TOXICITY OF INORGANIC ALUMINIUM IN HUMIC STREAMS

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Doctoral thesis in Applied Environmental Science

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The river that flows in you also flows in me.

Kabir

ABSTRACT

Aluminium (Al) has been recognised as a main toxic factor alongside pH in acidified water ecosystems. The toxic effect of Al has been attributed to inorganic Al (Al_i), though there are few in situ studies in ambient humic waters which are the focus of this thesis.

The aim was to estimate Al_i toxicity and thus also Al_i concentrations in Swedish humic streams. Subsequently it is necessary to analyse Al_i correctly, which was studied by modelling and method intercalibrations. The hypothesis was that the effect of Al_i could be followed via physiological effects and Al accumulation, as well as by mortality. Toxicity was studied by in stream exposures of brown trout (*Salmo trutta* L.) and two salmonid prey organisms (*Gammarus pulex* and *Baetis rhodani*) during spring flood.

The modelling of the Al_i fraction was performed using monitoring data covering all of Sweden with satisfactory results. The essential variables for Al_i modelling were determined; Al, DOC, pH and F, while Fe, Ca and Mg had less effect. The automated analytical procedure for Al_i (with cation exchange followed by complexation with pyrocatechol violet) was modified and validated and showed to be the preferred method for laboratory analyses.

To avoid detrimental effects for brown trout Al_i should be <20 µg/L and pH >5.0; mortality was high when the Al_i was above 50 µg/L. The invertebrates were more sensitive, as mortalities occurred at pH <6.0 and Al_i >15 µg/L for *G. pulex*, and at pH <5.7 and Al_i >20 µg/L for *B. rhodani*. It is prudent to use a wide view and let the most sensitive species set the tolerance limits; a pH above 5.7-6.0 and Al_i below 15-20 µg/L allows the stream ecosystems to thrive.

Today, as waters are recovering from acidification, the aim of mitigating liming is to carefully adjust dosage to avoid suboptimal water quality. The thresholds found in this thesis can be used to efficiently but carefully decrease liming, as both Al_i and pH levels have to be balanced to sustain the recovering aquatic biota.

SAMMANFATTNING

Aluminium (Al) kan bli giftigt i försurade vattenekosystem. Al och pH kan tillsammans samverka eller motverka varandras toxicitet (giftighet) beroende på vattenkvaliteten. Den toxiska effekten av Al brukar tillskrivas oorganiskt Al (Al_i), men få fältstudier har utförts i bruna, humösa vatten. Effekten av antropogen försurning kan vara mer uttalad och bygga på den organiska surheten i humösa vatten, vilka denna avhandling fokuserar på. Det finns ett behov av ökad kunskap om effekten av Al_i i höghumösa vatten då främst klara vatten har undersökts hittils. Målet för min forskning har varit att uppskatta Al_i's toxicitet i humösa vatten och därmed även Al_i koncentrationer i de svenska bruna vattendragen.

I avhandlingen studeras Al_i i humösa vatten både analytiskt och eko-toxikologiskt för att bidra till förståelsen av förekomst och effekter av Al_i. Resultaten baseras på validerade och noggranna Al_i bestämningar tillsammans med en grundlig undersökning av Al_i toxicitet. Toxiciteten har studerats och, vid sidan av dödlighet, genom orsak-verkan-kedjor kopplats till fysiologiska responser i öringen (vatten-gälar-blod). Därutöver har toxiciteten undersökts med analyser av helkroppsinnehåll av både baskatjoner (Ca, Mg, Na, K) och metaller (Al, Fe, Mn, Zn) i makroevertebrater (vattenlevande insekter) i väl tilltagna dataset.

Detta har varit mina frågeställningar:
Finns det ett tillförlitligt sätt att analysera och modellera Al_i i sura humösa vatten?
- Kan Al-fraktionerna modelleras samt validera de analytiska bestämningarna av Al_i? (papper I)
- Vilket är det optimala sättet att analysera Al_i ? (papper II)

Vilka halter av oorganiskt Al tål ekosystem i humösa vatten vilka i denna studie representeras av:
- öring (*Salmo trutta* L.) (papper III - V)
- makroevertebraterna (*Gammarus pulex* and *Baetis rhodani*) (papper VI)

Inledningsvis visades att det var möjligt att modellera Al_i fraktionen för vanliga miljöövervakningsdata från hela Sverige. Den automatiserade analysmetoden för Al_i-PCV (katjonbyte, pyrokatekolviolett-PCV) modifierades och validerades. De olika metodvarianterna som vanligen används jämfördes, systematiska skillnader i analysresultaten identifierades och även orsaker till dessa. Det visades att resultat från Al_i-PCV metoden stämde väl överens med resultat av modellerat Al_i och även med resultat för ultra-filtrerade Al_i-HQ (katjonbyte, 8-hydroxiquinolin). Al_i-PCV metoden passar bäst för lämpligast för laboratorieanalyser av övervakningsprover, men att vid fältförsök i instabila vatten är det bättre att använda Al_i-HQ.

Tröskelnivåer bestämdes för öring i bruna humösa vatten både genom kontrollerade exponeringar och med burförsök i bäckar under vårfloden. Öring behöver ett pH-värde över 5.0 och Al_i under 20 µg/l för att undvika skadliga

effekter. Dödligheten var alltid hög då Al_i var över 50 µg/l, dessa halter ses som akut toxiska; medan 20-50 µg Al_i/l representerar en suboptimal vattenkvalitet. Även tröskelnivåer för två födoorganismer för laxfiskar fastställdes. Dessa makroevertebrater var känsligare än öringen för surt och Al-rikt humöst vatten; dödlighet uppstod vid pH <6.0 och Al_i >15 µg/l för *G. pulex* och vid pH <5.7 och Al_i >20 µg/l för *B. rhodani*. Det är viktigt att vara aktsam och sätta gränsvärden utifrån de känsligaste organismerna; pH bör vara över 5.7-6.0 och Al_i under 15-20 µg/l för att skydda vattendragsekosystemen.

Gränsvärdena för Al_i och pH som fastställts i denna avhandling kan användas till att effektivt men försiktigt reducera kalkningsdoser då båda nivåerna behöver vara i balans så att inte tillfrisknande akvatisk biota skadas. Även om Al_i är starkt kopplat till pH så kan varierande Al halt i jord- och bergarter likväl som lokala förhållanden ge varierande Al_i halter vid samma pH-värde. Därför är det tillrådligt att övervaka och analysera Al_i och inte endast förlita sig till modellerat Al_i. Nu återhämtar sig de akvatiska ekosystemen från försurningen, men trots att det sura nedfallet har minskat efter ett halvt århundrade med surt regn kan marken lokalt fortsätta läcka Al. Man bör vara aktsam när kriterierna tillämpas eftersom nivåerna kanske inte säkert skyddar känsliga utvecklingsstadier eller exempelvis fiskstammar med lägre surhetstolerans. Det är nödvändigt att ha ett vitt synfält när kalkningsmål definieras. Förrutom fisk bör även dess födoorganismer inkluderas, då även den känsligaste organismen behöver skydd så att livet i vattendragen kan blomstra.

CONTENTS

ALUMINIUM IN SHORT

A basic understanding of aluminium (Al) terminology is needed to understand the studies presented in this thesis. Therefore, a simple fractionation scheme is presented (Fig. 1). The main focus of this text is on labile monomeric Al, which is commonly called inorganic aluminium (Al_i).

Total Al (total reactive Al, acid digested or eluted at pH 1.5) (Al)		
Total monomeric Al (no acid addition or digestion) (Al_m)		**Acid soluble Al**
Labile monomeric Al (retained in ion exchange) (Al_i)	**Stable monomeric Al** (cation exchanged eluate) (Al_o)	
* free aluminium * sulphate, hydroxide and fluoride complexes * possibly small Al-humus complexes	* monomeric organic complexes * possibly negative inorganic Al-complexes (pH >6) and Al-humus complexes	* colloids, polymers * strong organic complexes

Figure 1. Fractionation scheme for aluminium slightly modified from Driscoll (1984). In brackets are the abbreviations that are commonly found in publications and are used in this thesis.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I.   Cory, N.; Andrén, C. M.; Bishop, K., 2007. Modelling inorganic Aluminium with WHAM in environmental monitoring. Applied Geochemistry. **22**, 1196–1201.

II. Andrén, C. M. & Rydin. E., 2009. Which aluminium fractionation method will give true inorganic monomeric Al results in fresh waters (not including colloidal Al)? Journal of Environmental Monitoring, **11**, 1639 - 1646.

III. Andrén, C. M., Kroglund, F. & Teien, H-C, 2006. Controlled exposure of brown trout to a limed acid and aluminium-rich humic water. Verh. Internat. Verein. Limnol. **29**, 1548-1552.[†]

IV. Teien, H-C, Andrén, C. M. & Kroglund, F., 2005. Changes in gill reactivity of aluminium species following liming of acid and aluminium-rich humic water. Verh. Internat. Verein. Limnol, **29**, 837-8401.[†]

V.   Andrén, C. M. & Rydin. E., 2012. Toxicity of inorganic aluminium at spring snowmelt - in-stream bioassays with brown trout (*Salmo trutta* L.). Sci. Total. Env. **437**, 422-432.

VI. Andrén, C. M. & Eriksson Wiklund, A-K., Effects of springtime acid episodes - in-stream bioassays with two macro-invertebrates, manuscript.

My contribution to the papers:

I. Organised Al fractionation in the national surveys and took part in modelling, evaluation and writing.

II. Planned and arranged intercalibration tests (administration, bottling and distribution), performed a major part of the evaluation, statistical analysis and writing.

III. Planned and performed the controlled exposures, performed a major part of the samplings, statistical analysis and writing.

IV. Planned and performed the controlled exposures, contributed to the writing.

V. Planned and performed the field exposures, performed a major part of the samplings, Al fractionation, statistical analysis, evaluation and writing.

VI. Planned and performed the field exposures, performed a major part of the samplings, Al fractionation, statistical analysis, evaluation and writing.

INTRODUCTION

Aluminium (Al) has been identified as one of the main toxic factors in acidified water ecosystems. The toxic effect of Al has been attributed to inorganic aluminium Al_i, although there are only a few field studies on humic waters, which are common in Sweden. Waters with high humic content can be found in productive boreal forests on podzolised acid soils in the northeast USA, south eastern Canada, and Europe, where acid rain has been a cause for concern. Even if the active toxic agent is inorganic Al, it is not certain that the effects are similar in humic waters as organic matter may ameliorate this toxicity. In lakes, Al has time to stabilise and form mainly organic complexes, whereas in running waters, more short-lived and potentially toxic Al complexes are formed when soil water, groundwater and rain water are mixed.

Liming has been used to mitigate the harmful effects of acidification in Sweden and Norway. In the first phase, the goal of liming was direct: saving aquatic communities from acute toxicity and chronic acidification. Today, as waters are recovering from acidification, the aim is to carefully adjust lime dosage to avoid suboptimal water quality (e.g., acid episodes) that can harm biology. The recovery from acidification is predicted to take a long time, and as future climate change can render a more variable and intense precipitation and increased temperatures, the process might be hampered and prolonged. Thus, well-defined acid toxicity thresholds will be needed in the years to come.

Manual methods for Al speciation were tested and applied during the 1980s at the Trace Metal Laboratory of the Swedish Environmental Protection Agency (Swedish EPA). In 1990, an automated Al fractionation system was set up and applied to projects studying the effects of acidification and liming beginning in 1991. Today, more than 4000 samples/year are analysed in national programs such as the Integrated Studies on Effects of Liming in Acidified Waters (ISELAW) as well as regional monitoring projects. During the 1990s, the results were mainly evaluated in relation to other water quality variables and also correlated to the biological data (fish, macro-invertebrates) in the ISELAW project. Thus, the next logical step was moving from inductive statistical studies to deductive studies with designed experiments to confirm Al_i toxicity. It is valuable to have a number of indicators to estimate toxicity in an ecosystem, preferably at several levels in the food web.

OBJECTIVE

The principal objective was to estimate Al_i toxicity and thus also Al_i concentrations in humic streams. To be able to set critical limits, the chemical determination has to be accurate and preferably validated and proven. I arranged intercalibrations for the Scandinavian laboratories that analysed Al fractions to compare and confirm the analytical results. Moreover, Al_i modelling was used not only for comparison and to confirm analytical results but also to estimate the

relative importance of complex ligands. Next, I performed field trials in humic streams with brown trout (*Salmo trutta* L.), which is a key organism for mitigating liming activities, and two salmonid prey organisms (the benthic macro-invertebrates *Gammarus pulex* and *Baetis rhodani*). My hypotheses were that in addition to the acute mortality, the toxicity of Al_i in humic streams could be followed by measuring Al accumulation and also via physiological effects in the organisms. I postulated that it was possible to accurately measure Al_i in humic waters even though organic ligands were abundant and that Al_i has toxic effects in Swedish humic waters.

I addressed the following research questions:

Is there a reliable way to determine and model Al_i in acidic humic waters?

- o Can Al fractionation be modelled and validate analytical determinations of Al_i? (paper I)
- o What is the optimal way to determine Al_i? (paper II)

What levels of inorganic Al can ecosystems in humic streams tolerate? In addition to mortality, Al_i toxicity was assessed by several 'endpoints' in:

- o brown trout (*Salmo trutta* L.) (papers III - V)
- o the benthic invertebrates (*G. pulex* and *B. rhodani)* (paper VI)

The determined thresholds can be used as biological goals for liming, which are important for liming strategies during recovery from acidification.

BACKGROUND

ACIDIFICATION - LIMING

Acidification was identified as an environmental problem in northern Scandinavia by Odén (1968) and in North America by various authors. although the acidifying emissions already began around 1800 with the rise of industrialisation. Low pH dissolves aluminium (Al) in soil and increases Al water solubility, leading to increased levels of inorganic monomeric Al (Al_i): a form of Al that is highly toxic to fish (Driscoll and Schecher, 1990; Gensemer and Playle, 1999). In the early days of acidification research, toxicity to fish in soft waters was observed internationally (Cronan and Schofield, 1979; Driscoll et al., 1980) and also in Scandinavia (Dickson, 1978; Muniz and Leivestad, 1980). Al and pH toxicity can be synergistic or antagonistic depending on the water chemistry (Havas and Rosseland, 1995).

