INORGANIC ALUMINIUM IN STREAMS
—
BIOAVAILABILITY AND TOXICITY

Cecilia Andrén
Inorganic Aluminium in Streams – Bioavailability and Toxicity

Literature synthesis

Cecilia Andrén
CONTENTS

SAMMANFATTNING............................................................................................................................... 3

INTRODUCTION ........................................................................................................................................... 6

BENTHIC ALGAE .......................................................................................................................................... 8

BENTHIC ALGAE AND ACIDIFICATION ............................................................................................... 8
  Chlorophyll and Biomass ....................................................................................................................... 8
  Species Composition .......................................................................................................................... 9

STUDIES ON ALGAE IN ACIDIFIED STREAMS ................................................................................ 10
  Field studies ....................................................................................................................................... 10
  Artificial channels ............................................................................................................................ 10

CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BENTHIC ALGAE .............................................. 12

BENTHIC INVERTEBRATES ....................................................................................................................... 13

GENERAL ASPECTS ON BENTHIC INVERTEBRATES AND ACIDIFICATION ........................................ 13

BIOACUMULATION .................................................................................................................................. 14

SUBLETHAL EFFECTS .............................................................................................................................. 15
  Ion Regulation ..................................................................................................................................... 15
  Reproduction ....................................................................................................................................... 16
  Respiration .......................................................................................................................................... 17

TOXICITY/MORTALITY ............................................................................................................................ 17
  Bivalvia ................................................................................................................................................ 18
  Gastropoda ......................................................................................................................................... 18
  Crustacea ............................................................................................................................................ 18
  Diptera .................................................................................................................................................. 19
  Ephemeroptera ................................................................................................................................... 19
  Odonata ............................................................................................................................................... 20
  Plecoptera .......................................................................................................................................... 20
  Trichoptera ......................................................................................................................................... 20

COMMUNITY EFFECTS (THE AMBIENT FAUNA) .................................................................................. 21
  Drift ..................................................................................................................................................... 21

Community Composition - Biomass and Density ............................................................................... 22

CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BENTHIC INVERTEBRATES ................................. 25

FISH - BROWN TROUT .............................................................................................................................. 27

HISTORICAL VIEWS OF ACIDIFICATION EFFECTS ON FISH ................................................................ 27

TOXICITY MECHANISMS TO FISH ......................................................................................................... 27

CONDITIONS AFFECTING THE TOXIC EFFECT OF ALUMINIUM ON FISH ............................................. 29
  The Effect Of Water Quality On Aluminium Toxicity To Fish ............................................................ 29
    Calcium and ionic strength ............................................................................................................... 29
    Fluoride and Silicon .......................................................................................................................... 29
    Humus ............................................................................................................................................... 30
    Mixing zones ...................................................................................................................................... 30

THE EFFECT OF TEMPERATURE AND SEASON ON ALUMINIUM TOXICITY TO FISH ......................... 31

DIFFERENT SENSITIVITY OF FISH SPECIES AND STRAINS TO ALUMINIUM ....................................... 32

VARYING SENSITIVITY TO ALUMINIUM OF TROUT AT DIFFERENT AGES ........................................... 33

THE EFFECT OF ALUMINIUM ON FISH BODY DEVELOPMENT ................................................................ 34

THE EFFECT OF ALUMINIUM ON PLASMA COMPOSITION AND RESPIRATION IN FISH .................... 36

ACCUMULATION OF ALUMINIUM ON TROUT GILLS ........................................................................... 37

EFFECTS OF ALUMINIUM ON BROWN TROUT POPULATIONS ................................................................ 39

CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BROWN TROUT .................................................... 41

REFERENCE LIST ...................................................................................................................................... 42

APPENDIX, TABLE OVER STUDIES ON THE EFFECT OF (INORGANIC) ALUMINIUM AND BENTHIC INVERTEBRATES IN ACIDIC FRESHWATERS ......................................................................................... 51

2(59)
SAMMANFATTNING

**Bentiska Alger**

Effekten av försurning på algernas biomassa är oklar beroende på hur den uppskattas (vanligen används klorofyll a och/eller vikt) och hur andra faktorer i miljön (näring, ljus...) och nivåer i näringskedjan i det försurade ekosystemet påverkats. Biomassan ökar ofta med avseende på vikt men produktionen eller kvaliteten minskar (klorofyll-a innehåll) i försurade jämfört med mindre sura system. Då många makroinvertebrater försvinner minskar betoningstrycket vilket gynnar algtillväxten, och medan minskningen av mikrobiell nedbrytning ger ett mindre näringsförråd så ger depositionen ökat tillgång till kväve och svavel och fosfor är mer biotillgängligt i sura vatten – vilket gör att den totala effekten på algbiomassan beror på interaktioner och balansen i ekosystemet. Mindre tvetydiga resultat skulle troligen kunna fås genom att mäta biovolymen vilket bara två forskare har gjort (Mulholland et al., 1986; Planas et al., 1989.).

Algernas artsammansättning är tydligt påverkat – de dominanta arterna är andra i försurade vatten (kiselalger dominerar och ofta ökar gröna trådformade alger) och artrikedomen minskar. Aluminium tillsammans med väte joners ansas orsaka förändringarna i artsammansättningen och toxicitetstester med bentiska alger efterfrågas. Diatoméer har använts för att prediktera pH, TOC och monomert aluminium i sjöar och kan troligen användas även till att ta fram aluminium nivåer i rinnande vatten.

Studier på bioackumulation av metaller och oorganisk sammansättning i alger kan kanske binda samman och klargöra orsak och verkan för försurningseffekter på alger. Detta verkar som en väg till förståelse av de mekanismer som är inblandade och även kanske separera effekterna av 

**Bottenfauna**


Aluminium kan minska hemolymfans innehåll av makrojonerna i dagsländelarver (natrium), kräftdjur (natrium, kalcium) och nattsländelarver (natrium och kalium). Hos en

De många mortalitetsstudierna ger en något oklar bild, troligen orsakad av att studierna har genomförts vid skiftande förhållande; surt – mycket surt, med – eller utan organiskt material och resultaten bör därför ge en bimodal respons i åtminstone tre dimensioner. Aluminium är en viktig orsak till bottendjurens dödlighet huvudsakligen mellan pH 4 och 5,5, vid lägre pH skyddar Al mot H+ och vid högre pH polymeriserar aluminium (och blir då mindre giftigt för makroinvertebrater). Den analytiska fraktionen oorganiskt aluminium borde korrelera väl till toxiciteten men hindras av att försöken i denna studie utförts under mer än två decennier. Om oorganiskt aluminium har bestänts överhuvudtaget (och inte bara totalt aluminium) så har ofta olika fraktionsmetoder använts vilket ger inkompatibla resultat att relatera toxiciteten till.

Trotsallt finns det ett samband mellan dos och respons mellan oorganiskt aluminium och många dagsländelarver (t.ex. Baetis rhodani, Ecdyonorus venosus, Ephemerella ignita, Heptagenia sulphurea) och kräftdjur (Hyalella azteca, Gammarus pulex, Asellus intermedius) och även en nattslandelare (Psychopsycha guttifer), en mussla (Margaritifera margartifera), en snäcka (Gyraulus sp.) och ej artbestämda chironomider. Den toxiska effekten av aluminium på makroinvertebrater verkar vara kopplad till arten och inte till ordningen.

Flera studier har visat på en ökad drift, huvudsakligen för Baetidae men också för Heptagenidae och Simuliidae. Om den totala driften av invertebrater påverkas så kan det avspeglas att samhället innehåller en stor andel känsliga arter.

Öring

Aluminium verkar genom olika mekanismer; i sura aluminiumrika vatten ökar aluminium permeabiliteten hos gälarorna och ackumuleras intracellulärt i gälepitelceller. Detta förstör gälbarriären och fisken får jonregulatoriska störningar, respirationsproblem och osmoreglingen kollapsar. Den akut toxiska effekten av aluminium i vatten med ett pH över 5 eller vid instabila förhållanden (som kalkning) kan bäst förklaras genom polymerisering av aluminium på gälarorna och påföljande syrebrist.

Aluminiums toxicitet modifieras av andra komponenter i vattnet som reducerar giftverkan; påverkan av organiskt material, kalcium, fluorid, kisel och jonstyrka har undersökts. Temperaturen inverkar på själva aluminiumspecieringen men reglerar också lösligheten av syre och är korrelerad med fiskens utvecklingsstadium och metabolism. Lax var den mest känsliga fisken och öring följt av harr de minst känsliga i en jämförande studie med sju arter. Det finns skillnader i känslighet hos olika öringstammar; öring från sura vatten är mest tålig mot aluminium.

Uppsimmande yngel är väldigt känsliga för aluminium, de och årsgamla öringar, som förblivit känsliga, är goda biomonitorer då de fortfarande är stationära. Rommen är mest känslig före den blivit ögonpunktrad, därefter kan perivitellinevätskan buffra mot omkringliggande vatten tills dess gulesäcken förbrukats. Exponering för aluminium försämrar utvecklingen av rom och yngel och kroppsinnehållet av natrium, kalium och kalcium reduceras.

Hos årsungar och äldre fisk (där man kan ta blodprover) reducerar aluminium plasmans innehåll av blodelektrolytarna (Na, Cl och Ca) då Al och H-joner flödar in när gälbarriären bryts ner. Nivåerna av blodsocker (en stressrespons) och hemoglobin ökar; för att motverka den reducerade upptägningen av syre – av samma skäl ökar även andningsfrekvensen. Även om i en sammanställning av data från väldigt olika studier är korrelationen mellan oorganiskt aluminium i vatten och ackumulerat aluminium på gälarorna stark (r= 0.77). Ackumulation på gälarorna över 500 µg Al/g ts är dödligt för öring i dessa studier.

Vid fältinventeringar, är korrelationen god mellan tätheter av ung öring och pH, oorganiskt Al, ANC/alkalinitet och kalcium. Då detta är ett indirekt sätt att studera toxicitet är det känsligt för datautval och behandling, speciellt då vattenkemivariablerna inte är oberoende av varandra.
Jag har många människor att tacka för granskning, synpunkter och stöd i mitt arbete med denna sammanställning.

Mina handledare i forskarutbildningen Anders Broberg och Emil Rydin (Uppsala Universitet) och Hans Borg (Stockholms Universitet) tackas för upprepade granskningar och synpunkter. Biologerna Maria Kahlert (alger), Pär-Erik Lingdell (bottenfauna) och min man Paul Andersson (fisk) tackas för biologisk granskning av respektive avsnitt. Min projektledare Torbjörn Svensson (Naturvårdsverket) tackas för sitt stora stöd och tillit till mitt arbete.

Och sist men inte minst ett stort tack till alla ni andra kolleger och vänner som finns med mig i livet.
INTRODUCTION

This synthesis covers the effects of inorganic aluminium on organisms living in streams, beginning with benthic algae and invertebrates and ending with brown trout that will be viewed as a representative for stream water fishes.

In order to understand the studies presented some basic understanding of aluminium terminology is needed and in figure 1 a basic fractionation scheme is presented. The correct term for inorganic aluminium should be labile monomeric aluminium (Ali) and when I write aluminium or only Al that refers to total aluminium.

<table>
<thead>
<tr>
<th>Total Al</th>
<th>(total reactive Al, acid digestive or eluated at pH 1.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total monomeric Al</td>
<td>(no acid addition or digestion)</td>
</tr>
<tr>
<td>Labile monomeric Al</td>
<td>(Al)</td>
</tr>
<tr>
<td>(Ali)</td>
<td></td>
</tr>
<tr>
<td>* free aluminium</td>
<td></td>
</tr>
<tr>
<td>* sulphate, hydroxide and fluoride complexes</td>
<td></td>
</tr>
<tr>
<td>* possibly small Al-humus complexes</td>
<td></td>
</tr>
<tr>
<td>Stabile monomeric Al</td>
<td>(Al)</td>
</tr>
<tr>
<td>(Ali)</td>
<td></td>
</tr>
<tr>
<td>* monomeric organic complexes</td>
<td></td>
</tr>
<tr>
<td>* possibly negative inorganic Al-complexes (pH &gt; 6) and Al-humus complexes</td>
<td></td>
</tr>
<tr>
<td>Acid soluble Al</td>
<td></td>
</tr>
<tr>
<td>(Alo)</td>
<td></td>
</tr>
<tr>
<td>* colloids, polymers</td>
<td></td>
</tr>
<tr>
<td>* strong organic complexes</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Fractionation scheme for aluminium slightly modified after Driscoll (1984). In brackets are the commonly used abbreviations that can be seen in publications and in this text.

For more details about the chemistry of aluminium I refer to some of the many reports that have been published, see Bloom and Erich (1996) on quantitation of aqueous aluminium and Driscoll on aluminium chemistry in the environment (1985, 1990) and Gensemer and Playle (1999) about bioavailability and toxicity of aluminium.
BENTHIC ALGAE

Probably ‘most species of attached algae are available at all times and they flourish when conditions becomes suitable’ (Hynes 1970). Many genera occur in waters of varying pH while others are restricted to either soft, acid water or alkaline waters. Because of the multitude of species adapted to different environmental conditions benthic algae are suitable to use as indicators, which have been noted by many researchers e.g. Battarbee (1994) or Steinberg and Putz (1991), for a review see Lecointe et al. (1993). There is one commercial software ‘Omnidia’ (Lecointe et al. 1993) which renders eleven diatom indices, diversity and three different formulas calculating pH-values. When organisms are sensitive to acidification (frequently documented as pH) it is reasonable to believe that they respond also to Al - the parallel major toxic agent in acidified waters. In two large acidification programmes (SWAP, The Surface Water Acidification Programme (Europe) and PIRLA, The Paleolimnological Investigation of Recent Lake Acidification (USA)) diatoms are used to infer tolerance levels also of total Al besides pH and DOC (Battarbee 1994;Camburn and Charles 2000). The correlation between observed total Al and inferred total Al based on weighted averaging regression was fairly good (r= 0.777 and n= 126) (Battarbee 1994). The North American project has used paleolimnological diatoms to assess trends in fishery resources and lake water monomeric aluminium (Kingston et al. 1992) besides publishing tables with abundance weighted means and tolerance levels for more than 200 diatom species (Camburn and Charles 2000).

BENTHIC ALGAE AND ACIDIFICATION

CHLOROPHYLL AND BIOMASS

Three experimental acidification studies have documented increased stream periphyton biomass (measured as chlorophyll a or dry mass per unit area) (Hall et al. 1980;Allard and Moreau 1986;Planas and Moreau 1986). The increases in benthic algal biomass observed in acidic ecosystems (pH<5 for extended periods) are not necessarily due to an increase in primary production (Elwood and Mulholland 1989). Planas and Moreau (1986) studied the algal uptake of P and S and showed that the increase in algal biomass under acidified (episodic or continuous) conditions could probably be due to a greater availability of P (even though there is no detectable increase of the nutrient in water) or to an increased utilization of S in the elevated levels of sulfate in the water. Primary production and respiration ratios of periphyton declined when pH fell below 6.2 (from 4 to 1.5 at pH 4.8) - the increase in respiration for epilithipython was much greater than in gross production (Schindler 1990). The
effects of acidification on the benthic algal community cannot be attributed solely to increased concentrations of H+ or aluminium – indirect effects on environmental conditions like carbon, nutrients, light and temperature and changes in the ecosystem (grazing pressure and substratum availability) are also important.

