects of aluminium. The highest effect was obtained by the addition of Na^+, even though the ionic strength increase was the same in Al+Ca and Al+Na water. The reason for this difference could be that Ca^{2+} and Na^+ do not influence Al-chemistry in the same way. However, our Al-fractionation data reveal that there are no differences in any of the measured or calculated Al-fractions of the two treatments (Tab. 2). Furthermore, it was no difference in the saturation indexes regarding known solid Al-phases (estimated by the ALCHEMI-Version 4.0, Schecher & Driscoll 1987, 1988). All Al-solutions seem to be close to saturation with respect to micro-crystalline gibbsite (log*K_s = 9.35). Another explanation is that Ca^{2+} and Na^+ mitigate the Al-toxicity by their effect on the ability for aluminium to interact with the gill surface. However, this does

not necessarily mean that Na^+ more effectively ameliorate the Al-toxicity than Ca^{2+}, because, on equivalent basis about a 1.5 times higher amount of Na^+ is needed to obtain the same ionic strength increase as by Ca^{2+}. Thus, the possibility of a Na^+-ion to be present near the surface of the fish gill should be higher compared to Ca^{2+}. On the other hand, Ca^{2+} is a divalent ion and should therefore have better qualitative properties in the competition for cation exchange sites than the monovalent Na^+, like substituting aluminium present on negatively charged sites at the gill surface. However, Ca^{2+} as a divalent ion will have a thicker hydration shell than Na^+, which may prevent Ca^{2+} to come as close to the gill surface as Na^+. In general cation exchangers exhibit low or moderate preference for one cation species compared to another (Bolt 1979), a preference that is further reduced when the temperature is low (Boyd 1970) as in our experiments. All together, this may explain why the Al-toxicity was lower in the Al+Na water compared to the Al+Ca water.

The interaction between aqueous aluminium and the gill surface seems to be of major importance for the acute toxicity of aluminium in fish (Exley *et al.* 1991; Poléo 1995; Exley *et al.* 1996; Poléo & Bjerkely 2000). Al-accumulation and hypoxia seem to be very important for the effects of aluminium under non-steady state conditions (Poléo 1995; Poléo *et al.* 1995; Poléo & Bjerkely 2000). Ionic strength should therefore play a key role for the Al-toxicity in fish because it is essential for the chemistry of all aquatic surfaces, including the fish gill.

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