Since the 1970s, Swedish surface waters have been treated with lime to mitigate the effects of acidification on animals and plants. In total, over 445 million EUR (or 4 billion SEK) has been spent on liming, which makes liming one of the biggest environmental protection efforts ever performed. Liming has sustained ecosystems in thousands of acidified lakes and streams for several decades. The cost for liming in Sweden is 19 million EUR annually (Swedish EPA, 2010). Knowledge of how liming can be reduced or terminated is therefore an important economic consideration, and cost-effective approaches are currently being sought.

There is an on-going debate if acidity/acidification in some Swedish waters is of anthropogenic (mineral, S and N) or natural (organic) origin. There are numerous Swedish studies covering the question of natural and anthropogenic acidification/acidity (e.g. Cory et al., 2006; Erlandsson et al., 2011; Laudon et al., 1999). However, it is not easy to define "natural", as recently reported by Bishop (2012), who in the 1980s highlighted the cost of liming and its impact.

The Swedish environmental objective, "Only Natural Acidification," states "The acidifying effects of deposition and land use must not exceed the limits that can be tolerated by soil and water; Forestry will be adapted to the susceptibility of each site to acidification". Podzolised acidic soils with productive boreal forests can be found in the northeastern USA, southeastern Canada, Scandinavia and central Europe, and they are highly sensitive to acidification (Eshleman and Hemond, 1985; Krug and Frink, 1983). The objective "Flourishing Lakes and Streams" states that "Lakes and watercourses will achieve good surface water status with respect to species composition and chemical and physical conditions", as defined in the EU Water Framework Directive (European Union, 2000). The results from this thesis can hopefully support these objectives with knowledge of the effects of acidification, particularly Al_i, in Swedish humic waters.

REDUCED EMISSIONS AND RECOVERY – REDUCING LIME TREATMENT

The anthropogenic emissions of sulphur (S) have decreased by almost 80% and nitrogen (N) emissions have decreased by 40% over the last two decades in Europe and the USA, although in China and India, both S and N emissions have increased (Granier et al., 2011). Although long-range transboundary air pollution has decreased, N emissions from international sea transports have increased and are expected to continue to increase. Forestry also contributes to anthropogenic acidification, and its relative contribution to acidification has increased. More buffering substances are taken out of the forests as the forest yield has increased (by N air bound fertilisation), and the demand for biofuels has increased. Currently, whole trees are removed, as are branches, tops and stumps, which further diminish the natural return of buffering substances to the soil. The contributions from forestry to acidification are expected to increase and prolong the recovery phase (Swedish EPA, 2012).

Freshwaters in base-poor areas in northern Europe and North America have been shown to recover chemically from acidification (Skjelkvale et al., 2005; Stoddard et al., 1999). Acid deposition still exceeds the critical load that nature can tolerate in 22% of the total catchment areas in Sweden and in 19% of the total forest areal (Swedish EPA, 2012). The large store of organic S and discrepancy in the rates of decrease between deposition and runoff S makes a considerable time lag that can delay recovery for decades (Morth et al., 2005; Wright et al., 2005). Particularly in areas with thick soil, the effects of acidification might be prolonged because soils store S.

In Sweden, the share of acidified lakes decreased from 19.1% in 1980 to 12.1% in 2010. In the future, only a small recovery is estimated to occur with 11.9% acidified lakes in 2020 (pending if decided actions will be implemented). The largest recoveries have occurred in eastern, middle and northern Sweden, where the number of acidified lakes decreased to 5-10%. In the south western parts, the number of acidified lakes has decreased from 64% to 50% from 1980 to 2010 (Folster and Kohler, 2011). These estimates of acidification are according to Swedish Environmental Criteria for Acidification (Folster, 2007; Swedish EPA, 2007). In these criteria, a reduction of 0.4 pH units in present water quality compared to MAGIC-modelled preindustrial conditions is considered anthropogenic acidification. Streams have variable water quality and are directly affected by changes in flow, while in lakes (reservoirs) high flows can be balanced and dampened. At low flow, stream water is buffered by contact with groundwater and bedrock. At high flow, however, the water follows more superficial paths with less buffering capacity, leading to acid episodes. Fifteen per cent of the running distances of Swedish streams are estimated to be acidified, which increases if you include streams with small catchment areas (2 km2, Folster and Kohler, 2011). The recovery rate is more uncertain than in lakes, as streams will be more strongly affected by future climate change.

Climate change might cause larger variation in precipitation patterns, increasing snowmelts and rainstorms, which create hydrological episodes (Arnell, 1999; Bergstrom et al., 2001). The predicted shift in rainfall patterns and

rising temperatures can further prolong and hinder the recovery process. In addition, annual precipitation is predicted to increase by 15% and annual temperature to rise up to 2.5°C (means 1961-1990 relative to 2071-2100, European Environment Agency, 2012a; European Environment Agency, 2012b). The rising temperatures combined with declining acid deposition have coincided with marked increases in dissolved organic carbon (DOC) concentrations (Evans et al., 2005; Monteith et al., 2007).

Temperature increases can also decrease Al solubility and transport (Kram et al., 2009; Vesely et al., 2003) depending on catchment characteristics. As organic acidity increases, decreasing mineral (S) acidity may be buffered (Evans et al., 2008). These organic acids can be considered effective, but partial buffers to acidity change in organic soils and need to be considered when assessing and modelling recovery from acidification. The overall effect of climate change on Al_i is hard to predict as rising temperatures not only decrease Al solubility but also release more DOC carrying Al. Meanwhile, hydrological episodes might give trigger acid surges and form more Al_i.

Few records of biological recovery have been documented outside of Norwegian clear waters (Raddum et al., 2001) – mainly catchments draining bedrock with thin soil layers, which can assist in early recovery. The water quality in northern Europe and North America generally improves from chronic acidification but still poses a risk to biological organisms as episodic acidification can have a severe effect on remaining or recolonising populations (Kowalik et al., 2007; e.g. Lepori et al., 2003; Lepori and Ormerod, 2005). In North American field exposures with brook trout (Baldigo et al., 2007), similar mortality was recorded for 2001-03 and for 1984-5, 1988-90 and 1997, suggesting no appreciable change in stream water quality.

As a consequence of declining acidification, liming has decreased by 40% compared to the maximum dosage used in Sweden around 2000. However, liming may be continued with reduced doses to avoid surges of Al_i in streams with salmonid fish populations. Given that Al availability varies locally, knowledge is required to plan and balance the lime dose to avoid the environmental effects of pH-Al toxicity. This is even more important in the present recovery phase because surviving biota might be weakened by acidification (Ormerod and Jenkins, 1994). In addition to detrimental water quality, trout recovery can be hindered by lack of dispersal of macro-invertebrates (Monteith et al., 2005) and biotic interactions (Ledger and Hildrew, 2005). Relevant and sufficient knowledge seems to be lacking regarding the impact of acidification impact and the recovery processes of fish and invertebrates in streams after liming (Herrmann et al., 1993). The response of aquatic communities to decreased acidity in the recovery phase is more complex than to the acidity in the impairment phase (Johnson and Angeler, 2010).

MATERIALS AND METHODS

This thesis is based on data from three different projects presented below:

1) Data from the Swedish national lakes and rivers surveys in 1995 and 2000 (**paper I**)

2) Data from controlled exposures of brown trout to acid and Al-rich water limed to a gradient pH and Ali (**paper II, III and IV**)

3) Data from field exposures in six streams of
   - brown trout (*Salmo trutta* L.) in spring 2002 (**paper V**)
   - *G. pulex* in spring 2001 (**paper VI**)
   - *B. rhodani* in spring 2002 (**paper VI**)

DATASET 1 – NATIONAL SURVEYS

National surveys are designed to give a spatial representation of the status of Swedish surface waters based on water bodies chosen in a stratified random sampling design. Two surveys, conducted in the autumn of 1995 and 2000, were used in this study. All water samples underwent a comprehensive chemical analysis that included major constituents, nutrients and selected metals (Wilander et al., 1998; Wilander et al., 2003). A subgroup of the samples were analysed for Al fractions using the cation exchange method (Andrén and Rydin, 2009) modified from Driscoll (1984). These samples were randomly selected but excluded neutral and alkaline water where Al fractionation was of less interest. Only samples present in both years and with total Al and Al fractionation data were used in this study, amounting to 315 lakes (1995 and 2000) and 213 streams (only 2000). Both rivers and lakes showed a distinct difference between the two surveys in the high flow of autumn, 2000, which caused the water table to raise and activated more superficial, organic rich flow pathways.

DATASET 2A - CONTROLLED EXPOSURES (LIMED HUMIC WATERS)

An experiment was carried out in October 2002 with water from Lake Kopparen, located in southern Sweden. The changes in distribution of Al_i species following liming of acid water high in organic compounds was monitored and then compared to previous experiments in water low in DOC (Teien et al., 2004). Furthermore, a focus was on the identification of gill reactive Al species. Two different strains of brown trout were used to evaluate strain-dependent relationships to acid tolerance. Brown trout were exposed to stream setups differing with respect to pH and time after liming. Every setup contained one channel with two to three cages followed by three tanks and mimicked a stream and a liming pH target. To increase the toxicity of the lake water, a mixture of sulphuric and nitric acid was added simultaneously with aluminium (Al) to

reduce the pH to 4.6 and increase the total Al concentration to 268 µg L^{-1} (matching levels recorded in precipitation and acid streams monitored nearby).

Water was limed with a CaOH$_2$ slurry to four different pH values (4.9, 5.2, 5.5 and 6.0). Within each of the five channel segments, rapid metal transformations and parallel biological responses were followed, while the tanks enabled observation for an extended time after liming. In addition to an acidified reference with pH 4.6, fish were exposed to untreated water from Lake Kopparen (pH 5.6). A total of 27 sampling stations were established within this experimental design, with five or six sampling stations for each pH treatment. The experiment was designed as a two-dimensional test of Al toxicity both within streams (same pH, increasing water age) and between streams (different pH levels, same water age).

DATASET 2B – SUBSET FOR INTERLABORATORY COMPARISON

The results from five samples from an interlaboratory comparison of Al fractionation, with data from 14 facilities (Andrén, 2003), were used for this analysis. These data provided the opportunity to study the effect on Al$_i$ yield of different measurement methods at different sample pHs and resultant Al$_i$ concentrations. The selected samples represent a dilution series from a field exposure experiment with brown trout (dataset 2a). The source water control was excluded from his evaluation. The samples were collected on 21 October 2002 on the fourth day of the experiment and stored at 4°C until 2 December 2002, when they were then sent to different laboratories for analysis within one week.

DATASET 3 – FIELD EXPOSURES
(UNMODIFIED GRADIENT OF pH AND AL$_I$)

These studies were performed in humic streams on coniferous hills located in a base-poor area in the middle of Sweden with low acid deposition (Fig. 2). The catchments' buffering capacities were low considering their composition of granite and gneiss bedrock overlain by a thin layer of till. During springtime when the snow melts, the flood has low pH and high Al$_i$. All streams had minor wetlands upstream of the exposure sites in addition to forest (Table 1). The streams were pristine besides some foresting activities; however, downstream of the Havssvalgsbäcken site, lime was spread until 2000. The streams were chosen based on known water quality (Andrén, 1995) to obtain a gradient for the cage exposures, from acidic to slightly acidic to neutral conditions during the snowmelt. All streams have or have had native brown trout populations, with densities ranging from 0 to 77 trout/100 m^2 estimated by electrofishing in the autumn of 2002 (Table 1). There were no natural populations of *G. pulex* in these brooks, while *B. rhodani* was found in high densities in the three neutral brooks but sparingly in brook 4041 and not at all in brooks 4081 and 4070, as estimated by quantitative Surber samplings (1969) in May 2002.
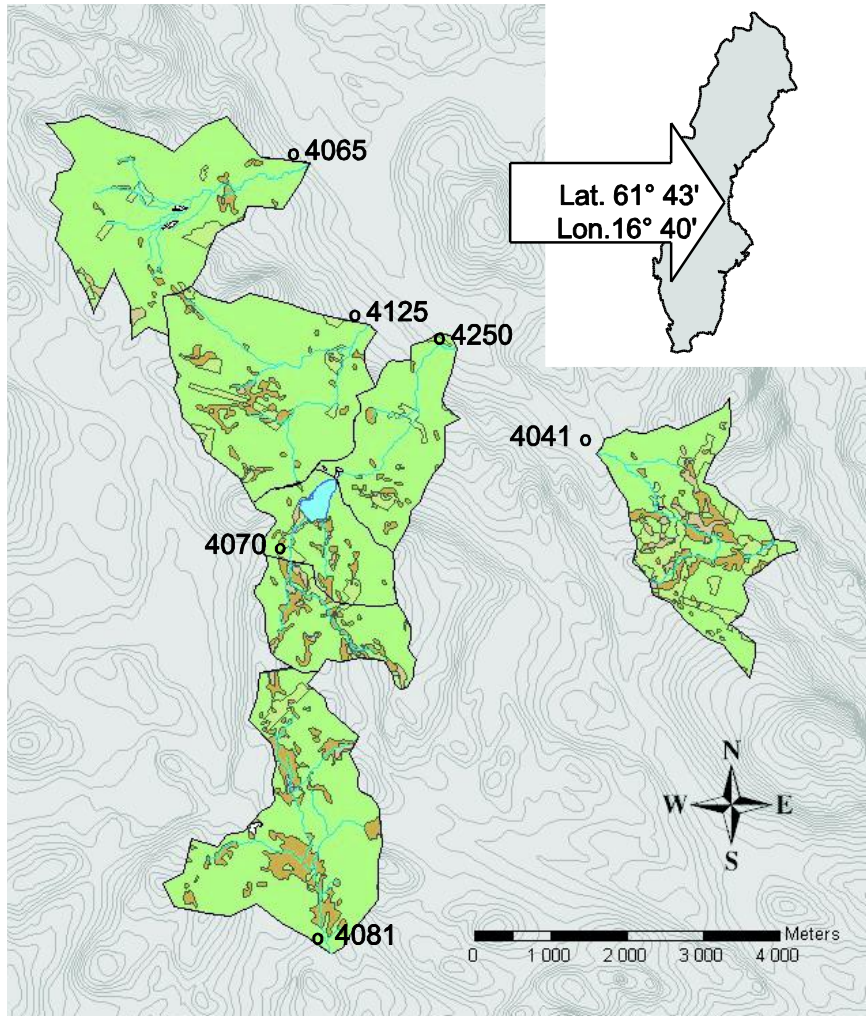
Figure 2. Catchment areas and stream exposure sites, with forest (green) and wetland (brown), brooks and Lake Örvallssjön (blue). The inserted map shows the location of the study area in Sweden.