That many important conditions for the benthic algal community themselves are affected by acidification making the resulting community biomass and production a combined outcome makes it hard to test hypothesis independently. Planas (1996) concludes that heightened light transparency and water temperature together with changes in nutrient availability (C, N, P) and microbial interactions and herbivore control are responsible for changes in productivity.

**Species Composition**

In acidified streams the species richness decreases and the community composition changes (Planas 1996). The reduction in species richness and diversity can, apart from the natural reduction in numbers of alkaliphilic and alkalibiontic species, be caused by increased competition for space when a few species grow dense or changes in epiphytic substrate (aquatic plants). With decreasing pH there is generally an increase in benthic filamentous green Zygnemataceae (particularly Zygogonium, Mougeotia, Spirogyra and Zygnema but also Ulothrix and Oedogonium can become abundant). In acidified more turbulent lotic waters the diatoms generally are the most abundant group with several acidophilic species present (particularly species of the genus Eunotia, Fragilaria, Navicula, Neidum and Tabellaria). Mayfly larvae (which are reduced and ultimately lost by acidification) are effective diatom grazers (Elwood and Mulholland 1989) and diatom abundance is inversely related to grazing (Lamberti et al. 1987). Blue-greens are the most sensitive algal group to acidification (they are scarce below pH 4 to 5), it has been suggested that the lack of internal cell structures render the chlorophyll vulnerable to acidic degradation.

The diatoms seem thus to be the algal group that has the potential to be relevant indicators in acidified fast-flowing streams. And even if no studies are available regarding the toxicity of aluminium to benthic algae, Planas (1996) concludes that hydrogen ions and aluminium could be directly responsible for changes in species composition.
STUDIES ON ALGAE IN ACIDIFIED STREAMS

FIELD STUDIES
Dominance by diatoms and some green algae have been noted in acidic sites (Mulholland et al. 1986). The algae at these sites also had a greater total cell volume, chlorophyll-a density and areal productivity and with some variation also lowest values of chlorophyll-a specific productivity. Sites with higher pH (> 5.7) were dominated by blue-green algae and chrysophytes. Also Hall et al. (1980) found a significant increase in algal biomass as expressed by chlorophyll-a per cm² or mg AFDW (ash free dry weight) in the experimentally acidified section compared to the untreated but a lowered production rate. Hall suggested that this was caused by (1) the decreased grazing pressure, (2) less microbial decomposition, (3) successional changes of acid-tolerant species or a combination of all three.

A general trend seem to be a lowered P/B ratio in acidified waters, where reduced grazing, increased light together with changes in nutrient availability might explain this phenomena.

ARTIFICIAL CHANNELS
In studies without grazers present acidification renders decreased colonization rates, measured by total biomass and a shift in community composition (Maurice et al. 1987). They propose the former response is due to reduced nutrient availability but that also elevated Fe/Al concentrations could exert an additional negative influence. The shift in community compositions was most likely due to both an intolerance of blue-green algae to acidic conditions and to the tolerance of particular eucaryotic genera for acidic conditions. When more combinations were tested the responses differ (Genter 1995); the total algal biomass estimated by chlorophyll-a and dry weight both decrease in acid channels and increase in Al-channels but in the combined channel (acid + Al) only chlorophyll-a decreases while the dry weight was similar to the control. The latter response may indicate a shift from a primary producer to a heterotrophic community, or may also be attributed to decreased chlorophyll-a content per algal cell. The bioaccumulation of Al and leakage of Ca and Mg may have reduced the algae’s stress tolerance and led to relatively lower chlorophyll-a content. Genter and Amyot (1994) noted that added Al in acid water was much more harmful than acid alone to the abundances of diatoms and green and blue-green algae. They found a larger reduction in chlorophyll-a when the acidified channels also contained Al.

Planas et al. (1989) verified that the suggested reduced grazing pressure existed: the experimentally controlled acidification reduced the macrobenthic community to
almost one third of that in the control; mainly collector-gatherer-species that exploit poorly attached periphyton species persisted. This decrease of grazing pressure in the acidified troughs could be the cause of the observed multi layered growth (also resulting in an increased biomass) but also the increase in phosphorus could favor this growth. There was no difference between acid only and acid+Al on the abundance of the dominant species algae but increased H⁺ may have directly or indirectly favored the growth of *Eunotia*. In the discussion Planas states two possible interpretations: differential sensitivity to H⁺ion can make some species shrivel while others prosper and/or this is an effect of interspecific competition as a result of food web modifications. The changes in the periphytic community are better described by changes in dominant species than by reduction in richness.

*The experimental results vary depending on which factors are included in the experiment, both abiotic (H⁺, Al and the effect of humus is not even tested), and biotic (the inclusion of other levels of the ecosystem). Nevertheless the periphytic community composition changes when the grazing pressure decreases but it is hard to discern differences in response between exposures with only H⁺ and Al together with H⁺. In the absence of grazers acidification reduces chlorophyll-a by the combined effect of H⁺ and Al, the total biomass expressed as dry weight is either reduced or unaffected. The difference in responses of biomass can be caused by differences in the original community composition, so the acidification effects are better reflected in the species composition of the periphytic community than in terms of production or biomass.*
CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BENTHIC ALGAE

The effect of acidification on biomass is ambiguous depending on how it is measured (usually chlorophyll a and or weight have been used) and how other environmental factors (nutrients, light…) and other levels in the food web of the acidified ecosystem interfere. The biomass often increases (weight) but the production or quality decreases (chlorophyll-a content) in acidified compared to less acid systems. The decrease in grazing pressure when many macro invertebrates disappear favors algal growth, and while the decrease in the microbial decomposition renders a smaller nutrient supply, deposition increases nitrogen and sulphur accessibility and phosphorus is also more bioavailable - so the total effect on algal biomass depends on the interactions and the balance in the ecosystem. However, only two researchers (Mulholland et al., 1986; Planas et al., 1989) have used biovolume, which might render less ambiguous results.

The community composition is clearly affected – the dominant species are changed in acidified waters (there is a dominance of diatoms and often an increase in filamentous green algae) and the richness decreases. Aluminium together with hydrogen ions is thought to be responsible for changes in species and toxicity tests with benthic algae are demanded. Diatoms have been used to predict pH, TOC and monomeric aluminium in lakes and can probably be used to infer aluminium levels in streams.

The results from algal studies on bioaccumulation of metals and inorganic constituents can maybe link and clarify cause and effect of acidification on algae. This seems like a way to begin to understand the mechanisms involved and maybe separate the effects of H+ and Al.
**Benthic Invertebrates**

Benthic invertebrates are regularly used in environmental monitoring; there are indices for many different conditions and disturbances (for a comprehensive overview see Rosenberg and Resh 1993). In the following pages, studies of the effects of aluminium on benthic invertebrates in streams will be summarized, for details of the studies please consult the table in the appendix.

**General Aspects on Benthic Invertebrates and Acidification**

The effects of acidification on benthic animals have generally been studied from four main approaches; 1) Time-trend studies, 2) Field experiments, 3) Laboratory experiments and 4) Field surveys (Okland and Okland 1986). The effect of acidification in streams usually are a decrease in number of species and biomass but the effect on biomass can be counteracted by large numbers of newly hatched larvae of pH-tolerant midges in the most acid streams (ibid). Several species of crayfish and most of other crustaceans as well as snails and mussels/clams are very sensitive to low pH (ibid). The acid-sensitivity can actually mask a deficiency of calcium or other covariables, ions that are essential to these organisms (Lonergan and Rasmussen 1996). Many acid-sensitive species can also be found among mayflies (Ephemeroptera) and some among midges (Chironomidae), stoneflies (Plecoptera) and caddisflies (Trichoptera) (Okland and Okland 1986). Invertebrates that are sensitive to low pH have an impaired ion balance while tolerant species maintain a stable hemolymph sodium/chloride balance at low pH (ibid).

Herrmann (1987a;1993) proposes that aluminium itself acts on the osmoregulation/ion balance and the respiration; 1) mechanically by aluminium precipitates or mucus-production and 2) chemically by deterring the ion transport, which leads to a decreased efficiency in the oxygen transportation. Aluminium can reduce the negative impact of acidity at very low pH (4.0) and several ways for this action is proposed; reducing membrane permeability for H+, changing Na and Ca fluxes and buffering pH (ibid).

In a recent comprehensive review on bioavailability and toxicity of aluminium Gensemer and Playle (1999) conclude that aquatic invertebrates are less sensitive to Al compared to fish. The toxic effects are considered to be additive to the ion regulatory effect of hydrogen ions and respiratory effects are considered uncommon, possibly with the exception of Odonata nymphs. Aluminium accumulates on ionoregulatory surfaces but there is no biomagnification in aquatic invertebrates.
In a hazard assessment Sparling and Lowe (1996) state that the effects of aluminium on aquatic organisms are confounded by pH and may vary depending on invertebrate species and water quality.

Crustaceans, snails and mussels are designated as particularly sensitive to low pH-although this might be an indirect effect. Low pH and high Al, which can disturb the sodium and chloride levels in the hemolymph, damage the ion balance in sensitive species. The osmoregulation and respiration can also be affected by aluminium that precipitate or hinder the oxygen transportation. The ephemeropterans I think actually are harmed by the hydrogen and aluminium ions (probably concurrently) even if the exact mechanism is unclear.

**Bioaccumulation**
No evidence sustained biomagnification’s of aluminium in the aquatic ecosystem and accumulation were considered to be small (Wren and Stephenson 1991). Later studies have shown that aluminium accumulated mainly in the exuvium of invertebrates and also internally – which has been demonstrated in the laboratory for *Heptagenia sulphurea* (Frick and Herrmann 1990b) and for *Hydropsyche pellucidula* (Vuori and Kukkonen 1996) collected in streams. Vuori and Kukkonen (1996) also found significant differences in the amount of aluminium in larvae of *Hydropsyche pellucidula* with darkened anal papillae (2 mg/g dw) and normal larvae (0.3 mg/g dw). For other trichopterans Vuori (1996) found a decreasing aluminium-sensitivity in *Hydropsyche angustipennis* (most sensitive), *Hydropsyche siltalai* and *Arctopsyche ladogensis* (least sensitive), which coincided with decreased numbers of individuals with darkened anal papillae. Vuori (1996) also found some individuals with abnormal tracheal gills but not significantly related to the measured environmental parameters.

In acidic episodes with elevated aluminium levels and liming (McCaon *et al.* 1989) there was a significant increase in body burden of aluminium over a 24-hour period for both *Isoperla grammatica* (10 – 50 µg/g dw) and *Gammarus pulex* (0.2 – 1.2 µg/g dw). Two ephemeropterans, *Baetis rhodani* and *Ecdyonorus venosus*, exposed for a simulated episode of acidity accumulated aluminium (McCaon *et al.* 1987). Staining with solochrome azurine displayed aluminium over the entire exoskeleton including gill plates and the gut. This suggests that aluminium physically occludes the main respiratory surface, the integument, leading to an increased respiration rate and subsequent death.

Ephemeropterans transplanted between sites differing in pH (6.4 v 5.0) rendered an increased mortality of organisms (*Stenonema* sp. and *Epeorus pleuralis*) placed at low pH sites (Rosemond *et al.* 1992). In a second transplant experiment differences in
mortality were not observed, but surviving invertebrates (*Drunella conestee*) had x10 higher body burdens of aluminium (462 ppm dw at pH 5.4 compared with 39 ppm dw at pH 6.0).

Aluminium accumulates to some degree in aquatic invertebrates and the question is in which way it affects the invertebrates. At experimental acidic episodes with crustaceans and ephemeropterans the body burden of aluminium increased, with and without liming. The body burden of aluminium was higher at sites with low pH and elevated aluminium levels in the water. It would be interesting to learn if the accumulation is active or passive, i.e. absorption or adsorption and if the accumulated aluminium interacts with the hemolymph, for instance.

**Sublethal Effects**

**Ion Regulation**
The effect of aluminium on the hemolymph sodium concentration has been studied for several ephemeropterans in laboratory experiments. In a two week test with *Heptagenia sulphurea* and *Ephemera danica* Herrmann (1987b) found no significant differences for pH 4 and 4.8 but a larger loss of sodium in synthetic medium (with no organic material binding the aluminium) than in soft stream water at different levels of aluminium. In a shorter 24h-test (Frick and Herrmann 1990a), with the same organisms, a reduction of pH (6.5 to 4) resulted in an increased outflow of sodium. The most acid sensitive species - *Ephemera danica*, showed the largest outflow of sodium. Addition of aluminium reduced the effect of low pH to a smaller outflow raise – to a larger extent for the acid sensitive *Ephemera danica* than for the more acid tolerant *Heptagenia sulphurea*. At neutral pH aluminium increased the sodium outflow for *Heptagenia sulphurea* but did not affect *Ephemera danica*.

In a flow through experiment Kroglund (1998) used two parallel channels to find the principal toxic components in stable freshwaters for acid resistant *Brachioptera risi*. The reference channel had pH 6.5 and no added Al, the experiment channel had pH 4.2, 4.8 and 5.4 combined with additions of 0, 150 and 350 µg Al/l and hemolymph samples (n=4–17) was collected from each test after 2 and 24 hour long exposures. The conclusion was that pH and aluminium are cooperating toxic agents. However, at low pH and high aluminium levels, aluminium seemed to counterbalance the effect of pH.

In the same study the effects of aluminium on hemolymph concentrations of sodium and potassium in unstable mixing zones was studied for one crustacean: *Gammarus lacustris* and two trichopterans: *Hydropsyche pellucidula* and *Hydropsyche siltalai*. The crustacean responded with lower hemolymph sodium levels in acid and aluminium rich waters than in the neutral reference and the mixing zones. The
trichopterans were more affected by sublethal stress in both the acid and aluminium rich water and the early mixing zone (20 sec.) than the neutral reference. The levels of both sodium and potassium hemolymph content were lowered for *H. pellucidula* while only the potassium content was reduced in *H. siltalai*.

The water bug *Corixa punctata* exposed at low pH (Witters *et al.* 1984) decreased the radioactively marked sodium influx when pH sank from 4 to 3 in bog lake waters. The sodium influx was halved when the Al concentration rose from 0.3 to 10 mg/l. In spite of this, the content of sodium in the hemolymph increased significantly which could be a result from a net-flux from tissue compartment to hemolymph or a decreased sodium efflux because of adsorbed aluminium.

A crustacean, *Orconectes virilis*, exposed to different pH and aluminium levels (Malley and Chang 1985) responded to reduced pH with reduced calcium uptake. The addition of aluminium caused a further reduction of the calcium uptake at pH 5.5 (but not at pH 7) – but there was no further response when the aluminium concentration was increased from 500 to 1000 µg/l.

In a summary of freshwater biomonitoring studies on the ion regulatory of water insects the response of crustacea and bivalvia to acid stress was designated as special, they decreased the hemolymph concentrations of sodium and chloride but increased the calcium concentrations by mobilizing the ion from their exoskeleton, shell and mantle (Johnson *et al.* 1993). In contrast, in other aquatic insects, concentrations of most major ions appear to decrease or remain unchanged when exposed to acid stress – with little consistency in the results (ibid).