Table 1. Brook cage site characteristics and water quality (means, number of observations) for the two years of exposure.

Brook site	Catchment area (km2)	Altitude (m a.s.l.)	Length (km)	Land use				Population 2002		Year	pH	Ali (µg/L)	TOC (mg/L)	Ca (meq/L)	BC (meq/L)	n
				Forest	Wetland	Water	Other	Brown trouts/100 m²	Baetis rhodani/m²							
Lövåsb.	4,82	160	6,27	92%	8%	0%	0%	77	1985	2001	6,3	14	12	,109	,244	10
4125										2002	6,3	6	13	,110	,232	12
Stängmyrb.	4,84	111	6,67	97%	2%	0%	1%	26	2540	2001	6,3	18	10	,087	,212	10
4065										2002	6,3	8	11	,081	,205	10
Örvallsb.	6,60	114	6,49	86%	12%	2%	0%	19	3005	2001	6,8	6	13	,225	,329	11
4250										2002	6,5	4	14	,143	,247	10
Brånvallsb.	4,32	153	3,27	83%	17%	0%	0%	22	25	2001	5,6	24	15	,078	,175	11
4041										2002	5,6	20	16	,075	,173	15
Häståb.	4,69	263	6,91	80%	20%	0%	0%	22	0	2001	5,5	26	14	,087	,181	11
4081										2002	5,5	25	15	,086	,176	15
Havssvalgsb.	1,98	317	2,19	74%	26%	0%	0%	0	0	2001	4,8	41	16	,051	,128	11
4070										2002	4,7	51	17	,053	,130	22

ALUMINIUM FRACTIONATION METHOD

Aluminium was measured as total monomeric Al (Al_m) and organic monomeric Al (Al_o) by the colorimetric reaction of pyrocatechol violet (PCV), a modified Al standard method (Swedish Standard, 1992). An AutoAnalyzer (I or II) system (®Bran Luebbe/Seal Analytical) was used in combination with cation exchange and a column flow rate of 2.7 mL/(min∗mL resin) (Driscoll, 1984), an adaptation from Rögeberg and Henriksen (1985). The system was a continuous segmented flow analyser and consisted of a sampler, a pump and chemistry manifolds with flow cell photometers linked to a writer or computer (Fig. 3). This on-line cation exchange with a high precision pump followed by automatic introduction and mixing of the reagents gave high accuracy and repeatability, which is needed for a complex analytical procedure. The cation exchange procedure distinguished the determination of Al_o from Al_m. Organic monomeric Al (Al_o) passed through the column while the potentially toxic fraction, inorganic monomeric Al (Al_i), was retained by the cation exchange resin (and hence could be calculated as the difference between Al_m and Al_o). The analytical method is described in detail in the **supplemental information to paper II**.

For **papers I-II**, the samples were analysed by an AutoAnalyzer II in duplicate with simultaneous determination of pre- and post-cation exchange aluminium on the two channels (Al_i-PCV). The results were evaluated as peak heights with AutoAnalyzer Control and Evaluation Software (AACE, Seal Analytical). For the controlled exposures with lime-adjusted pH (and Al_i) in **papers III** and **IV**, in situ fractionation was used. The cation exchange was preceded by size filtration (0.45 µm) and ultrafiltration (10 kDa) and followed by complexation and extraction (LMM Al_i-HQ, Teien et al., 2004). To avoid potential changes in fractionation caused by transportation and storage time in the cage exposures (**papers V** and **VI**), the samples were analysed within 24 h on a single channel AutoAnalyzer I, detecting one fraction after the other (Al_m and Al_o) and evaluated manually by peak height in the chromatogram. The analytical method (Al_i-PCV) was revised between the two years of field exposures because the flow through the ion-exchange resin was found to be too slow. Therefore, year 2002 samples were run both with the old and new methods to revise the results for year 2001 (Pearson correlation, r^2=0.95, new Al_i = old Al_i∗0.74-7.28, n=139).
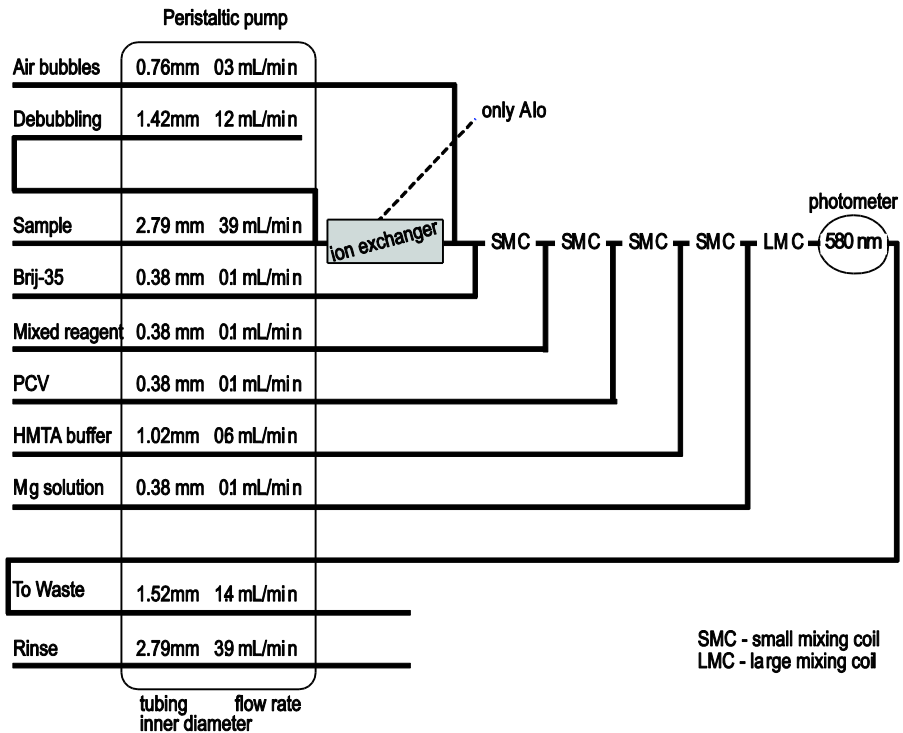
Figure 3. Al fractionation AutoAnalyzer manifold with on-line cation exchange Amberlite IR 120 resin and colorimetric PCV complexation measured in a flow cell photometer. Two parallel setups were made following the scheme, with and without cation exchanger (providing Al_o and Al_m). The noted inner diameter of tubing used on the AutoAnalyzer pump shows the flow rates and correct reaction times and reagent concentrations.

AL_I MODELLING PROGRAMS

The Windermere Humic Aqueous Model (WHAM) 6.0 is a mechanistic, equilibrium computer modelling program (Tipping, 1994). The model works by calculating the amount of Al bound to each available ligand. The binding of Al to inorganic ligands is relatively well defined, but Al binding to organic ligands is the more difficult and critical part of the model. The extreme heterogeneous nature of organic molecules makes it difficult to present a generalised binding capacity for a "typical organic molecule". The samples were measured for total organic carbon (TOC), which in water samples is assumed to be fully dissolved (TOC=DOC). The model was then calibrated by adjusting the fraction of DOC "active" in binding cations (e.g., Al).

In **paper I**, calibration was undertaken by systematically varying the percentage of humus assumed "active" and recording the subsequent Al speciation. Optimised values were obtaining by minimising the root of mean squared deviation (RMSD) between the measured and calculated inorganic cationic Al (Al_i^+). The data fell into clear pH categories, which was not unexpected due to the strong relationship between pH and Al solubility. Therefore, the model, which was first divided into three pH classes, then received individual calibrations for each class. In **paper II**, the model was run with default input values set to fulvic acids at 1.3*DOC (Bryan et al., 2002). This was considered more correct in this study because the intercalibration samples had the "same" humus, but different pHs through liming, and the model herein was run to produce results for these samples and not to calibrate the model. A second model was included in **paper II** to assess consistency in the modelled results.

Visual MINTEQ version 4.0 is a chemical equilibrium model for the calculation of metal speciation and the solubility equilibrium for natural waters. Metal-humic complexation was simulated using the Stockholm Humic Model (Gustafsson, 2001). Default input values were used, which set the fulvic acid concentration at 1.5*DOC (personal recommendation by Gustafson).

FIELD EXPOSURES

Brown trout yearlings (**paper V**) and two key prey organisms, *G. pulex* and *B. rhodani* (**paper VI**), were exposed during the spring flood in humic water with an authentic Al_i and pH gradient to determine when Al_i and pH become toxic. Thresholds for acid toxicity (pH and Al_i) were estimated by mortality and physiological responses. The Al effect on brown trout was traced via Al accumulation on the gills and the consequential physiological effects. For the invertebrates, the whole body content of base cations (BC; Ca, Mg, Na, K) and metals (Al, Fe, Mn, Zn) was measured to document loss or accumulation. The exposure cages were checked at least bi-weekly following the water samplings and sampled one to two times per week based on apparent organism responses. These cages were checked more often in the more acidic streams.

Standard analytical methods were used for the water chemical analyses performed following quality assurance and control procedures. In the brown trout, haemoglobin and glucose levels were determined on site with microcuvettes (Hemocue, Ängelholm, Sweden). Blood was centrifuged, the supernatant plasma was frozen, and plasma chloride was determined with a titrator (Radiometer, Copenhagen, Denmark). The second gill arch on the left side was excised from the dead fish and frozen in a pre-weighed, acid-washed plastic bottle. The gill arch was freeze-dried, weighed and digested in 10% HNO_3. The Al in the gill (gill-Al) was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES), and the results were reported as µg/g gill dry weight (dw). The dried invertebrate samples were acid digested with HNO_3, and single element analyses were performed. Base cations were determined with flame atomic absorption spectroscopy (FAAS) and metals with graphite furnace atomic absorption spectrometry (GFAAS).

RESULTS

AL$_I$ – CHEMICAL PERSPECTIVE

MODELLING

In **paper I** it was shown that it was possible to model Al fractionation in national survey data of Swedish lakes and watercourses. The results of the Al$_i$ modelling were satisfactory bearing in mind that the data were obtained from a large number of surface waters. The model calibration of Al$_i$ was relatively successful in the lake samples from 2000 (rho=0.59, n=299), but less so in 1995 (rho=0.50, n=299) and poor in the river samples from 2000 (rho=0.19, n=203). The correlations were strongly influenced by a few high Al$_i$ samples (acid waters), which is a bias that inflated the correlation coefficients. However, in environmental monitoring, the main purpose is to correctly identify and classify waters at risk for Al$_i$ toxicity as low (<30 µg/L) intermediate (30-50 µg/L) or high (>50 µg/L). The modelled Al$_i$ levels were placed in correct toxicity classes for 89-95% of the samples by matching modelled Al$_i$ with analysed Al$_i$.

The modelling showed that on average, 70% of the total Al was colloids, polymers and strong organic Al complexes (acid soluble), while 30% was monomeric Al complexes (more reactive and easily dissolved). The main part of the monomeric Al was organically bound (Al$_o$), while only 5% (of total Al) was in inorganic forms (Al$_i$) that are potentially toxic. The modelled Al$_i$ was comprised of sulphate, hydroxide and fluoride complexes, although complexes with fluoride dominated (almost 80%, Fig. 4). In general, Al solubility is governed by pH, and this speciation reflects the fact that Al is bound by organic matter and held in solution by fluoride.
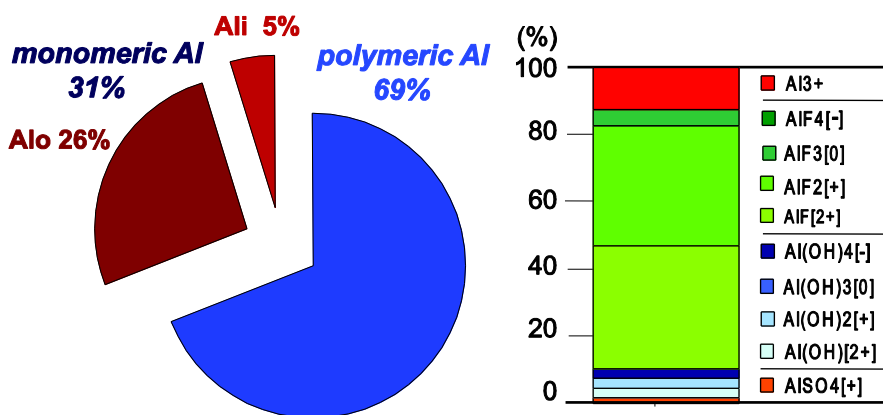

Figure 4. Average distribution of Al fractions and Al$_i$ species modelled with WHAM for the national survey of rivers in 2000.

A sensitivity analysis was performed to identify the dominant ligands binding Al by creating a mean sample and then systematically varying each chemical variable to the minimum and maximum observed values. The most essential variables were Al, pH, DOC and F, which all deviate the modelled Ali from the mean sample by 100% or more (Fig. 5). Fe, Ca and Mg were also significant in the modelling, but to a lesser extent. Furthermore, deviations below 10% from the mean sample were found for K, Na, Cl and SO_4. The minimum variable set required to model Al_i based on the variables causing the most distortion in the modelling are Al, DOC, pH, F, Fe, Ca and Mg.
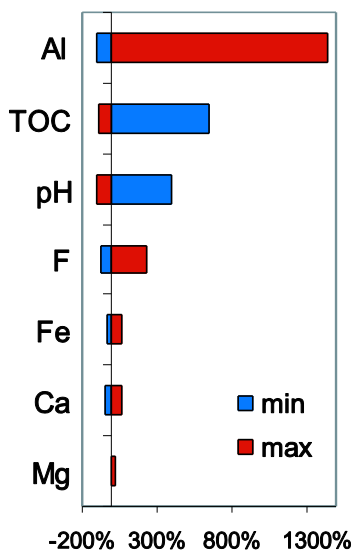
Figure 5. Results of sensitivity analysis; proportional change in Al$_i$ from the mean sample with minimum and maximum values.