The ion balance or osmoregulation is affected by hydrogen and/or aluminium ions for most aquatic insects, which lose major ions in acid waters. This has been demonstrated for sodium and in some studies also potassium, by measuring the hemolymph or whole body concentration or in the sophisticated studies by measuring in- and/or outflow of the elements. For most invertebrates, exposure to acid waters make the outflow to increase and the inflow to decrease with a reduced ion concentration in the hemolymph as a result, the effects are more pronounced in acid sensitive species. The crustacean and bivalvia (which cannot proliferate in acid waters) can, when exposed to acid waters, use their internal reserve of calcium as a defense.

**REPRODUCTION**

The ephemeropteran *Cloeon triangulifer* was exposed to different pH, aluminium and calcium levels (Tabak and Gibbs 1991) and the main negative effects were caused by pH, which decreased successful hatches and increased partial hatches. An increase of aluminium was detrimental while increased calcium levels reduced the toxic effect of
low pH. Aluminium was found to have little or no independent toxicity, however extended exposures of previously acid-stressed eggs could cause detrimental aluminium & pH interactions with a subsequent reduction or failure of recruitment.

**Respiration**
Studies of respiration in ephemeropterans gave no response to a decrease in pH from 4.8 to 4 (Herrmann and Frick 1986). Instead, at this low pH elevated aluminium levels caused significant increases in respiration. *Ephemera danica* was most affected while *Heptagenia fuscogrisea* and *H. sulphurea* had a less pronounced increase in respiration. The main reason for this increase is suggested to be difficulties with regulation and transport of ions.

Two odonata studies show diverging results concerning the effect of aluminium, but both suffered lower respiration at low pH. The respiration of *Libellula julia* (Rockwood et al. 1990) decreased further more when aluminium was added – this can be caused by the appearance of denatured mucus/aluminium precipitates (which in the confined rectal gill cannot be cleared as easily as the mayfly gills). In *Somatochlora cingulata* (Correa et al. 1985) low pH alone seems to bring out the same reduction in respiration as low pH and aluminium.

A trichopteran, *Limnephilus sp.*, displayed no significant effects on the respiration at low pH but the ammonium excretion increased (Correa et al. 1986). The additions of aluminium only seemed to reduce the effect of low pH on the trichopteran and not add to it (ibid.).

*The effects of aluminium on respiration are disparate; at low pH ephemeropterans increased the respiration when aluminium was added, while odonatas decreased the respiration when aluminium was added. The different defense strategies can originate in the differences in anatomy; mayflies have mobile gills and try to clear them by increased movement while odonatas with confined motionless gills try to prevent clogging by decreasing the exposure to the acid aluminium rich water.*

**Toxicity/Mortality**
This is the most popular effect to study – maybe because it is easier to detect and quantify – 30 species have been exposed in acute toxicity tests to different levels of aluminium and pH. The benthic fauna orders, which mostly have been studied, are crustacea and ephemeroptera – an interest probably reflecting their susceptibility to acidification.
**BIVALVIA**

*Pisidium spp.* had a LC50 for pH (in a laboratory test) at 3.5 but tolerated more aluminium (>1.0 mg/l) than was tested (Mackie 1989). *Margaritifera margaritifera* was affected by lower ambient aluminium concentrations (0.15 –0.65 mg/l, with 60% in the inorganic Al-form) in field studies (Henrikson 1996). The freshwater pearl mussel had a strong correlation between survival and pH when exposed in cages in acidified and limed streams, however pH alone cannot explain the difference in population densities of mussels recorded by field inventories (ibid). Downstream lime-dosers, which elevated calcium levels, the survival and densities of adult mussels was improved and in streams, with pH less than 5, mussels were able to survive if the level of inorganic aluminum was low (ibid).

**GASTROPODA**

*Amnicola limosa* had a LC50 for pH (in a laboratory test) at 3.5 but tolerated more aluminium (>1.0 mg/l) than was added in the experiment (Mackie 1989).

There was no significant increase in mortality for *Physella heterostropha* when aluminium was added to an acid (pH 4) laboratory stream – but fewer specimens survived (Burton and Allan 1986).

In an acute toxicity test (48h) *Gyraulus sp.* had higher mortality when aluminium was added (87%) to the acid test water (55%) (Havens 1993).

**CRUSTACEA**

*Hyalella azteca* (Mackie 1989) exhibited an inverse relationship between mortality and pH with only a small additional effect of aluminium (France and Stokes 1987). They considered the hydrogen toxicity sufficiently severe to cause mortalities with no independent effect of aluminium - neither toxic nor ameliorating. In another study aluminium significantly increased the mortality caused by low pH (Havas and Likens 1985b).

*Gammarus pulex* was susceptible to aluminium in two stream studies, but pretreatment with aluminium enhanced the survival (Ormerod *et al.* 1987) and complexion of aluminium with citrate reduced the mortality to zero (McCaohon and Pascoe 1989). The latter authors also demonstrated that the mortality was predisposed by pretreatment and animal origin and/or culture conditions. In a laboratory study *G. pulex* was intolerant of pH 4 and the mortality was greatly increased by aluminium ions but no mortalities occurred above pH 4.5 irrespective of aluminium concentrations (Storey *et al.* 1992). In a cage-study with *G. pulex* (Hargeby 1990) there was no correlation between inorganic labile aluminium and the mortality (only a correlation to pH), but the aluminium was measured in samples of similar pH collected one year later. All *G. lacustris* survived in a stream-channel test with unstable mixing zones for 69 h (Kroglund 1998).
Asellus aquaticus survived in cages in streams (ranging from pH 4.3 to 7.5) but the physiological status (=body water content) was affected and negatively correlated to pH (Hargeby 1990), while A. intermedius also in a long term test (28d) had 60% higher mortality for pH 4 compared to 7 (Burton and Allan 1986). The presence of aluminium (500 µg/l) added to the effect of H+ especially in the absence of organic material (0 and 45 mg/l DOC was tested). Burton and Allan (1986) concluded that high percentage monomeric aluminium increased the mortality of A. intermedius. For Orconectes rusticus, O. propinquus and Cambarus robustus no significant effect of aluminium was detected in a laboratory test (Berrill et al. 1985), they were sensitive to pH below 5 and the response was dependent on the life stage of the organisms.

DIPTERA
Aluminium was demonstrated to significantly increase the mortality and add to the toxic effect of low pH for unidentified Chironomids (Havens 1993). But Chironomus anthrocinus (Havas and Likens 1985b) was not sensitive to either low pH or high aluminium and C. riparius registered an equal mortality (10%) caused by pH and aluminium (Ormerod et al. 1987). Chaoborus punctipennis was also exposed in the former study and did not respond to either low pH or aluminium.

EPHEMEROPTERA
Baetis rhodani is the species which most authors consider especially sensitive to acidification, with harmful effects of aluminium in addition to those of low pH (McCa hon and Pascoe 1989; McCa hon et al. 1987; Merrett et al. 1991; Ormerod et al. 1987). In an experiment with short-term acidification in a stream with pH 4.9 the mortality was 0%, at the same pH together with 0.113 mg/l inorganic monomeric aluminum the mortality was 77-100%, the mortality decreased to 0% when aluminium was complexed with citrate (McCa hon and Pascoe 1989). Ormerod et al (1987) also found an increased mortality with addition of aluminium and they suggest that sublethal factors could explain the absence of B. rhodani from streams with low pH and high aluminium. Contradictory, the results from tray-experiments in acid and limed streams (Raddum and Fjellheim 1987) indicated that labile aluminium was of minor importance for invertebrate mortality. In laboratory streams, Kroglund (1998) found no increase in mortality in unstable mixing zones, but 100% mortality in incoming acid aluminium rich water and also 60% mortality in incoming neutral reference water.

The survival of Caenis sp. at pH 4.5 was enhanced by addition of aluminium at first (12-24h) but no additional effect of aluminium was noted after 48 h (Havens 1993). The reduction of pH-related toxicity was explained by the involvement of pH-dependent aluminium dynamics at sensitive target tissues. Havens (1993) noted that this had previously been proposed to occur when soluble aluminium ions dominate
(at low pH) and which may act like calcium and decrease membrane permeability and protect the animals from ion losses caused by H⁺ stress (Muniz and Leivestad 1980; Havas and Likens 1985a).

The addition of aluminium in acid streams increased the mortality of *Ecdyonorus venosus* (McCa hon *et al.* 1987) while the survival was enhanced for specimens pre-exposed to a low level of aluminium (Ormerod *et al.* 1987).

*Ephemerella ignita* showed an increased survival when aluminium was complexed with citrate at pH 4.9, but a larger post-exposure mortality with added free aluminium compared to only acid waters (McCa hon and Pascoe 1989).

*Heptagenia sulphurea* had the same mortality (30%) in mixing zones at pH 6.3 with unstable aluminium chemistry (Krog Lund 1998) as in acid water (pH 5.2), both were higher than in neutral water, pH 7 (20%).

The mortality for *Rhitrogena semicolorata* increased when the duration of the acid episodes increased (from 6 - 12h to 18 - 24 h) - no separation was made between effects caused by low pH or high aluminium (Merrett *et al.* 1991).

**ODONATA**

*Agria sp.* survived at all conditions when acid (pH 5.2), neutral (pH 7) and mixed (pH 6.3) water channels were used in an experiment designed to evaluate the effects of unstable aluminium chemistry (Krog Lund 1998).

The mortality was reduced for *Enallagma sp.* when aluminium (200 µg/l) was added in a short laboratory test at pH 4.5 (Havens 1993). The caudal lamellae of *Enallagma* (an organ for gas exchange with great surface area) are proposed by Havens to be the target for aluminium. The LC50 value was determined to be >1.0 mg/l at pH 3.5 (Mackie 1989).

**PLECOPTERA**

There was no mortality connected to acidity when *Amphinemura sulcicollis* was exposed to chronic and episodic acidification in streams (Merrett *et al.* 1991). The mortality of *Dinocras cephalotes* was low (<10%) both for acid and acid and aluminium streams (Ormerod *et al.* 1987). But *Nemoura sp.* was sensitive to aluminium in acidified laboratory streams, especially in the absence of organic material (Burton and Allan 1986).

**TRICHOPTERA**

Several *Hydropsyche* species have been used in acute toxicity studies, *H. angustipennis* and *H. instabilis* seems to lack acid sensitivity (Ormerod *et al.* 1987; Merrett *et al.* 1991) which indicates that the occurrence in acid streams is limited by trophic conditions but it cannot be excluded that they might be affected by prolonged acid exposures during sensitive stages (Merrett *et al.* 1991). In the acid and mixed zones in a
laboratory channel experiment for unstable aluminium chemistry there was no mortality for *H. pellucidula* while the mortality of *H. siltalai* increased (Kroglund 1998). Significant additional mortality was obtained for *Pycnopsyche guttifer* when aluminium was added in acidified laboratory streams, especially in the absence of organic material (Burton and Allan 1986).

There is a dose-response relationship between inorganic aluminium and above all several ephemeropterans (*Baetis rhodani, Ecdyonorus venosus, Ephemera ignita, Heptagenia sulphurea* for instance) and crustaceans (*Hyalaella azteca, Gammarus pulex, Asellus intermedius*) but also one trichoptera (*Pycnopsyche guttifer*), one bivalvia (*Margaritifera margaritifera*), one gastropod (*Gyraulus sp.*) and chironomids unidentified to taxa. The toxic effect seems to be species and not order dependent. In several studies it has been shown that organic material reduces the harmful effect of aluminium so it must be the inorganic form of aluminium that is responsible for the toxic effect. But when the pH is 4 or lower aluminium can reduce the toxic effect of H+, probably by stabilizing the cell membrane and act as a barrier towards hydrogen ions.

The mechanisms for aluminium toxicity to invertebrates can be both extracellular (increasing the permeability which alter the transmembrane ion fluxes) and intracellular (accumulation which disrupts the cytosolic calcium homeostasis). The invertebrates seems not to be affected by aluminium polymerization on gills or other sensitive organs, since no greater mortalities were observed in unstable mixing waters compared to acid waters. Polymerization of aluminium is the most probable mechanism for the acute toxicity of aluminium to fish in non steady state situations.

In conclusion aluminium is an important source to invertebrate mortality mainly between pH 4 and 5.5, at lower pH Al can act ameliorating and at higher pH aluminium can polymerize which is a less effective toxic action to invertebrates.

**COMMUNITY EFFECTS (THE AMBIENT FAUNA)**
There are two ways to study the effect of acidification on the community of benthic invertebrates; artificial acidification of streams (usually episodic) or field inventories of acidified streams - in both cases the use of neutral references is essential. The stream water quality relates to the sampled benthic community expressed as composition, biomass, density and/or changed behavior such as disturbed drift.

**DRIFT**
In an experiment with artificial channels Allard and Moreau (1986) noted a reduced density but no effect on drift was observed in the acid or acid + aluminium channel. Several experiments have also been conducted directly in streams with simulated
acid/aluminium episodes of different duration and concentration. Merrett et al. (1991) found a significant increase in total invertebrate drift at exposure to multiple episodes of low pH and high aluminium. The drift of Baetidae and Heptageniidae was enhanced during the episodes especially *Baetis rhodani* and *Rhithrogenia semicolorata*. Hall et al. (1987) found a greater drift for mayfly nymphs and blackfly and chironomid larvae from HCl- and AlCl3-treated sections than from corresponding reference areas in first- and second-order streams. They also observed that overall more aquatic insects drifted at pH 5.0 during aluminium addition (>0.28 mg/l) than at pH 5.0 with a low aluminium concentration (0.012 mg/l). In another experiment, trying to separate the effects of hydrogen and aluminium ions, Ormerod et al. (1987) documented a significant increase in total invertebrate drift in both acid and acid + aluminium zones of the stream. Drift densities (specimens/m3 or hour) of *Dixa puberula*, *Protonemura meyeri*, *Ephemerella ignita* and *Dicranota* sp. increased in the acid+aluminium zone. The drift of Simuliidae increased in both the acid and the acid+aluminium zone but the most pronounced response was the 8.4 fold increase in drift density of *Baetis rhodani* in the aluminium+acid zone during the episode (also in the acid zone the drift increased 1.4 times). Wheatherley et al. (1988) further sophisticated the experimental design by adding a fourth zone with low pH and high aluminium but bound in a complex with citrate – to discern the effect between different forms of aluminium. Only *Baetis rhodani* showed a significant drift response: drift density was not affected by flow (reference zone) or by organically bound aluminium but increased x6 in both the acid and acid + 'labile’ aluminium zone. Some samples were lost in the two last zones so it was difficult to separate the responses. The total invertebrate densities in the four zones show the same trend (significant reductions only in acid and acid + 'labile’ aluminium zone with reservation for lost samples).

**COMMUNITY COMPOSITION - BIOMASS AND DENSITY**

In two studies using artificial channels (Allard and Moreau 1986;Allard and Moreau 1987) low density was observed in the acidified channels and high density was observed in the control channel, reversed observations of the biomass was only a
trend but not significant. The differences originated from lack of colonization in the acidified channels and the biomass was mainly affected by the colonization of big larvae of Microtendipes (Chironomidae) in all channels. The percentage of ephemeropterans was significantly smaller in the acidified channels. There was no detectable effect of the addition of aluminium to the acidified channel.

Brown streams studied in New Zealand (Winterbourn and Collier 1987) with a wide pH range (3.5-8.1) displayed no strong association between physicochemical factors and benthic communities. Low pH alone could not regulate the distribution of fauna, which mainly seemed influenced by geographical barriers, causing streams in close proximity to have similar faunas. The invertebrates were adapted to the commonly occurring brown and acid waters and no effect of pH was detected above pH 4.5, aluminium mainly occurred as organic complexes.

When benthic invertebrates were studied by quarterly samplings in woodland streams in Adirondack Mountains (Smith et al. 1990), pH and benthic organic matter (loss on combustion for material >250 µm after the invertebrates were removed) were the most important environmental parameters correlated to density and richness of the total benthic fauna and the diversity index values were only significantly correlated to pH. However, in multiple regressions both pH (positive) and different forms of aluminium (negative) were correlated to ephemeroptera and the feeding groups collector-gatherers and scrapers.

A comparative study (Mulholland et al. 1992) between the previous mentioned study in the Adirondack Mountains and a similar study in the Southern Blue Ridge Mountains was performed. The same pH range was covered (4.5-6.4) but there was a greater seasonal variation in pH and higher monomeric aluminium levels in the Adirondack streams. The stronger correlation between pH and densities of Ephemeroptera and scrapers in the Adirondack streams may be caused by their higher Al concentrations. Monomeric Al may be important in limiting Ephemeroptera abundance in acidic streams but less so in controlling the number of taxa in these streams. It was suggested that there may be some adaptation among a few acid/aluminium tolerant species in the seasonally more constant Southern Blue Ridge streams as the slopes between Ephemeroptera densities and all forms of Al were steeper in the more variable Adirondack streams. Based on differences in slope of regressions between Ephemeroptera density and different forms of Al it was suggested that aquo+hydroxy Al is most toxic to invertebrates.

In a similar approach, streams in the Great Smoky Mountains (Rosemond et al. 1992) was studied. Invertebrate density (total and densities of different orders) was generally more strongly correlated to pH than to inorganic monomeric Al. The density of Ephemeroptera was more highly correlated to pH than other taxa of the benthic fauna. Richness of Ephemeroptera and Trichoptera was positively correlated to pH and Trichopteran richness was also negatively correlated to inorganic
monomeric Al concentrations. The results indicate that the direct effects of pH and Al (affecting survival) were more important than the indirect effects of food availability in determining changes in invertebrate community structure between sites.

*The results of the American field studies seem reliable: they suggest that aquo+hydroxy Al is the most toxic form and that the effect is dependent on invertebrate species. The hydrogen ions are considered to decrease the number of species and high aluminium levels add to that effect by diminishing the numbers of individuals.*
CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BENTHIC INVERTEBRATES

Ephemeropterans, trichopterans and crustaceans have in studies been shown to accumulate aluminium – but mainly in the exuvium. An important question is if this is a passive effect – plain adsorption or if there also is an active import of aluminium. The accumulation is generally considered to be small but may still at some situations be important. The sublethal and toxic effects of aluminium are often regarded as dependent of the pH-effect in itself – this is not surprising since inorganic aluminium is present at acid pH (<5.5) and should be regarded as a coincident effect. Sometimes at lower pH (<4) aluminium instead ameliorates the effect of the hydrogen ions.

Aluminium can decrease hemolymph concentrations of macro constituents in ephemeropterans (sodium), crustaceans (sodium, calcium) and trichopterans (sodium and potassium). For a water bug sodium content in the hemolymph increased but this could be a result of changed fluxes within the insect. Aluminium has no independent effect on hatching but extended acid exposure is suggested to harm ephemeropterans by detrimental aluminium and pH interactions. Diverging results exist of the effect on benthic fauna respiration; at low pH ephemeropterans increased the respiration when aluminium was added, while odonatas decreased the respiration when aluminium was added. In the first case the reason was suggested to be problems with ion regulation/transport and in the second the confined rectal gill was supposed to be clogged by denatured mucus/aluminium precipitates. Maybe they have different defense strategies; mayflies clear their mobile gills by increased movement while odonatas try to reduce exposure of the immobile gills by decreased respiration.

The many studies of mortality give an unclear picture, probably because the studies were conducted at varied conditions; acid - very acid conditions, with - without organic matter and the results therefore should render a bimodal response pattern in at least three dimensions. Aluminium was an important source to invertebrate mortality mainly between pH 4 and 5.5, at lower pH Al can act ameliorating and at higher pH aluminium polymerizes (which is a less effective toxic cause to invertebrates). The analytical fraction inorganic aluminium should correlate to the responses but cannot since the studies in this review span over at least two decades. If inorganic aluminium was measured at all (and not only total aluminium) too dissimilar fractionation methods have been employed rendering incompatible results to relate the toxicity to. Nevertheless there is a dose-response relationship between inorganic aluminium and mainly several ephemeropterans (e.g. Baetis rhodani, Ecdyonorus venosus, Ephemera ignita, Heptagenia sulphurea) and crustaceans (Hyalella azteca, Gammarus pulex,
Asellus intermedius) but also one trichoptera (Pycnopsyche guttifer), one bivalvia (Margaritifera margaritifera), one gastropod (Gyraulus sp.) and unidentified chironomids. **The toxic effect on the invertebrates seems to be species and not order dependent.**

Several studies have demonstrated an increased drift, mainly of Baetidae but also of Heptagenidae and Simuliidae. If the total drift of invertebrates were affected that may reflect that a large proportion of sensitive species are present.

The effect on the community of invertebrates was also species dependent – monomeric aluminium reduced the number of individuals (abundance) of a certain species of ephemeropterans - aluminium had none or little additional effect to low pH itself on diversity (number of species). But in the light of how extremely species poor acidic waters are and the difficulty separating the effects of pH from that of aluminium this viewpoint is questionable. **Therefore it is best to look upon aluminium and hydrogen ions as dual toxic forces in acidic waters.**
FISH - BROWN TROUT

This summary will concentrate on papers concerning brown trout and different effects of inorganic aluminium. Brown trout will be used as a representative for fish in Swedish stream waters; sometimes studies of other species will be included to give a wider view.

HISTORICAL VIEWS OF ACIDIFICATION EFFECTS ON FISH
In the late 70’s and early 80’s researchers began to realize that aluminium was just as important as pH for the harmful effects of acidification (Muniz and Leivestad 1980).

Studies by (Cronan and Schofield 1979) showed that aluminium was toxic at the low concentrations that naturally occur, the toxicity depends on pH with a maximum effect around pH 5. At this pH the hydrogen ions themselves give no physiological stress (Muniz and Leivestad 1980). At the same time similar conditions for fish kills were observed in Sweden; a “safe” pH 5.5 but still high levels of free aluminium just after liming affected newly stocked rainbow trout (Dickson 1978).

Driscoll et al. (1980) pointed out that the speciation of aluminium is important for understanding the toxicity; both the aqueous aluminium chemistry and the effect of different forms of aluminium are variable. The stream water varies with regard to organic carbon and hydrogen ion concentration, the organically complexed Al dominates but pulses of inorganic (labile) aluminium during snowmelt and heavy rainfall are potentially lethal to fish eggs and fry.

The focus of acidification research on toxic effects on fish has now widened from hydrogen ions to total aluminium and further on to inorganic aluminium and their effects through the last 2-3 decades. And as we shall see below it now includes studies of the effect of polymerized aluminium.

TOXICITY MECHANISMS TO FISH
In the following the mechanism of aluminium toxicity according to Exley et al. (1991) is introduced. The principal target organ for acute aluminium toxicity is the gill, where macroscopic and/or microscopic damage can be seen in the gill tissue. Death is caused by a combination of dysfunctions which are detected by several symptoms; ionoregulatory disturbances (measured as net loss of plasma electrolytes, Na, Cl and Ca and a net gain of plasma H+ and Al); respiratory dysfunction characterized by
plasma acidosis and concomitant hypoxia and hypercapnia and osmoregulatory breakdown resulting in a net flux of water into the fish. Aluminium binds to functional groups both apically located at the gill surface and intracellularly located within lamellar epithelial cells disrupting the barrier properties of the gill epithelium (see fig. 2).

Figure 2. A schematic representation of the potential sites of aluminium interaction at the lamellar epithelium of the gill, from Exley et al. (1991). Numbers denote interaction sites; 1) Apical surface of chloride cell, 2) Basally-located active transport system, 3) Narrow apical junction connecting chloride and accessory cells, 4) Wide apical junction connecting accessory and epithelial cells, 5) Polyanionic mucous layer including enzyme carbonic anhydrase, 6) Apical surface of epithelial cell, 7) Intracellular effects on intracellular junctions, 8) Intracellular accumulation of aluminium, 9) Apically-located active transport system, 10) Apical membrane channels, 11) Extracellular effect on intracellular junctions.

Aluminium exerts two principal effects:
- induces an increase in gill epithelium permeability with consequent alternations in transmembranal ion fluxes.
- accumulates intracellularly in epithelial cells and on approaching a toxic threshold illicits deleterious disruptions in cytosolic calcium homeostasis. Subsequent accelerated cell death exacerbates the breakdown in the barrier properties of the gill and the death of the fish results. Aluminium effects on the gill membrane permeability resulting in accelerated cell death may be general features of aluminium toxicity in cells.
Poleo (1995) propose that the process of Al polymerisation (with subsequent hypoxia) is the mechanism that gives the best explanation for the acute toxicity of aqueous aluminium to fish, especially above pH 5.0 or after a rise in pH in mixing zones of acid and neutral/limed waters. This is supported by experiments with anoxia tolerant crucian carp, *Carcassius carassius*, to which aluminium was not acutely toxic in situations that was favourable for Al polymerisation (Poleo et al. 1994).

Aluminium toxicity cannot be explained in terms of one single mechanism, and which mechanism is most important in any given situation is probably dependent on pH, organic matter and temperature. The major mechanisms in acid waters are increased permeability of gill epithelium and intracellular accumulation – both results in ionoregulatory disturbances and respiratory dysfunction and osmoregulatory breakdown. In non-equilibrium situations when Al can polymerize on the fish gills, it is acutely toxic and hypoxia leads to an imminent death.

**CONDITIONS AFFECTING THE TOXIC EFFECT OF ALUMINIUM ON FISH**

**THE EFFECT OF WATER QUALITY ON ALUMINIUM TOXICITY TO FISH**

**Calcium and ionic strength**

It is well documented that high Ca-concentrations can reduce Al-toxicity to fish (Brown 1983; Playle and Wood 1989). This mitigating effect has most often been attributed to Ca$^{2+}$ per se, through its effect on the permeability of fish gills. But Ca-concentrations and water ionic strength is correlated. Therefore, recently Lydersen et al. (2002a) tested the influence of water ionic strength on the toxicity of aluminium to salmon parr with additions of Ca$^{2+}$ and Na$^+$ in non steady state aluminium rich water. Increasing the ionic strength with Na reduced the mortality to a larger extent than the corresponding increase in ionic strength by the addition of Ca. Calcium does not play a unique role in decreasing aluminium toxicity, rather ionic strength seems to be important probably for the interaction between aluminium and the gill surface, reducing the possibility for positively charged aluminium species to bind to negatively charged sites. Whether ionic strength decrease aluminium toxicity in the same way in more stabile acid, high aluminium waters, in which other mechanisms of aluminium dominates remains to be investigated.

**Fluoride and Silicon**

Other complexing agents can also change predicted toxicity of aluminium. The presence of fluoride (1-10uM) makes aluminium less toxic than without fluoride but
more toxic than predicted by calculated Al³⁺-concentrations (Wilkinson et al. 1990). Thus the free ion activity model does not adequately predict the toxicity of aluminium, causing increased mortality to salmon even in the presence of excess fluoride (ibid.).

Silicon can also reduce Al toxicity to fish, Birchall et al. (1989) suggested that the formation of hydroxy-aluminosilicate species, enhanced by the alkaline gill environment, blocks the binding or precipitation of Al-OH species on the gills.

**Humus**

The organic binding of aluminium is strongest at higher pH-values, which is explained by H⁺ competition for binding sites (=protonation of binding sites) at low pH and evident by the predominance of Al³⁺ and higher solubility of Al at low pH. Dissolved organic matter, DOM, has received a lot of interest as it protected fish from aluminium toxicity (when aluminium was represented by total aluminium measurements). But as analytical methods have been developed, it has been clear that the best predictor of aluminium toxicity is free/inorganic monomeric aluminium. Even so it has been shown that the protective effect of DOM is greater than expected due to just the reduction of available Al that can interact with the gills. In fact it took twice as much inorganic Al to be toxic to salmon in the presence of 2-3 mg/l fulvic acids. Roy and Campbell (1997) who conducted the experiment thought it was possible that DOM itself interacted at fish gills. Gensemer and Playle (1999) speculated that it was possible that the presence of DOM can slow or prevent the precipitation or polymerization of aluminium in the more alkaline gill environment.

**Mixing zones**

Several studies have shown that the non-equilibrium situations that exist in the mixing zones between acidic aluminium-rich water and neutral and/or limed water are extremely toxic to salmonid fish (Rosseland et al. 1992; Verbost et al. 1995; Poleo et al. 1994). There is a highly increased rate of cell death by apoptosis (= physiologically controlled cell death, characterized by cellular shrinkage and densification of nuclei, mitochondria and cytoplasm) and necrosis (= accidental cell death, characterized by rupture of membranes and swelling of cell compartments) in mixing zones (Wendelaar Bonga et al. 1990).

Gill cells was affected even if the pH in the mixing zone was high, above 6, but the toxicity was transient and decreased with age of the mixed water (the time from confluence was 20 to 340 seconds)(Verbost et al. 1995). The intracellular spaces became enlarged and many leucocytes penetrated in these spaces and mucus release was stimulated to depletion.
In another mixing zone experiment Witters et al. (1996) determined the mortality to 98% after 48h in a simulated mixing zone (pH 6.4, 76 µg Al/l) compared to 60% in acid water (pH 4.6, 184 µg Al/l).

To understand the chemistry in dynamic freshwater systems containing aluminium in situ measurement of the aluminium fractions (with respect to both size and charge) were used (Lydersen et al. 1994). The use of in situ aluminium analyses made it possible to relate fish toxicity to analytical observations of Al-polymerization.

The toxicity of aluminium is affected by other components in the water – the most important in Swedish waters is probably the organic matter, which reduces aluminium toxicity beyond the mere reduction of available aluminium. But the reducing influences of calcium, fluoride, silicon and the ionic strength on aluminium toxicity are also of importance. A lot of research, especially in Norway has concerned mixing zones where aluminium can polymerize and be acutely toxic to salmonids. If this is a widespread problem also in Sweden remains to be seen, the topography of the drainage areas and used liming techniques are quite different.

The Effect of Temperature and Season on Aluminium Toxicity to Fish

The effect of temperature on aqueous aluminium chemistry and survival of fingerlings and smoltifying Atlantic salmon Salmo salar was studied (Poleo and Muniz 1993; Poleo et al. 1991). Mortality was correlated to the concentration of inorganic aluminium and increased with rising temperature. The degree of ongoing Al-polymerisation is thought to be important for the temperature-dependent Al-toxicity observed.