VALIDITY OF ANALYTICAL METHOD

In **paper II**, the results are compared for Ali analysis with the three major variations of Al fractionation applied to freshwater samples in Scandinavia and elsewhere. Although the actual ion exchange process (Driscoll, 1984) does vary somewhat between laboratories, the same ion-exchange resin (Amberlite IR120) is used by all laboratories; thus, the main difference was assumed to come from the detection method.

1) Cation exchange followed by determination of monomeric Al using the weak Al complexing agent pyrochatechol violet (Dougan and Wilson, 1974), (**PCV** - primarily a laboratory technique).

2) Cation exchange followed by determination of total Al using graphite furnace atomic absorption spectrometry (GFAAS) or inductively coupled plasma optical emission spectrometry (ICP-OES), (**Total** - primarily a laboratory technique).

3) Cation exchange followed by complexation with the strong complexing agent 8-hydroxy-quinoline (Barnes, 1975), (**HQ** - primarily a field technique).

The total and HQ methods not only produced significantly higher Al_i concentrations than PCV but also resulted in greater data variability over the entire tested pH range. The inclusion or exclusion of outliers detected in the interlaboratory comparison did not affect the statistical results (Fig. 6a). The magnitude of the differences among methods was evident when the mean values of each method were plotted together and analysed using a linear regression (Fig. 6b). All three Al_i methods correlated well with pH, implying that the differences between the methods were systematic and not affected by random errors. Al_i concentrations determined with HQ were significantly reduced by ultra-filtration performed during the field experiment. The smallest size fraction, LMM- Al_i (i.e., the ultra-filtered Al_i fraction) produced results that were similar to the PCV determination (Fig. 6c).

The two models that predicted Al_i concentrations produced results that were also very similar to the mean Al_i concentrations determined by the PCV method. The correlations between results from the two Al_i models, the PCV method and pH are shown in Fig. 6d. The analytical reliability for Al_i measurements was at least equivalent to other variables (such as calculated charge-balance ANC or modelled Al_i) commonly used as indicators of acidification. In **paper II,** it was found that Al_i measurements were accurate and that the level of precision was approximately 25% (RSD), which was similar to calculated ANC and modelled Al_i. Based on the results of **paper II** for routine laboratory analyses, the PCV method for fractionation (**supplemental information paper II**) following cation exchange (Driscoll, 1984) is recommended. For field measurements, the HQ method combined with ultra-filtration after Driscoll's cation exchange procedure is recommended (Teien et al., 2004).
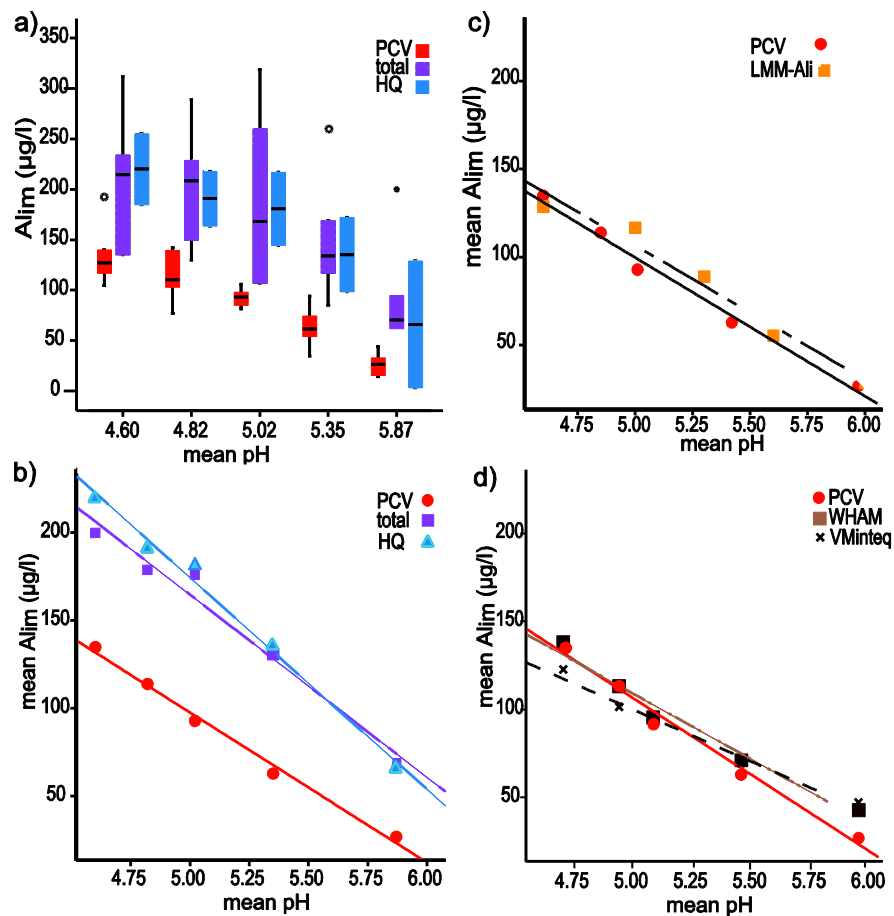
Figure 6. Al$_i$ measurement results vs. the pH for the three methods in the inter-laboratory comparison: PCV = pyrocatechol violet; HQ = 8-hydroxy-quinoline; total = measurement by GFAAS or optical ICP. Best-fit linear regression model lines are shown. a) Box plot of all Al$_i$ results. Lines in boxes are the means, ends of boxes are the quartiles, dots represent individual values, and whiskers show the range of values. Outliers are marked with circles and extreme values with asterisks. b) Mean Al$_i$ concentrations for three methods. Outliers with random errors were excluded. c) Mean Al$_i$ concentrations determined with PCV and the ultra-filtered fraction LMM-Al$_i$ complex bound with HQ in the field experiment. d) Mean Al$_i$ concentrations determined with PCV and modelled Al$_i$ from WHAM and visual MINTEQ.

AL$_I$ – BIOAVAILABILITY AND TOXICITY

BROWN TROUT – CONTROLLED EXPOSURES IN A LIMED PH & AL GRADIENT

The bioavailability of Al decreased both within and between streams after liming. The acidified lake water stream (pH 4.6) was semi-stable, and fish started to die after 40 h of exposure, with >900 µg Al/g of gill. Insignificant mortality was observed in two limed channels (pH 4.9 and 5.2) where a few fish died at the end of the 7-day exposure (**paper III**). Liming reduced the Al accumulation on gills in a pH- and time-dependent manner. This change in Al accumulation could be explained by changes in Al speciation (**paper IV)** (Fig. 7a). Directly after liming (at pH 6.0), the reduced Al accumulation on gills was attributable to the reduced presence of LMM Al$_i$ (low molecular mass – ultra-filtered fraction).

In more stabilised (older) water, Al accumulation on gills is primarily attributable to LMM Al$_i$, which was transformed via HMM Al$_i$ to HMM Al$_o$ (high molecular mass fractions - not passing through the ultra-filter). However, the Al accumulation on gills was higher at low pH (Fig. 7b), which was attributed to slower kinetics and that the fraction of hydrolysed LMM Al$_i$ was reduced (**paper IV**). There was a linear correlation between decreasing plasma chloride and increasing glucose as gill-Al increased (**paper III**).

The kinetics involved in the present high TOC limed water appeared to be fast. The transformation of LMM Al$_i$ to HMM Al$_i$ occurred much more rapidly, less than one minute in the present mixing zone, compared to low organic, non-equilibrium systems (Teien et al., 2004). The decreasing accumulation of Al implied occurrence of within stream processes (transformation of bio-available Al to non-available forms with increased water age) and between stream responses (transformation rates related to pH). No fish died in the limed streams
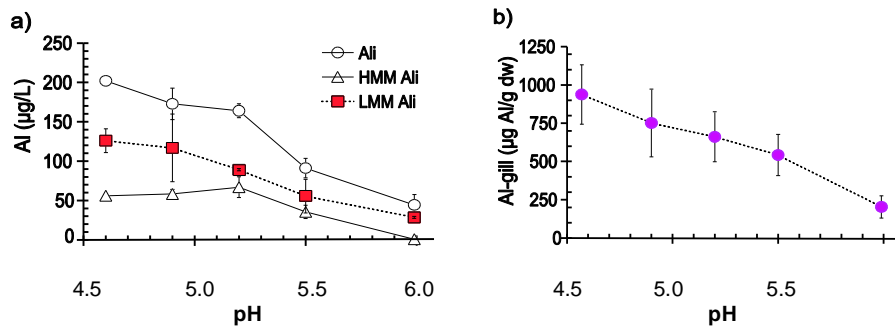

Figure 7. pH dependent changes a) in concentration of Al$_i$-species (LMM, HMM, total Al$_i$) in water 140 min after liming, and b) Al concentrations on fish gills (µg/g dw) after 3 d exposure to pH 4.6, 4.9, 5.3, 5.5 and 6.0 water. Standard deviation is indicated for Al$_i$-species (n≥2) based on replicate sampling during the 3 d exposure of fish prior to sampling of gills from groups of 6 fishes.

only in the stream with acidified and Al-enriched lake water, which indicated a major improvement in water quality through liming.

Fish contained in a flow through tank of ultra-filtered water (pH 4.9) accumulated Al on the gills at the same magnitude as fish exposed in unfiltered water (Fig. 8b). Thus, particular, colloidal and organic Al-species in the unfiltered water did not influence Al accumulation on fish gills (**paper IV**) as the ultrafiltration had removed a total of 300 µg Al/L and 7 mg TOC/L (Fig. 8a).
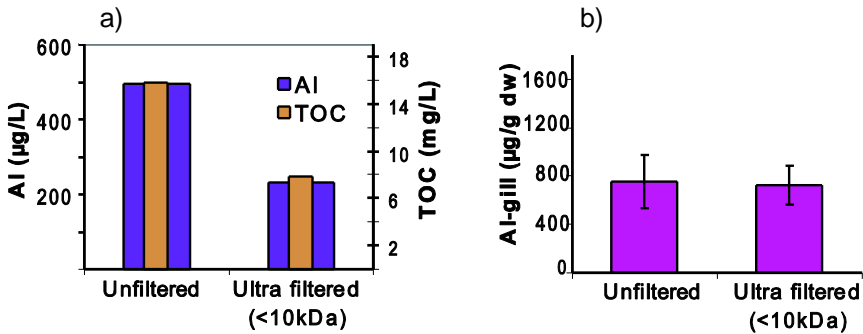
Figure 8. a) Total Al (µg/L) and TOC (mg/L) in unfiltered and ultra-filtered water (pH 4.9). b) Accumulated gill Al (µg/g dw) in fish exposed in unfiltered and ultra-filtered water.

There was a considerable difference in mortality between the two exposed trout strains Heligeå and Konnevesi (Fig. 9a). The higher and faster mortality for the Konnevesi strain seemed to be caused by a higher susceptibility to Al accumulation (Fig. 9b).
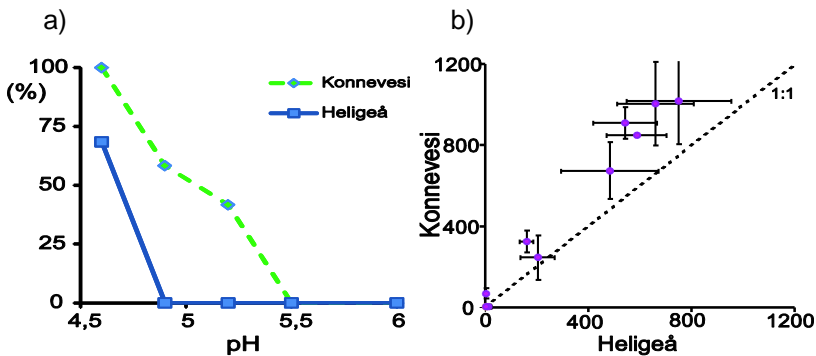
Figure 9. a) Mortality of the two exposed trout strains after 72h and b) gill Al concentrations (means and standard deviation) for the two strains sampled at the same time (ca. 72 and 168 h).

BROWN TROUT – IN-STREAM EXPOSURE IN A NATURAL pH & AL GRADIENT

During the snow melt, the overall water quality encompasses a wide pH (4.6 - 6.8) and Al_i (0 - 69 µg/L) range, which varied both over time and between sites. As a general trend, it was found that when the pH dropped, the Al_i increased. Al_i was found to be harmful at the slightly acidic sites and deadly to brown trout at the most acidic site. All fish survived in five of the six streams even if their exposure time covered the entire snowmelt period (37 days). In the acidic stream, mortality varied between 10 and 100% for five consecutive rounds of exposure (maximum exposure length nine days) and was associated with low pH and elevated levels of Al_i. The water acidity was in turn correlated with Al accumulated on the gills. When Al accumulated on the gills, haemoglobin levels increased, plasma chloride levels decreased, and glucose levels increased.

The fish results were divided into Al_i ranges of 10 µg/L (0-10, 10-20, and so on). Fish swimming erratically and hypoventilating (i.e., affected, moribund fish) had more Al accumulated on their gills compared to unaffected fish within the same Al_i level (Fig. 10a). The haemoglobin levels followed increasing Al_i levels up to 50 µg Al_i/L, with significantly higher levels for affected fish at the same Al_i levels. However, above 50 µg Al_i/L the haemoglobin levels dropped (Fig. 10b). When the results were divided into pH classes, more Al accumulated in affected fish compared to unaffected fish within two classes (pH <4.6 and pH 5.0-5.2, Fig. 10c), which could be expected because Al_i is tightly linked to pH. There was a trend of lower plasma chloride in the affected fish as pH decreased (Fig. 10d). However, there were too few cases (as affected fish had thickened blood and not enough plasma for Cl analysis) to statistically test the difference in plasma chloride. Moribund fish with affected behaviour had similar levels of accumulated Al as dead fish.