Seasonal variation in mortality of brown trout in an acidic aluminium rich lake was studied by (Lydersen et al. 2002b). The mortality was highest during spring (for small fish) and summer (for larger fish). Water temperature and fish length could explain most of the seasonal variation in mortality. It is suggested that the dependence of water O₂-solubility and fish metabolism upon temperature is of importance for the temperature-dependent mortality observed. To some extent, this was supported by the higher mortality in large fish than in small fish, due to a lower gill surface area/body weight ratio. Temperature as well as pH affects the solubility, the hydrolysis and molecular weight distribution of aqueous Al species (Lydersen et al. 1990). There is less sedimentation and a higher proportion of high molecular weight Al species present as colloids in colder waters (2 compared to 25 °C).
Temperature is important in several ways; it is correlated to the life stage of the fish and it affects the solubility of oxygen and the metabolism of fish but also the actual speciation of aluminium.

DIFFERENT SENSITIVITY OF FISH SPECIES AND STRAINS TO ALUMINIUM

Poleo et al. (1997) exposed seven common Scandinavian fish species to acidic Al-rich, acidic Al-poor and approximately neutral Al-poor water as a control. The experiments took place in flow-through channels for 11-42 days depending on the mortality of the fish. The relative sensitivity to aluminium was decreasing in the following order: Atlantic salmon (Salmo salar); then roach (Rutilus rutilus); minnow (Phoxinus phoxinus); perch (Perca fluviatilis); grayling (Thymallus thymallus); brown trout (Salmo trutta) and Arctic char (Salvelinus alpinus).

Vuorinen et al. (1999) compared the sensitivity of yolk sac fry of brown trout and grayling and confirmed the sensitivity order, at pH 5 EC50 was 200 µg Al/l for grayling and 700 µg al/l for trout.

In the Norwegian ReFish-project brown trout and atlantic salmon has been studied to find out if there exists physiological strains of the fish species in relation to acid waters (Rosseland 1994; Dalziel et al. 1995). The project included trout from five areas and studied restocking and subsequent test fishing in 13 acid lakes and laboratory toxicity testing with eggs and fry. The results from the field and laboratory are unanimous; there are differences between trout strains in the ability to tolerate acid water. Trouts from acidic areas have developed the largest tolerance to acidic conditions. Experiments with calcification of fry showed that the most acid tolerant fry had less calcified skeletons but equivalent total amount of Ca in the body. They ‘borrowed’ calcium from the skeleton to keep the gill membrane integrity intact in acidic surroundings, later they returned it to the skeleton and was fully calcified (Dalziel et al. 1995). Atlantic salmon from five areas, different life-stages and year-classes was studied at the laboratory. There was a tendency to strain difference; salmon from not-acid waters had the largest acid tolerance. The local selection pressure is probably less hard on salmon because of a larger genetic flow with not-adapted genes (Rosseland 1994).

Fish species have a different sensitivity to aluminium probably based on differences in metabolism and oxygen dependence; Atlantic salmon is the most sensitive species. Difference in acid tolerance is pronounced for trout strains, trout from acid waters have developed the largest acid tolerance. There is only a tendency to strain difference for salmon (inversed compared to trout, salmon from acid waters is least acid tolerant) probably because of a greater genetic flow that reduces the selection pressure.
VARYING SENSITIVITY TO ALUMINIUM OF TROUT AT DIFFERENT AGES

Al appears to mitigate deleterious action of low pH on trout ova reared in soft water (Sayer et al. 1991). The egg stage (the different terms describing fish development is shown in figure 3) is considered to be susceptible to H+ toxicity although acidosis is reduced by the buffering capacity of the perivitelline fluids (of eyed eggs) (Peterson et al. 1980; Kugel and Peterson 1989).

The mortality increased during or immediately following hatch (Sayer et al. 1991), resulting in partial hatching and or prolonged hatching. This can possibly be caused by early (before the eyed stage, 60 degree days pre-hatch (Sayer et al. 1991)) inactivation of the hatching enzyme chorionase, caused by a reduction in pH in the perivitelline fluid (Peterson et al. 1980).

Swim up fry (>36 days) was more sensitive to pH/aluminium than yolk sac fry and year-old fish had an increased sensitivity (Brown 1983). Reader et al. (1991) found that eggs and yolk sac fry was less sensitive (and best protected by larger yolk) than swim up fry with no yolk left. They also found swim up fry more sensitive than 1-2yr juveniles of brown trout to episodic exposures in soft, acidic aluminium rich waters and argued that the divergence with Brown’s results (1983) could be explained by a difference in classification (swim up fry with a little yolk left would be less sensitive than juveniles).
Trout eggs are relatively tolerant to low pH and high aluminium concentrations (Weatherley et al. 1990). Alevins are highly sensitive with LC50 values of >20 µg Al tm/l (‘pre swim-up’, 28d) and >15 µg Al tm/l (‘post swim-up’, 42d), whilst parr (3 months old) are slightly more resistant based on acute toxicity ignoring sublethal effects. Trout eggs exposed in different streams for 13 days (from 7 days before hatching) had a hatching frequency between 70 and 98 %, not correlated to water chemistry (Norrgren and Degerman 1993). The exposure continued for 77 days and the total fry mortality was 94% in the acidic stream (pH 5.1, 203 µg Alr/l) and between 4-25% at the better localities (pH > 5.7, < 49 µg Alr/l).

Early life stage (ELS) toxicity tests with fish have been developed as a supplement and alternative to acute and chronic fish toxicity tests. Compared to adult fish, sublethal effects can be detected much easier in early ontogenetic stages due to an increased sensitivity and several end-points. Luckenbach et al. (2001) demonstrated the embryo toxic potential for brown trout (exposed from fertilization to alevin stage) in a complexly polluted stream. Mortality, developmental rate and body growth were the most significant endpoints with respect to pollution effects in this study.

In a study which used brown trout as biomonitor and linked heavy metal concentrations in two abiotic compartments (water and sediment) and one biotic; brown trout (liver), only one-year-old juveniles were useful to study between-location differences – older trout are probably to mobile (Linde et al. 1998). When correlating stream conditions to fish conditions, one-year-old juveniles or younger fish should be used because they are stationary.

Trout eggs are most sensitive before they become eyed, which happens during late autumn. After that the yolk sac with perivitelline fluid buffers towards the acidity of the surrounding water. When the yolk sac has been consumed by the swim up fry (>40 days since hatching) and the fry has left the protecting ground layer of the stream, the fry are very sensitive. This can coincide with the snowmelt and severe damage to trout populations can occur. The fry is more sensitive than older fish, both fry and year-old juveniles are good biomonitors since they are still stationary and do not escape when the water quality deteriorates.

**THE EFFECT OF ALUMINIUM ON FISH BODY DEVELOPMENT**
Concentrations of 54-216 µg Al/l impaired net uptake of calcium, potassium and sodium and lightly increased the net loss of magnesium (Reader et al. 1988).
At the ‘swim up’ stage surviving fry had lower whole body calcium, sodium and potassium when exposed to 162 µg Al/l compared to control fish (Sayer et al. 1991).

Al-treated yolk-sac fry exposed to low ambient Ca concentrations from 200-300 degree-days post hatch, suffered high mortalities regardless of pH (Sayer et al. 1991). There is no evidence that impaired mineral uptake and/or enhanced mineral loss are a direct result of Al or H+ in the ambient medium – instead they may be a consequence of some other disorder which in itself is responsible for the larval mortality.

Reader et al. (1991) found reduced sodium and potassium concentration for whole body tissue digests of early and late yolk sac fry sampled after a 168h-recovery period after episodic acid and aluminium exposures than in control fry. Yolk-sac fry had significantly lower whole body concentrations of sodium and potassium when exposed in an acidic stream than in neutral streams (Norrgren and Degerman 1993). Fry exposed in humic-rich streams (average pH 5.7 and 6.6 respectively) also contained reduced sodium concentrations and for the first stream also potassium was reduced compared to the more neutral streams.

Major body development and skeletal calcification was impaired by Al (54-216 µg/l) (Reader et al. 1988; Sayer et al. 1991), the effect was reduced by higher ambient Ca concentration (Sayer et al. 1991). The growth of yearling trout was retarded by aluminium (inorganic Al 0-100 µg/l) at pH below 5.5 – low pH itself (4.3) had little effect on growth (Sadler and Lynam 1987; Sadler and Turnpenny 1986). In one study the mortality of yearlings also increased besides the suppression of growth, (Sadler and Lynam 1988). In the studies in which Ca was added, its presence reduced the toxic effect of Al. (Sayer et al. 1991; Sadler and Lynam 1988).

The chemical body composition of yolk-sac fry is affected by exposure to acid and aluminium rich waters; sodium, potassium and calcium body content is reduced compared to references. The mortalities are high even if only eggs were exposed but there is no evidence that the changed mineral composition is directly related to the exposure to Al and H-ions. Exposure to aluminium impairs major body development and skeletal calcification; this can also be seen as retarded or suppressed growth of fry or young trout. Calcium reduces the deleterious effect of aluminium on body development; low pH alone had little effect on body development.
THE EFFECT OF ALUMINIUM ON PLASMA COMPOSITION AND RESPIRATION IN FISH
A decrease in plasma sodium levels was observed in caged brown trout held in three acidified reaches (pH 5.0 and Al 0.37 mg/l, pH 4.5 and 0.40 respectively 0.67 mg/l filterable Al) compared to one limed reach (pH 7.2 and 0.13 mg Al/l) of a welsh stream (Weatherley et al. 1989). In the limed reach the plasma sodium levels were higher but not as high as in the reference stream, which may have been due to pre-treatment of the fish in the naturally acidic water. Increased ventilation frequencies accompanied the sodium decreases at the acidified locals. The proposed toxic mechanisms are penetration of hydrogen ions into the blood with ion regulatory disturbances by hydrogen and aluminium.

Aluminium accumulation, mucus production and damage to gill epithelium reduced the gill diffuson capacity and caused the ventilatory stress for trout in laboratory mixing zones (Witters et al. 1996). The fish was stressed (increased glucose and cortisol levels) but the increased ventilation frequency and the blood hematocrit levels gave support to the thesis that acute fish mortality in mixing zones could be caused by respiratory dysfunction.

There was no additive effect of aluminium on plasma ion concentrations at pH 4.7 and Al 280 and 450 µg/l (Laitinen and Valtonen 1995) however aluminium had a clear additive effect on respiratory stress (based upon ventilatory and cardiovascular data together with severe hyperglycemia).

Morris and Reader (1990) used controlled chemical episodes and found a similar response pattern in all sublethal episodes; fish respond by decreasing sodium influx to the plasma and increasing outflux during initial stages of the episodes. The outflux gradually decreased and reached its original rate by the end of the episode while the influx also partly recovered but did not reach its original rate after the recovery period at high aluminium levels. The level of plasma sodium was significantly correlated to measured water aluminium levels (0-486 µg/l). The authors measured oxygen consumption during the episodes (pH 4.5, 307 µg Al/l) compared to the artificial soft-water medium (pH 5.6, 0.8 mg Ca/l) and concluded that short acid or aluminium acid episodes have little deleterious effect on fish respiration other than an irritant effect on some individuals. But at higher pH (5.4) and aluminium (756 µg/l) the oxygen consumption rose during and following the episode exposure.

Surviving trout juveniles from episodic acid and aluminium exposures had lower sodium and chloride levels than the controls and the hematocrit level was
higher (Reader et al. 1991). Dying animals sampled close to death during a 60-h acid plateau (pH 4.5, 323 µg Al/l) had sodium levels only a little lower than the controls and chloride levels were markedly lower. Hematocrit levels in the dying fish were much higher than in the controls and the surviving juveniles and potassium levels were higher than in the controls.

Dietrich and Schlatter (1989) exposed one and two year old trout to low ionic strength water with moderate pH (5.4) and moderate Al (121 µg/l) and found acute toxic symptoms. They found extremely high plasma hematocrit levels – the dying fish was depleted of plasma chloride and sodium and there was also 6 times more Al in the plasma compared to control fish.

When fish is stressed by exposure to acid and aluminium rich water the glucose level increases and accumulated aluminium on the gills causes the fish to produce more hemoglobin (higher hematocrit levels) and increase ventilation frequency to oxygenate the important organs. Blood electrolytes are affected by changes in permeability of the gill and intrusion of hydrogen ions whereas sodium, chloride and calcium ions flow out.

ACCUMULATION OF ALUMINIUM ON TROUT GILLS
Karlsson-Norrgren et al. (1986) used light- and electron microscope examination to disclose two major types of gill lesions characterized by chloride cell hyperplasia in the secondary lamellar epithelium and enlargement of the intercellular spaces in the secondary lamellar epithelium. These occurred in farmed trout grown in limed fish farms where the water was circumneutral but still contained high levels of aluminium (27 and 80 µg Al/l) (probably polymerising).

One summer old trout obtained from two different streams was used by Stoner et al. (1984) and exposed for 17 days in October in 8 streams. There was a positive correlation between mortality rate and gill aluminium concentration (r=.96, p>0.05). One and two year old trout was exposed by Dietrich and Schlatter (1989) to low ionic strength water with moderate pH (5.4) and moderate Al concentration (121 µg/l) with acute toxic symptoms. Mucous clogged the gills and the epithelial was damaged mainly by epithelial lifting, fusion of the secondary lamellae, necrosis and hyperplasia.

Trout was exposed in limed mixing zones in Wales with high mortalities correlated to Al (<0.22µm) and Fe in the water and Al in the gills (Weatherley et al. 1991). Iron on the gills was well correlated to aluminium on the gills and hence indirectly to mortality. The highest accumulation took place at the limed reaches
where Al was polymerising and the measured Al-concentration in water was low (only the values for the acid locals is plotted in figure 4 as the Al-polymerisation makes the correlation between water inorganic Al and gill aluminium irrelevant).

In a project studying episodic acidification in northern Sweden, mainly one-year-old trout was exposed in troughs during snowmelt (Laudon et al. 2001). The mortality was best correlated to the proportion \([\text{ANC}/H^+]\) (since there were analytical problems with the fractionation of aluminium in these humic streams) – but still when the results are plotted together with other studies they fall in quite nicely in the correlation between aluminium in the water and on the gills (fig. 4).

![Graph showing the relationship between inorganic aluminium in water (x-axis) and accumulated aluminium on the gills (y-axis).](image)

*Figure 4. The relationship between inorganic aluminium in water (x-axis) and accumulated aluminium on the gills (y-axis). The correlation is strong and significant even though the studies and the methods used are very diverse – both concerning the aluminium fractionation and the conditions for the trout in the streams studied. There was also a correlation between accumulated aluminium and fish mortality (filled symbols); over 500 µg Al/g dw on the gills is mortal.*
EFFECTS OF ALUMINIUM ON BROWN TROUT POPULATIONS

Trout status and water chemistry in the Norwegian Thousand Lake Survey was statistically analysed (Bulger et al. 1993). The results of analysis of variance, principal components analysis and discriminant analyses converge on the variables pH, monomeric inorganic aluminium and ANC as the most strongly related to trout status (= “healthy, marginal or extinct”). Lakes with extinct populations have an average aluminium concentration of 133 µg/l, an ANC of –34 µeq/l and a pH of 4.8 versus 11 µg Al/l, + 27 µeq/l and 6.0 respectively for healthy lake populations. Calcium and sulfate influenced trout status moderately as individual variables, but quite strongly in combination. The variation in their concentrations is the major determinant of alkalinity in these lakes and reflects the acidic deposition.

Trout status and water chemistry in 1192 lakes in Norway (some also used in Bulger et al. 1993) was evaluated by a stepwise linear regression (Muniz and Walloe 1990). Their conclusion that pH, labile aluminium and lake altitude were the main determinants of fish status (and that calcium is unimportant) is challenged by (Bulger et al. 1993). The statistical treatment by (Muniz and Walloe 1990) is said to be biased and ignorant of the fact that the contribution of an independent variable to a multiple regression is very much dependent on the other variables in the model; calcium provided no further predictive information but should still be considered important.

The density of young brown trout in lake tributaries and water chemistry and habitat variables in acidic soft-water river systems in Norway was examined (Hesthagen et al. 1999). The concentration of inorganic Al was too low to influence the fish, instead the brown trout density was best correlated positively to calcium content and to the ratio Ca²⁺/H⁺. The influence of habitat variables such as altitude, mean water temperature or habitat heterogenity was much smaller than the influence of water chemistry.

An evaluation of data on trout populations and water chemistry in streams in Sweden with non-parametric statistical analysis revealed that pH above 6, alkalinity above 0.05 mekv/l and inorganic aluminium below 30 µg/l was necessary for successful reproduction (Andren and Bergquist 2000). The density of young of the year trout was significantly correlated both with mean and extreme values of pH, alkalinity and inorganic aluminium, while the density of older trout only correlated significantly with mean values of the same variables.

Field studies in 61 acidic and circumneutral streams in England and Wales showed a strong relationship between water quality and standing crop of 1+ brown
trout (Sadler and Turnpenny 1986). The measured pH levels were too high to be toxic but heavy metal and Al concentrations could account for low or no brown trout biomass in the more acidic streams.

Stoner et al. (1984) surveyed 13 streams in Wales and found no trout populations in waters that had a mean pH <5.5 and mean soluble Al > 15 µg/l.

Several field inventories have been done to study the density of trout in relation to water chemistry and habitat variables. The correlation is best between young trout and pH, inorganic Al, ANC/alkalinity and calcium. Since the water chemistry variables are correlated themselves, the importance assigned to the individual chemical variables by the statistical method depends on how the dataset is treated and the selection of streams.
CONCLUSIONS ABOUT ALUMINIUM TOXICITY AND BROWN TROUT

Aluminium acts through different mechanisms; in acid aluminium rich waters aluminium induces an increase in the permeability of the gills and accumulates intracellular in gill epithelium cells. This destroys the gill barrier and the fish experiences ionoregulatory disturbances, respiratory dysfunctions and osmoregulatory breakdown. The acutely toxic action of aluminium in waters with a pH above 5 or in non steady state situations (as liming) can best be explained by the process of polymerization of aluminium on the gills and subsequent hypoxia.

The toxicity of aluminium is affected by other components in the water reducing the toxicity; the influence of organic matter, calcium, fluoride, silicon and ionic strength has been studied. Temperature affects the speciation itself but also regulates the solubility of oxygen and is correlated to the development stage and metabolism of the fish. Atlantic Salmon was the most sensitive fish species and Brown trout followed by Arctic char as the least sensitive in a comparative study with seven species. There exists strain differences for brown trout; trout from acid waters are most acid tolerant.

Swim up fry are very sensitive to aluminium, they and year-old-trout, which have retained some sensitivity, are good biomonitors since they still are stationary. The eggs are most vulnerable before the eyed stage, then the perivitelline fluid buffers against surrounding waters until the yolk sack is consumed. Exposure to aluminium impairs the development of eggs and fry and the body mineral content of sodium, potassium and calcium is reduced.

In yearlings and older fish (where blood sampling can be performed) aluminium reduces the plasma content of the blood electrolytes (Na, Cl and Ca) as Al and H-ions flows in when the gill barrier is breaking up. The levels of glucose (a stress response) and haemoglobin rise; to counteract the reduced oxygen uptake area – for the same reason also the ventilatory frequency increases. Even if I have found data from very dissimilar studies of the correlation between inorganic aluminium in water and accumulated aluminium on the gills the relationship is strong (r= 0.77). Accumulation on the gill over 500 µg Al/g dw is lethal for trout in these studies.

In field inventories, good correlations between density of young trout and pH, inorganic Al, ANC/alkalinity and calcium have been found. Since this is an indirect way to study toxicity it is also sensitive to data selection and treatment, especially as the studied water chemistry variables are not independent themselves.
REFERENCE LIST


Herrmann, J. (2001) Aluminium is harmful to benthic invertebrates in acidified waters, but at what threshold(s)? Water, Air and Soil Pollution, 130, 837-842.


# Appendix. Table over studies on the effect of (inorganic) aluminium and benthic invertebrates in acidic freshwaters.

**GENUS; Bi=Bivalvia, Cr=Crustacea, D=Diptera, Ep=Ephemeroptera, Ga=Gastropoda, He = Hemiptera Plecoptera, Od = Odonata, Oi = Oligochaeta, Tr = Trichoptera.**

Response type: Mortality M, Ion regulation I, Respiration R, Bioaccumulation A, Physiological status PS, Energy metabolism EM.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Type of study</th>
<th>Experiment conditions</th>
<th>Response type/time</th>
<th>pH-effect</th>
<th>Al-effect</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bi</strong></td>
<td>Pisidium spp.</td>
<td>Lab/Jar</td>
<td>pH: 4, 4.5, 5.5, 5.5</td>
<td>LC50</td>
<td>96h</td>
<td>&lt; pH3.5</td>
<td>Toxic levels were more representative of the species than of their functional group. Not likely that Al levels in Ontario are acutely toxic to most invertebrates.</td>
</tr>
<tr>
<td><strong>Bi</strong></td>
<td>Margaritifera margaritifera (L.)</td>
<td>Field/Stream</td>
<td>pH: 4.2-7.0</td>
<td>M</td>
<td>transplanted in cages</td>
<td>Correlation between lowest pH and mussel survival after 241 d</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Asellus aquaticus</td>
<td>Field/Stream</td>
<td>pH: 4.3-7.5</td>
<td>M</td>
<td>25d exposure, water chem at start and end</td>
<td>PS (water content %) sign. negatively correlated to pH, no M</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Asellus intermedius</td>
<td>Lab/Stream</td>
<td>pH: 4, 7</td>
<td>M</td>
<td>28d</td>
<td>60% Additive effect to pH, especially in the absence of organic material</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Cambarus robustus</td>
<td>Lab/Jar</td>
<td>pH: 4.7, 5.7</td>
<td>M</td>
<td>10-14d</td>
<td>Not sensitive to pH &lt; 5.0</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Rivulogammarus lacustris</td>
<td>Lab/Stream</td>
<td>pH: 5.2, mix: 20, 330 sec.</td>
<td>M</td>
<td>Na, K hemolymph</td>
<td>Sublethal stress- lower Na in specimens from acid, Al-rich than in reference and mix waters.</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Gammarus pulex</td>
<td>Field/Stream</td>
<td>pH: 7.2, 4.5</td>
<td>M</td>
<td>72h</td>
<td>M 25%, pretreatment with .05 Al enhanced survival</td>
<td></td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>Gammarus pulex</td>
<td>Field/Stream</td>
<td>pH: 7.2, 4.9, +0.085</td>
<td>M</td>
<td>72h postexp. 5% M 6-13%, 72 h postexp. 6-16%, M 0% after 15h in citrate-complexed Al at low pH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Morphological abnormalities AB, Reproduction RE, Drift D, Biomass&Density BD, Composition C

<table>
<thead>
<tr>
<th>pH-limit,</th>
<th>SEPA-report</th>
<th>pH-limit</th>
<th>Johnson et al. 1993</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.50</td>
<td>4.7</td>
<td>&gt;5.0</td>
<td>&gt;5.5</td>
</tr>
<tr>
<td>&gt;4.50</td>
<td>&gt;4.7</td>
<td>&gt;5.0</td>
<td>&gt;5.0, 4.7</td>
</tr>
<tr>
<td>&gt;5.00</td>
<td>&gt;5.5</td>
<td>&gt;6.00</td>
<td>&gt;5.5</td>
</tr>
</tbody>
</table>