The gill Al affected physiology, as demonstrated by the changes in haemoglobin, plasma chloride and glucose levels. There was a statistically significant effect for haemoglobin that could reflect the gas exchange linked to Al_i, while pure pH effects could not be confirmed statistically. The pH effect on the salt balance was not statistically significant, even if the plasma chloride generally dropped when the water was more acidic (Fig. 10). The decrease in plasma chloride could not be tested because less blood was available, which might indicate that circulation collapsed at low plasma chloride levels. Separate toxic effects of increased Al_i and decreased pH levels could not be differentiated by fish mortality, although they could partially be discerned by combining fish status with fish physiology.
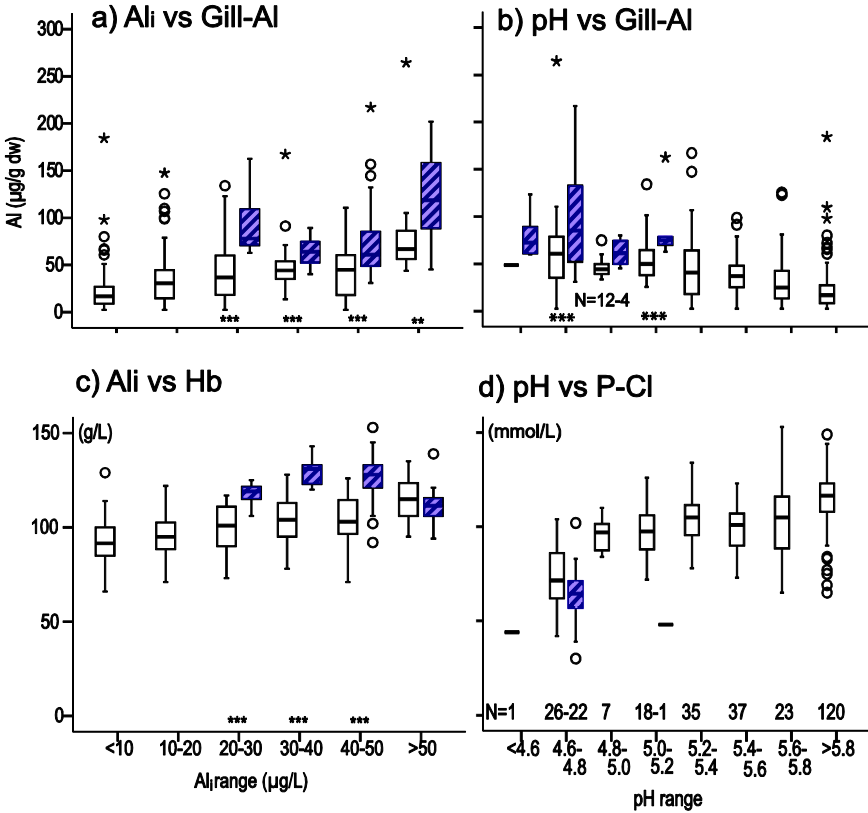
Figure 10. Box plots with fish response and condition plotted against water quality (split into ranges of Al_i and pH). Affected fish (black hatched bars) had slow movements, were barely breathing or swam upside down. Ali range and a) Al accumulated on the gills, b) haemoglobin and pH range, c) Al accumulated on the gills and d) plasma chloride.

The gill accumulation of Al is summarised in a three-dimensional graph with pH and Al_i (Fig. 11) with three discernible groups: no effect, physiological effects and mortality. To avoid detrimental effects, pH levels should be above 5.0 and Al_i below 20 µg/L. These levels are suggested for optimal water quality for brown trout populations. The middle group (Al_i 20-50 µg/L) had little mortality, but significant physiological effects and was considered to reflect sub-optimal conditions from which the fish could recover but where growth was limited. When the Al_i concentration was >50 µg/L, the mortality was always high.
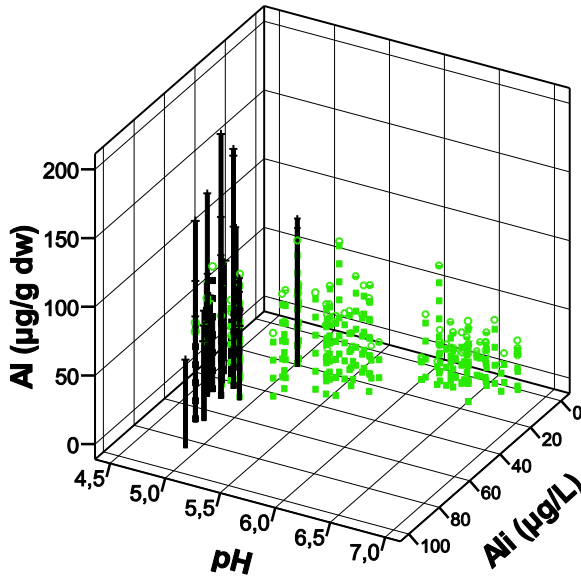
Figure 11. Mean values for groups of fish from each sampling point (6 living trout/sampling) for accumulated gill Al correlated to pH and Ali. Green circles and dashed lines represent healthy fish groups (from exposures where no fish died), and crosses and solid lines are fish groups where death occurred.

GAMMARUS PULEX AND BAETIS RHODANI – IN-STREAM SPRING EXPOSURE

In the neutral brooks, all animals survived, while the mortality was intermediate in the slightly acidic brooks. However, nearly all individuals of both species died in the acidic brook. For *G. pulex*, the accumulated mortality was higher (54% and 88%) than for *B. rhodani* (22% and 40%) in the two slightly acidic streams, and death also seemed to occur faster for *G. pulex* than for *B. rhodani* (Fig. 12). A survival analysis (Cox regression) was performed to determine the causes of mortality in both external water quality and internal response variables. The primary external influence was pH for *G. pulex* and Al_i for *B. rhodani*, and for both organisms, the major response in body composition was Na content.
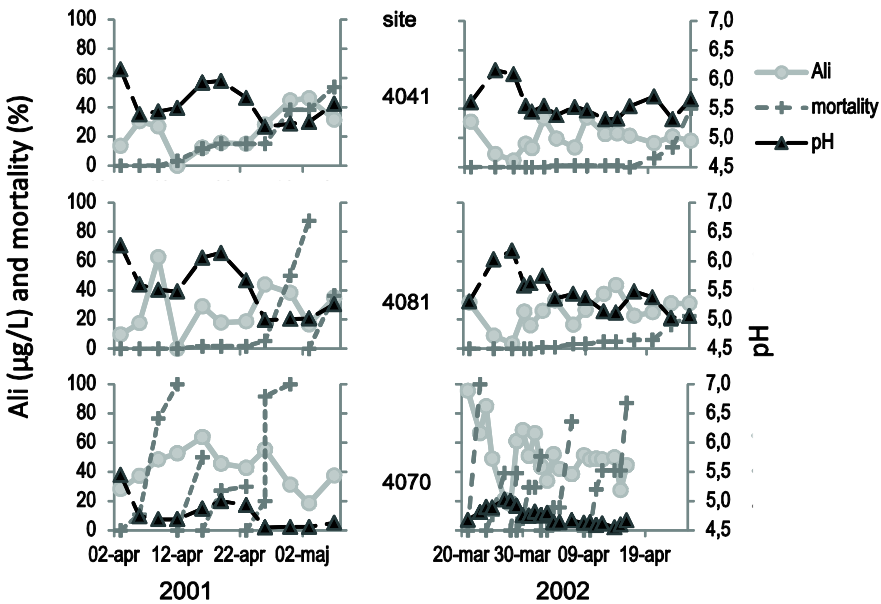
Figure 12. Water quality and invertebrate mortality during the spring floods of 2001 (left panels) and 2002 (right panels) in the three most acidic brooks with invertebrate mortality. Site numbers are shown in between the two columns of panels. pH (black triangles and solid line) on the right hand y-axis and on the left side y-axis, mortality (%, crosses and dashed line) and Al_i concentrations (ug/L, grey circles and solid line).

Mortality of *G. pulex* chiefly occurred below pH 6.0 and above 15 µg/L Al$_i$, but for the somewhat less sensitive *B. rhodani*, below pH 5.7 and above 20 µg/L Al$_i$ (Fig. 13a and b). Similar borders for mortality could also be discerned from decreasing Na body content in the two species (Fig. 13c).
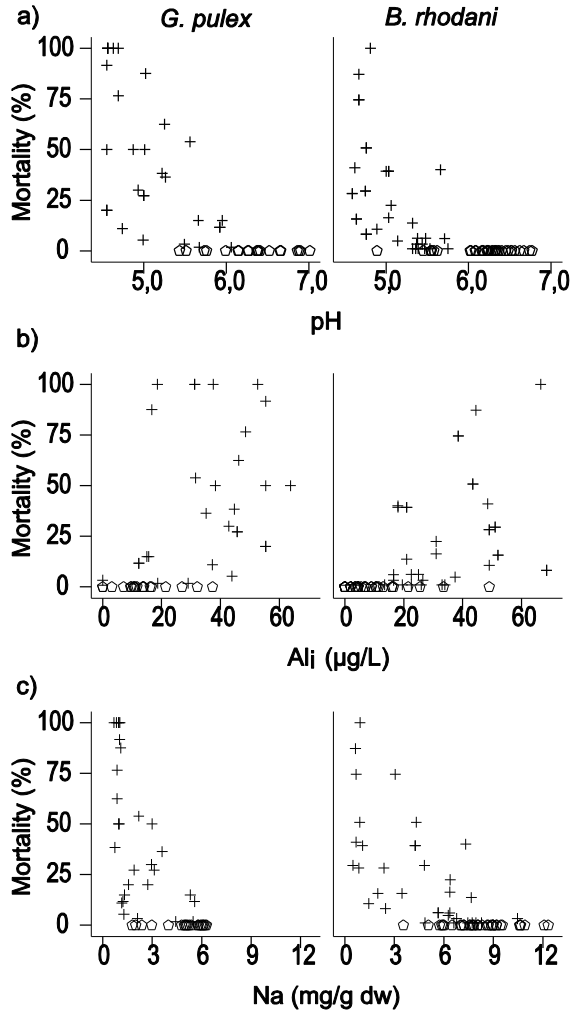

Figure 13. Scatterplot for water acidity; a) pH, b) Al$_i$ and c) Na body content in live invertebrate sample in relation to categorical accumulated mortality. Circles represent samples from healthy invertebrate cages, and crosses samples from cages where death has occurred at some time from the start of the exposure.

DISCUSSION

MODELLED AND MEASURED AL$_I$ CONCENTRATIONS

Paper I showed that it was possible to model and hence simulate Al$_i$ concentrations using data from national monitoring programs. Therefore, Al$_i$ modelling can be used as a tool in operational monitoring through prediction of Al$_i$ levels and trends in surface waters. Previously, WHAM was applied to replicate laboratory batch titrations involving Al in organic soil, but poor results were obtained (de Wit et al., 2001). Recently, more Swedish studies modelling Al speciation have been undertaken, including a combined geochemical speciation of Al and concurrent modelling of pH (Sjostedt et al., 2010) and a more specific application for liming (Sjostedt et al., 2009). Modelling could also be used to recognise Al$_i$ occurrence and predict in which waters Al toxicity can be expected. As liming is reduced, it is essential to identify waters where special attention and precautions for acidic episodes should be taken.

Significant differences in the levels of apparently retained Al$_i$ measured by the ion exchange column were found for the three fractionation methods in the intercalibration of **paper II**, and causes to these differences were revealed. The deviating results were unsatisfactory, although not surprising, especially because a consensus does not exist on which is the most appropriate method for Al fractionation to use in acidification research and monitoring. As a result of this methodological confusion, it has not been possible to harmonise cross-study results, even though comparisons of Al fractions have been included in ICP waters intercalibrations. However, data have not been evaluated since Hovind (2001) stated that Al$_i$ results were not relevant because they were dominated by systematic errors.

Previously, mainly pairwise comparisons on variations of this method have been performed (Berden et al., 1994; Fairman et al., 1994; Wickstrom et al., 2000). Previous inter-method comparative studies (e.g. Salbu et al., 1990) did not clarify the causes of variability. A thorough statistical assessment of the forms of Al actually included by different variations of the cation exchange technique was thus lacking. By comparing experimental results with modelled Al$_i$ (**paper II**), the PCV measurements were shown to be the most accurate. It was further verified that, as colloids can erroneously be included in the Al$_i$ results, the detection method did influence the result. Either ultra-filtration to give LMM-Al$_i$ (low molecular mass fraction) has to be performed prior to the ion exchange, or a soft detection method has to be used to obtain correct Al$_i$ results. Therefore, the method used to measure Al$_i$ influences the results of aluminium fractionation by unintentional inclusion of Al that is retained by the exchange column due to factors other than cation binding. To obtain reliable results for Al$_i$, choosing both a correct fractionation procedure and detection method is important. Stronger detection methods require more cautious pre-treatment by ultra-filtration to avoid inclusion of colloidal Al in the inorganic monomeric (Al$_i$) fraction.

The results in **paper II** were contradictory to those presented in Sjostedt et al. (2010), where determinations with the PCV-method overestimated Al_i and ICP (total) determinations underestimated Al_i compared to modelled Al_i. In **paper II**, the humic material was the same in all samples; thus, Al_i speciation was not dependent on either humic character or model calibration, and only pH was changed (by liming). My study comprised only a few but identical samples analysed by several laboratories, while the other study modelled thousands of different samples from several projects. The dissimilar findings might be explained by differences in the water qualities analysed by the two methods in that study; samples analysed with the PCV-method had less Al and TOC as well as higher pH than the samples analysed with ICP. Furthermore, different constants were used in the modelling to approximate available fulvic acids.

The suggestion that the PCV-method overestimates Al_i by performing the ion exchange at a speed that is too low is less plausible. The effect of pump speed was tested when the method was revised in 2002 and found to be adequate because the level of Al in the resin eluate reached a plateau state. In **paper II**, the analytical results were directly linked to toxicity, as relevant test waters were used (coming from a gradient with fish exposure). Nonetheless, the results were intriguing as the two major Swedish laboratories fractionating Al participated in both studies.

Based on the results in **paper II**, the PCV method is recommended for routine laboratory analyses using an automatic fractionation following cation exchange protocol (Driscoll, 1984) described in the supplementary material. For field measurements in mixing zones or other unstable situations, 8-hydroxy-quinoline complexation (Barnes, 1975) combined with ultra-filtration before Driscoll's cation exchange procedure (Teien et al., 2004) is recommended.

BIOLOGICAL THRESHOLDS

Through the controlled exposures in **papers III and IV** that mimicked previous low DOC exposure setups with salmonids (e.g. Teien et al., 2004), three findings concerning Ali occurrence and toxicity were revealed:

- o Liming transformed low molecular mass (LMM) Al_i to high molecular mass (HMM) Al_i much more rapidly in this humic (high DOC) mixing zone than in low DOC, non-equilibrium systems.

- o Organic compounds had no major influence on products formed during transformation of LMM-Al_i following liming. In the same way as in mixing zones in water of low DOC, LMM-Al_i and HMM-Al_i decreased as the concentration of HMM-Al_o increased.

- o Particular, colloidal and organic Al species did not influence Al accumulation on fish gills. The concentration of Al on gills was instead dependent on the concentration of LMM-Al_i species present because the accumulation was the same for fish exposed to Al in ultra-filtered water as in unfiltered water.

The decrease in Al gill concentration followed decreases in LMM-Al$_i$ levels, just as in previous salmon exposures simulating liming in low DOC waters where gill active Al species decreased (Kroglund et al., 2001). The rapid transformations implied that mixing zones were less widespread and much smaller in typical Swedish high DOC waters than in waters with low DOC. Additionally, lime is usually spread higher up and at multiple locations in the Swedish catchments compared to liming in large Norwegian salmon rivers. In Norway, lime is often applied by fewer and larger automatic dosing systems that might create mixing zones when the limed mainstreams meet acid tributaries (e.g. Kroglund et al., 2001; Poleo et al., 1994; Rosseland et al., 1992). Therefore, mixing zones and problems associated with these types of water (high toxicity and rapid transformations) are of lesser importance in Sweden so in situ fractionation is not often required.

Intercalibration (**paper II**) showed that the ultra-filtered fraction (LMM-Al$_i$) gave consistent results with the laboratory method routinely used in Sweden, PCV-Al$_i$. In the controlled exposures of 11-cm yearlings of brown trout (**paper III and IV**), LMM-Al$_i$ was shown to account for Al gill accumulation (tested by fish exposure in ultra-filtered water). Therefore, PCV-Al$_i$ provides a realistic estimate of Al$_i$ bioavailability and hence toxicity. In these controlled exposures, acceptable water quality over a 7-day period was obtained in water having <33 µg Al$_i$/L at pH 5.5 in stabilised limed waters (recalculated from total Al$_i$ reported in **paper IV**).

Moving on to even more relevant exposures in ambient waters at spring flood (**paper V**), the pH and Al$_i$ were further confirmed by resemblance in both physiological responses and Al$_i$ thresholds. Normally, brown trout mortality was associated with low pH, elevated levels of Al$_i$ and significantly increased gill Al. The levels of Al on the gills were lower than in **papers III and IV**, as well as in other simulated mixing zone studies (with transient Al polymers up to 1.5 mg/g dw, e.g. Kroglund et al., 2001). In these high humic waters, evidence was found of Al toxicity as follows:

o The elevated levels of Al on the gills and Hb in the blood indicated respiratory disturbances.

o The more pH-related ion regulatory disturbances were of less importance.

o Al$_i$ occurred in detectable and toxic concentrations in these humic boreal brooks.

The physiological responses of the trout in these field exposures (**paper V**) were similar to the results in the controlled exposures (**papers III and IV**) with the exception of Al accumulation. Gill Al seemed to accurately reflect the toxicity, although as a relative measure and not as an absolute dose as has been proposed. Higher amounts of Al accumulated on the gills in the simulated unstable mixing zone than at the spring flood (**paper V**). This behaviour indicated that more reactive complexes occurred in the mixing zone and deposited on the gills even though the Al$_i$ levels, and hence the bioavailability, were similar.

In **paper V**, two thresholds for Al_i toxicity to brown trout were found: high mortality above 50 µg/L and low mortality with physiological effects above 20 µg/L. In a Scottish study (McCartney et al., 2003), similar critical limits were found, as streams with Al_i <20 µg/L held sustainable trout populations, while those with >50 µg/L were fishless. The Al_i limits established (<30 µg/L in the controlled exposure and <20 µg/L in the in-stream exposures) were further validate by the bounds predicted in prior inductive statistical studies correlating water quality and brown trout populations in Norway (11 µg/L, Bulger et al., 1993), the USA (22 µg/L, Baldigo and Lawrence, 2001), Sweden (30 µg/L, Andrén and Bergquist, 2000), and the UK (40 µg/L, Sadler and Turnpenny, 1986). The fact that the thresholds were of similar magnitude confirmed the assumption that conditions with no physiological effects during an exposure could be equated with water qualities that could support the residing fish.

Today, the guidelines for liming in brown trout waters are pH >5.6 and Al_i <50 µg/L, and for natural salmon waters, they are pH >6.0 and Al_i <30 µg/L (Swedish EPA, 2010). However, there seems to be little difference in the tolerance levels for Al_i at different levels of TOC, as these worldwide studies span over different humus concentrations. Thus, these population-based limits do not support the possible ameliorating effect of TOC proposed by Roy and Campbell (1997). The amending effect of organic matter on Al toxicity has been pursued without any conclusive findings (Liebich et al., 2011; e.g. Winter et al., 2005; Witters et al., 1990). Al complexation by organic matter definitely decreases the toxic effects of Al, but the existence of an additional protective property of OM is uncertain (Winter et al., 2005). Liebich et al. (2011) suggested that moderate to high DOC was protective to some degree for Al (*total*), although at the same time, the control of the inorganic fraction (Al_i) was said to be highly complex and dependent on many aspects of stream chemistry (Liebich et al., 2011). However, my studies did not indicate any difference in Al_i effects (*inorganic Al*) between low DOC and high DOC waters. This conclusion was shown in **paper III**, where identical Al gill concentrations were measured in exposed trout in both ultra-filtered water and unfiltered water (reducing TOC and total Al levels by 50%).

The presence of Al_i has been proposed as an unambiguous indication of acidic deposition (Lawrence et al., 2007). My results do not contradict this hypothesis because the toxicity in the exposures matched the trout densities, which in turn reflected the development of acidification. High Al_i concentrations and mortalities occurred in the brook where trout are scarce today and where they apparently vanished when the acidic deposition increased. Lawrence's proposal is debated in Sweden in terms of strong (inductive) correlations of Al_i to fluoride (Sjostedt et al., 2010) or catchment size and where the occurrence of mature Norway spruce (Lofgren et al., 2010) have been shown. In a prediction of the effect of lime reductions for the 3 043 limed Swedish lakes (Sjostedt et al., 2012 in prep), a clear geographical pattern emerged with the highest Al_i levels in the south and the lowest pH in south western Sweden. This distribution corresponded to known S deposition patterns.

Using a wider approach, the occurrence of Al_i mirrored acidification, even though it is hard to select a background level. Other factors in the catchment

areas and water chemistry clearly affect Al_i occurrence, but acidification pressure has a large impact. Even if the response (presence/absence) of biota can be considered as a relevant benchmark for acidification, it is impractical because reliable historical records are difficult to find. Al_i concentrations below 10 µg/L (demonstrated in this thesis to be tolerated by biota) might be considered to reflect 'natural' background levels. Additionally, concentrations above 10 µg Al_i /L can be considered to be analytically accurate because this is the reporting limit according to current analytical quality protocols.

In **paper VI**, the survival analyses indicated that Al_i was the most important toxic factor for *B. rhodani*, while pH seemed to be more important for toxicity to *G. pulex*. Although Al and H^+ ions are concurrent forces in acidified waters, it is interesting that Al_i was indicated for *B. rhodani* in these in-stream exposures. The acid toxicity affected Na levels (i.e., ion regulation), which was found to be significantly altered by the survival analysis on body elemental composition in both species. However, there was no Al accumulation for any of the species, which supported the suggestion of a poor relationship between body content and toxicity of Al (Havas and Hutchinson, 1983).

The same species were previously exposed in short-term experimental acidification studies in Wales (McCahon et al., 1987; McCahon and Pascoe, 1989; Merrett et al., 1991; Ormerod et al., 1987), where mortality and effects on feeding and drift were shown. Hall et al. (1980) concluded from an experimental field test from New Hampshire that fluctuating Al concentrations in acidic episodes in low-order streams might alter the biology and geochemistry in poorly buffered waters. The accumulation of Al reported by McCahon et al. (1987) might have been caused by Al variations in unstable waters, as staining showed that Al accumulated over the whole of the invertebrates (possibly a surface adsorption with little physiological effect). It is not clear if Al toxicity in those trials impacted ion regulation or respiration via the gills, as no measurement of ventilation or Na concentration was performed.

In **paper VI**, both Al and Na body contents were determined. Al is usually considered to add to pH stress itself despite Al possibly ameliorating low pH toxicity (pH <4) (e.g. Frick and Herrmann, 1990; Herrmann et al., 1993). The additive effect of Al_i (to H+ ions) on ion regulation was stronger for *B. rhodani* than *G. pulex*. This finding might suggest that Al acts on the intracellular channels, reducing ion uptake and increasing ion effluxes, as has been proposed for fishes (reviewed by Gensemer and Playle, 1999). In addition, Na concentrations appeared to indicate disturbances in several functions, as reduced Na levels in *G. fossarum* were correlated to stream mean pH (Felten and Guerold, 2004); increased mortality (Felten and Guerold, 2006); and decreased osmolality, ventilation and locomotor activity in *G. pulex* (Felten et al., 2008). Previous in vitro experiments in acid- and Al-rich waters showed a possible additive effect for Al (with H+) in mayflies (Frick and Herrmann, 1990; Herrmann, 1987) and in *G. fossarum* (Felten et al., 2008; Felten and Guerold, 2001) by decreasing Na levels.

Unfortunately, many studies have not specified actual TOC levels, which might indicate that they not are decidedly humic. Nonetheless, the results in my studies are from highly humic waters (approximately 15 mg TOC/L). Therefore, it is difficult to conclude whether TOC ameliorated toxicity, as was proposed for fish (Roy and Campbell, 1997). However, an effect for Al_i was found in these highly humic waters that were toxic at moderate pH levels (pH 5-6), a pH range where Al toxicity is considered to be important for fish.

Some notable reverse relationships between water concentration and body content in **paper VI** indicated that active transport mechanisms must be involved, perhaps as a defence to acidity. In *B. rhodani*, the regulation of bodily Mg and Zn appeared to collapse when Na levels were low; the relationships to Na were no longer linear, Mg increased and Zn decreased while mortalities occurred. The regulation of Ca in *G. pulex* also collapsed when the Na body content decreased (Ca levels increased in *G. pulex* when Na levels decreased). This could be a defensive mechanism similar to that observed in another aquatic crustacean (*Calinectes sapidus*) by Cameron (1985). In a similar way, dragonfly nymphs (*Libulla julia* Uhler) lost their ability to regulate K when Na levels were reduced by 60% in acid- and Al-rich exposures (Rockwood and Coler, 1991). Critical Na levels were roughly half of maximum levels in both species exposed.

IMPLICATIONS OF THE THRESHOLDS

Fish populations are commonly restored by introducing farmed trout to streams where the populations have disappeared or by transplanting trout to supplement weakened populations. Introduction of fishes should be performed in a way that will not influence the biological diversity at gene, species and ecosystem levels negatively according to Swedish guidelines (Degerman and Pakkasmaa, 2005). When reintroducing or reinforcing, local trout strains (endemic) should be chosen, but this can still lead to a decrease in the genetic pool. This occurs when only a few breeders are used and they are selected based on criteria other than natural selection. In **paper IV**, for example, two farmed strains were exposed to Al: Finnish Konnevesi trout and Heligeå trout from Småland. The difference in sensitivity was not surprising. The Finnish trout is a fast growing but more sensitive strain coming from clear waters with more neutral pH than the Swedish strain, which originates from browner, acidic water. In **paper V**, a locally farmed strain of Mellan-Ljusnan trout was used, which is more comparable to the Heligeå strain, both native to humic waters.

Different fish species have different tolerance to acidity (e.g. Poleo et al., 1997) which is also true among the salmonid species (e.g. Rosseland and Skogheim, 1984) and also among different trout strains (Rosseland and Skogheim, 1987). In brown trout, the physiological basis for strain differences in acid tolerance has been suggested to originate from Ca metabolism (Dalziel et al., 1995). Brown trout have a larger genetic variation and seem to be more genetically pre-adapted

to environmental changes than salmon (Rosseland et al., 2001). The differences in tolerance to acidity in trout strains implies that variability in organism sensitivity needs to be considered when critical limits are set and when new strains are introduced in recovering waters.

Additionally, critical limits for acidity also need to protect the more sensitive life stages at reproduction and hatching (e.g. Brown, 1983; Norrgren and Degerman, 1993; Reader et al., 1991). In my studies, only young-of-the-year brown trout were exposed. It is further necessary to consider the autecological aspects of an organism when extrapolating between different hierarchical levels and circumstances (Douben and Siepel, 1993), although the ecosystem aspect might be even more important for acidity thresholds. Not only do brown trout need to proliferate and flourish for a sustainable population, but the whole food web with prey organisms also needs to be available in sufficient quantities. In **paper VI**, more restricting thresholds were thus introduced for the two prey species: pH above 5.7-6.0 and Al_i below 15-20 µg/L. The levels found in **paper VI** were comparable to those found in studies linking water quality to population data (Otto and Svensson, 1983). My results implied that in addition to direct effects of harmful water quality, indirect effects on food sources could also create suboptimal conditions for fish in streams recovering from acidification.

Conclusions

In this thesis, I evaluated Al_i in highly humic waters both analytically and toxicologically, which adds to the understanding of Al_i occurrence and effects. My findings are based on validated and accurate Al_i determinations coupled with thorough tests of Al_i toxicity. Mortalities were linked through cause and effect chains to physiological measurements in brown trouts (water–gill–blood), as well as whole body elemental contents in macro-invertebrates using satisfactorily large datasets.

At the outset, I showed that it was possible to model the Al_i fraction in common monitoring data from all over Sweden. I modified and validated the automated analytical procedure for Al_i-PCV (cation exchange-PCV). I also compared the various analytical methods and identified differences and reasons for these deviations. I found that the Al_i-PCV method agreed with modelled Al_i and also with ultra-filtered Al_i-HQ analyses. I determined that the Al_i-PCV method was the preferred method for routine laboratory analyses of monitoring samples, while for field experiments with unstable conditions, it was better to use Al_i-HQ.