51(59)
<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Type of study</th>
<th>Experiment conditions</th>
<th>Response type /time</th>
<th>pH-effect</th>
<th>AI-effect</th>
<th>Discussion</th>
<th>pH-limit, SEPA-report</th>
<th>pH-limit, Johnson et al. 1993</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr.</td>
<td>Gammarus pulex</td>
<td>Field/Stream</td>
<td>pH: 4.3-7.5 Al:&lt;0.1-42 C: 8-280 mgPt/l</td>
<td>M PS cages in 20 streams 25d exp., water chem start &amp; end</td>
<td>M &amp; PS (water content %) sign. neg. corr. to pH</td>
<td>Labile Al (sampled next year) not correlated to M.</td>
<td>pH is considered the only significant predictor of M and water content- other variables have small influence.</td>
<td>&gt;5.50</td>
<td>-</td>
<td>Hargeby 1990</td>
</tr>
<tr>
<td>Cr.</td>
<td>Gammarus pulex</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 5, 5.5, 6.9 Al:0, .01, 0.1, 1.0 C: pond water</td>
<td>M 168h 70% after 168 h</td>
<td></td>
<td>90-100% at pH4 with increasing Al levels after 168 h</td>
<td>The mortality of Gammarus pulex at pH4 was greatly elevated by the presence of Al3+ ions.</td>
<td>&gt;5.50</td>
<td>-</td>
<td>Storey et al. 1992</td>
</tr>
<tr>
<td>Cr.</td>
<td>Hyalella azteca</td>
<td>Lab/Jar</td>
<td>pH: 4.3, 4.8, 5.3, 6.5 Al: 0.5, 25, .4, .7 C: synt</td>
<td>M 8d</td>
<td>Inverse relationship between mortality and pH.</td>
<td>Dose-response effect at pH 4.8 and 4.3.</td>
<td>1) Al-toxicity was dependent on the dominating influence of H+. 2) The H+ toxicity was sufficiently severe. 3) No evidence of ameliorating Al-effects.</td>
<td>-</td>
<td>&gt;4.7</td>
<td>France &amp; Stokes 1987</td>
</tr>
<tr>
<td>Cr.</td>
<td>Hyalella azteca</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 5.5 Al: 0.02, 0.05, .1, .2, .4, 1 C: 5DOC</td>
<td>M LC50 96h pH 4.3-4.9</td>
<td>&gt;1.0 at 5.0</td>
<td>Toxic levels are more representative of the species than of their functional group. Not likely that Al levels in Ontario are acutely toxic to most invertebrates.</td>
<td>-</td>
<td>&gt;4.7</td>
<td>-</td>
<td>Mackie 1989</td>
</tr>
<tr>
<td>Cr.</td>
<td>O. propinquus</td>
<td>Lab/Jar</td>
<td>pH: 4.7, 5.7 Al: 0, &gt;.3 C: -</td>
<td>M 10-14d Sensitive to pH &lt;5.0</td>
<td>No significant effect</td>
<td>Different responses depending of lifestages.</td>
<td>-</td>
<td>-</td>
<td>Berrill et al. 1985</td>
<td></td>
</tr>
<tr>
<td>Cr.</td>
<td>Orconectes rusticus</td>
<td>Lab/Jar</td>
<td>pH: 4.7, 5.7 Al: 0, &gt;.3 C: -</td>
<td>M 10-14d Sensitive to pH &lt;5.0</td>
<td>No significant effect</td>
<td>Different responses depending of lifestages.</td>
<td>-</td>
<td>-</td>
<td>Berrill et al. 1985</td>
<td></td>
</tr>
<tr>
<td>Cr.</td>
<td>Orconectes virilis</td>
<td>Lab/Jar</td>
<td>pH: 5.5 Al: 0.2, 0.5, 1.0 C: tap</td>
<td>M 12h Reduced Ca-uptake by 30%. Reduced Ca-uptake slightly more 20%, no relation to Al-conc.</td>
<td>-</td>
<td>-</td>
<td>Malley &amp; Chang 1985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr.</td>
<td>Hyalella azteca</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 7 Al: 0.2 C: deion. Medium</td>
<td>M 24 - 48h 88%</td>
<td>Al significantly increased mortality.</td>
<td>Possibly affected through the gills.</td>
<td>-</td>
<td>&gt;4.7</td>
<td>Havens 1993</td>
<td></td>
</tr>
<tr>
<td>Di.</td>
<td>Chaoborus punctipennis</td>
<td>Lab/Jar</td>
<td>pH: 3.5, 4, 5, 6.5 Al: 0.02, 0.32, 1.0 C: 3 DOC</td>
<td>M 180h Not shown sensitive</td>
<td>Not shown sensitive</td>
<td>Neither Al nor H+ ion concentrations normally encountered in acidic lakes, appeared to affect the mortality.</td>
<td>-</td>
<td>&lt;4.50</td>
<td>Havas &amp; Likens 1985</td>
<td></td>
</tr>
<tr>
<td>Di.</td>
<td>Chironomids</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 7 Al: 0.2 C: deion. medium</td>
<td>M 24 - 48h 6%</td>
<td>Al significantly increased mortality.</td>
<td>Adds to the low pH effect itself</td>
<td>&lt;4.50</td>
<td>&lt;4.7</td>
<td>Havens 1993</td>
<td></td>
</tr>
<tr>
<td>Di.</td>
<td>Chironomids</td>
<td>Field/Stream</td>
<td>pH: 6.6, 4.0 Al: 0.17, 18 C: 6 TOC</td>
<td>BD D 73d</td>
<td>Similar percentage, total biomass in all channels mainly due to the big larvae microtendipes. Less taxa in acidified ch.</td>
<td>Similar percentage, total biomass in all channels mainly due to the big larvae microtendipes. Less taxa in acidified ch.</td>
<td>&lt;4.50</td>
<td>&lt;4.7</td>
<td>Allard &amp; Moreau 1986 &amp; 1987</td>
<td></td>
</tr>
<tr>
<td>Di.</td>
<td>Chironomus riparius</td>
<td>Field/Stream</td>
<td>pH: 4.7; 4.2; 4.5 Al: 0.046; 0.052; 3.47 C: 2 DOC</td>
<td>M 72h M&lt; 10%</td>
<td>M&lt; 10%</td>
<td>-</td>
<td>-</td>
<td>Ormerod et al. 1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di.</td>
<td>Chironomus anthrocinus</td>
<td>Lab/Jar</td>
<td>pH: 3.5, 4, 5, 6.5 Al: 0.02, 0.32, 1.0 C: 3 DOC</td>
<td>M 180h Not shown sensitive</td>
<td>Not shown sensitive</td>
<td>Neither Al nor H+ ion concentrations normally encountered in acidic lakes, appeared to affect the mortality.</td>
<td>-</td>
<td>&lt;4.50</td>
<td>Havas &amp; Likens 1985</td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type /time</td>
<td>pH-effect</td>
<td>AI-effect</td>
<td>Discussion</td>
<td>pH-limit, SEPA-report</td>
<td>pH-limit, Johnson et al. 1993</td>
<td>Reference</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>------------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------------</td>
<td>----------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Odagmia ornata</td>
<td>Lab/Jar</td>
<td>pH:4.8</td>
<td>At:0.15 C: 300-600 mg Pt/l</td>
<td>M 96h</td>
<td>Humus-rich (300-600 mg Pt/L) water counteracts Al effect. Effect of low pH will dominate.</td>
<td>Simuliidae: &lt;4.50</td>
<td>-</td>
<td>Petersen et al. 1986</td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Orthocladiinae</td>
<td>Field/Stream</td>
<td>pH:6.4-6.5</td>
<td>Al:0.15 C: 2 DOC</td>
<td>D 3-6h pulses</td>
<td>No effect, at pH 6.4</td>
<td>Fluctuating Al-levels at pH's not toxic to biota (6.4-6.5) may be an important factor regulating abundance and distribution of biota.</td>
<td>Simuliidae: &lt;4.50</td>
<td>-</td>
<td>Hall et al. 1987</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Simulium decorum</td>
<td>Lab/Jar</td>
<td>pH:4.8</td>
<td>At:0.15 C: 300-600 mg Pt/l</td>
<td>M 96h</td>
<td>Humus-rich (300-600 mg Pt/L) water counteracts Al effect. Effect of low pH will dominate.</td>
<td>Simuliidae: &lt;4.50</td>
<td>-</td>
<td>Petersen et al. 1986</td>
<td></td>
</tr>
<tr>
<td><strong>Ep</strong></td>
<td>Baetis rhodani</td>
<td>Field/Stream</td>
<td>pH:5.3-5.7</td>
<td>Al:0.01-.06 C: -</td>
<td>M D trays fed by acid resp.limed streams</td>
<td>pH &lt; 5.5 lethal, increased drift</td>
<td>Mortality lower for Baetis rhodani than for young atlantic salmon. Labile Al is indicated to be of minor importance for the invertebrate mortality.</td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>McCahon &amp; Pascoe 1989</td>
</tr>
<tr>
<td><strong>Ep</strong></td>
<td>Baetis rhodani</td>
<td>Field/Cage</td>
<td>pH:7.4-7.2</td>
<td>Al:0.04-.84 C: -</td>
<td>M D 72h</td>
<td>M 25 %, D 9x increased</td>
<td>Mortality lower for Baetis rhodani than for young atlantic salmon. Labile Al is indicated to be of minor importance for the invertebrate mortality.</td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>Merrett et al. 1991</td>
</tr>
<tr>
<td><strong>Ep</strong></td>
<td>Baetis rhodani</td>
<td>Field/Cage &amp; Stream</td>
<td>pH:7.5-7.4</td>
<td>mix:8.3 Al:0.05-.35 C: 1 TOC</td>
<td>M 24h, 60h</td>
<td>100% mortality in acidic Al-richwater but also 60% in neutral reference.</td>
<td>Designed to find adverse effect of unstable Al chemistry in freshwater mixing zones on invertebrates. Fish may be the preferred alternative when sub-lethal responses are studied - especially for short-term exposures.</td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>Kroglund M, 1998 (exp II)</td>
</tr>
<tr>
<td><strong>Ep</strong></td>
<td>Baetis rhodani</td>
<td>Field/Stream</td>
<td>pH:7.4-7.2</td>
<td>Al:0.046-.052 C: 2 DOC</td>
<td>M D 72h</td>
<td>M 100% after pretreatment with .05 Al, D x1.4</td>
<td>Possible that sublethal factors could explain the absence of this species from streams with low pH and high Al (since large Al-effect on drift and similar on mortality for pH and Al).</td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>Ormerod et al. 1987</td>
</tr>
<tr>
<td><strong>Ep</strong></td>
<td>Baetis rhodani</td>
<td>Field/Stream</td>
<td>pH:7.3-4.3</td>
<td>Al:0.046-.052 C:</td>
<td>M 24h</td>
<td>M 20% A 1200 ug/g dw (control 175)</td>
<td>Al staining showed Al over the entire exoskeleton including gill plates and the gut. This suggests that Al physically occludes the main respiratory surface, the integument, leading to an increased respiration rate and subsequent death.</td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>McCahon et al. 1987</td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type /time</td>
<td>pH-effect</td>
<td>AI-effect</td>
<td>Discussion</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ep</td>
<td>Baetis rhodani</td>
<td>Field/Stream</td>
<td>pH: 7.2, 4.9x3 Al: 0.05; 0.05; 27.0 (0.23 Alt) C: citrate i #4</td>
<td>D BD -24h pre, 24 h postepisode</td>
<td>D 6xcontrol (samples lost). No effect of Al complexed with citrate. BD sign. effect of Al treatments. BD sign. reductions of both all taxa and Baetis rhodani for both Al-treatments - but some samples lost.</td>
<td></td>
<td></td>
<td>&gt;4.50</td>
<td>Weatherley et al. 1988</td>
<td></td>
</tr>
<tr>
<td>Ep</td>
<td>Caenis sp.</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 7 Al: 0.2 C: deion. medium</td>
<td>M 24 - 48h</td>
<td>98% Al enhanced survival.</td>
<td>Possibly affected through the gills.</td>
<td></td>
<td>&gt;4.50</td>
<td>4 C. species: pH &gt;5.5, &gt;5.0</td>
<td>Havens 1993</td>
</tr>
<tr>
<td>Ep</td>
<td>Cloeon triangulifer</td>
<td>Lab/Jar</td>
<td>pH: 4, 5.5 Al: 0.1, 0.5 C: tap water Ca: 0, 10, 100</td>
<td>M D 72h</td>
<td>M 25%, pretreatment with 0.5 Al enhanced survival</td>
<td>Increased Al detrimental, elevated Ca ameliorating</td>
<td></td>
<td>&gt;4.50</td>
<td>Cloeon sp.: pH &gt;4.7</td>
<td>Tabak &amp; Gibbs 1991</td>
</tr>
<tr>
<td>Ep</td>
<td>Ecdyonurus venosus</td>
<td>Field/Stream</td>
<td>pH: 7, 4.2, 4.5 Al: 0.046, 0.052, 0.347 C: 2 DOC</td>
<td>M A 24h</td>
<td>M 5.3% incr. to 53% 48 h post exp. A 500 ug/g dw (contr 125) M 14.3% incr post exp 43% A 3175 ug/g dw</td>
<td>Al staining showed Al over the entire exoskeleton including gill plates and the gut. This suggests that Al physically occludes the main respiratory surface, the integument, leading to an increased respiration rate and subsequent death.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Ormerod et al. 1987</td>
</tr>
<tr>
<td>Ep</td>
<td>Ephemeroptera sp.</td>
<td>Field/Stream</td>
<td>pH: 7.2, 4.9x3 Al: 0.28 C: 2 DOC</td>
<td>M 3-6h pulses 72h postexposure</td>
<td>No effect, at pH 5. Greater drift when also Al levels were elevated and fluctuating.</td>
<td>Fluctuating Al-levels at pHs not toxic to biota (5.5-5.2) may be an important factor regulating abundance and distribution of biota.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Hall et al. 1987</td>
</tr>
<tr>
<td>Ep</td>
<td>Ephemera danica</td>
<td>Lab/Jar</td>
<td>pH: 4, 4.8 Al: 0.0, 0.5, 2 C: stream, synt. I: Na 10d</td>
<td>no significant difference 4:4.8</td>
<td>Increased 101 resp 100%</td>
<td>Dose-response tendency</td>
<td></td>
<td>&gt;6.00</td>
<td>&gt;5.0</td>
<td>Hermann &amp; Andersson 1986</td>
</tr>
<tr>
<td>Ep</td>
<td>Ephemera danica</td>
<td>Lab/Jar</td>
<td>pH: 4, 4.8 Al: 0.0, 0.5, 2 C: stream, synt. I: Na 14d</td>
<td>no significant difference 4:4.8</td>
<td>Larger loss of Na in synthetic water (0 DOC)</td>
<td>Greater net loss at low pH with amelioration by Al-addition</td>
<td></td>
<td>&gt;6.00</td>
<td>&gt;5.0</td>
<td>Hermann 1987</td>
</tr>
<tr>
<td>Ep</td>
<td>Ephemerella ignita</td>
<td>Field/Stream</td>
<td>pH: 7.2, 4.3x3 Al: 0.06, 0.113, 0 C: citrate i #4</td>
<td>M 24h dosing - 72h postexposure</td>
<td>M 0%</td>
<td>Complexation of Al with organic compounds (citrate) decreased its toxicity. Clearly fish are more sensitive to Al at low pH than the invertebrates. Mortalities of the invertebrates was influenced by animal origin and pretreatment/culture conditions</td>
<td></td>
<td>&gt;4.50</td>
<td>&gt;5.5, &lt;4.7</td>
<td>McCaugh &amp; Pascoe 1989</td>
</tr>
<tr>
<td>Ep</td>
<td>Ephemeroptera sp.</td>
<td>Field/Stream</td>
<td>pH: 6.6, 4.0 Al: 0.71, 0.18 C: 6 TOC</td>
<td>BD D 73d</td>
<td>Sign. smaller percent of the community in the acidified channel. Same percent of the community as in the acidified channel.</td>
<td>Acidification acted directly on the invertebrates. The addition of Al had no effect on total invertebrate density and biomass.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Allard &amp; Moreau 1986 &amp; 1987</td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type /time</td>
<td>pH-effect</td>
<td>Al-effect</td>
<td>Discussion</td>
<td>pH-limit, SEPA-report</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Epaflephemopterans</td>
<td>Field/Stream</td>
<td>pH: 4.5-6.8 Al: 0.003-197 Al</td>
<td>4 sites, 1 year, 4 quarterly samplings, chemistry monthly.</td>
<td>Density more highly correlated to pH, than to inorganic monomeric Al. Also correlated to species richness.</td>
<td>Density also correlated to Al.</td>
<td>Invertebrate density (total and of different orders) was generally more strongly correlated to pH than to inorganic monomeric Al. Differences not based on changes in food abundance.</td>
<td>-</td>
<td>Rosemond et al. 1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia fusocugrisea</td>
<td>Lab/Jar</td>
<td>pH: 4.8, Al: 0, 0.5, 2 C: stream, synt.</td>
<td>R 10d no significant difference 4.48</td>
<td>Increased 54 resp 95%</td>
<td>Mortality in low pH local transplant of Stenomena sp. &amp; Epopeorus pleuralis.</td>
<td>pH-limit, SEPA-report 4343</td>
<td>&lt;4.50</td>
<td>&lt;4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.8, Al: 0.5 - 2 C: stream, synt.</td>
<td>M 14d no significant difference 4.48</td>
<td>Larger loss of Na in synthetic water (0 DOC)</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>-</td>
<td>Hermann &amp; Andersson 1986</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.5, Al: 0, 0.5, 2 C: stream, synt.</td>
<td>R 10d no significant difference 4.48</td>
<td>Increased 59 resp 68%</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>pH-limit, SEPA-report 4343</td>
<td>&lt;4.50</td>
<td>&lt;4.7, &lt;4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.5, Al: 0, 0.2, 2 C: stream, synt.</td>
<td>M 24h, 69h</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Designed to find adverse effect of unstable Al chemistry in freshwater mixing zones on invertebrates. Equally or more sensitive to short-term exposure in mixing zone than brown trout.</td>
<td>&gt;4.50</td>
<td>Hermann 1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.5, Al: 0.5, 2 C: stream, synt.</td>
<td>R 10d no significant difference 4.48</td>
<td>Al:0.2, 0.4, 1 C: boglake water</td>
<td>Accumulation mainly in the exuvium, but also an internal accumulation occurred</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>&gt;4.50</td>
<td>Hermann &amp; Andersson 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.5, Al: 0.5, 2 C: stream, synt.</td>
<td>M 24h, 69h</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>&gt;4.50</td>
<td>Hermann &amp; Andersson 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafHeptagenia sulphurea</td>
<td>Lab/Jar</td>
<td>pH: 4.5, Al: 0, 0.2, 2 C: stream, synt.</td>
<td>M 24h, 69h</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>Mortality caused by mixing zone (20-330), caused by different Al-chemistry.</td>
<td>&gt;4.50</td>
<td>Hermann &amp; Andersson 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpafRhithrogena semicolorata</td>
<td>Field/Cage &amp; Stream</td>
<td>pH: 4.8, 7.2 Al: 0.04-84 C: -</td>
<td>M D Chronic30d Episod; 6+12+18h; tot 96h</td>
<td>M Chr: 90%, epi:25% D 8x increased</td>