There is a need for increased knowledge on Al_i in high DOC waters, as mainly low humic waters have been studied so far. Thus, I estimated thresholds for brown trout in typical high DOC waters for Sweden both by controlled exposures and with in-stream exposures at snowmelt. Brown trout need the levels of pH to be above 5.0 and Al_i to be below 20 µg/L to avoid detrimental effects. The mortality was always high when Al_i was >50 µg/L; therefore, these levels are acutely toxic, while Al_i between 20-50 µg/L represents suboptimal water quality. I also estimated thresholds for two salmonid preys. The invertebrates were more sensitive than brown trout to acid- and Al-rich humic water; mortalities occurred at pH <6.0 and Al_i >15 µg/L for *G. pulex* and at pH <5.7 and Al_i >20 µg/L for *B. rhodani*. It is prudent to be cautious and to set water limits for the more sensitive species; the pH should be above 5.7-6.0 and Al_i below 15-20 µg/L to protect the stream ecosystem.

The thresholds found in this thesis can be used to cautiously and effectively decrease liming because both Al_i and pH levels have to be balanced to prevent harm to the recovering aquatic biota. Although Al_i is strongly linked to pH, varying Al concentrations in the soil and bedrock, as well as local conditions, can give deviations in Al_i levels. Therefore, it is prudent to also monitor and determine Al_i and not only model Al_i However, care should be taken when applying these criteria to streams, as they might not protect more sensitive life stages of fish. Further differences in strain acid tolerance were shown that should be considered when critical limits are set. It is necessary to apply a wide view when defining liming goals and besides fish include their food sources as the most sensitive organism and life stage also needs protection to allow the stream ecosystem to thrive.

FUTURE RESEARCH SUGGESTIONS

Relevant and sufficient knowledge seems to be lacking regarding the impact of acidification and the recovery processes of fish and invertebrates in streams after liming and/or receding acidification. The studies should focus on examining the response in stream ecosystems to changes in water quality and community structure. Inductive studies, correlating population data to water quality as well as designed experiments are needed and modelling of Al_i can be used to elucidate the occurrence and influence of Al_i. The acquired knowledge will illuminate the direct and indirect effects of Al_i on different organism groups and stream ecosystems (e.g., algae, macro-invertebrates and fish). Benthic diatoms are a good candidate as biological indicator organisms of Al_i integrating seasonal changes that would be interesting to explore more fully.

In the future, I hope we can more fully understand the occurrence of Al_i and its linkage to water quality, especially pH, TOC, fluoride and total Al. The effect of catchment characteristics (such as bedrock, soil and land use) on Al_i occurrence are also important to investigate. Furthermore the influence of all aspects of forestry needs to be examined, different kind forests i.e. species and age composition and intensity in management needs to be examined.

Additionally the effect of the on-going climate change needs to be assessed and included in future scenarios of Al_i occurrence and impact on aquatic ecosystems.

TACK

REFERENCES

Andrén,C.M.: 1995, 'Aluminium speciation; Effects of sample storage', *Water, Air, Soil Pollut.* **85,** 811-816.

Andrén, C. M. Provningsjämförelser av Aluminiumfraktioner 2001 & 2002. Interlaboratory comparisons of Aluminiumfractionation 2001 & 2002. (In Swedish with English summary). ITM-report 117: 44 p. 2003. Stockholm University, Sweden, Department of Environmental Science.

Andrén, C. M. and Bergquist, B. C. Aluminium and damage to fish populations in limed streams. Acid rain 2000, 6th International Conference on Acidic Deposition, 10-16 december 2000, Tsukuba, Japan , 321. 2000. Kluwer Academic Publishers.

Andrén,C.M. and Rydin,E.: 2009, 'Which aluminium fractionation method will give true inorganic monomeric Al results in fresh waters (not including colloidal Al)?', *J. Environ. Monit.* **11,** 1639-1646.

Arnell,N.W.: 1999, 'The effect of climate change on hydrological regimes in Europe: a continental perspective', *Global Environmental Change-Human and Policy Dimensions.* **9,** 5-23.

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

Baldigo,B.P. and Lawrence,G.B.: 2001, 'Effects of stream acidification and habitat on fish populations of a North American river', *Aquat. Sci.* **63,** 196-222.

Barnes,R.B.: 1975, 'Determination of Specific Forms of Aluminum in Natural-Water', *Chem. Geol.* **15,** 177-191.

Berden,M., Clarke,N., Danielsson,L.G. and Sparen,A.: 1994, 'Aluminum speciation - variations caused by the choice of analytical method and by sample storage', *Water, Air, Soil Pollut.* **72,** 213-233.

Bergstrom,S., Carlsson,B., Gardelin,M., Lindstrom,G., Pettersson,A. and Rummukainen,M.: 2001, 'Climate change impacts on runoff in Sweden - assessments by global climate models, dynamical downscaling and hydrological modelling', *Climate Research.* **16,** 101-112.

Bishop,K.: 2012, 'Naturligt - lätt att säga, omöjligt att definiera', *Skog & Framtid.* 15-16.

Brown,D.J.A.: 1983, 'Effect of Calcium and Aluminium Concentrations on the Survival of Brown Trout (*Salmo trutta*) at Low pH', *Bull.*

Environ. Contam. Toxicol. **30,** 582-587.

Bryan,S.E., Tipping,E. and Hamilton-Taylor,J.: 2002, 'Comparison of measured and modelled copper binding by natural organic matter in freshwaters', *Comp. Biochem. Physiol. Part C Toxicol. Pharmcol.* **133,** 37-49.

Bulger,A.J., Lien,L., Cosby,B.J. and Henriksen,A.: 1993, 'Brown trout (*Salmo trutta*) status and chemistry from the Norwegian 1000 lake survey - statistical-analysis', *Can. J. Fish. Aquat. Sci.* **50,** 575-585.

Cameron,J.N.: 1985, 'Compensation of Hypercapnic Acidosis in the Aquatic Blue-Crab, *Callinectes sapidus* - the Predominance of External Sea-Water Over Carapace Carbonate As the Proton Sink', *J. Exp. Biol.* **114,** 197-206.

Cory,N., Buffam,I., Laudon,H., Kohler,S. and Bishop,K.: 2006, 'Landscape control of stream water aluminum in a boreal catchment during spring flood', *Environ. Sci. Technol.* **40,** 3494-3500.

Cronan,C.S. and Schofield,C.L.: 1979, 'Aluminium Leaching Response to Acid Precipitation: Effects on High-Elevation Watersheds in the Northeast', *Science.* **204,** 304-306.

Dalziel,T.R.K., Kroglund,F., Lien,L. and Rosseland,B.O.: 1995, 'The refish (restoring endangered fish in stressed habitats) project, 1988-1994', *Water, Air, Soil Pollut.* **85,** 321-326.

de Wit,H.A., Groseth,T. and Mulder,J.: 2001, 'Predicting aluminum and soil organic matter solubility using the mechanistic equilibrium model WHAM', *Soil Sci. Soc. Am. J.* **65,** 1089-1100.

Degerman, E. and Pakkasmaa, S. Fish in wrong waters Ecological consequences of fish introductions (in Swedish). 2005:9, 1-78. 2005. Swedish National Board of Fisheries. Finfo.

Dickson,W.: 1978, 'Some effects of the acidification of Swedish lakes', *Verh. Int. Ver. Theor. Angew. Limnol.* **20,** 851-856.

Douben,P.E.T. and Siepel,H.: 1993, 'Extrapolation from laboratory to field and from individual to populations: pitfalls to avoid', *Sci. Total Environ.* **Supplement 1993,** 1025-1036.

Dougan,W.K. and Wilson,A.L.: 1974, 'Absorptiometric determination of aluminum in water - comparison of some chromogenic reagents and development of an improved method', *Analyst.* **99,** 413-430.

Driscoll,C.T.: 1984, 'A procedure for the fractionation of aqueous aluminum in dilute acidic waters', *Int. J. Environ. Anal. Chem.* **16,** 267-283.

Driscoll,C.T., Baker,J.P., Bisogni,J.J. and Schofield,C.L.: 1980, 'Effect of aluminium speciation on fish in dilute acidified waters', *Nature.* **284,** 161-164.

Driscoll,C.T. and Schecher,W.D.: 1990, 'The Chemistry of

Aluminum in the Environment', *Environ. Geochem. Health.* **12,** 28-49.

Erlandsson,M., Cory,N., Folster,J., Kohler,S., Laudon,H., Weyhenmeyer,G.A. and Bishop,K.: 2011, 'Increasing Dissolved Organic Carbon Redefines the Extent of Surface Water Acidification and Helps Resolve a Classic Controversy', *Bioscience.* **61,** 614-618.

Eshleman,K.N. and Hemond,H.F.: 1985, 'The Role of Organic-Acids in the Acid-Base Status of Surface Waters at Bickford Watershed, Massachusetts', *Water Resour. Res.* **21,** 1503-1510.

European Environment Agency. Annual Precipitation Change 2021-2050, http://climate-adapt.eea.europa.eu/viewaceitem?aceitem_id=4571. 1-8-2012a.

European Environment Agency. Annual Temperature Change 2021-2050, http://climate-adapt.eea.europa.eu/viewaceitem?aceitem_id=3973. 8-1-2012b.

European Union: 2000, 'Directive 2000/60/EC of the European Parliament and the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. ', *Official Journal of the European Communities.* **327,** 1-73.

Evans,C.D., Monteith,D.T. and Cooper,D.M.: 2005, 'Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts', *Environ. Pollut.* **137,** 55-71.

Evans,C.D., Monteith,D.T., Reynolds,B. and Clark,J.M.: 2008, 'Buffering of recovery from acidification by organic acids', *Sci. Total Environ.* **404,** 316-325.

Fairman,B., Sanzmedel,A., Gallego,M., Quintela,M.J., Jones,P. and Benson,R.: 1994, 'Method Comparison for the Determination of Labile Aluminum Species in Natural-Waters', *Anal. Chim. Acta.* **286,** 401-409.

Felten,V., Charmantier,G., Charmantier-Daures,M., Aujoulat,F., Garric,J. and Geffard,O.: 2008, 'Physiological and behavioural responses of *Gammarus pulex* exposed to acid stress', *Comp. Biochem. Physiol. Part C Toxicol. Pharmcol.* **147,** 189-197.

Felten,V. and Guerold,F.: 2001, 'Hyperventilation and loss of hemolymph Na+ and Cl- in the freshwater amphipod *Gammarus fossarum* exposed to acid stress: a preliminary study', *Diseases of Aquatic Organisms.* **45,** 77-80.

Felten,V. and Guerold,F.: 2004, 'Haemolymph [Na+] and [Cl-] loss in *Gammarus fossarum* exposed in situ to a wide range of acidic streams', *Diseases of Aquatic Organisms.* **61,** 113-121.

Felten,V. and Guerold,F.: 2006, 'Short-term physiological responses to a severe acid stress in three macroinvertebrate

species: A comparative study', *Chemosphere.* **63,** 1427-1435.

Folster, J. Förslag till Bedömnings-grunder för försurning i sjöar och vattendrag. 2007:9, -28p. 2007. Uppsala, Institutionen för miljöanalys, SLU.

Folster, J. and Kohler, S. Försurningsläget i Sveriges ytvatten 2010. 2011:24, -50p. 2011. Uppsala, Institutionen för vatten och miljö, SLU.

Frick,K.G. and Herrmann,J.: 1990, 'Aluminium and pH effects on sodium ion regulation in mayflies', in B.J.Mason (ed.), *Surface Water Acidification Programme, Proceedings from the Final Conference in London, March 19-23, 1990,* Cambridge University Press, London, pp. 409-412.

Gensemer,R.W. and Playle,R.C.: 1999, 'The bioavailability and toxicity of aluminum in aquatic environments', *Crit. Rev. Environ. Sci. Technol.* **29,** 315-450.

Granier,C., Bessagnet,B., Bond,T., D'Angiola,A., van der Gon,H.D., Frost,G.J., Heil,A., Kaiser,J.W., Kinne,S., Klimont,Z., Kloster,S., Lamarque,J.F., Liousse,C., Masui,T., Meleux,F., Mieville,A., Ohara,T., Raut,J.C., Riahi,K., Schultz,M.G., Smith,S.J., Thompson,A., van Aardenne,J., van der Werf,G.R. and van Vuuren,D.P.: 2011, 'Evolution of anthropogenic and biomass burning emissions of air pollutants at global and regional scales during the 1980-2010 period', *Climatic Change.* **109,** 163-190.

Gustafsson,J.P.: 2001, 'Modeling the acid-base properties and metal complexation of humic substances with the Stockholm Humic Model', *J. Colloid Interface Sci.* **244,** 102-112.

Hall,R.J., Likens,G.E., Fiance,S.B. and Hendrey,G.R.: 1980, 'Experimental acidification of a stream in the Hubbard Brook experimental forest, New Hampshire', *Ecology.* **61,** 976-989.

Havas,M. and Hutchinson,T.C.: 1983, 'Effect of low pH on the chemical composition of aquatic invertebrates from Tundra Ponds at the Smoking Hills, Nwt, Canada', *Can. J. Zool.* **61,** 241-249.

Havas,M. and Rosseland,B.O.: 1995, 'Response of zooplankton, benthos, and fish to acidification: An overview', *Water, Air, Soil Pollut.* **85,** 51-62.

Herrmann,J.: 1987, 'Sodium levels of lotic mayfly nymphs being exposed to Aluminium at low pH ,a preliminary report', in H.Witters and O.Vanderborght (eds.), *Ecophysiology of acid stress in aquatic animals,* pp. 181-188.

Herrmann,J., Degerman,E., Gerhardt,A., Johansson,C., Lingdell,P.E. and Muniz,I.P.: 1993, 'Acid-Stress Effects on Stream Biology', *Ambio.* **22,** 298-307.

Hovind, H. Intercomparision 0115: pH, K_{25}, HCO_3, NO_3 + NO_2, Cl, SO_4, Ca, Mg, Na, K, total aluminium, aluminium - reactive and nonlabile, TOC, COD-Mn. Fe, Mn, Cd, Pb, Cu, Ni and Zn. NIVA-Report

4416, ICP Waters report 64/2001. 2001.

Johnson,R.K. and Angeler,D.G.: 2010, 'Tracing recovery under changing climate: response of phytoplankton and invertebrate assemblages to decreased acidification', *J. North. Am. Benthol. Soc.* **29,** 1472-1490.

Kowalik,R.A., Cooper,D.M., Evans,C.D. and Ormerod,S.J.: 2007, 'Acidic episodes retard the biological recovery of upland British streams from chronic acidification', *Global Change Biology.* **13,** 2439-2452.

Kram,P., Hruska,J., Driscoll,C.T., Johnson,C.E. and Oulehle,F.: 2009, 'Long-term changes in aluminum fractions of drainage waters in two forest catchments with contrasting lithology', *Journal of Inorganic Biochemistry.* **103,** 1465-1472.

Kroglund,F., Teien,H.C., Rosseland,B.O. and Salbu,B.: 2001, 'Time and pH-Dependent detoxification of aluminum in mixing zones between acid and non-acid rivers', *Water, Air, Soil Pollut.* **130,** 905-910.

Krug,E.C. and Frink,C.R.: 1983, 'Acid-Rain on Acid Soil - A New Perspective', *Science.* **221,** 520-525.

Laudon,H., Kohler,S. and Bishop,K.H.: 1999, 'Natural acidity or anthropogenic acidification in the spring flood of northern Sweden?', *Sci. Total Environ.* **234,** 63-73.

Lawrence,G.B., Sutherland,J.W., Boylen,C.W., Nierzwicki-Bauer,S.W., Momen,B., Baldigo,B.P. and Simonin,H.A.: 2007, 'Acid rain effects on aluminum mobilization clarified by inclusion of strong organic acids', *Environ. Sci. Technol.* **41,** 93-98.

Ledger,M.E. and Hildrew,A.G.: 2005, 'The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient', *Environ. Pollut.* **137,** 103-118.

Lepori,F., Barbieri,A. and Ormerod,S.J.: 2003, 'Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams', *Freshwat. Biol.* **48,** 1873-1885.

Lepori,F. and Ormerod,S.J.: 2005, 'Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests', *Freshwat. Biol.* **50,** 1568-1577.

Liebich,T., McCormick,S.D., Kircheis,D., Johnson,K., Regal,R. and Hrabik,T.: 2011, 'Water chemistry and its effects on the physiology and survival of Atlantic salmon *Salmo salar* smolts', *J. Fish Biol.* **79,** 502-519.

Lofgren,S., Cory,N. and Zetterberg,T.: 2010, 'Aluminium concentrations in Swedish forest streams and co-variations with catchment characteristics', *Environ. Monit. Assess.* **166,** 609-624.

McCahon,C.P. and Pascoe,D.: 1989, 'Short-term experimental

acidification of a Welsh stream - toxicity of different forms of aluminum at low pH to fish and invertebrates', *Arch. Environ. Contam. Toxicol.* **18,** 233-242.

McCahon,C.P., Pascoe,D. and McKavanagh,C.: 1987, 'Histochemical observations on the salmonids *Salmo salar* L and *Salmo trutta* L and the ephemeropterans *Baetis rhodani* (Pict) and *Ecdyonurus venosus* (Fabr) following a simulated episode of acidity in an upland stream', *Hydrobiologia.* **153,** 3-12.

McCartney,A.G., Harriman,R., Watt,A.W., Moore,D.W., Taylor,E.M., Collen,P. and Keay,E.J.: 2003, 'Long-term trends in pH, aluminium and dissolved organic carbon in Scottish fresh waters; implications for brown trout (*Salmo trutta*) survival', *Sci. Total Environ.* **310,** 133-141.

Merrett,W.J., Rutt,G.P., Weatherley,N.S., Thomas,S.P. and Ormerod,S.J.: 1991, 'The response of macroinvertebrates to low pH and increased aluminum concentrations in Welsh streams - multiple episodes and chronic exposure', *Arch. Hydrobiol.* **121,** 115-125.

Monteith,D.T., Hildrew,A.G., Flower,R.J., Raven,P.J., Beaumont,W.R.B., Collen,P., Kreiser,A.M., Shilland,E.M. and Winterbottom,J.H.: 2005, 'Biological responses to the chemical recovery of acidified fresh waters in the UK', *Environ. Pollut.* **137,** 83-101.

Monteith,D.T., Stoddard,J.L., Evans,C.D., de Wit,H.A., Forsius,M., Hogasen,T., Wilander,A., Skjelkvale,B.L., Jeffries,D.S., Vuorenmaa,J., Keller,B., Kopacek,J. and Vesely,J.: 2007, 'Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry', *Nature.* **450,** 537-5U9.

Morth,C.M., Torssander,P., Kjonaas,O.J., Stuanes,A.O., Moldan,F. and Giesler,R.: 2005, 'Mineralization of organic sulfur delays recovery from anthropogenic acidification', *Environ. Sci. Technol.* **39,** 5234-5240.

Muniz,I.P. and Leivestad,H.: 1980, 'Acidification - effects on freshwater fish', *Proc. Int. conf. ecol. impact acid precip., SNSF project,* Norway, pp. 84-92.

Norrgren,L. and Degerman,E.: 1993, 'Effects of different water qualities on the early development of atlantic salmon and brown trout exposed in situ', *Ambio.* **22,** 213-218.

Odén,S.: 1968, 'The acidification of air and precipitation and its consequences on the natural environment.', *Swed. Nat. Sci. Res. Council, Ecol. Comm. Bull.* **1,** 1-68.

Ormerod,S.J., Boole,P., McCahon,C.P., Weatherley,N.S., Pascoe,D. and Edwards,R.W.: 1987, 'Short-term experimental acidification of a Welsh stream - comparing the biological effects of hydrogen-ions and aluminum', *Freshwat. Biol.* **17,** 341-356.

Ormerod,S.J. and Jenkins,A.: 1994, 'The biological effects of acid episodes', in C.E.W.Steinberg and R.F.Wright (eds.), *Acidification of Freshwater Ecosystems: Implications for the Future,* John Wiley & Sons Ltd., pp. 259-272.

Otto,C. and Svensson,B.S.: 1983, 'Properties of acid brown water streams in south Sweden', *Arch. Hydrobiol.* **99,** 15-36.

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

Poleo,A.B.S., Ostbye,K., Oxnevad,S.A., Andersen,R.A., Heibo,E. and Vollestad,L.A.: 1997, 'Toxicity of acid aluminium-rich water to seven freshwater fish species: A comparative laboratory study', *Environ. Pollut.* **96,** 129-139.

Raddum,G.G., Fjellheim,A. and Skjelkvale,B.L.: 2001, 'Improvements in water quality and aquatic ecosystems due to reduction in sulphur deposition in Norway', *Water, Air, Soil Pollut.* **130,** 87-98.

Reader,J.P., Dalziel,T.R.K., Morris,R., Sayer,M.D.J. and Dempsey,C.H.: 1991, 'Episodic exposure to acid and aluminum in soft-water - survival and recovery of brown trout, *Salmo trutta* L', *J. Fish Biol.* **39,** 181-196.

Rockwood,J.P. and Coler,R.A.: 1991, 'The effect of aluminum in soft-water at low pH on water-balance and hemolymph ionic and acid-base regulation in the dragonfly *Libellula julia* Uhler', *Hydrobiologia.* **215,** 243-250.

Rogeberg,E. and Henriksen,A.: 1985, 'An automatic method for fractionation and determination of Aluminium species in fresh-waters', *VATTEN.* **41,** 48-53.

Rosseland,B.O., Blakar,I.A., Bulger,A.J., Kroglund,F., Kvellestad,A., Lydersen,E., Oughton,D.H., Salbu,B., Staurnes,M. and Vogt,R.D.: 1992, 'The mixing zone between limed and acidic river waters - complex aluminum chemistry and extreme toxicity for salmonids', *Environ. Pollut.* **78,** 3-8.

Rosseland,B.O., Kroglund,F., Staurnes,M., Hindar,K. and Kvellestad,A.: 2001, 'Tolerance to acid water among strains and life stages of Atlantic salmon (*Salmo salar* L.)', *Water, Air, Soil Pollut.* **130,** 899-904.

Rosseland,B.O. and Skogheim,O.K.: 1984, 'A comparative study of salmonid fish species in acid aluminum-rich water. II. Physiological stress and mortality of one- and two-year-old fish', *Rep. Inst. Freshw. Res. Drottningholm.* **61,** 186-194.

Rosseland,B.O. and Skogheim,O.K.: 1987, 'Differences in sensitivity to acidic soft-water among strains of brown trout (*Salmo trutta*-L)',

Ann. Soc. Roy. Zool. Belg. **117,** 255-264.

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

Sadler,K. and Turnpenny,A.W.H.: 1986, 'Field and laboratory studies of exposures of brown trout to acid waters', *Water, Air, Soil Pollut.* **30,** 593-599.

Salbu,B., Riise,G., Bjornstad,H.E. and Lydersen,E.: 1990, 'Intercomparison study on the determination of total aluminium and aluminium species in natural fresh waters', in B.J.Mason (ed.), *The Surface Waters Acidification programme,* Cambridge University Press, Cambridge, pp. 251-254.

Sjostedt,C., Andrén,C.M. and Gustafsson,J.P.: 2012, 'Modelling of pH and inorganic aluminium after termination of liming in 3 000 Swedish lakes', *manuscript.*

Sjostedt,C., Wallstedt,T., Gustafsson,J.P. and Borg,H.: 2009, 'Speciation of aluminium, arsenic and molybdenum in excessively limed lakes', *Sci. Total Environ.* **407,** 5119-5127.

Sjostedt,C.S., Gustafsson,J.P. and Kohler,S.J.: 2010, 'Chemical equilibrium modeling of organic acids, pH, aluminum, and iron in Swedish surface waters', *Environ. Sci. Technol.* **44,** 8587-8593.

Skjelkvale,B.L., Stoddard,J.L., Jeffries,D.S., Torseth,K., Hogasen,T., Bowman,J., Mannio,J., Monteith,D.T., Mosello,R., Rogora,M., Rzychon,D., Vesely,J., Wieting,J., Wilander,A. and Worsztynowicz,A.: 2005, 'Regional scale evidence for improvements in surface water chemistry 1990-2001', *Environ. Pollut.* **137,** 165-176.

Stoddard,J.L., Jeffries,D.S., Lukewille,A., Clair,T.A., Dillon,P.J., Driscoll,C.T., Forsius,M., Johannessen,M., Kahl,J.S., Kellogg,J.H., Kemp,A., Mannio,J., Monteith,D.T., Murdoch,P.S., Patrick,S., Rebsdorf,A., Skjelkvale,B.L., Stainton,M.P., Traaen,T., Van Dam,H., Webster,K.E., Wieting,J. and Wilander,A.: 1999, 'Regional trends in aquatic recovery from acidification in North America and Europe', *Nature.* **401,** 575-578.

Surber,E.W.: 1969, 'Procedure in taking stream bottom samples with the stream square foot bottom sampler.', *Proc.23'd Ann. Conf. S. East. Assoc. Game and Fish Comm.,* pp. 587-591.

Swedish EPA. Bedömningsgrunder för sjöar och vattendrag. Bilaga A till Handbok 2007:4. 2007. Stockholm, Sweden, Swedish Enviromental Protection Agency.

Swedish EPA. Liming of lakes and streams. Handbook 2010:2. Utgåva 1. 90 pp. 2010. Stockholm, Sweden, Swedish Enviromental Protection Agency.

Swedish EPA. Miljömålen– fokus på förutsättningarna, Årlig uppföljning av miljökvalitetsmålen 2012. Naturvårdsverket diarienr NV-08927-11, -45p. 2012. Stockholm, Swedish EPA.

Swedish Standard: 1992, *SS 282 10,* Water analysis - Determination of acid soluble aluminium in water - Photometric method.

Teien,H.C., Salbu,B., Kroglund,F. and Rosseland,B.O.: 2004, 'Transformation of positively charged aluminium-species in unstable mixing zones following liming', *Sci. Total Environ.* **330,** 217-232.

Tipping,E.: 1994, 'WHAM - a chemical-equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site electrostatic model of ion-binding by humic substances', *Comput Geosci.* **20,** 973-1023.

Vesely,J., Majer,V., Kopacek,J. and Norton,S.A.: 2003, 'Increasing temperature decreases aluminum concentrations in Central European lakes recovering from acidification', *Limnol. Oceanogr.* **48,** 2346-2354.

Wickstrom,T., Clarke,N., Derome,K., Derome,J. and Rogeberg,E.: 2000, 'Comparison study of five analytical methods for the fractionation and subsequent determination of aluminium in natural water samples', *J. Environ. Monit.* **2,** 171-181.

Wilander,A., Johnson,R.K. and Goedkoop,W.: 2003, *Riksinventering 2000 - En synoptisk studie av vattenkemi och bottenfauna i svenska sjöar och vattendrag*, Department of Environmental Assessment, SLU, Uppsala, Sweden.

Wilander,A., Johnson,R.K., Goedkoop,W. and Lundin,L.: 1998, *Riksinventering 1995 - En synoptisk studie av vattenkemi och bottenfauna i svenska sjöar och vattendrag*, Swedish Environmental protection Agency, Stockholm, Sweden.

Winter,A.R., Nichols,J.W. and Playle,R.C.: 2005, 'Influence of acidic to basic water pH and natural organic matter on aluminum accumulation by gills of rainbow trout (*Oncorhynchus mykiss*)', *Can. J. Fish. Aquat. Sci.* **62,** 2303-2311.

Witters,H.E., VanPuymbroeck,S., Vangenechten,J.H.D. and Vanderborght,O.L.J.: 1990, 'The effect of humic substances on the toxicity of aluminum to adult rainbow trout, *Oncorhynchus-Mykiss* (Walbaum)', *J. Fish Biol.* **37,** 43-53.

Wright,R.F., Larssen,T., Camarero,L., Cosby,B.J., Ferrier,R.C., Helliwell,R., Forsius,M., Jenkins,A., Kopacek,J., Majer,V., Moldan,F., Posch,M., Rogora,M. and Schopp,W.: 2005, 'Recovery of acidified European surface waters', *Environ. Sci. Technol.* **39,** 64A-72A.