<td>Mayfly species were particularly sensitive to low pH and elevated Al.</td>
<td>Mortality in low pH local transplant of Stenomena sp. &amp; Epopeorus pleuralis.</td>
<td>&lt;4.50</td>
<td>Frick &amp; Herrmann, 1990b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GaafAmnicola limosa</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 5, 5, 5 Al: 0.01, 0.025, 0.04, 1 C: 5DOC</td>
<td>LC50 96h &lt;pH3.5</td>
<td>D 8x increased</td>
<td>Toxic levels are more representative of the species than of their functional group. Not likely that Al levels in Ontario are acutely toxic to most invertebrates.</td>
<td>Toxic levels are more representative of the species than of their functional group. Not likely that Al levels in Ontario are acutely toxic to most invertebrates.</td>
<td>&lt;4.0</td>
<td>Barkley &amp; Allain 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GaafPhysella heterostropha</td>
<td>Lab/Stream</td>
<td>pH: 4.5, 7 Al: 0, 0.5 C: 0, 45 DOC</td>
<td>M 28d 40%</td>
<td>D 8x increased</td>
<td>Interactive effects between pH, Al and organic matter are important in determining susceptibility of invertebrates to acidification.</td>
<td>Interactive effects between pH, Al and organic matter are important in determining susceptibility of invertebrates to acidification.</td>
<td>-</td>
<td>Burton &amp; Allain 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GaafGyraulus sp.</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 7 Al: 0.2 C: deion. medium</td>
<td>M 24, 48h 45%</td>
<td>Al significantly increased mortality.</td>
<td>Adds to the low pH effect itself</td>
<td>Adds to the low pH effect itself</td>
<td>-</td>
<td>Havens 1993b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeafCorixa punctata</td>
<td>Lab/Jar</td>
<td>pH: 3.4 Al: 0.1-50 C: boglake water</td>
<td>I Na haemolymph conc.</td>
<td>Decrease in Na-influx by 50% with increasing Al-conc.</td>
<td>Decrease in Na-influx by 50% with increasing Al-conc.</td>
<td>Decrease in Na-influx by 50% with increasing Al-conc.</td>
<td>&lt;4.50</td>
<td>Witters et al. 1984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type /time</td>
<td>pH-effect</td>
<td>AI-effect</td>
<td>Discussion</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Agria sp.</td>
<td>Lab/Stream</td>
<td>pH: 7, 5.2, mix:8.3 Al:0.05, .35, mix 20, 330 sec. C: 1 TOC</td>
<td>M 24h, 69h</td>
<td>No mortality.</td>
<td>No mortality.</td>
<td>Designed to find adverse effect of unstable Al chemistry in freshwater mixing zones on invertebrates. Fish may be the preferred alternative when sub-lethal responses are studied - especially for short-term</td>
<td>Kroglund M, 1998 (exp II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Enallagma sp.</td>
<td>Lab/Jar</td>
<td>pH: 4.5, 7 Al:0.2 C: deion. medium</td>
<td>M 24 - 48h</td>
<td>80% Al enhanced survival.</td>
<td>Possibly affected through the caudal lamellae.</td>
<td>-</td>
<td>&gt;4.7 Havens 1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Enallagma sp.</td>
<td>Lab/Jar</td>
<td>pH:4, 4.5, 5.5 Al:0.025, .05, .1, .2, .4, 1 C: DOC</td>
<td>M LC50 96h &lt; pH3.5 &gt;1.0 at 3.5</td>
<td>Toxic levels are more representative of the species than of their functional group. Not likely that Al levels in Ontario are acutely toxic to most invertebrates.</td>
<td>-</td>
<td>&gt;4.7 Mackie 1989</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Libellula julia</td>
<td>Lab/Jar</td>
<td>pH: 2.3 Al: 30 C: bog water</td>
<td>PS: weight (wet&amp;ash), I: Na, Ca 96h</td>
<td>ash w. loss sign. loss of all measured variables</td>
<td>Na is the critical element controlling fluid secretion by the Malphigian tubes (Nicholls,1985)</td>
<td>-</td>
<td>&lt;4.50 Libellulinae: 4.7 Rockwood et al. 1988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Libellula julia</td>
<td>Lab/Jar</td>
<td>pH: 4 Al: 0.3, 3, 30 C: bog water</td>
<td>R 96h</td>
<td>resp.down</td>
<td>resp.down markedly &gt;3 mg/l Al</td>
<td>Respiration down because denatured mucus/Al precipitates. The confined rectal gill can’t be cleared as easily as mayfly-gills.</td>
<td>-</td>
<td>&lt;4.50 Libellulinae: &gt;4.7 Rockwood et al. 1990</td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Libellula julia</td>
<td>Lab/Jar</td>
<td>pH: 2.3, 4 Al: 0.3, 3, 30 C: bog water</td>
<td>PS: wet w., hemolymph v. I: Na,K,Ca,Mg 96h &amp;192h</td>
<td>waterbalance, inoac regulation, acid-bas balance</td>
<td>at high Al (30) the pH-effects became significantly affected.</td>
<td>Depressed hemolymph volumes resulted from both a loss of water and a shift from the extracellular to intracellular compartment.</td>
<td>L.sp.: &lt;4.50 Libellulinae: &gt;4.7 Rockwood et al. 1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od</td>
<td>Somatochlora cingulata</td>
<td>Lab/Jar</td>
<td>pH:3.6, 4.2, 6.75 Al:0.1, 10, 20, 30 C: -</td>
<td>R, EM 96h</td>
<td>Respiration down, restricted utilization of protein, synthesising glutamate</td>
<td>Low pH alone seems to elicit the same responses as low pH and Al</td>
<td></td>
<td>L.sp.: &lt;4.50 Libellulinae: &gt;4.7 Rockwood et al. 1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>Amphinemura sulcicollis</td>
<td>Field/Cage&amp;Stream</td>
<td>pH:4.8 - 7.2 Al:0.048, 0.064 C: -</td>
<td>M D Chronic30d Episod; 6+12+18h; tot 96h M Chr no relation to chem., epi 5%</td>
<td>M &lt;10%</td>
<td>M &lt;10%</td>
<td>There was a cumulative effect of repeated episodes.</td>
<td>Merrett et al. 1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>Brachyptera risi</td>
<td>Lab/Stream</td>
<td>pH: 4.2, 4.8, 5.4 Al:0.03, 15, .4 C: 1 TOC</td>
<td>I Na hemolymph 2h/24h</td>
<td>Al and H+ are concurrent causes to reduced Na at 2h. At 24h a dynamic adjustment shifted Na towards reference levels.</td>
<td>Al and H+ are concurrent causes to reduced Na at 2h. At 24h a dynamic adjustment shifted Na towards reference levels. But at low pH high Al seemed to counterbalance the effect of pH.</td>
<td>Designed to find principal toxic component for stable freshwater with different pH and Al levels.</td>
<td>&gt;4.50 - Kroglund M, 1998 (exp I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>Dinocras cephalotes</td>
<td>Field/Stream</td>
<td>pH:7; 4.2; 4.5 Al:0.46;0.052,347 C:2 DOC</td>
<td>M D 72h</td>
<td>M&lt; 10%</td>
<td>M&lt; 10%</td>
<td></td>
<td>&gt;5.50 &gt;5.0 Ormerod et al. 1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type / time</td>
<td>pH-effect</td>
<td>Al-effect</td>
<td>Discussion</td>
<td>pH-limit, SEPA-report</td>
<td>pH-limit</td>
<td>Reference</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>----------</td>
<td>-----------</td>
<td>------------</td>
<td>----------------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Pi</td>
<td>Nemoura sp.</td>
<td>Lab/Stream</td>
<td>pH: 4, 7; Al: 0.5; C: 0</td>
<td>M</td>
<td>28d</td>
<td>50%</td>
<td>Additive effect to pH, especially in the absence of organic material</td>
<td>High percentage of monomeric Al appeared to increase mortality of this species.</td>
<td>-4.50</td>
<td>Johnson et al. 1993</td>
</tr>
<tr>
<td>Tr</td>
<td>Arctopsyche ladogensis</td>
<td>Lab/Stream</td>
<td>pH: 5; Al: 0, 0.625, 1.25, 2.5, 5 C: 40 mg Pt/l</td>
<td>AB: dark anal papillae abnormal tracheal gills</td>
<td>96h</td>
<td>-</td>
<td>EC-50: 1.0 Al sign. increased numbers of ind. with dark-ened anal papillae</td>
<td>Labile Al highest (0.5) at 1.250 nominal Al concentration.</td>
<td>-4.50</td>
<td>Vuori et al. 1996</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche angustipennis</td>
<td>Lab/Stream</td>
<td>pH: 5; Al: 0, 0.625, 1.25, 2.5, 5 C: filt. stream</td>
<td>AB: dark anal papillae abnormal tracheal gills</td>
<td>96h</td>
<td>-</td>
<td>EC-50: 2.4 Al sign. increased numbers of ind. with dark-ened anal papillae</td>
<td>Labile Al highest (0.5) at 1.250 nominal Al concentration.</td>
<td>-4.50</td>
<td>Vuori et al. 1996</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche angustipennis</td>
<td>Field/Stream</td>
<td>pH: 7; 4.2; 4.5; Al: 0.046; 0.052; 0.347 C: 2 DOC</td>
<td>M D</td>
<td>72h</td>
<td>M&lt; 10%</td>
<td>M&lt; 10%</td>
<td>Lacks acid sensitivity - indicates that it is limited in acid streams by trophic conditions or prolonged exposures possibly during sensitive stages.</td>
<td>-4.50</td>
<td>Ormerod et al. 1987</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche instabilis</td>
<td>Field/Cage &amp; Stream</td>
<td>pH: 4.8 - 7.2; Al: 0.04 - 0.84 C: -</td>
<td>M D</td>
<td>Chronic - 30 &amp; 3 episodes; 6+12+18h; tot 96h</td>
<td>M 7%</td>
<td>-</td>
<td>Suggests that accumulation of Al significantly contributes to abnormalities. There is both an accumulation and surface adsorption of Al. Fe is mainly adsorbed on surface of larval exocuticle.</td>
<td>-4.50</td>
<td>Merrett et al. 1991</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche pellucidula</td>
<td>Field/Stream</td>
<td>pH: 5.3, 5.7; Al: 1.9, 1.4 C: 150 mg Pt/l</td>
<td>A: Al, Fe, Cu, Cd, Zn, Pb; AB: dark anal papillae</td>
<td>24h, 69h</td>
<td>Sublethal stress- lower Na, K in specimens from acid,Al-rich and mix 20 sec than in reference water. No mortality.</td>
<td>Sublethal stress- lower Na, K in specimens from acid,Al-rich and mix 20 sec than in reference water. No mortality.</td>
<td>Suggests that accumulation of Al significantly contributes to abnormalities. There is both an accumulation and surface adsorption of Al. Fe is mainly adsorbed on surface of larval exocuticle.</td>
<td>-4.50</td>
<td>Vuori et al. 1996</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche pellucidula</td>
<td>Lab/Stream</td>
<td>pH: 7; 5.2, mix: 6.5; Al: 0.05, 0.35, mix 20, 330 sec. C: 1 TOC</td>
<td>M I Na, K hemolymph</td>
<td>24h, 69h</td>
<td>Sublethal stress- lower Na, K in specimens from acid,Al-rich and mix 20 sec than in reference water.</td>
<td>Sublethal stress- lower Na, K in specimens from acid,Al-rich and mix 20 sec than in reference water. No mortality.</td>
<td>Designed to find adverse effect of unstable Al chemistry in freshwater mixing zones on invertebrates. Fish may be the preferred alternative when sub-lethal responses are studied - especially for short-term exposures.</td>
<td>-4.50</td>
<td>Kroglund M, 1998 (exp II)</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche siltalai</td>
<td>Lab/Stream</td>
<td>pH: 5; Al: 0, 0.625, 1.25, 2.5, 5 C: filt. Stream</td>
<td>AB: dark anal papillae abnormal tracheal gills</td>
<td>96h</td>
<td>-</td>
<td>EC-50: 1.4 Al sign. increased numbers of ind. with dark-ened anal papillae</td>
<td>Labile Al highest (0.5) at 1.250 nominal Al concentration.</td>
<td>-4.50</td>
<td>Vuori et al. 1996</td>
</tr>
<tr>
<td>Tr</td>
<td>Hydropsyche siltalai</td>
<td>Lab/Stream</td>
<td>pH: 7; 5.2, mix: 6.3; Al: 0.05, 0.35, mix 20, 330 sec. C: 1 TOC</td>
<td>M I Na, K hemolymph</td>
<td>24h, 69h</td>
<td>Sublethal stress- lower K in specimens from acid,Al-rich and mix 20 sec than in reference water. Mortality caused by mixing zone (20&gt;330), caused by different Al-chemistry.</td>
<td>Sublethal stress- lower K in specimens from acid,Al-rich and mix 20 sec than in reference water.</td>
<td>Designed to find adverse effect of unstable Al chemistry in freshwater mixing zones on invertebrates. Equally or more sensitive to short-term exposure in mixing zone than brown trout.</td>
<td>-4.50</td>
<td>Kroglund M, 1998 (exp II)</td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type/time</td>
<td>pH-effect</td>
<td>AI-effect</td>
<td>Discussion</td>
<td>pH-limit, SEPA-report</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>----------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Tr</td>
<td>Limnephilus sp.</td>
<td>Lab/Jar</td>
<td>pH: 4.6.75, Al: 0.3, C: -</td>
<td>R, EM 96h</td>
<td>No significant effect on respiration, increased NH4 excretion</td>
<td>No significant effect on respiration. Reduction in NH4-excretion rates comp to low pH.</td>
<td>Al seems to reduce effect of low pH.</td>
<td>4.50 &gt;4.7</td>
<td>Correa et al. 1986</td>
<td></td>
</tr>
<tr>
<td>Tr</td>
<td>Trichopterans</td>
<td>Field/Stream</td>
<td>pH: 4.5-6.8, Al: 0.03 - 0.197, C: -</td>
<td>BD 4 sites, 1 year, 4 quarterly sampling, chemistry monthly.</td>
<td>Density and species richness correlated to pH</td>
<td>Density and species richness correlated to Al.</td>
<td>Invertebrate density (total and of different orders) was generally more strongly correlated to pH than to inorganic monomeric Al. Differences not based on changes in food abundance.</td>
<td>Rosemond et al. 1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tr</td>
<td>Pycnopsyche guttifer</td>
<td>Lab/Stream</td>
<td>pH: 4, 0.5, Al: 0, 0.5, C: 0, 45 DOC</td>
<td>M 20-30%</td>
<td>Additive effect to pH, especially in the absence of organic material</td>
<td>Interactive effects between pH, Al and organic material are important in determining susceptibility of invertebrates to acidification.</td>
<td></td>
<td>Burton &amp; Allan 1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>Total Invertebrates</td>
<td>Field/Stream</td>
<td>pH: 4.9-6.2, Al: 0.07 - 0.38, C: 1-12 DOC</td>
<td>BD C 8 sites, 1 year, 4 quarterly samplings, chemistry monthly.</td>
<td>Total invertebrate density, generic richness and diversity were not sign. lower at the most acidic site. The two latter variables were however correlated to pH.</td>
<td>Ephemeroptera, collector-gatherers &amp; scrapers were correlated to pH and Al.</td>
<td>Benthic organic matter and pH were the most important environmental parameters correlated to invertebrate density and richness.</td>
<td>Smith et al. 1990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>Total Invertebrates</td>
<td>Field/Stream</td>
<td>pH: 3.5-8.1, Al: 0 est., C: 1-15 DOC</td>
<td>BD C 34 sites, 1 summer sample incl chemistry</td>
<td>No reduction in species richness above pH 4.5.</td>
<td>The lack of correlation to pH &lt; 4.5 is suggested to be caused by the total complexation of Al by humic acids present.</td>
<td>Cannot compare brown naturally acidified streams with those affected by acid rain. Functional group designations not considered appropriate to use.</td>
<td>Winterbourn &amp; Collier, 1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Type of study</td>
<td>Experiment conditions</td>
<td>Response type /time</td>
<td>pH-effect</td>
<td>Al-effect</td>
<td>Discussion</td>
<td>pH-limit, SEPA-report</td>
<td>pH-limit, Johnson et al. 1993</td>
<td>Reference</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>----------------------</td>
<td>-----------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>TI</td>
<td>Total Invertebrates</td>
<td>Field/Stream</td>
<td>pH: 4.5-6.4, Al: &lt; 0.1, C: 5-7 DOC</td>
<td>BD C</td>
<td>17 sites - 2 areas, 1 year, 4 quarterly samplings, chemistry monthly.</td>
<td>Negative effects of stream acidity on stream communities over the long term may be primarily on the composition and taxa richness than on total density or biomass.</td>
<td>Monomeric Al may be important limiting Ephemeroptera abundance in streams but less so in controlling the number of taxa. The negative effect of Al might be higher in the most recently acidified area with higher seasonal variations of Al/pH. The difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>Total Invertebrates excl Chironomidae</td>
<td>Field/Stream</td>
<td>pH: 4.5-6.8, Al: 0.003-1.97, C: -</td>
<td>BD C</td>
<td>4 sites, 1 year, 4 quarterly samplings, chemistry monthly.</td>
<td>Density and species richness correlated to pH.</td>
<td>No significant correlation to density but to total species richness for Al.</td>
<td>Invertebrate density (total and of different orders) was generally more strongly correlated to pH than to inorganic monomeric Al. Differences not based on changes in food abundance.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I got inspiration to this table from a presentation at Acid Rain conference in Japan 2000: Jan Herrmann, *Aluminium is harmful to benthic invertebrates in acidified waters,* 2001, Water, Air and Soil pollution, 130, 837-842.

The pH-limits are from these two publications, all the other references can be found in the reference list.

